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Ellis, Philip P.
Everett, William G.
Falls, Harold F.
Fink, Austin I.
Forbes, Max

Fraunfelder, Frederick T.
Frayer, William C.
Freeman, H. MacKenzie
Gass, J. Donald M.
Glew, William B.
Grayson, Merrill
Guerry III, Dupont
Hagler, William S.
Hamilton, Ralph S.
Harley, Robison D.
Hedges Jr., Thomas R.
Henderson, John Warren
Henderson, John Woodworth
Hiatt, Roger L.
Hollenhorst Sr., Robert W.
Howard, Rufus O.
Hyndiuk, Robert A.
Irvine, Alexander R.
Jarrett II, William H.
Jones, Ira S.
Kearns, Thomas P.
Kennedy, Robert E.
Knobloch, William H.
Kolker, Allan E.
Latties, Alan M.
Lawwill, Theodore
Levene, Ralph Z.
Little, Hunter L.
Lloyd, Lois A.
Macdonald Jr., Roderick
Manchester Jr., P. Thomas
McCulloch, Clement
McDonald, James E.
Meyer, Roger F.
Miranda Jr., Manuel N.
O'Connor, G. Richard
O'Rourke, James
Okun, Edward
Owens, William C.
Parks, Marshall M.
Patz, Arnall
Pico Sr., Guillermo
Pollack, Irvin P.
Regan, Ellen F.
Richards, Richard D.
Robb, Richard M.
Schultz, Richard O.
Schwartz, Ariah
Sears, Marvin L.
Shaffer, Robert N.
Sherman, Arthur E.
Small, Robert G.
Snell, Albert C.
Members

Spalter, Harold F.
Spaulding, Abbot G.
Spencer, William H.
Spivey, Bruce E.
Straatsma, Bradley R.
Streeten, Barbara W.
Swan, Kenneth C.
Taylor, Daniel M.
Thompson, H. Stanley
Troutman, Richard C.
Truhlsen, Stanley M.
Veronneau-Troutman, Suzanne
von Noorden, Gunter K.
Waller, Robert R.
Watzke, Robert C.
Weinstein, George W.
Wetzig, Paul C.
Wilson Sr., Fred M.
Wilson, R. Sloan
Wirtschafter, Jonathan D.
Wolff, Stewart M.
Wong, Vernon G.
Yanoff, Myron

HONORARY MEMBERS

Zimmerman, Lorenz E.

Members

214

Emeritus Members

115

Honorary Members

1

Total Membership

330
NECROLOGY
In Memorium

Frank D. Carroll, Elected 1943
Robert Day, Elected 1957
Edward A. Dunlop, Elected 1967
John C. Locke, Elected 1963
George R. Merriam, Elected 1956
J. Reimer Wolter, Elected 1965
Frank D. Carroll MD was born March 4, 1907, in New Bedford, Massachusetts to John and Mary Carroll, both originally from Kilkenny County, Ireland. A natural athlete, he became a competitive tennis player while attending the New Bedford High School. At Harvard College, he rowed a single scull, ran track, played tennis, and completed his bachelor’s in three years by attending summer school. During summers at Yale Medical School, he helped pay for his education by working as a tennis pro at a private club in Rye, New York, where he later settled. After graduating from Yale in 1932, he interned at the Rhode Island Hospital in Providence, R.I., and served as resident in ophthalmology at the Massachusetts Eye and Ear Infirmary from 1933-35. In January 1935, he accepted an invitation from John D. Wheeler, the first director of the Edward S. Harkness Eye Institute at Columbia, to become a research fellow.

During residency, Frank had become interested in “tobacco-alcohol amblyopia.” At the Eye Institute, he hospitalized patients with this condition, permitting their customary amounts of both alcohol and tobacco, and supplemented their usually deficient diet with vitamin B complex. His patients recovered as well as or better than patients who simply abstained from alcohol and tobacco, and he summarized his clinical research in his 1943 AOS thesis, “The Etiology and Treatment of Tobacco-Alcohol Amblyopia.” In the era before formal sub-specialization, this work helped to define his reputation as an authority on disorders of the optic nerve. During his long association with the Institute, he helped to define the visual manifestations of digitalis toxicity, the manifestations of optic neuritis in adults and children, the hereditary optic neuropathies, ischemic optic neuropathy following cataract extraction, the use of corticosteroids in thyroid optic neuropathy, and the occurrence of centrocecal scotomas in glaucoma. Dr. Carroll created the Optic Nerve Clinic, the forerunner of the neuro-ophthalmology service of today. He belonged to a legendary generation of astute clinicians who could work with simple tools and never miss a diagnosis. In particular, he was a master at eliciting and mapping subtle central scotomas at the tangent screen. A warm and dignified man, he taught the art of perimetry to generations of residents.

In addition to teaching and seeing patients at the
Institute, Dr. Carroll maintained a busy private office in Rye, New York. For 28 years, he was in charge of the Eye Department of the United Hospital in Port Chester, New York, where he supervised a free eye clinic. When he retired from active clinical practice in 1990, Dr. Carroll was Clinical Professor Emeritus of Ophthalmology at Columbia University.

A devoted member of the AOS, Dr. Carroll served on the Committee on Theses during 1964-66. He was also a founder and the first President of the New York State Ophthalmological Society. During his long clinical career, he continued to enjoy tennis, as well as sailing and golf. He died peacefully at his home in Rye on July 24, 2002.

His wife, Shirley Ann, a daughter, Anne Claire Furman of Albany, New York, a son, Edward John Carroll of Rye, New York, and three grandchildren survive Dr. Carroll.
Robert Day was born on October 4, 1915, in Washington D.C. He received his education at Hotchkiss and Harvard and his medical degree from Johns Hopkins in 1943. He interned on the Osler service and then served in a medical unit of the Salt Lake County Hospital of the University of Utah Medical School. In 1944, he joined the Navy, serving as a medical officer in the Pacific and at Charleston Naval Base. He returned to Hopkins in 1946 to enter the ophthalmology residency at the Wilmer Eye Institute where he became chief resident for 1950-51 after a year of research with Dr. Jonas Friedenwald.

Bob enjoyed golf and reminisced while playing with friends about the influence of his mentors: Dr. Allan Woods, his chief at Wilmer; Dr. Max Weintrobe, a co-worker in Salt Lake; and Dr. Friedenwald. Though successful in his research on experimental histoplasmosis and on artificial corneal implants, his professional interests were to be most satisfied by clinical practice and teaching.

He entered the Washington practice of Dr. John Burke and Dr. Harold Downey and became a highly respected teacher to residents and medical students at the Old Episcopal Eye Hospital, the Washington Hospital Center, and George Washington Medical School. A revered doctor to thousands of his patients, he was awarded the Gold-Headed Cane Award as the outstanding physician at Washington Hospital Center in 1983.

He was elected Chairman of the Eye Department at WHC for the years 1968-70, and subsequently served four years as chairman of Training and Education, during which he wrote a much-used pocket manual for eye residents. As chief of the cornea clinic at Episcopal and at the Hospital Center, he performed many transplants. He also served as consultant to the National Institutes of Health.

Bob had a fine bass voice and was active in the Cathedral Choir, the Washington choral society, and the Augmented Eight informal singing group. He was an enthusiastic member of the University Club, the Middleburg Tennis Club, the Bethesda Country Club, and the Middleburg Players. His memberships included the Osler Society, the American Academy of Ophthalmology, and the American Board of Ophthalmology, where he served as an examiner.
Necrology

He relished sharing his sense of humor and conversing with the wide spectrum of patients who sought his care at the historic Blaine Mansion at 2000 Massachusetts Avenue. When he died at 88 years of age after a brief illness in October 2003, he had practiced ophthalmology for 52 years and had continued to see patients several days a week until August of that year.

He is survived by his wife Paula Peak Day of Arlington as well as four children from his first marriage to Joan Ellis Godin, John Anthony Day of Chapel Hill, N.C., Robert Barton Day of McLean, Francis Bigelow Day of Arlington, Deborah Day of Charlotte—and seven grandchildren.
Edward Dunlap was born November 11, 1911, in McKeesport, Pennsylvania. He died March 27, 2004, in New Wilmington, Pennsylvania in a retirement community near his beloved alma mater, Westminster College. He and his two sisters were raised by their mother, Maud Dunlap, who as a single parent struggled to support her children.

In 1932, during the depths of the Great Depression, Dr. Dunlap graduated from Westminster and moved to Western Reserve Medical School, graduating in 1935. Following an internship at Gorgas Hospital in the Canal Zone, Panama that began his specialization in EENT, he became John M. McLean’s first resident at New York Hospital, Cornell Medical Center. At this time, McLean was the youngest chief of an ophthalmology department in the United States. But Ed’s residency was interrupted by Pearl Harbor and the United States’ entry into World War II. On December 31, 1941, he enlisted in the US Army and returned to Gorgas Hospital as an Army physician. He returned to New York Hospital when he finished his tour of duty and completed his residency in 1946. McLean appointed him as the second geographic faculty member of the Eye Division in 1947 and Ed remained at Cornell until he retired in 1972.

Although a very competent ophthalmologist in all areas, Ed’s real talent was treating strabismus. In particular, his clinical skills found their most artful expression taking care of children. He loved the children; he was completely at ease with them and they with him. He authored 69 publications on strabismic topics, culminating in his use of Supramid™ sleeves and caps in strabismus surgery, which was the subject of his AOS thesis published in the Transactions in 1967. He was justifiably proud of this contribution, having pioneered the topic from selection of the proper plastic material and its dimensions to the animal work proving its tolerance in animals, and finally, its introduction into clinical practice as a solution to scarring following multiple strabismus operations. His good friend Phillip Knapp used the material and was its most ardent advocate in selected cases.

Following John McLean’s death in 1968, Ed became acting chief of the Ophthalmology Division. He made a number of faculty appointments critical to maintaining the viability of the division and invested extraordinary time and effort in the Alumni Association. He sustained the endowment of the John McLean Professorship and
Chair and thus preserved the McLean legacy at Cornell for the future. Both were tasks for which he got little credit, but as was always the case with him, it made no difference if the task was well done.

During his retirement years Ed became an avid rock and seashell collector. He became a self-taught expert in the preparation, polishing, preserving and mounting of these artifacts in suitable frames. His quiet sense of elegance and beauty were reflected in these collections. Many of us possess examples of his skills in both these hobbies.

Though Ed had no children, he was happily married to his second wife, Mary Elizabeth Maoney, until her death in 1999. In many ways, Ed’s children were the residents under his tutelage. John McLean was an austere and, to many, a remote individual, although a wonderful companion when he overcame his innate shyness. Ed was the counterpoint to McLean’s persona. He became friend and confident to many residents who trained under him. He taught ocular motility, always an arcane and difficult subject, and he taught it well. Ed’s special contribution was teaching the “art” of our profession, the art he exemplified in the way he cared for children.

Ed was a close and precious friend to those of us privileged to know him. One could not help but admire his intellectual honesty and courage in holding to his convictions. He was somebody you could trust in the small and great needs of life. All of us have lost a wonderful human being. We learned much from him, and we will never forget him.
JOHN CRAIG (JACK) LOCKE MD
BY Froncie Gutman MD

John Craig (Jack) Locke MD, a member of the American Ophthalmological Society since 1963, died October 30, 2003, in Naples, Florida.

Dr. Locke was born December 7, 1918, in Winnipeg, Manitoba, Canada and moved to Montreal at the age of 5. After attending Selwyn House (Montreal) and Upper Canada College (Toronto) on full scholarships, Dr. Locke received his medical degree from McGill University. He immediately entered active military service as a lieutenant in the Royal Canadian Army Medical Corps. After his military service in 1947, he began his residency training at Columbia Presbyterian Eye Institute in New York City and subsequently was awarded the degree of Doctor of Medical Science for postgraduate research in bacteriology and retinopathy of prematurity.

In 1952, Dr. Locke returned to Canada and established an ophthalmological practice at the Royal Victoria Hospital in Montreal, where he continued his studies on retinopathy of prematurity. In 1956, he was presented with the Annual Medal in Medicine by the Royal College of Physicians of Canada for his research contributions and appointed Ophthalmologist-in-Chief and Chairman of the Ophthalmology Department at McGill University. He served as chairman for 14 years.

His honors, appointments, and offices included chairman of the Examining Board for Certification in Ophthalmology; member of The Club Jules Gonin, president of the Montreal Ophthalmological Society, and honorary member of the Chilean Ophthalmological Society.

Dr. Locke retired from practice in 1990 and enjoyed his hobbies of travel, golf, and duplicate bridge competition. He was known as a compassionate physician who was deeply religious with a love for learning and a wonderful sense of humor. Dr. Locke is survived by his wife, Frances Duncan Locke; his daughter, Barbara; and his stepchildren, John, Gaylen, Gordon, Jennifer and Jamie.
George R. ("Bud") Merriam, Jr. died January 13, 2004, at Columbia University Medical Center in Manhattan, where he had worked from 1937 until his retirement in 1993. He was 90.

Dr. Merriam was born in Harrisburg, Pennsylvania, on May 22, 1913, the son of the Reverend George R. Merriam, a Baptist minister who devoted much of his life to the YMCA. His mother, Harriet Lombard, died in the 1918 influenza pandemic, and Bud spent much of his childhood with loving aunts until his father remarried. He finished high school in Melrose, Massachusetts, where he was quarterback of the football team, then attended Brown University in Providence. There, he met his future wife, Martha Carlson, excelled at lacrosse, and studied economics. After graduation in 1934, he worked for Montgomery Ward and the Bemis Corporation before deciding on a career in medicine and enrolling at Columbia’s College of Physicians and Surgeons. In 1936, Bud and Marty were married by his father. Bud began a surgical internship at Lenox Hill Hospital after graduating from Columbia in 1941 and he was in the OR when news of the attack on Pearl Harbor arrived. Physicians and surgeons from Lenox Hill became the Army’s 12th Evacuation Hospital, based in England to care for the Air Force. The unit landed in Normandy one week after D-Day, served at the Battle of the Bulge, and followed Patton into Germany. When the Army discharged him, he was a Captain.

He studied radiation therapy and head and neck oncology at Memorial Hospital before beginning his residency at the Institute of Ophthalmology in 1946. Afterward, he worked with the institute’s director, John Dunington, for six months before opening his own practice.

At the time, the Eye Institute had an active Tumor Clinic, established by Algernon Reese. Bud ran the Radiotherapy unit, located in the basement of the Institute, and tried to refine radiotherapy protocols for children with retinoblastoma, as well as for patients with other benign and malignant disorders, to minimize complications. Even modest doses led to cataract, and the biology of radiation cataract became a lifelong interest. Using clinical data from the radiotherapy clinic, he helped to establish the radiation sensitivity

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of the lens, work which he summarized in his 1956 AOS thesis, “A Clinical Study of Radiation Cataracts.”

With collaborators from medical physics and cell biology, Bud complemented his clinical observations with experimental studies in animals. The launch of the U.S. manned space program led to concerns over the potential health effects of exposure to radiation during space travel outside the protection of the Earth’s atmosphere. In 1964, Bud became a consultant to the Space Sciences Board of the National Academy of Sciences and to NASA’s Radiobiological Advisory Panel and focused his research on the effects of space radiation. To study these types of radiation, he and colleagues used the Brookhaven National Laboratory on Long Island and later, the Lawrence Radiation Laboratory in Berkeley, California. Bud considered himself first and foremost a clinician and maintained an active medical and surgical practice with a special interest in cataract and plastic surgery. His patients were attracted by his optimism and warmth, and he made each day easier for his patients and for himself by having fun with them.

During his long tenure at the Institute, he served as Chief of the Ophthalmology Clinic, Director of the Surgical Service, and member of the Board of Surgeons while also consulted at the Memorial Hospital and the Harlem Hospital. When the New York-Presbyterian Hospital opened an outpatient facility in midtown Manhattan, the clinic devoted to ophthalmology was named the “George R. Merriam, Jr., M.D., Clinical Research Center.” Bud was a member of many professional organizations, including the American Academy of Ophthalmology, the American College of Surgeons, the American Radium Society, the New York Ophthalmological Society, the New York Academy of Medicine, and the Association for Research in Vision and Ophthalmology.

Bud’s father and grandfather were both ministers, but his grandmother was the daughter of the captain of a sailing ship that traded between Boston and Australia. From her, he may have inherited his love of sailing and the sea. He mastered celestial navigation, and he participated in offshore sailing cruises and races, including several Newport-to-Bermuda races. He belonged to several yachting organizations, including the New York Yacht Club, the Corinthians, and the United States Power Squadron.

He retired from clinical practice one month after his 80th birthday. He remained active in the P&S Alumni Association and learned that he had been awarded the medical school’s Gold Medal for Meritorious Service one month before his death. Dr. Merriam is survived by his wife, Martha; by two sons, George III, an endocrinologist at the University of Washington in Seattle, and John, who joined him in practice at Columbia; by two daughters, Charlotte Cole, a teacher, and Susan; and by seven grandchildren.
J. Reimer Wolter MD, Professor Emeritus in the Department of Ophthalmology and Visual Sciences at the University of Michigan, died May 30, 2003, in Ann Arbor. He was 79. He was elected to the AOS in 1965.

Dr. Wolter was born in Halstenbeck, Germany in 1924. His formal education was obtained in Hamburg, culminating in his medical degree in 1949 and further training as an ophthalmologist at the University of Hamburg. In 1952, Dr. Wolter married Lieselotte Schlote, a physical therapist, and the next year, the two immigrated to the United States after he accepted a research appointment at the University of Michigan. After two years, the Wolters returned to Germany, but quickly decided life in Ann Arbor was more to their liking. Dr. Wolter then returned to Michigan in 1956 with a primary faculty appointment in Ophthalmology and a joint appointment in Pathology. Dr. and Mrs. Wolter became U.S. citizens in 1959 and Dr. Wolter rose quickly through the academic ranks, becoming a full professor in 1964.

Dr. Wolter served the University of Michigan in many capacities. Foremost was his founding of the Ophthalmic Pathology Laboratory, which he directed for more than 30 years. In this role, Dr. Wolter not only provided histopathologic patient services, but he taught ophthalmic pathology to the residents and conducted a pathology research laboratory where he welcomed medical students and residents who wanted a research experience. Many of these projects resulted in publications for the students and residents, and many medical students chose ophthalmology as their specialty due to the positive experience in Dr. Wolter’s laboratory.

In addition to his work in ophthalmic pathology, Dr. Wolter was Chief of Ophthalmology at the Ann Arbor Veterans Administration Hospital from 1962 to 1987 where he helped train the residents during their rotations there. Nearly 150 former residents owe their early cataract surgical training to Dr. Wolter.

During his long and distinguished career, Dr. Wolter was always willing to fill a gap in the clinical needs of the Department of Ophthalmology. For example, before ophthalmology subspecialty training became common, Dr. Wolter was the expert in retinal and laser surgery. He was
Necrology

proud to have been with Professor Gerd Meyer Schwickerath in Germany during development of the Xenon laser. Later, when there was need for additional expertise in orbital surgery, Dr. Wolter assumed responsibility for this clinical demand and coupled this interest nicely with his ophthalmic pathology pursuits. Surgical specimens obtained during orbital surgery were assiduously studied for their histopathologic characteristics, and many of Dr. Wolter’s scientific publications reflected this dual interest. Perhaps Dr. Wolter’s most important clinical interest was in general ophthalmology, however, and he was always willing to see patients who did not necessarily have a referring physician but who wanted to be cared for at the university.

A keen sense of observation and a curious mind led Dr. Wolter to make a number of important and very basic clinicohistopathologic correlations. He became an expert in silver staining, relating many of his early papers to this technique. For example, he was the first to demonstrate that a cotton-wool spot represented ganglion cell necrosis and not an exudate, as was commonly believed at the time. He had a long-standing interest in ocular neuroanatomy and in neuropathology. In his later career, Dr. Wolter became interested in the histopathology of intraocular lenses and published a number of papers on the subject. He had nearly 300 scientific articles to his credit and contributed book chapters as well. He had an interest in pediatric ophthalmology and was the first editor of the Journal of Pediatric Ophthalmology and Strabismus, serving from 1967 to 1981.

Dr. Wolter’s interests beyond medicine included ice-skating, sailing, windsurfing, rowing, and oil painting. He was preceded in death by his wife, Lotte, and is survived by his daughter, Maria, and by his three sons, Klaus (spouse Deborah), Jan (spouse Valerie Mates), and Niels, as well as by five grandchildren. His family has lost a devoted and loving father and grandfather. Ophthalmology has lost a renaissance ophthalmologist and ophthalmic pathologist who contributed important fundamental knowledge to our field that continues to stand the test of time.
MINUTES OF THE PROCEEDINGS
The ONE HUNDRED AND FORTIETH ANNUAL MEETING of the American Ophthalmological Society (AOS) was held at The Homestead in Hot Springs, Virginia on May 23-26, 2004. President Froncie A. Gutman called the opening session to order on Monday, May 24. The program began with a symposium on Targeted Drug Delivery in Ophthalmology, as follows:

Symposium: Targeted Drug Delivery in Ophthalmology
1. “New Ways to Achieve Therapeutic Drug Levels in the Vitreous and Retina,” Henry F. Edelhauser PhD
4. “Variables in Transscleral Drug Delivery,” Timothy Olsen MD
5. “Cytoskeletal, Cell Adhesion, and Cell Contractility in the Trabecular Meshwork as Targets for Glaucoma Therapy,” Paul L. Kaufman MD

The meeting was continued with the following scientific program:
1. “Chemoreduction for Retinoblastoma. Analysis of Tumor Control and Risks for Recurrence in 457 Tumors,” Carol L. Shields MD, Arman Mashayekhi MD, Abdallah Shelil, Jacqueline Cater PhD, Anna T. Meadows MD, Jerry A. Shields MD
3. “Corneal Endothelium and Postoperative Outcomes Fifteen Years After Penetrating Keratoplasty,” Sanjay V. Patel BMBS, David O. Hodge MS, William M. Bourne MD

Executive Session, May 18

President Froncie A. Gutman called the Annual Executive Session of the American Ophthalmological Society to order at 11:30 AM. He appointed Dr Edward Raab to be the Parliamentarian.

A motion to approve the minutes of the Executive Session, which were published in Volume CI of the

TRANSACTIONS OF THE AMERICAN OPHTHALMOLOGICAL SOCIETY, were made, seconded, and approved.

The following reports were submitted and approved.

Secretary-Treasurer Report

CHARLES P. WILKINSON MD. I am happy to report that the Society is in a much healthier financial position than was the case one year ago. This is primarily due to improvements in the market and the influence of the Knapp Fund.

In 1997, the Knapp Fund became a supporting organization of the AOS. In the past few years the Knapp Fund has continued to fund second-year fellows. More recently, the Fund partnered with RPB to provide funding for a pathology fellowship training program. Unfortunately this latter effort did not bare appropriate fruit.

At their last meeting, the Board of Trustees of the Knapp Fund made several suggestions of great importance to our Society:

• A recommendation to fund the Annual Symposium at this meeting. This is to be known as the “AOS-Knapp Symposium”.
• To provide support for the printing of the hard copy of the 2004 Transactions.
• The Board voted to amend the Knapp Bylaws. This essentially turned over control of the Fund to the Council with administrative support to be provided by the administrative offices of the AOS. Beginning January 1, 2005, the Council members will replace the Knapp Board of Trustee members.

At its meeting yesterday, the Council agreed to fund both an annual AOS-Knapp Symposium and also the printing of a hard copy of the Transactions in the years ahead, assuming that investments and our financial status remain sound.

The Council acknowledges and sincerely thanks the members of the Knapp Board of Trustees for all of their efforts. And in particular, the Council thanks the tireless Secretary-Treasurer of the Knapp Trust Fund, our current President, Froncie Gutman.

Finally, the Council voted to have annual dues for Active members remain unchanged.

Editor’s Report

THOMAS J. LIESEGANG MD. The authors and discus-
sants were all relatively diligent in their participation in the publishing process with the 2003 TRANSACTIONS being printed one month late.

The challenges this past year related to an initiative to restrict the TRANSACTIONS to a web-based journal with improved online graphics and appearance of the papers, theses, proceedings, and figures. The Instructions for Authors and the templates were reformatted so that the TRANSACTIONS contents would be presented in an XML environment with all the benefits that an online journal can offer, including MEDLINE links, high-quality digital images, hyperlinked references, and cleaner appearance. An exclusive digital environment was envisioned for text and figure transmission between authors, editorial office, copyeditors, publisher, and, finally, the website. There is a different formatting and flow process between preparation for a digital environment online and preparation for the print medium. Late into the process this year, the AOS Council was able to allocate financial resources and decided to continue the print journal. Because it is not financially viable to support preparation of both a complete XML digital environment on the web and a print version, the Author Instructions were again rewritten in response to continuation of the print issue. The online TRANSACTIONS for 2003 were in a Portable Document Files (PDF) format, as in the prior three years, and will be the same in 2004. The papers, theses, abstracts, discussion, poster abstracts, and reports are now being submitted and transferred in a total digital environment with no hard copy required, except for the theses committee review.

The 2003 TRANSACTIONS OF THE AMERICAN OPHTHALMOLOGICAL SOCIETY, Volume CI, was mailed in February 2004. The TRANSACTIONS included the Minutes of the Proceedings of the Executive Session, 7 obituaries, 26 papers, 6 poster abstracts, and 10 theses. A Verhoeff Lecture delivered by Thaddeus Dryja and a Symposium on Ocular Genetics were presented during the 2003 Annual Meeting but there were no resulting submitted papers for inclusion in the TRANSACTIONS. The print issue was 524 pages (compared to 438 the prior year) with many high quality illustrations and tables.

The Proceedings of the meeting, including the spontaneous secondary discussions and responses of the presenter for each paper delivered at the Annual Meeting, and the Banquet comments are recorded on audiotapes and transcribed. These are edited to shorten and clarify readability. Each discussant and presenter is given the opportunity to further clarify the exchange prior to publication. The final product presents a more concise and scientific TRANSACTIONS.

In addition to the print volume, the papers, theses, and poster abstracts from the 2003 TRANSACTIONS are available as an accessible referral source for the public on the AOS website in a PDF format. The entire TRANSACTIONS is also available under the members section of the AOS website in a PDF version just as it appears in the print version. The web availability has enhanced our visibility to the medical and scientific communities; as Editor, I receive several requests for reprints and for copyright permission throughout the year.

Enhancements for the TRANSACTIONS this year include a simplification of the author byline to denote the AOS member and the presenter, a simplification of the city of the author, and a standardization of the listing of authors in the Program Abstracts and Proceedings. In this new digital environment, figures were requested to be submitted in very specific format as listed in the Author Instructions in order to ensure the best print quality. Unfortunately it has been my experience that authors are not aware of the many nuances of digital formatting (95% submitted incorrectly), so it will be necessary to continue to require standard 5x7 glossy prints for each figure in order to keep the TAOS on schedule.

The new set of Instructions for Authors of Papers, Instructions for Authors of Theses, and Instructions for Authors of primary discussions, and Instructions for Authors of Poster Abstracts were formulated this year and are on the AOS website. The Instructions now cover a number of policies, including deadlines, authorship criteria, and submission rules for theses or papers to another journal. The TRANSACTIONS permits papers or theses to be formatted for another journal provided it is published between the Annual Meeting in May and the publication of the TRANSACTIONS in December. The individual Editors of the journals must also understand and accept these special nuances. The specific copyright issue related to this policy is outlined in the Instructions. Last year at least four of the TAOS manuscripts were also published in toto or part in another Journal.

Report of the Program Committee

ROBERT RITCH MD. The four members of the Program Committee for the 2004 Annual Meeting were Drs Douglas D. Koch, Robert Ritch, Kent W. Small, and C. Gail Summers.

A total of 35 abstracts were submitted. Three were withdrawn because of scheduling conflicts with other meetings. Each abstract was reviewed independently by the four members of the Program Committee and assigned a grade from 1 to 5, with 1 being the highest and 5 the lowest. Because of the addition of the poster session to the program, all of the abstracts submitted were accepted: 21 for paper presentations and 11 for poster presentations. Of the poster presentation acceptances, all authors but one assented to participate in the program.
Because poster presenters feel that presenting a poster does not have the same cachet as presenting a paper, the Program Committee has suggested steps which would equalize poster and paper presentations.

Because the amount of information that can be presented on a poster is more limited than can what be presented in a paper, the Program Committee is experimenting with allowing laptop presentations of supplemental material along with the poster. This allows more clinical and histological photographs and even videos to be shown in conjunction with the poster.

At the 2004 meeting, there were four new member paper presenters: Drs. Chi-Chao Chan, Daniel Schwartz, John Thompson and Marco Zarbin. There were two new member poster presenters: Drs. M. Gilbert Grand and Christopher Rapuano, and three new member paper discussants: Drs. Richard Abbott, Gilbert Grand, and Christopher Rapuano. There were two non-member paper presenters by invitation and 15 member paper presenters in addition to the new members for a total of 19 member paper presenters.

The Program Committee would like to thank the members and guests for their help and assistance in developing the scientific program of the annual meeting. I would like to extend my personal thanks to Drs. Douglas Koch, Kent Small and C. Gail Summers for their aid in organizing the scientific meeting and their help in inviting the primary discussants. I would also like to thank Lisa Brown, the AOS Meeting Manager, for her great efforts on our behalf.

Report of the Committee on Theses

JOEL MINDEL MD. The Committee on Theses consisted of Joel Mindel as Chairman, James Bobrow and Richard Parrish. The Theses Committee was operating under a new set of guidelines, which required that no thesis be rejected. The thesis would either be accepted or returned for revision.

Twenty-five theses were submitted and reviewed by each of the committee members. Each thesis had a written evaluation by each committee member, and each thesis was verbally evaluated during a conference call. There was ultimately unanimous agreement about each thesis evaluation.

Seven theses were accepted and 18 were returned for revision.

The number of theses has increased dramatically over the years. Two years ago, there were 11 theses submitted, last year there were 21, and this year 25. The new policy of no rejections will further increase the number reviewed each year. It was suggested that the deadline date for submission of theses be made earlier to allow more time for their evaluation.

Report of the Membership Committee

LEE M. JAMPOL, MD The Committee on Membership is a relatively new committee. You may not know exactly what it does, but I can summarize our charge by saying our job is to ensure the present and future leaders of American ophthalmology join and participate in the Society.

I want to recognize the only previous chair of this committee, Mel Rubin, who was instrumental in bringing this committee into existence. The other members of the committee at the present time, besides me, are Mark Mannis, Jane Kivlin and Julia Haller.

We want to have the present and future leaders apply to this Society, so we have developed criteria for being the present and future leaders. That was submitted to the Council, which is fine-tuning it. I expect that you will receive a final copy of that relatively soon. If you have any questions, I’m sure my committee or the Council would be glad to address them. We really need to define, using those criteria, who we want as members of the Society. Once we have those criteria, we want to spread the word about the AOS, that it is worthy of participation and that people should be joining and participating. We informally do that by talking to people. As a result the number of theses submitted has increased over the last three years. This is a reflection of the actions of this committee.

Once the people are nominated for the Society, then the applications are submitted to the membership committee, which evaluates those applications and recommends to the Council who we feel should submit theses to the Society. You’ve already heard that the theses are not currently rejected, so the deliberations of the membership committee in advising the Council are crucial in setting the bar as to who probably will get into the Society. For the year 2003, there were 29 nominations, and there were 26 final applications received. The committee reviewed those and gave our recommendations to the Council, which, in general, accepted these recommendations. Twenty four people were invited to submit theses.

In 2004 so far, there are 22 nominations that I’m aware of and those applications will now be solicited and the committee will review them in the coming months and give a recommendation to the Council at its fall meeting. I have very much enjoyed participating in this committee and I would like to thank Lisa Brown for the great support she has given the committee.

Report of the Archivist/Photographer

RALPH C. EAGLE JR, MD More than 350 photos were
taken at the 2003 meeting of the American Ophthalmological Society at the Four Seasons Biltmore in Santa Barbara, California. Eleven were included in the 2003 volume of the Transactions of the AOS. These included photos of President Marilyn Miller and her spouse Dr. Ron Fishman, Howe Medalist Alfred Sommer, Verhoeff Lecturer Thaddeus P. Dryja and other participants in the Ocular Genetics Symposium, and group photographs of the Council and new members. All photos at the 2003 meeting were taken with a Nikon D100 SLR digital camera and archived on CD-ROM. Selected prints were prepared from the digital images and distributed to new members, officers and selected members. The photos for the TAOS were cropped and converted to black and white using Adobe Photoshop and submitted in digital format. In prior years photos were taken using 35 mm print film and the negatives were commercially digitized. The Society’s photo archives now contain more than 1800 digitized images.

Last year I prepared Microsoft Powerpoint slide shows for each meeting from 1996 to 2002. CD ROMs containing all seven of these archival slide shows were distributed in Santa Barbara. An updated CD including the 2003 meeting has been prepared. A limited number of CD’s were made available at the Registration desk.

Report of the Emeritus Committee

ROBERT WALLER MD. The American Ophthalmological Society has an Emeritus membership of 101 members. I am sorry to report the deaths of five of our members since the last meeting of the Society:

<table>
<thead>
<tr>
<th>Name</th>
<th>Residence</th>
<th>Year Inducted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Robert Day</td>
<td>Washington, DC</td>
<td>Joined 1957</td>
</tr>
<tr>
<td>Edward A. Dunlap</td>
<td>New Wilmington, PA</td>
<td>Joined 1967</td>
</tr>
<tr>
<td>John C. Locke</td>
<td>Quebec, Canada</td>
<td>Joined 1963</td>
</tr>
<tr>
<td>George R. Merrian, Jr</td>
<td>Tenafly, NJ</td>
<td>Joined 1956</td>
</tr>
<tr>
<td>J. Reimer Wolter</td>
<td>Ann Arbor, MI</td>
<td>Joined 1965</td>
</tr>
</tbody>
</table>

In accordance with our constitution, any Active Member who has been a member for 25 years, or is 70 years of age, or has completely retired from active practice or from gainful occupation, may upon written request become an Emeritus Member of the Society. Such request is subject to the recommendation of the Council and the affirmative vote of three quarters of the members present at the Executive Session of an Annual Meeting.

New applicants for Emeritus membership this year are:

Max Forbes
Frederick T. Fraunfelder

Hal Freeman
Allan Kolker
Alan M. Laties
Jonathan Wirtschafter

With the addition of these members, the Society will now have 107 Emeritus Members.

The Emeritus Members and guests will have their luncheon on Tuesday, May 25, 2004, from 12:00 to 2:00PM in the Dominion Room of The Homestead. Hot Springs, Virginia.

Report of the Representative to the Pan American Association of Ophthalmology

SUZANNE VÉRONNEAU-TROUTMAN MD. Since the detailed report on the March 2003 PAAO meeting in Puerto Rico, the XIII Pan-American Regional Course in Ophthalmology took place March 11-13, 2004 in Managua, Nicaragua. One-hundred and eighty-three ophthalmologists from 8 countries attended. Additional regional courses are scheduled to take place in June 2004 in Santo Domingo, Dominican Republic, and in August 2004 in La Paz, Bolivia.

During 2003-2004, The Pan-American Foundation awarded $55,000 to fund 15 Fellowships. Alice McPherson, MD, of Houston, Texas, chairs the Fellowships Committee, which has disbursed to date approximately $765,000 to 127 candidates and that continues its support of educational and cultural exchanges throughout the Western hemisphere.

Two new scholarships were announced: The Tim and Judith Sear Scholarship Award for $4,000, for a Latin American ophthalmologist to participate in advanced training and the David and Julianne Pyott Scholarship Award, for $20,000, for a Latin American ophthalmologist to study in North America for a year.

During 2003, The Visiting Professors Program, the longest running PAAO educational program, sent 8 visiting professors to four countries.

Also during 2003, the Pan-American Council of University Professors of Ophthalmology (PACUPO), under the leadership of Juan Verdaguer, MD, of Santiago, Chile, began a new program where training institutions in North and Latin America will exchange junior faculty for one week. The purpose of this program is to unite and standardize university training programs throughout Latin America.

Full preparations are underway for the XXV Pan-American Congress, under the presidency of AOS member Bronwyn Bateman, MD, of Denver, Colorado that will take place March 18-21, 2005 in Santiago, Chile.
four named lecturers have been announced: Griddle Lectures - Engenio Maul, MD, Santiago, Chile; Pan-American Lecture - Rubens Belfort Jr., MD, Sao Paulo, Brazil; AJO Lecture - Bruce E. Spivey, MD, San Francisco, CA; Academia Ophthalmologica Internationalis Lecture - William Tasman, MD, Philadelphia, PA.

**Report of the Representative to the American College of Surgeons Board of Governors**

BARRETT G. HAIR MD FACS. The Advisory Council for Ophthalmic Surgery of the American College of Surgeons (ACS) is chaired by Dr. Barrett G. Haik and includes Council representatives Drs. Mark P. Hatton, James W. Karesh, Arysol S. Niffenegger, A. Raymond Pilkerton, Jr., David T. Tse, Robert P. Tucker III, and regent William H. Coles. As of January 1, 2004, ophthalmic surgeons constituted 6 percent of the active membership of the ACS.

At the October 2003 Board of Governors Annual Meeting, the ACS Committee on Physician Competency and Health announced that it will proceed with a pilot study in the area of competency relating to practice-based learning and improvement. The Committee will also work with the Office of Evidence-based Surgery to facilitate means of individual practice evaluation and the development of supportive educational programs focusing on these areas.

The ACS Committee on Blood-borne Infection and Environmental Risk completed two manuscripts, one on the biological agents of terrorism and one on the chemical agents of terrorism. The Committee also recommended that the Division of Education develop an educational module on disaster planning and weapons of mass destruction that could be made available to fellows via the ACS Web site and proposed that weapons of mass destruction and mass casualty training be encouraged in surgical residency training.

The Chair of the Committee on Socioeconomic Issues reviewed the activity of the Political Action Committee (PAC) of the ACS and strongly urged the Governors to become involved with the PAC.

At its adjourned meeting on October 22, 2003, the Board of Governors approved a formal procedure for nominating ACS representatives to the American Medical Association House of Delegates to give the growing number of Fellows interested in serving this capacity an opportunity to do so.

At its June 2003 meeting, the ACS Board of Regents approved the College’s continued participation in an effort led by Health Coalitions on Liability and Access and approved a contribution to aid the efforts toward professional liability reform led by the Coalition for Affordable and Reliable Health Care.

At its October 2003 meeting, the Board of Regents approved a document outlining 10 core patient safety principles that should govern office-based procedures involving moderate sedation, deep sedation, or general anesthesia. Both the ACS and the American Medical Association have formally adopted these patient safety principles. The Board of Regents also approved a business plan that will allow the ACS to expand the National Surgical Quality Improvement Program (NSQIP) into additional private-sector hospitals (96 within the first year). This program supports the College’s mission of promoting the highest standards of surgical care through the evaluation of surgical outcomes in clinical practice and promises to improve the quality of surgical care.

At its February 2004 meeting, the ACS Board of Regents approved the ACS Statement on Universal Health Insurance. The statement was developed by the Health Policy Steering Committee as a step toward achieving universal health insurance in the United States. The ACS also has partnered with the Association of Program Directors in Surgery to develop a Web-based system that would assist surgery program directors in assessing the six core competencies of residents required by the Accreditation Council for Graduate Medical Education.

At the February 2004 American College of Surgeons Professional Association (ACSPA) Board of Directors meeting, the recent formation of the Doctors for Medical Liability Reform (DMLR) was announced. This coalition of organizations (including the ACSPA) representing high-risk medical and surgical specialties was created to conduct an aggressive public education campaign on the need for federal medical liability reforms on a state-by-state basis.

At their October 22 meeting, the Advisory Council for Ophthalmic Surgery focused on increasing the relevance of the ACS to ophthalmologists.

The Subcommittee on Resident Education of the ACS, in collaboration with the ACS Subcommittee on Medical Student Education, is in the process of revising the Prerequisites for Graduate Surgical Education: A Guide for Medical Students and PGY-1 Surgical Residents. The revised document will be used to develop a curriculum for medical students that will help them prepare for residency training.

The American College of Surgeons continues to serve the educational, clinical, and research interests of ophthalmologists, and continued cooperation with the ACS is beneficial to the mission of the American Orthoptical Society.

**Report of the Representative to the American Orthoptic Council**
EDWARD L. RAAB MD. The American Orthoptic Council (AOC) consists of representatives of several sponsoring organizations, including the American Ophthalmological Society. The Society reappointed Drs. Thomas France, David Weakley and Edward Raab as its representatives to the Council during the past year.

Dr. France, a former President of the AOC, continued as AOC representative to the Canadian Orthoptic Council and to the Joint Commission on Allied Health Personnel in Ophthalmology, and as Editor of the American Orthoptic Journal. He also serves on the Accreditation, Bylaws, Long Range Planning, and Public Relations Committees.

Dr. Weakley is Chair of the Continuing Education Committee and serves on the Accreditation, Editorial, Examination, Program, and Program Development Committees.

Dr. Raab, also a former President of the AOC, is a member of the Accreditation, Bylaws, Ethics, Long Range Planning, and Program Development Committees.

Our representatives additionally are active on the Council’s various committees and as examiners of candidates taking the certifying examination. Under current AOC bylaws, one serves by designation on the Council’s Nominating Committee to select officers.

Certification of an orthoptist is time limited and must be renewed every three years by demonstration of sufficient continuing education in offerings approved by the Council. As a new development in the education of orthoptic students, ability to refract will have to be demonstrated on the certification practical examination. Teaching programs have been informed that this requirement will be in place within the next two years.

Its small numbers and its unlicensed status disadvantage the profession of orthoptics. A major focus of the Council is to increase awareness of the profession among college students so that the increasing demand for these skills can be addressed. Possible sharing of the applicant pool with Canadian programs, facilitated by our close collaboration with the Canadian Orthoptic Council, is being explored. An informational video for college students has been informed that this requirement will be in place within the next two years.

The Council is investigating the possibility of accreditation and certification processes.

The Council co-sponsored, along with the American Academy of Ophthalmology (AAO) and the American Association of Certified Orthoptists, a well attended Symposium: “Strabismus Secondary to Trauma”, co-chaired by Dr. Weakley, at the Academy’s 2003 Annual Meeting. The program can be found on the American Orthoptic Journal website, www.aoj.org. The offering for the 2004 Annual Meeting of the AAO is “Eye Movement Disturbances and Neurological Disease Made Ridiculously Simple.”

Your representatives encourage continuing AOS representation on the American Orthoptic Council, in support of activities that promote this important health care profession.

Report of the Representative to the Council of the American Academy of Ophthalmology

JOHN F. O’NEILL MD. The Council of the American Academy of Ophthalmology (AAO) meets in formal session twice yearly, first during the fall annual meeting and then jointly with the AAO Mid-Year Forum in April. The focus of the spring Forum is to address the political and economic issues facing the membership, both current and future, and is generally held in Washington D.C. After being briefed on the Academy’s top legislative priorities and counseled on relationship building with their congressional representatives, many AAO members visited Capitol Hill and the offices of their personal members of congress. The overriding and major issue of concern this year was the efforts of organized Optometry to expand its scope of practice to include surgery and the legislated authorization for optometrists to perform laser surgery in the Veterans Administrations Hospitals in Oklahoma.

In conjunction with the AAO, a major effort has been initiated in congress through a bill sponsored by Representative John Sullivan of Oklahoma to counteract this optometric incursion. Rep. Sullivan introduced HR 3473, the Veterans Eye Treatment Safety (VETS) Act with more than 60 co-sponsors to insure that only medical doctors with specific surgical training, i.e. ophthalmologists, perform surgical procedures on the eyes of Veterans Hospital patients. Rep. Sullivan was greeted by acclamation and spoke briefly to the assembled Mid-Year Forum and Council members. The Council made strong recommendations to the AAO Board that resulted in the “Members Alerts” sent to all AAO members limiting Optometric attendance at our annual meeting and particular exclusion from any “skills-transfer” courses. Dr. Hoskins, Executive Vice President, also emphasized the importance of AAO members supporting our OPHTH-PAC to assist these legislative efforts.
The primary issue for consideration at the fall AAO Council Meeting resulted in a Council Advisory Recommendation to the AAO Board of Trustees addressing the formal request by the American Society of Ophthalmic, Plastic and Reconstructive Surgery (ASOPRS) for sub-specialty certification. Following extensive discussion, position papers were requested from the 52 state societies and 21 sub-specialty organizations. There are six special-interest organizations (including AOS) that are non-voting members. The official responses indicated 14 in favor of sub-specialty certification, 30 opposed and 11 expressed no opinion. The request for sub-specialty certification therefore was rejected at this time.

**Report of the Chairman of the Council**

JOHN G. CLARKSON MD. I would like to acknowledge the work of the Council members who will not be making presentations today, but have worked hard in the past year to move the organization forward: Dan Jones, Susan Day, Travis Meredith and George Bartley.

The Council recommends the following appointments for 2004-2005:

**Officers**
- Dr J. Brooks Crawford, President
- Dr Daniel Albert, President-Elect
- Dr C.P. Wilkinson, Secretary-Treasurer
- Dr Thomas Liesegang, Editor

**New Members Committee**
- Dr Douglas D. Koch, Chair

**Athletic Director**
- Dr Woodford Van Meter

**Archivist/Photographer**
- Dr Ralph C. Eagle

**AAO Councilor**
- Dr John O’Neil
- Dr C.P. Wilkinson (alternate)

**American College of Surgeons Board of Governors Representative**
- Dr Barrett Haik
- Dr Malcolm Mazow (alternate)

**Pan-American Association of Ophthalmology Representative**
- Dr Suzanne Véronneau-Troutman

The Council accepted the report of the Committee on Theses and has proposed the following candidates for membership in the American Ophthalmological Society:
- Dr M. Edward Wilson, Jr
- Dr Steven E. Feldon
- Dr Dennis P. Han
- Dr Mary Gilbert Lawrence
- Dr Evelyn A. Paysse
- Dr Elias Traboulsi
- Dr Terri L. Young

**Report of the President**

FRONCIE A. GUTMAN MD. Thank you, John and the Council, for doing a terrific job. I would like to present to you the presidential appointments. Historically, the president has made these appointments and, I think over the years, they’ve been good appointments. But, we’ve recognized a need to see that the Council is able to look at the needs of the organization and lead the appointments. So, the traditional appointments of the president now are really Council appointments. The Council serves as the nominating committee for the organization, so I’m bringing to you the presidential appointments, but I want you to know that these really are Council appointments. New members are indicated with an asterisk.

**Council**
- Dr Dan B. Jones, Chair
- Dr Susan H. Day
- Dr Travis A. Meredith
Dr George Bartley
Dr Lee Jampol *

Emeritus Committee
Dr Robert R. Waller

Committee on Prizes
Dr William S. Tasman
Dr George L. Spaeth
Dr Ronald Smith*

Committee on Theses
Dr James C. Bobrow
Dr Richard K. Parrish
Dr. Hans Grossniklaus*

Committee on Programs
Dr C. Gail Summers
Dr Kent W. Small
Dr Mark J. Mannis
Dr Richard Mills *

Committee on Membership
Dr Jane D. Kivlin
Dr Mark J. Mannis
Dr Julia A. Haller
Dr Barrett Haik *

American Orthoptics Council Representatives
Dr Edward L. Raab
Dr David R. Weakly, Jr
Dr Thomas France *

JCAHPO
Dr Robert L. Stamper

The AOS is permitted two representatives to JCAHPO and if anyone is interested in joined Robert Stamper as a representative to JCAHPO, please speak with me.

I am declaring the Executive Session of 140th Meeting in recess until tomorrow evening at the banquet.

Tuesday Morning, May 19

The scientific program continued with the following papers:


5. “von Hippel-Lindau (VHL) Gene Deletion and Ubiquitin Expression in Optic Nerve Hemangioma,” Chi-Chao Chan MD, Younsou Lee MD, Zhengping Zhuang MD PhD, Joseph Hackett, and Emily Y. Chew MD

6. “A Model of Spectral Filtering to Reduce Photochemical Damage in Age-Related Macular Degeneration,” Sanford M. Meyers MD, Mikhail Ostrovsky PhD DSc, and Robert F. Bonner PhD


10. “Aggressive Retinal Astrocytomas in 4 Patients with Tuberous Sclerosis Complex,” Jerry A. Shields MD, Ralph C. Eagle Jr MD, Carol L. Shields MD, and Brian P. Marr MD

11. “Human Intraocular Penetration Pharmacokinetics Of Moxifloxacin 0.5% via Topical and Collagen Shield Routes of Administration,” Seenu M. Hariprasad MD, William F. Mieler MD, Gaurav K. Shah MD, Kevin J. Blinder MD, Rajendra S. Apte MD, Nancy M. Holekamp MD, Matthew A. Thomas MD, Brett Rosenblatt MD, Jingduan Chi PhD, and Randall A. Prince PharmD

12. “Presumed Sinus Related Strabismus,” Irene H. Ludwig MD, and Joe Frank Smith MD FACS FARS


15. “Comparison of Intraocular Lens Power Calculation Methods in Eyes That Have Undergone Laser In-Situ Keratomileusis,” Li Wang MD PhD, Marc A. Booth MD, and Douglas D. Koch MD

**Tuesday Evening Banquet, May 19**

DR JOHN G. CLARKSON, COUNCIL CHAIR. Good evening and welcome to the 2004 Presidential Dinner/Dance. We’ve had an outstanding scientific program, I think outstanding social and athletic events, and I am certain that this evening’s presidential banquet will continue in that outstanding mode. Please enjoy your meal. If you’re so inspired, get up and dance, and we’ll be back later.

JOHN G. CLARKSON, MD. (Following dinner) I think it is now a good time for us to continue with our program and the continuation of the Business Meeting. It has been my privilege to serve as Council chair this year. I’d like to introduce the other hardworking members of the Council who have, in my opinion, done an outstanding job: Dan Jones, Susan Day, Travis Meredith, and George Bartley.

One of the privileges of the Council chair is to have the opportunity to introduce our president. This is a distinct honor for me, for I, as many of you, have worked with Dr. Gutmans in several different ophthalmic organizations and on several different projects over the years and have a great appreciation for his leadership style and his effectiveness. Business author James Collins has written extensive-
Minutes of the Proceedings

Monday. It’s my pleasure to call to the podium reports from the most important aspects of AOS life, starting with the Committee on Athletics and Woody Van Meter.

Report of the Athletic Committee

WOODFORD S. VAN METER, MD. When Froncie was quarterback at Purdue, the timing wasn’t exactly right for him to influence the Vietnam War, but he has always said that it’s too bad he was never sent to North Vietnam, because he could overthrow anybody.

Committee on Athletics report:
The winners of the trophies for the athletic events are:

<table>
<thead>
<tr>
<th>Event</th>
<th>Trophy</th>
<th>Category</th>
<th>Winner</th>
</tr>
</thead>
<tbody>
<tr>
<td>Golf</td>
<td>Mishima-Michels Trophy</td>
<td>Men’s Low Gross</td>
<td>Jay Erie</td>
</tr>
<tr>
<td></td>
<td>Canada-McCullough Cup</td>
<td>Men’s Low Net</td>
<td>David Berler</td>
</tr>
<tr>
<td></td>
<td>Truhlsen Trophy</td>
<td>Men’s Low Gross &gt;65</td>
<td>John Clarkson/</td>
</tr>
<tr>
<td></td>
<td>Knapp Memorial Trophy</td>
<td>Men’s Low Net Team</td>
<td>Taylor Ashbury</td>
</tr>
<tr>
<td></td>
<td>Ellsworth Trophy</td>
<td>Ladies Low Gross</td>
<td>Carolyn Lichter</td>
</tr>
<tr>
<td></td>
<td>Homestead-Calloway Cup</td>
<td>Ladies Low Net</td>
<td>Sandra Berler</td>
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<tr>
<td></td>
<td></td>
<td>Men’s Long Drive (16)</td>
<td>Jay Erie</td>
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<tr>
<td></td>
<td></td>
<td>Men’s Long Drive 65+ (7)</td>
<td>John Clarkson</td>
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<td>Men’s Closest to Pin (15)</td>
<td>John Bullock</td>
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<tr>
<td></td>
<td></td>
<td>Men’s Closest to Pin 65+ (18)</td>
<td>Taylor Ashbury</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Women’s Long Drive (12)</td>
<td>Carolyn Lichter</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Women’s Closest to Pin (14)</td>
<td>Dorene Shipley</td>
</tr>
<tr>
<td>Tennis</td>
<td>EVL Brown Bowl</td>
<td>Men’s Doubles Winners</td>
<td>Froncie Gutman/</td>
</tr>
<tr>
<td></td>
<td>EVL Brown Trophy</td>
<td>Men’s Doubles Runner-up</td>
<td>George Spaeth/</td>
</tr>
<tr>
<td></td>
<td>Wilkinson Trophy</td>
<td></td>
<td>Sloan Wilson/Men’s &gt;65 Winner</td>
</tr>
<tr>
<td>George Spaeth</td>
<td>Lewis-Perera Bowl</td>
<td>Women’s Doubles Winners</td>
<td>Alice Wilkinson/</td>
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<tr>
<td></td>
<td>Hughes Bowl</td>
<td>Women’s Doubles Runner-up</td>
<td>Gretchen Bullock/</td>
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<tr>
<td></td>
<td>Wong-MacDonald Trophy</td>
<td>Mixed Doubles Winners</td>
<td>Deena Laties</td>
</tr>
<tr>
<td></td>
<td>Wilson Trophy</td>
<td>Mixed Doubles Runner-up</td>
<td>Woody Van Meter</td>
</tr>
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<td></td>
<td>Dorothy Van Meter</td>
</tr>
<tr>
<td>Fly Fishing</td>
<td>McCaslin-Fralick-Kimura Trophy</td>
<td></td>
<td>Cheryl Frueh</td>
</tr>
<tr>
<td>Skeet Shooting</td>
<td>Beetham Trophy</td>
<td></td>
<td>Richard Abbott</td>
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</tbody>
</table>

Weekend Warrior Award goes to Chris Rapuano who dislocated his shoulder playing tennis.
who were here were also involved in the program. I would like to have each person stand with their spouse as I give your name.

Dr Richard L. Abbott, San Francisco, with his wife Chita
Dr Chi-Chao Chan, Bethesda, with her husband Chung
Dr Jay Erie, Rochester, Minnesota, with his wife Janet
Dr M Gilbert Grand, Saint Louis, with his wife Judy, who was not present
Dr Christopher J Rapuano, Philadelphia, with his wife Sara
Dr Gary L. Rogers, Columbus, Ohio, with his wife Ellen
Dr Daniel Schwartz, San Francisco
Dr John Thompson, Baltimore, with his wife Mary Ann
Dr Marco Zarbin, Newark, with his wife Susan
Dr Edwin M Stone, Iowa City, was unable to attend the meeting.

Report of the Committee on Prizes

BRUCE E. SPIVEY MD. President, AOS Members, and Guests:
First awarded in 1922 to Karl Koller, who established the anesthetic effect of topical cocaine, the Howe Medal has a rich tradition. In the first 25 years of the Howe Medal, from 1922 to 1946, there were only 13 recipients. Six of the first twenty-five award winners were not AOS members or American citizens. This era began with Ernst Fuchs of Vienna in 1924 and concluded with Ida Mann of Australia in 1958. In between were Priestly Smith, Axenfeld, Parsons, and Duke Elder: three from England; one Australian; one Viennese; and one, German. Since 1959, the award has been given annually to an AOS member, a tradition we continue this year. Even some members may be surprised to learn that this medal was awarded in the members’ only executive session until 1991. Many consider this to be the most prestigious award in American Ophthalmology and the previous recipients heartily agree.

Our awardee of 2004 was born in Bismarck, North Dakota. His family later moved to California where they established a date packing business. Our recipient appeared to be the heir-apparent head packer based on his fascination with designing tools and his continuous pondering of how the dates could be better packed. Despite this, he sought a college degree, first in "criminal technology," where he felt he could adapt his interests in
photography, gadgets, and solving mysteries. As an undergraduate, he went to an Optometry Clinic for an eye exam. Because of the detective nature of the exam equipment, this experience proved a pivotal event that steered him toward eyes. After obtaining his degree in optometry, a wise teacher prodded him to go further with a career in medicine. After graduating from medical school, he took additional training in ophthalmology in London and Los Angeles, and then a research fellowship at the Karolinska Institute in Stockholm. His training was interrupted during World War II, where his refusal to re-use needles for vaccination of recruits led to his reassignment from state-side to the Philippines.

Our Howe Medalist later established a private practice in association with his residency alma mater. Although he had a subspecialty interest, he saw general ophthalmology patients as well. One in particular had difficulty with playing tennis, and our hero's management of this patient resulted in the patient's improved tennis. This very grateful patient then contributed to the birth of an Eye Institute. For over 3 decades, this Institute has had a notable reputation for quality research based in clinical care. For many years, it has ranked among the top 10 recipients of NEI funding despite the absence of university affiliation. It has served as a center for postgraduate work for hundreds of MD's and PhD's. Despite the temptation to expand in size and scope that often comes with success, the director of this prestigious institution has passionately maintained its mission to balance emphasis in clinical, laboratory, and rehabilitation while maintaining its small size that engenders collegiality and a sense of family. As the institute evolved, expertise of both clinical and research nature was concentrated first in ocular motility producing basic contributions toward our understanding of extra-ocular muscle physiology. It went on to expand into amblyopia and vision development research as well as visual rehabilitation. Another AOS member received a Howe Medal for the development of Botox, work that was developed at this Institute.

By now, you probably know, that our Howe Medalist is none other than Dr. Arthur Jampolsky and his creation is the Smith Kettlewell Eye Research Institute.

Dr. Jampolsky's personal research endeavors increasingly focused on the clinical problems he encountered in his own practice. Quickly, he became known as the strabismus authority, particularly in regard to adult strabismus. On his patient days, the hallway was filled with patients from all corners of the world, many of VIP status, who never complained of the sometimes long waits and omnipresent students. Our awardee trained hundreds of fellows in strabismus who now can be found on virtually every continent. Residents from all Bay Area programs and the world eagerly sought the opportunity to observe Dr. Jampolsky's patients and surgery.

Jampolsky days in the operating room were always adventures with EMG recorders, video recording, and eager observers. There was never a doubt as to who was in control. The complexity of his strabismus cases was his hallmark. His surgical list was filled with thyroid myopathy, Duane Syndrome, re-operations referred from other subspecialists, retinal buckle restrictive problems, and lost muscles. Is it myth or reality that he never failed to locate a lost muscle over the course of his entire surgical career? Dr Jampolsky's intra-operative muscle force testing, video recording of effects of muscle weakening, and use of adjustable sutures resulted in operating rooms packed with spectators, yet there was never a doubt that the patient was the recipient of Jampolsky's riveted attention. Even after his retirement from clinical practice, AJ, as he is affectionately called, has maintained a presence at California Pacific Medical Center, with near-perfect attendance at weekly Grand Rounds. The precision of his questions and comments serves as a clear model of the essence of professionalism for residents, faculty, and community ophthalmologists: solid base of knowledge, ability to determine the key issues, continuing need to challenge assumptions, and delight in finding a way to do things better. That's AJ!

Dr Jampolsky's contributions to scientific literature have been in all major journals and number two hundred eighty refereed publications. Among his colleagues, he is well known for his performances at symposia and other scientific meetings. His wit, tenacity, and inclination to "call a spade a spade," and his pure and simple wisdom gained by having “been there, done that” would make even the other experts on the panel take careful note.

The Jampolsky sparring matches with Marshall Parks at the New Orleans Academy of Ophthalmology live in strabismus folklore. Each had a deity complex. Year after year, his AAO and AAPOS instructional courses would attract an audience of professors in his field.
In addition to Art’s focus on his local families at Smith-Kettlewell and California Pacific Medical Center, he was a strong presence in multiple capacities at the NEI, NIH, and ARVO. He has hosted major NEI-sponsored meetings on strabismus, vision development, and visual rehabilitation. Any meeting can find Dr Jampolsky attentively taking notes, questioning speakers, and perusing posters. As a co-founder of the American Association for Pediatric Ophthalmology and Strabismus, he alone is responsible for the addition of “strabismus” to the title and scope of the organization. He served as its President and has given its prestigious Costenbader Lecture. He is proud to maintain active membership (as opposed to his deserved category of emeritus) for this and other organizations in order to vote and voice his opinions.

Dr Jampolsky has a particular affinity for the blossoming of strabismus specialists in Latin America. He has regularly given named lectureships and flown thousands of miles at his own expense for the sake of collegiality and intellectual interchange. Sometimes, he operates on the premise, “If you’re not sure, just be positive.” Art holds strong feelings and forcefully advocates his beliefs. Art says that he thought he has been wrong only once. It was in 1959 when he thought he was wrong.

Art is a fiercely loyal individual, both to colleagues and to family. His conviction about the role a consultant should play as a resource for community ophthalmologists was as important a message to trainees as was his knowledge of strabismus. For meetings, Art would plan months in advance in order to include evenings with former fellows and their spouses.

Dr Jampolsky’s successful career is in no small measure due to his very special wife, Peggy. Art and Peggy met while she was on the job as a Pan Am Clipper stewardess, and have had 47 happy years of marriage since (as Art would say, 46 for him and 1 for her). They have 3 children and 5 grandchildren.

Art is a unique individual and has done it his way. Ayn Rand had Art in mind when she wrote Atlas Shrugged. Never pushing for himself, but ferociously standing on principle, science, and loyalty, Art Jampolsky is one of the Twin Pillars of world strabismus in the past half century.

Bill Tasman and George Spaeth, fellow members of the prizes committee, please escort our 2004 Howe Medal recipient Arthur Jampolsky to receive our recognition.

ARTHUR JAMPOLSKY MD. I am deeply grateful to my colleagues, friends, and the Society for awarding this prestigious prize to me. I thank you Bruce for not obtaining some other compromising pictures for his introduction. It is an unusual and odd experience to have your soul bared in public chronologically, and better, I suppose, than baring other things by photos. I’d like your permission to share this prize with my wife, Peggy. Bruce mentioned about the Smith Kettlewell, the fellows and the meetings. Those of you who have been there know about fundraising with principal donors and all the administration things that goes with it. Many are the weekends and evenings that Peggy has not been behind me, but beside me and in front of me in a one-to-one relationship, which really made those things possible. In my mind, and in my heart, it’s half yours, Peggy. Thank you again.

PRESIDENT GUTMAN. Dr. Jampolsky, the Howe Medal from the AOS is an honor to you, but you also bring honor to the Society. Congratulations.

I’m permitted a few remarks, and they’ll be very few. John Clarkson alluded to the retreat we held in 2000. We tried to make that a very broad-based group of people, including younger members and diversity within that group attending the retreat as well as past leadership of the AOS. As John mentioned, from this retreat we were able to identify some goals and strategic initiatives. Over the past four years since the 2000 retreat, the Council has worked steadily and effectively to strengthen our organization. Special attention has been given to membership issues, program content, financial solvency, and broadening the base of our leadership and trying to be a more inclusive organization. I believe that the strategic initiatives are bearing fruit. It takes time and patience to move an organization with the history of the AOS.

The AOS dates back to 1864, when it was the only game in town. Its historic roles have been assumed by many other organizations. Today it provides a unique, informal environment for social and collegial professional exchange and interaction. The AOS has an honored role in the history of American ophthalmology, which we certainly celebrate. My charge to the membership is that we do all we can do to maintain this very special organization for the current and future members.

Now, it’s my privilege and pleasure to make a few comments as I introduce your President-elect, Brooks Crawford. Brooks is a native of the San Francisco Bay area. He ricocheted between the East and West coasts for his education and medical training. He attended undergraduate school at Yale before medical school and residency at the University of California in San Francisco. He was on the East Coast for his internship at Columbia and appointment as clinical associate at the National Institute of Neurological Disease and Blindness and a two-year fellowship in ophthalmic pathology at the Armed Forces Institute of Pathology. Following this 16-year education journey, he returned to permanent residence in the Bay area. Brooks is currently professor and director of the Eye Pathology Laboratory at the University of California-San Francisco School of Medicine. Amongst his many offices
and appointments, Brooks has served as chairman of the American Board of Ophthalmology, president of the Verhoeff Society, editor of our own Transactions of the AOS, and associate secretary for programs with the American Academy of Ophthalmology.

I really got to know Brooks when we served together on the American Board of Ophthalmology. He is a modest, kind, and thoughtful individual who leads by example. If he is responsible for any task, you know it is going to be done promptly and well. All who know him respect him. However, this mild-mannered man is deceptive. How can you not stand in awe of someone whose recreation includes a San Francisco Bay afternoon outing in a kayak? Or, better yet, a three-week trek through the snow-covered glaciers of the Alps? Now, for answers on how Brooks keeps up with his diverse interests and responsibilities, you’ll have to ask Chrissie, his lovely, gracious partner, who is always at his side. Please join me in welcoming our President-elect and his wife, Chrissie, to the podium.

J. BROOKS CRAWFORD MD. Well, first of all, I want to congratulate Art for his wonderful honor. It’s certainly well deserved.

Thank you to members of the American Ophthalmology Society for this honor to me and to my wife, Chrissie, because we all know that our spouses play a crucial role in this Society. I also want to particularly thank Froncie because he, along with Bill Tasman, have been examples and mentors to me ever since we first met on the Written Committee of the American Board of Ophthalmology, when our carefully worded questions that we spent hours slaving over were thrown in the fireplace by members of the bad guys on the OKAP Committee, who thought they were wiser than we were. Froncie has been just a shining example to me, and his wisdom, his incredible self-deprecating sense of humor, his extraordinary leadership, and his incredible contributions he’s made to this Society, are truly inspiring. It’s just been a pleasure to know him, and it will be a pleasure to continue to know him. This is a special honor for me tonight because this was my father’s favorite society and the Homestead was his favorite place to meet. He first brought me and my beautiful bride of six months to the Homestead 39 years ago when we were at the National Institutes of Health. That was our first introduction to the AOS and I hope that I can serve you well. I’ll do my very best to do that. This concludes the Executive Session. Please resume dancing and enjoy the rest of this lovely evening.

Wednesday Morning, May 20

The scientific meeting concluded with the following papers:

16. “Efficacy and Efficiency of a New Involutional Ptosis Correction Procedure Compared to a Traditional Aponeurotic Approach,” Bartley R. Frueh MD, David C. Musch PhD, and Hector MacDonald MB FRCS
17. “Vascular Perfusion of Choroidal Melanoma by 3Tesla MRI,” Bruce Buerk MD, Jose S. Pulido MD MS, Ignacio Chiong, Robert Folberg MD, Deepak Edward MD, Mark Duffy MD PhD, and Keith Thulborn MD PhD
18. “Does Medical Treatment Influence the Success of Trabeculectomy?” Allan J. Flach MD
20. “Results of the Early Treatment for Retinopathy of Pre-maturity Study,” William V. Good MD
21. “Effects of Latrunculin B on Outflow Facility, Intraocular Pressure, Corneal Thickness, and Miotic and Accommodative Responses to Pilocarpine in Monkeys,” Mehmet Okka MD, Baohe Tian MD, and Paul L. Kaufman MD

The following members were present and registered at the meeting.

Members registered for the 2004 meeting. Thirteen professional guests are at the end of the list.

Active Abbott, Richard L.
Emeritus Alper, Melvin G.
Active Anderson Jr., W. Banks
Emeritus Annesley Jr., William H.
Emeritus Asbury, Taylor
Active Augsburger, James J.
Emeritus Baum, Jules L.
Active Beauchamp, George R.
Active Benson, William E.
Active Berler, David K.
Emeritus Berrocal, Jose A.
Active Biglan, Albert W.
Active Blankenship, George W.
Active Bobrow, James C.
Active Bourne, William M.
Active Brown, Gary C.
Active Bullock, John D.
Active Caldwell, Delmar R.
Active Cantor, Louis B.
Active Chan, Chi-Chao
Active Cibis, Gerhard W.
Active Clarkson, John G.
Emeritus Cox Jr., Morton S.
Active Crawford, J. Brooks
Minutes of the Proceedings

Active Day, Susan H.
Emeritus Drews, Robert C.
Emeritus Duke, James R.
Active Eagle Jr., Ralph C.
Active Elner, Susan G.
Active Elner, Victor M.
Active Erie, Jay C.
Active Farris, R. Linsy
Active Ferris, Frederick L.
Active Ferry, Andrew P.
Active Flach, Allan J.
Active Flanagan, Joseph C.
Active Flynn, John T.
Active Forbes, Max
Active Forster, Richard K.
Active Frank, Robert N.
Emeritus Fraenfelder, Frederick T.
Active Frayer, William C.
Active Friedman, Alan H.
Active Frueh, Bartley R.
Active Gaasterland, Douglas E.
Active Gardner, Thomas W.
Emeritus Glew, William B.
Active Godfrey, William A.
Active Gottsch, John D.
Active Grand, M. Gilbert
Active Grossniklaus, Hans E.
Active Gutman, Froncie A.
Active Guyton, David L.
Active Haik, Barrett G.
Active Hull, David S.
Active Iliff, Nicholas T.
Active Jabs, Douglas A.
Active Jaeger, Edward A.
Active Jampol, Lee M.
Active Jampolsky, Arthur
Emeritus Jarrett II, William H.
Active Jones, Dan B.
Active Katz, Barrett
Active Kaufman, Paul L.
Active Kelley, James S.
Active Koch, Douglas D.
Active Kreiger, Allan E.
Active Laibson, Peter R.
Active Landers III, Maurice B.
Active Laties, Alan M.
Active L'Esperance, Francis A.
Active Lichter, Paul R.
Active Liesegang, Thomas J.
Active Ludwig, Irene H.
Active Mannis, Mark J.
Active Mazow, Malcolm L.
Active Meredith, Travis A.
Active Merriam, John C.
Active Metz, Henry S.
Active Meyers, Sanford M.
Active Mieler, William F.
Active Miller, Marilyn T.
Active Mindel, Joel S.
Active Mitchell, Paul R.
Active Nork, T. Michael
Active Parrish II, Richard K.
Active Parver, Leonard M.
Active Payne, John W.
Active Pollack, Irvin P.
Active Pulido, Jose S.
Active Puro, Donald G.
Active Raab, Edward L.
Active Rapuano, Christopher J.
Active Ritch, Robert
Active Robb, Richard M.
Active Robertson, Dennis M.
Active Robin, Alan L.
Active Rogers, Gary L.
Active Rubin, Melvin L.
Active Schwab, Ivan R.
Active Schwartz, Ariah
Active Schwartz, Daniel M.
Active Sergott, Robert C.
Active Shields, Carol L.
Active Shields, Jerry A.
Active Spaeth, George L.
Active Spivey, Bruce E.
Active Stager Sr., David R.
Active Steinert, Roger F.
Active Summers, C. Gail
Active Tasman, William S.
Active Thompson, John T.
Active Townsend, William M.
Emeritus Tnhlsen, Stanley M.
Active Tso, Mark O. M.
Active Van Meter, Woodford S.
Active Vine, Andrew K.
Active Welch, Robert B.
Active Wilensky, Jacob T.
Active Wilkinson, Charles P.
Active Wilson II, Fred M.
Emeritus Wilson, R. Sloan
Active Yamnuzzi, Lawrence A.
Active Yee, Robert D.
Active Zarbin, Marco A.

Professional Gst Adamis, Anthony P.
Professional Gst Bonner, Robert F.
Professional Gst Edelhauser, Henry F.
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PAPERS
CHEMOREDUCTION FOR RETINOBLASTOMA: ANALYSIS OF TUMOR CONTROL AND RISKS FOR RECURRENCE IN 457 TUMORS

BY Carol L. Shields MD,* Arman Mashayekhi MD, Jacqueline Cater PhD, Abdallah Shelil MD, Anna T. Meadows MD, and Jerry A. Shields MD

ABSTRACT

Purpose: To evaluate individual tumor control following chemoreduction for retinoblastoma.

Methods: Prospective nonrandomized single-center case series of 457 retinoblastomas managed with six cycles of chemoreduction (vincristine, etoposide, and carboplatin). The tumors were then managed with chemoreduction alone (group A) or chemoreduction combined with thermotherapy (group B), cryotherapy (group C), or both thermotherapy and cryotherapy (group D). The main outcome measure was development of tumor recurrence.

Results: Of 457 retinoblastomas, 63 (14%) were in group A, 256 (56%) in group B, 127 (28%) in group C, and 11 (2%) in group D. The tumor was located in the macula in 33 (52%) of group A, 109 (43%) of group B, 3 (2%) of group C, and 1 (9%) of group D. Using Kaplan-Meier analysis, recurrence of the individual retinoblastoma at 7 years was found in 45% of group A and in 18% of combined groups B, C, and D. Treatment of the 93 tumor recurrences included thermotherapy, cryotherapy, or plaque radiotherapy in 62 cases (67%) and external beam radiotherapy or enucleation in 31 cases (33%). Risk factors predictive of tumor recurrence by multivariate analysis included macular tumor location for all groups and, additionally, female sex for group A and increasing tumor thickness for groups B, C, and D.

Conclusions: Chemoreduction alone or combined with cryotherapy and/or thermotherapy is effective for treatment of retinoblastoma, but tumor recurrence is greatest for those located in the macula and those with greater thickness. Globe salvage is usually achieved despite tumor recurrence.


INTRODUCTION

Chemoreduction plus focal consolidation treatment is an important therapeutic approach for retinoblastoma. The goal of such therapy is to reduce tumor size with chemotherapy and then consolidate the regressed tumor with thermotherapy or cryotherapy to ensure permanent control. This approach offers globe salvage for approximately 85% of eyes classified as Reese-Ellsworth groups I to IV, but it is less successful for group V because globe salvage is achieved in only 47% of eyes. Previous publications on the subject of chemoreduction have addressed various issues, some of which include tumor control with chemotherapy alone, tumor control with chemotherapy and focal consolidation, and risks for failure with need to resort to enucleation or external beam radiotherapy.

Chemotherapy alone has been found to control only 8% of eyes with retinoblastoma in various stages, on account of problems with tumor and related seed progression and recurrence. When assessing specifically only retinal tumor control using chemotherapy alone, without the confounding factors of vitreous or subretinal seed control, it was reported that 56 (72%) of 78 tumors responded favorably at a mean follow-up of 29 months. Similarly in this analysis, we evaluate specific tumor control of 457 retinoblastomas following chemoreduction, but further investigate the role of tumor consolidation with thermotherapy or cryotherapy in offering more complete tumor control.

METHODS

All patients with retinoblastoma who were treated initially
with chemoreduction (institutional review board approved CHP #582) on the Ocular Oncology Service, Wills Eye Hospital, Thomas Jefferson University, in conjunction with the Division of Oncology at The Children’s Hospital of Philadelphia, were identified. The eligibility criteria for treatment with chemoreduction were children with intraocular retinoblastoma in whom either eye would ordinarily require enucleation or external beam radiotherapy for cure of the disease based on published indications. Patients whose tumor(s) could be properly controlled with focal methods alone (cryotherapy, laser photocoagulation, thermotherapy, plaque radiotherapy) were not eligible for inclusion in the chemoreduction protocol. Exclusion criteria for treatment with chemoreduction included biomicroscopic evidence of iris neovascularization, neovascular glaucoma, tumor invasion into the anterior chamber, iris, optic nerve, choroid, or extraocular tissues as documented by clinical, ultrasonographic, and neuroimaging modalities. Exclusion criteria from a systemic standpoint were those patients with evidence of systemic metastasis, prior chemotherapy, prior treatment for retinoblastoma, or inadequate organ function of the kidney, liver, or auditory apparatus. The chemotherapeutic agents included intravenous vincristine, etoposide, and carboplatin (VEC), as shown in Table 1. The duration of treatment was planned for six monthly cycles. The potential risks and benefits of the chemoreduction protocol were discussed with the patient’s family, and informed consent was obtained.

Ocular oncologic follow-up was provided at examination under anesthesia every 1 to 2 months after initiation of chemoreduction until all tumors showed complete control. Thereafter, examinations were provided every 2 to 4 months as needed. At each examination, the status of the individual retinal tumors, vitreous seeds, subretinal seeds, and subretinal fluid was noted, and detailed retinal drawings and photographic documentation were performed. At cycle 1, all initial data was recorded and chemoreduction was instituted. At cycles 2 through 6, some tumors were managed with chemoreduction alone and no adjuvant treatment (group A), whereas other tumors received additional thermotherapy (group B), additional cryotherapy (group C), or additional thermotherapy and cryotherapy (group D). The patients were not randomized to treatment. Nearly all tumors outside the macular region received additional therapy (groups B, C, or D). The macula was defined as a circular area within 3 mm of the foveola. Patients with tumors anterior to the equator of the eye generally received additional cryotherapy (group C), and those posterior to the equator received additional thermotherapy (group B). If an inadequate response was achieved following thermotherapy, then cryotherapy was provided (group D). Tumors in the macular region were treated with chemoreduction alone (group A) if the opposite eye had severe visual deficit such as macular retinoblastoma or enucleation, or they were treated with chemoreduction plus additional thermotherapy (group B) if the macula in the opposite eye was intact with potential for good visual acuity.

Adjuvant thermotherapy was provided using the indirect ophthalmoscopic system using 1.2-mm spot size. Duration and power varied so that a light gray-white appearance could be achieved at the end of the session. The entire tumor was treated in a slow fashion over several minutes with adjustment of the power to reach a satisfactory appearance at end point. The only exception to treating the entire tumor was with macular tumors for which foveal-sparing thermotherapy was employed to minimize central vision loss. Cryotherapy was applied using transscleral triple freeze-thaw technique directly over the tumor with ophthalmoscopic visualization using a retinal cryoprobe.

All data were collected in a prospective fashion. At initial examination, each patient was evaluated for age at diagnosis, race, male or female sex, and hereditary pattern of the retinoblastoma (sporadic, familial). The eye was assessed for laterality of involvement (unilateral, bilateral) and Reese-Ellsworth classification (I, II, III, IV, V). The tumor was assessed for anteroposterior location (macula, macula to equator, equator to ora serrata), quadrant location (superotemporal, inferotemporal, inferonasal, superonasal), and size in basal dimension (mm) and thickness (mm). At final examination, each tumor was assessed for basal dimension (mm), thickness (mm), regression type (0, 1, 2, 3, 4), and tumor recurrence. Regression type 0 is complete disappearance with no remnant, type 1 is completely calcified remnant, type 2 is completely noncalcified remnant, type 3 is a combination of calcified and noncalcified remnant, and type 4 is flat chorioretinal atrophic scar but no tumor remnant. The interval from initiation of chemoreduction to the tumor recurrence was calculated. The tumor follow-up was continued until the date the patient was last examined or until the date of enucleation of the eye.

### Statistical Analysis
The clinical data were then analyzed with regard to the single outcome of retinoblastoma recurrence for each...
group (A, B, C, and D) and for the entire cohort of 457 tumors as a whole. The effect of each individual clinical variable recorded at the time of patient presentation on the Ocular Oncology Service and the effect of the treatment strategy on the development of this outcome were analyzed by a series of univariate Cox proportional hazards regressions. The correlation among the variables was determined by using Pearson correlations. All variables were analyzed as discrete variables (continuous variables were analyzed by grouping them into discrete categories). Variables that were significant on a univariate level \((P \leq 0.05)\) were entered first into the multivariate Cox regression analysis. For variables that showed a high degree of correlation, only one variable from the set of associated variables was entered at a time into subsequent multivariate models. A final multivariate model tested variables that were identified as significant predictors \((P \leq 0.05, \text{Wald's statistic or 95\% confidence interval [CI] of the relative risk})\) from the initial model as well as variables deemed clinically important for the outcome of retinoblastoma recurrence. In the final model, a predictor was considered a significant risk factor if the 95\% CI of its relative risk did not contain a risk value of 1. The time to retinoblastoma recurrence using Kaplan-Meier life table analysis was performed.

**RESULTS**

There were 457 retinoblastomas in 193 eyes of 125 patients managed with six cycles of this chemoreduction protocol (Table 1) between January 1995 and May 2003. Group A (chemoreduction alone) consisted of 63 tumors (14\%), group B (chemoreduction plus thermotherapy) consisted of 256 tumors (56\%), group C (chemoreduction plus cryotherapy) consisted of 127 tumors (28\%), and group D (chemoreduction plus thermotherapy and cryotherapy) consisted of 11 tumors (2\%). The demographic features of all 457 tumors are listed in Table 2. The mean patient age at treatment was 9 months, and the hereditary pattern was sporadic in 75\% and familial in 25\%. The Reese-Ellsworth classification of each eye is listed in Table 3.

A description of the clinical features of the retinoblastomas at initial examination is listed in Table 4. The tumor base and thickness were greater for those in group A (mean, 11 mm and 7 mm, respectively) than those in groups B (mean, 7 mm and 4 mm, respectively), C (mean, 3 mm and 2 mm, respectively), and D (mean, 5 mm and 3 mm, respectively). Macular retinoblastoma represented 52\% of group A, 43\% of group B, 2\% of group C, and 9\% of group D. Peripheral retinoblastoma near the ora serrata represented 11\% of group A, 5\% of group B, 72\% of group C, and 18\% of group D. A comparison of tumors in group A versus groups B, C, and D revealed that there were significant differences in patient age and race, tumor basal dimension and thickness, and tumor location (Table 5). A description of the retinoblastoma's appearance following therapy at last examination is listed in Table 6. Type 1 regression was noted in 30\% of group A, 9\% of group B, 2\% of group C, and 0\% of group D. Regression pattern was most commonly type 3 for group A (46\%) and type 4 for groups B (45\%), C (85\%), and D (82\%). Recurrence at last follow-up was found in 20\% of all 457 tumors. Kaplan-Meier estimates of recurrence by 5 years follow-up was 45\% for group A, 18\% for combined groups B, C, and D, and 22\% for the entire group of 457 retinoblastomas (Table 7). The Kaplan-Meier estimates

### Table 2. Demographic Features of Patients Treated with Chemoreduction for Retinoblastoma (n = 125)

<table>
<thead>
<tr>
<th>Age (mo)</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median (range)</td>
<td>7 (&lt;1-41)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>74 (59%)</td>
</tr>
<tr>
<td>Female</td>
<td>51 (41%)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>100 (80%)</td>
</tr>
<tr>
<td>African American</td>
<td>17 (14%)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>4 (3%)</td>
</tr>
<tr>
<td>Asian</td>
<td>4 (3%)</td>
</tr>
<tr>
<td>Heredity</td>
<td></td>
</tr>
<tr>
<td>Sporadic</td>
<td>94 (75%)</td>
</tr>
<tr>
<td>Faroïdal</td>
<td>31 (25%)</td>
</tr>
<tr>
<td>Laterality</td>
<td></td>
</tr>
<tr>
<td>Unilateral</td>
<td>35 (28%)</td>
</tr>
<tr>
<td>Bilateral*</td>
<td>90 (72%)</td>
</tr>
<tr>
<td>Eye affected†</td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>99 (51%)</td>
</tr>
<tr>
<td>Left</td>
<td>94 (49%)</td>
</tr>
</tbody>
</table>

*Of the 90 patients with bilateral retinoblastoma, 22 eyes were not entered into this study because they were initially treated with enucleation.
†No. of eyes = 193.

### Table 3. Reese-Ellsworth Classification of 193 Eyes Treated with Chemoreduction for Retinoblastoma

<table>
<thead>
<tr>
<th>REESE-ELLSWORTH GROUP</th>
<th>NO. OF EYES (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ia</td>
<td>8 (4)</td>
</tr>
<tr>
<td>Ib</td>
<td>5 (3)</td>
</tr>
<tr>
<td>IIa</td>
<td>34 (18)</td>
</tr>
<tr>
<td>IIb</td>
<td>19 (10)</td>
</tr>
<tr>
<td>IIIa</td>
<td>13 (7)</td>
</tr>
<tr>
<td>IIIb</td>
<td>18 (9)</td>
</tr>
<tr>
<td>IVa</td>
<td>5 (3)</td>
</tr>
<tr>
<td>IVb</td>
<td>52 (27)</td>
</tr>
<tr>
<td>Va</td>
<td>13 (7)</td>
</tr>
<tr>
<td>Vb</td>
<td>26 (13)</td>
</tr>
</tbody>
</table>
Treatment of 93 tumor recurrences included cryotherapy in 14 cases (15%), thermotherapy in 9 (10%), plaque radiotherapy in 39 (42%), external beam radiotherapy in 25 (27%), and enucleation in 6 cases (5%). Those eyes managed with external beam radiotherapy or enucleation usually had additional diffuse vitreous and/or subretinal seed recurrence. Factors predictive of tumor recurrence for the 457 tumors using multivariate analysis included non-Caucasian race, macular tumor location, and increasing tumor thickness (Table 8). Multivariate analysis for factors predictive of recurrence for the 63 patients in group A included female sex and tumor location in the macula (Table 9). Multivariate analysis for factors predictive of recurrence for the 256 patients in combined groups B, C, and D included macular tumor location and increasing tumor thickness (Table 8).

**DISCUSSION**

Chemoreduction with or without focal tumor consolidation treatment is now the most commonly employed conservative (nonenucleation) therapeutic regimen for retinoblastoma. Despite its popularity, long-term tumor control is often less than desired. Most eyes show favorable initial response within one or two cycles of chemotherapy, but over time tumor and associated vitreous and subretinal seeds can recur. We had noted that by 3 years following chemoreduction, tumor recurrence was found in 51% of eyes. Of those eyes with subretinal seeds at initial examination, subretinal seed recurrence was found in 46% of eyes, and of those with initial vitreous seeds, recurrence was detected in 62% of eyes by 3 years follow-up. These findings did not increase much by 5 years follow-up, so it was presumed that most recurrence following chemoreduction would be clinically visible by 3 years following treatment. These findings underscore the difficulty with this therapy and the need for long-term cautious monitoring of these patients by experienced clinicians. Even though these results seem unfavorable, recurrence is usually detected at an early stage and can be controlled with further salvage measures, avoiding enucleation and external beam radiotherapy.

Tumor change following two cycles of chemoreduction has been reported at approximately 35% reduction in basal dimension and 50% reduction in thickness. In this analysis, we found that tumor basal dimension reduction following completed six cycles and consolidation (if any) was 36% for group A, 43% for group B, 67% for group C, and 60% for group D. Tumor thickness reduction following completed six cycles and consolidation (if any) was 57% for group A, 50% for group B, 75% for group C, and 67% for group D. A few cases showed very little change in size following chemoreduction. It has been speculated that minimal response following chemoreduction may be a feature of well-differentiated retinoblastoma. Follow-up of such patients has documented that these poorly responsive tumors remain stable. Unpublished observations from our department have also identified that the presence of intratumoral cysts is a potential sign of tumor...
differentiation and less dramatic response to chemoreduction.

Following therapy, judgment of tumor response is based on reduction of tumor size, resolution of subretinal fluid, and change in tumor appearance. The appearance is classified into five regression patterns from type 0 to type 4, based on presence of tumor calcification and appearance of the residual tumor scar (defined in the “Methods” section). Tumor regression patterns depended on the initial tumor size and treatment. Retinoblastomas in group A most commonly showed regression type 3 (46%) and type 1 (30%), whereas those in group B showed regression type 3 (42%) and type 4 (45%). Tumors in groups C and D showed most commonly regression type 4 (85% and 82%, respectively). Regression type 4 is the most satisfying pattern to the clinician, because there is no tumor remnant and the site is atrophic without blood supply. As indicated in this report, this pattern was generally found following chemoreduction and cryotherapy (85%) and less often following chemoreduction and thermotherapy (45%) or chemoreduction alone (5%). Complete calcification of the retinoblastoma following therapy was found in 30% of group A, 9% of group B, 2% of group C, and 0% of group D. This is another pattern that provides satisfaction to the clinician because there is a completely calcified scar, implying tumor necrosis with dystrophic calcification.

### Table 5. Comparison of Clinical Features in Patients With Retinoblastoma at Initial Examination

<table>
<thead>
<tr>
<th>CLINICAL FEATURE</th>
<th>GROUP A: CHEMOREDUCTION ALONE (n = 24 PATIENTS, 63 TUMORS)</th>
<th>GROUPS B, C, D: CHEMOREDUCTION + THERMOTHERAPY, CRYOTHERAPY, OR BOTH (n = 101 PATIENTS, 394 TUMORS)</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient age (mo): mean (median, range)</td>
<td>13 (11, 1-41)</td>
<td>8 (6, &lt;1-39)</td>
<td>.02†</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian*</td>
<td>13 (54%)</td>
<td>87 (86%)</td>
<td>.04‡</td>
</tr>
<tr>
<td>African American</td>
<td>8 (33%)</td>
<td>9 (9%)</td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>2 (8%)</td>
<td>2 (2%)</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>1 (4%)</td>
<td>3 (3%)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>12 (50%)</td>
<td>74 (73%)</td>
<td>.4</td>
</tr>
<tr>
<td>Female</td>
<td>12 (50%)</td>
<td>27 (27%)</td>
<td></td>
</tr>
<tr>
<td>Heredity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sporadic</td>
<td>20 (83%)</td>
<td>72 (73%)</td>
<td>.3</td>
</tr>
<tr>
<td>Familial</td>
<td>4 (17%)</td>
<td>27 (27%)</td>
<td></td>
</tr>
<tr>
<td>Laterality</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unilateral</td>
<td>5 (21%)</td>
<td>30 (29%)</td>
<td></td>
</tr>
<tr>
<td>Bilateral</td>
<td>19 (79%)</td>
<td>71 (70%)</td>
<td>.8</td>
</tr>
<tr>
<td>Total number tumors per eye: mean (median, range)</td>
<td>3.3 (3, 1-9)</td>
<td>2.9 (3, 1-15)</td>
<td>.1</td>
</tr>
<tr>
<td>Base (mm): mean (median, range)</td>
<td>11 (11, &lt;1-25)</td>
<td>6 (6, &lt;1-23)</td>
<td>.0001‡</td>
</tr>
<tr>
<td>Thickness (mm): mean (median, range)</td>
<td>7 (7, &lt;1-15)</td>
<td>3 (3, &lt;1-13)</td>
<td>.0001‡</td>
</tr>
<tr>
<td>Anteroposterior location</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macula*</td>
<td>33 (52%)</td>
<td>113 (29%)</td>
<td></td>
</tr>
<tr>
<td>Macula to equator</td>
<td>23 (37%)</td>
<td>174 (44%)</td>
<td>.008†</td>
</tr>
<tr>
<td>Equator to ora serrata</td>
<td>7 (11%)</td>
<td>107 (27%)</td>
<td>.001†</td>
</tr>
<tr>
<td>Quadrant location</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macula*</td>
<td>33 (52%)</td>
<td>113 (29%)</td>
<td></td>
</tr>
<tr>
<td>Supertemporal</td>
<td>6 (10%)</td>
<td>49 (12%)</td>
<td>.021‡</td>
</tr>
<tr>
<td>Inferotemporal</td>
<td>12 (19%)</td>
<td>61 (16%)</td>
<td>.3</td>
</tr>
<tr>
<td>Inferonasal</td>
<td>5 (8%)</td>
<td>90 (23%)</td>
<td>.001†</td>
</tr>
<tr>
<td>Superoonasal</td>
<td>7 (11%)</td>
<td>81 (21%)</td>
<td>.005†</td>
</tr>
<tr>
<td>Proximity to optic disk (mm): mean (median, range)</td>
<td>1.2 (0, 0-16)</td>
<td>2.4 (0, 0-17)</td>
<td>.2</td>
</tr>
<tr>
<td>Proximity to foveola (mm): mean (median, range)</td>
<td>1.0 (0, 0-16)</td>
<td>2.5 (0, 0-19)</td>
<td>.2</td>
</tr>
</tbody>
</table>

*Reference variable.

†Indicates clinical features significantly different between the two groups.
The least satisfying tumor regression pattern is type 2, which appears as a noncalcified residua, sometimes quite similar to the original tumor. Type 2 pattern is also termed “fish flesh appearance” and has a grey, translucent minimally vascular surface that is different from an active tumor, which is usually more opaque, pink-white, and highly vascular. Type 2 regression pattern was found in 6% of group A, 4% of group B, 1% of group C, and 0% of group D. The lack of calcification or chorioretinal atrophy raises the question of tumor viability at each examination, but lack of change on follow-up suggests tumor regression. Even though it might be suspected that tumors showing regression type 2 were more likely to show ultimate recurrence, the statistical analysis did not support this conclusion. There was no relationship between tumor regression type and tumor recurrence. All regressed retinoblastomas, despite the tumor regression pattern, require meticulous periodic observation to monitor for recurrence.

In this analysis, we specifically evaluated control of each individual retinal tumor following chemoreduction. By 7 years follow-up, we found overall tumor control of 78%, without the need for external beam radiotherapy or enucleation. More specifically, by 7 years, tumors treated with chemoreduction alone showed 55% tumor control.

Table 6. Retinoblastoma Features at Last Examination

<table>
<thead>
<tr>
<th>Tumor Feature</th>
<th>Group A: Chemoreduction Alone (n = 63)</th>
<th>Group B: Chemoreduction + Thermotherapy (n = 256)</th>
<th>Group C: Chemoreduction + Cryotherapy (n = 127)</th>
<th>Group D: Chemoreduction + Cryotherapy (n = 11)</th>
<th>Total (N = 457)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base (mm)</td>
<td>Mean 7</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Median (range) 6 (0-20)</td>
<td>3 (0-18)</td>
<td>0 (0-13)</td>
<td>0 (0-10)</td>
<td>0 (0-20)</td>
</tr>
<tr>
<td>Thickness (mm)</td>
<td>Mean 3</td>
<td>3</td>
<td>&lt;1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Median (range) 3 (0-10)</td>
<td>1 (0-9)</td>
<td>0 (0-7)</td>
<td>0 (0-9)</td>
<td>0 (0-10)</td>
</tr>
<tr>
<td>Regression type*</td>
<td>0</td>
<td>8 (13)</td>
<td>4 (3)</td>
<td>0 (0)</td>
<td>14 (3)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>19 (30)</td>
<td>23 (9)</td>
<td>3 (2)</td>
<td>45 (10)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4 (6)</td>
<td>10 (4)</td>
<td>1 (1)</td>
<td>15 (3)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>29 (46)</td>
<td>107 (42)</td>
<td>11 (9)</td>
<td>149 (33)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>3 (5)</td>
<td>114 (45)</td>
<td>108 (85)</td>
<td>234 (51)</td>
</tr>
<tr>
<td>Recurrence</td>
<td>Yes</td>
<td>28 (44)</td>
<td>51 (20)</td>
<td>11 (9)</td>
<td>93 (20)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>35 (56)</td>
<td>205 (80)</td>
<td>116 (91)</td>
<td>364 (80)</td>
</tr>
</tbody>
</table>

*Type 0 = tumor has disappeared; type 1 = tumor is completely calcified; type 2 = tumor is noncalcified; type 3 = tumor is partly calcified; type 4 = tumor is flat with chorioretinal scar.

Table 7. Kaplan-Meier Estimates of Time to Recurrence of Retinoblastoma

<table>
<thead>
<tr>
<th>Time of Recurrence</th>
<th>Group A: Chemoreduction Alone (n = 63)</th>
<th>Group B: Chemoreduction + Thermotherapy (n = 256)</th>
<th>Groups B, C, D: Chemoreduction + Adjunct Therapy (n = 394)</th>
<th>Total: Chemoreduction With or Without Adjunct Therapy (n = 457)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 yr</td>
<td>39% (24/63)</td>
<td>18% (47/256)</td>
<td>13% (50/394)</td>
<td>17% (73/457)</td>
</tr>
<tr>
<td>2 yr</td>
<td>45% (27/63)</td>
<td>20% (49/256)</td>
<td>17% (61/394)</td>
<td>20% (88/457)</td>
</tr>
<tr>
<td>3 yr</td>
<td>45% (27/63)</td>
<td>21% (50/256)</td>
<td>17% (62/394)</td>
<td>21% (89/457)</td>
</tr>
<tr>
<td>4 yr</td>
<td>45% (27/63)</td>
<td>22% (51/256)</td>
<td>18% (64/394)</td>
<td>22% (91/457)</td>
</tr>
<tr>
<td>5 yr</td>
<td>45% (27/63)</td>
<td>22% (51/256)</td>
<td>18% (64/394)</td>
<td>22% (91/457)</td>
</tr>
<tr>
<td>6 yr</td>
<td>45% (27/63)</td>
<td>22% (51/256)</td>
<td>18% (64/394)</td>
<td>22% (91/457)</td>
</tr>
<tr>
<td>7 yr</td>
<td>45% (27/63)</td>
<td>22% (51/256)</td>
<td>18% (64/394)</td>
<td>22% (91/457)</td>
</tr>
</tbody>
</table>

*(No. of events/No. still in risk set.)
whereas those treated with chemoreduction combined with focal consolidation of thermotherapy or cryotherapy showed 82% control. In the overall group of 457 retinoblastomas, tumors most likely to recur were large tumors and those located in the macular region. It should be realized that in this nonrandomized study, tumors in group A were larger than those in groups B, C, and D, and this could have impacted tumor recurrence. Large tumors are not particularly amenable to tumor consolidation with thermotherapy or cryotherapy, because these focal modalities would affect only a small portion of the large mass and provide only partial treatment. Additionally, large retinoblastomas are often found in the macular region, and in many cases tumor consolidation is avoided to preserve vision for the child, especially if it is the patient’s only remaining eye. For these reasons, large retinoblastomas are occasionally managed with chemoreduction alone.

In a previous report, we specifically assessed tumor control of macular retinoblastoma in a group of 68 patients. We noted Kaplan-Meier estimates for tumor recurrence in 35% of those macular tumors treated with chemoreduction alone and only 17% of those treated with chemoreduction plus foveal-sparing thermotherapy. Surprisingly, tumor recurrence was most often found in smaller, relatively inactive tumors without subretinal or vitreous seeds. Gombos and colleagues found similar results in their analysis of chemotherapy alone for 78 retinoblastomas. In their report, tumor recurrence was greatest for small tumors, that is, those under 2 mm in diameter. We speculate that this occurs because small tumors might be more well differentiated and show less chemotherapy response, or they may receive smaller doses of chemotherapy on account of tiny feeder vessels.

In our analysis, all patients were treated with...
## TABLE 9. RISK FACTORS PREDICTIVE OF TUMOR RECURRENCE FOR INDIVIDUAL GROUP A AND GROUP B, AND COMBINED GROUPS B, C, AND D

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>No Recurrence</th>
<th>Recurrence</th>
<th>p Value</th>
<th>Risk*</th>
<th>Confidence Intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group A</strong></td>
<td>(n=11 patients, 36 tumors*)</td>
<td>(n=13 patients, 27 tumors)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Univariate analysis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex (female vs male†)</td>
<td>4 (36%)</td>
<td>8 (62%)</td>
<td>.025</td>
<td>2.92</td>
<td>(1.15,7.42)</td>
</tr>
<tr>
<td>No. of tumors per eye: mean (median, range)</td>
<td>4 (4, 1-9)</td>
<td>3 (3, 1-4)</td>
<td>.009</td>
<td>1.26</td>
<td>(1.06,1.49)</td>
</tr>
<tr>
<td>Tumor basal dimension (mm): mean (median, range)</td>
<td>9 (9, &lt;1-25)</td>
<td>14 (14, 1-25)</td>
<td>.007</td>
<td>1.08</td>
<td>(1.02,1.14)</td>
</tr>
<tr>
<td>Tumor thickness (mm): mean (median, range)</td>
<td>6 (5, &lt;1-15)</td>
<td>8 (9, &lt;1-15)</td>
<td>.035</td>
<td>1.10</td>
<td>(1.01,1.19)</td>
</tr>
<tr>
<td><strong>Tumor quadrant</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macula</td>
<td>19 (53%)</td>
<td>14 (52%)</td>
<td>&lt;.0001</td>
<td>NE</td>
<td>NE</td>
</tr>
<tr>
<td>Macula vs superotemporal†</td>
<td>6 (17%)</td>
<td>0 (0%)</td>
<td>NE</td>
<td>NE</td>
<td></td>
</tr>
<tr>
<td>Macula vs superonasal†</td>
<td>1 (3%)</td>
<td>4 (15%)</td>
<td>.011</td>
<td>2.83</td>
<td>(1.27,6.32)</td>
</tr>
<tr>
<td><strong>Multivariate analysis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex (female vs male†)</td>
<td>—</td>
<td>—</td>
<td>.025</td>
<td>2.76</td>
<td>(1.11,6.89)</td>
</tr>
<tr>
<td><strong>Group B</strong></td>
<td>(n = 66 patients, 205 tumors)</td>
<td>(n = 23 patients, 51 tumors)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Univariate analysis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor basal dimension (mm): mean (median, range)</td>
<td>7 (4, &lt;1-23)</td>
<td>9 (6, &lt;1-23)</td>
<td>.01</td>
<td>1.05</td>
<td>(1.01,1.09)</td>
</tr>
<tr>
<td>Tumor thickness (mm): mean (median, range)</td>
<td>4 (3, &lt;1-13)</td>
<td>5 (4, &lt;1-12)</td>
<td>.02</td>
<td>1.11</td>
<td>(1.02,1.20)</td>
</tr>
<tr>
<td>Tumor location (macula vs extramacular†)</td>
<td>79 (39%)</td>
<td>30 (59%)</td>
<td>.01</td>
<td>2.06</td>
<td>(1.18,3.61)</td>
</tr>
<tr>
<td><strong>Multivariate analysis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor location (macula vs extramacular†)</td>
<td>—</td>
<td>—</td>
<td>.01</td>
<td>2.06</td>
<td>(1.18,3.61)</td>
</tr>
<tr>
<td><strong>Groups B, C, D</strong></td>
<td>(n = 76 patients, 328 tumors)</td>
<td>(n = 25 patients, 66 tumors)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Univariate analysis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor basal dimension (mm): mean (median, range)</td>
<td>5 (3, &lt;1-23)</td>
<td>8 (6, &lt;1-23)</td>
<td>&lt;.001</td>
<td>1.08</td>
<td>(1.10,1.27)</td>
</tr>
<tr>
<td>Tumor thickness (mm): mean (median, range)</td>
<td>3 (2, &lt;1-13)</td>
<td>5 (4, &lt;1-13)</td>
<td>&lt;.001</td>
<td>1.18</td>
<td>(1.10,1.27)</td>
</tr>
<tr>
<td>Tumor location (macula vs extramacular†)</td>
<td>82 (25%)</td>
<td>31 (47%)</td>
<td>.001</td>
<td>2.45</td>
<td>(1.48,4.08)</td>
</tr>
<tr>
<td>Proximity to optic nerve (mm): mean (median, range)</td>
<td>3 (0, 0-17)</td>
<td>&lt;1 (0, 0-5)</td>
<td>.02</td>
<td>1.15</td>
<td>(1.02,1.29)</td>
</tr>
<tr>
<td>Proximity to foveola (mm): mean (median, range)</td>
<td>3 (0, 0-19)</td>
<td>&lt;1 (0, 0-7)</td>
<td>.009</td>
<td>1.23</td>
<td>(1.05,1.43)</td>
</tr>
<tr>
<td><strong>Multivariate analysis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor thickness (mm)</td>
<td>—</td>
<td>—</td>
<td>.001</td>
<td>1.13</td>
<td>(1.05,1.22)</td>
</tr>
<tr>
<td><strong>Tumor quadrant</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macula vs superotemporal†</td>
<td>—</td>
<td>—</td>
<td>.04</td>
<td>2.88</td>
<td>(1.03,8.10)</td>
</tr>
<tr>
<td>Macula vs superonasal†</td>
<td>—</td>
<td>—</td>
<td>.04</td>
<td>2.27</td>
<td>(1.02,5.02)</td>
</tr>
</tbody>
</table>

*Risk ratio computed from Cox proportional hazards model.
†Reference variable.

NE, nonestimable (zero cell).
multi-agent chemotherapy for retinoblastoma to minimize chemo-resistance in eyes with often extensive disease. Others have investigated single-agent chemotherapy for retinoblastoma using carboplatin. Lumbroso and coworkers found that carboplatin plus thermotherapy for selected small to medium-sized retinoblastomas (median tumor diameter, 3.5 mm) provided control in 89.5% of patients at 2 years follow-up. However, eyes with large tumors or those with localized or diffuse vitreous or subretinal seeding were excluded from their protocol owing to anticipated concerns for recurrence. Their selected cohort differs from ours in that we were more inclusive to provide a general picture of tumor control with chemoreduction strategies. We included eyes with small, medium, and large retinoblastomas, and we included eyes with localized and diffuse vitreous and subretinal seeding.

There are limitations to this study that should be realized. We specifically evaluated only retinal tumor control and did not evaluate vitreous seed or subretinal seed control. Seed recurrence is a common cause for chemoreduction failure, so this should be anticipated when treating patients with such features. We also did not evaluate for the development of new retinoblastomas, because this has been previously reported. In addition, many patients had more than one tumor per eye. There may be unknown factors within a single patient’s tumor(s) that could lead to excessive resistance or sensitivity to treatment and could bias the results. Finally, these tumors were not randomized to treatment, because treatment selection was based on many features of the patient, the eye(s), and the tumor(s). Randomization would have been difficult because of the many variables from case to case. This report was not designed to be a comparison of various treatment methods. Our goal was to assess the singular outcome of tumor recurrence following four chemoreduction strategies.

In summary, chemoreduction with or without focal tumor consolidation is effective therapy for retinoblastoma. Each treated tumor had approximately 22% risk for recurrence. Most recurrences were detected within 1 year of initiation of chemoreduction, and no recurrences occurred after 4 years follow-up. Meticulous examination in the first few years following chemoreduction and consolidation is critical to early detection of recurrence and salvage of the eye. Long-term follow-up is advised in all cases.

REFERENCES


DISCUSSION

Dr Barrett G. Haik. In the past decade, systemic chemotherapy has replaced external beam radiotherapy as the primary treatment for patients with multifocal intraocular retinoblastoma. Different combinations of carboplatin, vincristine, etoposide or teniposide, and cyclosporine form the basis of most current treatment regimens for intraocular retinoblastoma. Although no two institutions have had identical experiences, reports thus far have been encouraging. Just as chemotherapy protocols have varied, so have the methods and timing of consolidation for individual tumors. Even the need for consolidation is debatable: Some investigators have favored intensive, early focal consolidation while others have elected to defer it. This is due in part to difficulty in predicting an individual tumor’s response to chemotherapy. Lesions that demonstrate the classic patterns of regression described by Reese and Ellsworth following chemoreduction do not necessarily behave in a similar manner following chemotherapy.

We agree with Dr Shields and colleagues that primary systemic chemotherapy alone does not successfully treat most eyes with multifocal intraocular retinoblastoma. Consolidation with external beam radiotherapy, episcleral plaque brachytherapy, diode laser, argon laser photocoagulation, and/or cryotherapy is usually needed. However, we did observe individual tumors in those eyes that required no consolidation following chemotherapy. Those tumors were more often located in the posterior pole. Treatment of those lesions with chemotherapy alone spared visually sensitive areas from the destructive effects of consolidation, hopefully ensuring maximal preservation of vision.

Dr Shields and colleagues have analyzed local tumor control and factors predictive of recurrence in a group of 457 retinoblastomas occurring in 193 eyes of 125 patients. Twenty-eight percent of the patients had unilateral disease; 50 percent of the eyes had advanced retinoblastoma. The authors analyzed the effectiveness of different combinations of therapies for local control and concluded that chemotherapy alone or combined with focal treatments is effective, but large tumors and those occurring in the macula have higher rates of recurrence.

These are important observations. However, several of these observations deserve additional evaluation:

The use and type of focal treatments for each patient, each eye, and each tumor are functions of several factors: laterality and location of the tumor, intraocular group, age of the patient, expected visual outcome, compliance status, treatment given to other tumors in the same eye, tumors in the vicinity, growth, etc.

Although the authors describe the different treatment groups, it is not clear how the decisions were made regarding the treatment of an individual tumor. It appears that consolidation therapy may have been delayed or modified in the most visually sensitive areas of the retina. While we agree with this strategy, it makes some comparisons of tumor responses extremely difficult.

This study includes patients treated over an eight-year period. Treatment practices have evolved over those years.

The authors have provided valuable information on retinal tumor control and in this study did not evaluate vitreous or subretinal seed control. We look forward to additional studies discussing the clinical management of vitreous disease in retinoblastoma patients who have received chemotherapy, since these foci are often the source of tumor control failure and eye loss.

Dr John T. Flynn. These are three very toxic drugs and you are administering them to very small children. What have been the side effects? It also seems that retinoblastoma is an ideal tumor to study with a randomized clinical trial. Are the major centers that study or treat patients with retinoblastoma considering a randomized clinical trial or are each of you resolving your medications, dosages, and regimens of treatment independently of each other?

Dr Bartley R. Freuh. With rhabdomyosarcoma, the great advances that have been made have been by pooling cases. Recognizing different research groups are using different regimens, combining your data and then fully evaluating the data might advance the treatment of retinoblastoma more quickly.
DR DOUGLAS A. JABS. There is a potential treatment indication bias issue. The study demonstrated that recurrence was related to macular location, and your indication for chemotherapy alone was macular location. Your conclusion is that consolidation reduces the rate of recurrence, but you’re consolidating those who are less likely to recur. Did you do a multivariate analysis, and does the conclusion that consolidation reduces the rate of recurrence still persist after a multivariate analysis that corrects for tumor location?

DR CAROL L. SHIELDS. I’d like to thank Dr Haik for his wisdom and comments. He continues to provide us with a tremendous amount of information regarding therapy for retinoblastoma.

Regarding Dr Flynn’s question on the side effects, those of us who treat retinoblastoma with systemic chemotherapy are worried about side effects. Are we trading the side effects from external beam radiation for the side effects from chemotherapy? The side effects using carboplatin in children include ototoxicity and renal toxicity. In our series of patients, we have not seen these side effects, but we monitor the medication carefully and rarely give more than six cycles. There would be nothing worse than having a child who’s blind from the retinoblastoma and deaf from the chemotherapy. The main side effect from etoposide is induction of leukemia that usually occurs within the first five years after delivering the medication. Worldwide, there have been about 11 or 12 cases of secondary leukemia from etoposide. The main side effect from vincristine is neurotoxicity. We have had a few children develop neurotoxicity, usually manifested as a head droop. When we cut back on their vincristine dose, it usually resolves. I’m very interested in the carboplatin in the epibulbar gel that was discussed in the symposium today. There is a push toward delivering the chemotherapy locally to avoid these systemic side effects.

Regarding Dr John Flynn’s question about a clinical trial, we made an effort to start a national clinical trial on retinoblastoma called The Retinoblastoma Study. About five or six years ago, we all met at the NIH to discuss the design of that clinical trial. At that time, we were going to be using a trial that was instituted in Toronto, but later this evolved into a trial that was developed in Los Angeles and Philadelphia in a collaborative mode similar to treating advanced eyes with rhabdomyosarcoma. Advanced eyes with retinoblastoma would receive high dose chemotherapy, three agents plus subconjunctival carboplatin, plus low dose radiation. That trial is about to begin at eight major centers in the United States. The second part of The Retinoblastoma Study will be to evaluate intermediate retinoblastoma using two agents and avoiding etoposide, the drug that might induce leukemia. Then there are other side investigations that will be in The Retinoblastoma Study such as risk factors predictive of metastatic disease and some pathology-based factors. They will not be randomized because of other complex issues. Randomizing a child who has unilateral versus bilateral disease or has unifocal versus multifocal disease is difficult. These are going to be single-armed trials where we all pool our data and come to a consensus regarding the best approach for chemoreduction for retinoblastoma.

Dr Freuh raised the question about a clinical trial and suggested a retinoblastoma trial similar to the rhabdomyosarcoma trial. The rhabdomyosarcoma trial really made giant steps in our management of rhabdomyosarcoma back in the 1970s when only 30 percent of children survived with rhabdomyosarcoma. Currently, 93 percent of children survive. With the upcoming national study of retinoblastoma, we might obtain better results in the long term by collaborating with our colleagues.

DR JABS queried the design of our study. This was a retrospective study to assess our results regarding the need for tumor consolidation for children with retinoblastoma. As it turns out, tumors in the macular region tended to show the highest recurrence whether or not consolidation was performed. Our study and several other studies have now shown that macular retinoblastoma tends to be the site with the greatest recurrence. This nonrandomized retrospective analysis of a rare tumor would not allow us to select out the macular tumors and compare to extramacular tumor results.

We appreciate the interest and comments of the participants in our study on chemoreduction for retinoblastoma.
THE UTILIZATION OF EYE CARE SERVICES BY PERSONS WITH GLAUCOMA IN RURAL SOUTH INDIA

BY Alan L. Robin MD,* Praveen K. Nirmalan MD MPH, Ramasamy Krishnadas MD, Rengappa Ramakrishnan MD, Joanne Katz ScD, James Tielsch PhD, Ravilla D. Thulasiraj MBA, and David S. Friedman MD MPH

ABSTRACT

Purpose: To determine utilization of eye care services, in particular those relating to glaucoma, in a rural population of southern India aged 40 years or older.

Methods: A total of 5,150 subjects aged 40 years or older selected through a random cluster sampling technique from three districts in southern India underwent detailed ocular examinations for vision impairment, blindness, and ocular morbidity. Information regarding previous use of eye care services was collected from this population through a questionnaire administered by trained social workers prior to ocular examinations.

Results: One thousand eight hundred and twenty-seven persons (35.5%) gave a history of prior eye examinations, primarily from a general hospital (n = 1,073, 58.7%). Increasing age and education were associated with increased utilization of eye care services. Among the 3,323 persons who had never sought eye care, 912 (27.4%) had felt the need to have an eye examination but did not do so. Only one third of persons with vision impairment, cataracts, refractive errors, and glaucoma had previously utilized services. Of the 64 subjects diagnosed as having primary open-angle glaucoma, 32 (50%) had previously seen an ophthalmologist, but none had had an eye examination within 1 year before the study. Only six (19%) of the 32 had been diagnosed as having glaucoma (9% of all subjects found to have glaucoma in the survey). Thirteen (20.3%) of the 64 subjects were blind in either eye due to glaucoma, including one person who was bilaterally blind.

Conclusions: A large proportion of persons in a rural population of southern India who require eye care are currently not utilizing existing eye care services. Strategies to improve the uptake of services are required to reduce the burden of blindness due to glaucoma in southern India.


INTRODUCTION

Glaucoma is a major cause of global blindness. Regional and racial differences in the prevalence of glaucoma have been reported, with significant variations in visual loss that may apparently differ by ethnic group. India has over 1 billion people and has a huge burden of visual impairment and blindness. Studies have reported the prevalence of glaucoma from urban and rural populations in south India. In these populations, age-related cataract was the leading cause of bilateral blindness in those over 40 years of age. This accounts for 77.5% of bilateral blindness. Glaucoma was responsible for 10.2% and optic atrophy for 8.2% of bilateral blindness.

Various measures have been employed in an attempt to reduce the burden of blindness in the United States. For preventable blindness to be minimized, persons must first utilize the available eye care resources. Preslan and Novak found poor utilization of already existing resources in the pediatric age group. Following a primary school screening, when financial and transportation barriers were removed and free eyeglasses were provided, only 30% of children wore glasses and 80% failed the school screening 1 year later. Quigley and coworkers evaluated the utilization of eye care following screening...
held at churches and community centers during daytime, evenings, and weekends. They found that even in this ideal situation, where transportation was paid for, visits were free, and locations were convenient, only 17% of all who were asked to schedule a definitive examination did so. Only 60% diagnosed as having definite ocular pathology returned for follow-up. Seventeen subjects had been previously diagnosed as having glaucoma, prior to the study, and 24% of them had been lost to follow-up for at least a year. In the 27 subjects diagnosed as having glaucoma, 5 (29%) were lost to follow-up. In the group with suspected glaucoma, 3 of 7 (43%) who were told they needed drops actually returned. Likewise, 25 of 37 (68%) who were told they did not need drops, but were suspected of having glaucoma, were lost to follow-up.

The low level of utilization of eye care has been previously documented in prevalence surveys in developed nations. The Baltimore Eye Survey found that within a five-mile radius of the Wilmer Institute (Johns Hopkins Hospital), 35.8% of people older than 45 years were needlessly disabled by curable cataracts, 6.6% by diabetic retinopathy, and 4.7% by glaucoma. Had these individuals utilized available eye care, much of this disability might not be present.

The utilization of eye care is less than ideal in more developed nations. Little is known about the utilization of available eye care resources for glaucoma in less developed nations. Blindness is a major public health care problem in India despite relatively recent sustained efforts by the ophthalmic community that has seen a doubling of cataract surgical output to 3.5 million in 2000. However, we have found that more than 40% of those with bilateral blindness had never visited an eye doctor. Previous studies have reported on the barriers to eye care services in south India and have found that economic reasons and access to care (including transportation and lack of persons to accompany patients) were among the most important reasons that persons blind with cataract did not seek care. The ophthalmic community can address some of these identified barriers. However, a large proportion of individuals who have preventable visual disability require focused and sustained interventions, including, but not limited to, improving literacy, improving health education, and improving practical aspects of access to health care, such as transportation.

The high rates of blindness prevailing in India despite the sustained improved efforts of the ophthalmic community suggest that a larger national and community-based concerted effort is required to reduce blindness in India to manageable levels. Besides improving infrastructure and manpower, a major challenge will be to address the barriers currently preventing a large proportion of the blind population from utilizing existing services.

We have noted that almost three quarters of those older than 40 years had poor vision necessitating eye care, yet only 61% of those needing eye care services had ever previously sought such services. Diseases such as glaucoma can be treated if detected early enough, and the risk of visual disability or loss can be significantly minimized. There are many reasons for blindness due to primary open-angle glaucoma (POAG). These include, but are not limited to, inability to screen and diagnose glaucoma, inadequate or inaccurate therapy, lack of compliance, and nonutilization of available facilities.

To the best of our knowledge, there have been no previous reports on utilization of eye care services by persons with glaucoma in less developed nations. This study reports on the utilization of eye care services by subjects with glaucoma identified through a population-based survey in rural south India.

METHODS

The design and methodology of the Aravind Comprehensive Eye Survey (ACES) have been previously published. Briefly, ACES is a population-based cross-sectional survey carried out between November 1995 and February 1997 among rural residents aged 40 years or older in three districts of southern India (Madurai, Tirunelveli, and Tuticorin) to assess the burden of ocular morbidity and blindness (Figure 1). The sampling frame for this study consisted of a sample of typical rural areas (equivalent to counties within the United States) that are served by the Aravind eye hospitals that are located in both Madurai and Tirunelveli districts, India. During the period of the study, these hospitals provided free eye care to over 50% of all patients (Figure 2) and gave free surgical care to over 60% of all patients (Figure 3).
Subjects for the study were identified through a stratified systematic random cluster sampling technique. The sampling frame for this study consisted of a sample of typical rural districts in order to best reflect the rural population in the southern part of India. This sample is representative of rural areas in south India, but not necessarily of urban areas there or of rural or urban areas further north in India.

Comprehensive ocular examinations were offered to all subjects aged 40 and older who resided in selected geographic areas and were willing to be part of the study. Comprehensive ocular examinations were performed at the base hospital and included slit-lamp biomicroscopy, lens grading using the Lens Opacities Classification System III (LOCS III), applanation tonometry, gonioscopy, visual fields using automated perimetry, and dilated fundus examinations with indirect ophthalmoscopy and 90-diopter lens for all subjects. Visual acuity was measured with retroilluminated ETDRS charts, and refraction was performed for all subjects. Examiners were standardized for the study prior to the start of the study and at regular intervals during the study period.

Intraocular pressure (IOP) was measured using Goldmann applanation tonometry at the slit lamp with the patient under local anesthesia. Three consecutive measurements were taken and recorded, and the median measurement was considered as the IOP for analysis. A single-mirror Goldmann contact lens (Ocular Instruments Inc, Bellevue, Washington) was used for gonioscopy on all subjects, and the anterior chamber angle was graded using the Shaffer system of classification. The clock hours for each grade were also recorded. Angles were considered open if more than 10 clock hours were clearly visible up to the scleral spur in each eye. We also looked for changes in the angle, including increased pigmentation, pseudoexfoliation (PXF) deposition, and PXF material within the angle during gonioscopy. Visual field examination was deferred for participants who either refused or had visual acuity less than 6/30 in the eye to be tested. All eligible participants underwent a Humphrey central 24-2 full threshold visual field test by the Humphrey automated perimeter (Humphrey Instruments Inc, Dublin, California). If the visual field was determined to be abnormal, unreliable, or both, testing was repeated on a subsequent day or on the same day after the subject had adequate rest. Criteria used to determine abnormality included an abnormal glaucoma hemifield test and a corrected pattern standard deviation $P < .05$. Criteria used to determine unreliability of the fields included false positives 50% or greater, false negatives 33% or greater, and fixation losses 50% or greater.

The definition of glaucoma used in this study did not depend on IOPs and required one or both of the following: (1) changes in the appearance of the optic nerve head due to glaucoma and (2) perimetric defects in the nerve fiber bundle pattern typical of damage from glaucoma. Subjects with a vertical cup-to-disk ratio of $>0.8$ or a narrowest neuroretinal rim width of $<0.2$ (including classic notching) or asymmetry $>0.2$ between eyes coupled with a visual field defect in the matching location were considered as cases of glaucomatous optic nerve damage. When visual fields were not available because of a subject’s poor visual acuity or poor reliability, the presence of significant optic disk excavation compatible with glaucoma, or end-stage glaucoma with severe central vision loss, or total optic disk cupping was sufficient for diagnosing glaucomatous optic nerve damage. Subjects with symmetric, large optic cups and
eyes with IOP greater than 21 mm Hg but without
definite evidence of glaucomatous optic nerve damage
were characterized as glaucoma suspects and advised to
seek periodic ophthalmologic examination.

To be sure that individuals with mildly atypical
findings or inconsistencies or missing data were not
overlooked, all individuals with potentially abnormal
visual fields were reviewed again clinically by two
glaucoma specialists. Abnormal visual fields included
abnormal or borderline fields on the glaucoma hemifield
test and fields that were abnormal but incompatible with
glaucoma. Available visual fields of an individual were
compared with one another and with the appearance of
the optic disk for compatibility. None of the subjects who
were reviewed again met the definition of glaucomatous
optic nerve damage, that is, a vertical cup-to-disk ratio
>0.8 or a narrowest neuroretinal rim width <0.2
(including classic notching) or asymmetry >0.2 between
eyes.

Definite POAG was defined as angles open on
gonioscopy and glaucomatous optic disk changes with
matching visual field defects. Ocular hypertension was
defined as IOP greater than 21 mm Hg and open angles
on gonioscopy without glaucomatous optic disk damage
and visual field defects. Manifest primary angle-closure
glaucoma was defined as (1) glaucomatous optic disk
damage or glaucomatous visual field defects with the
anterior chamber angle either partly or totally closed,
appositional angle closure, or synechiae in the angle, and
(2) absence of signs of secondary angle closure. Secondary
glaucoma was defined as glaucomatous optic nerve
damage or visual field abnormalities, or both, suggestive
of glaucoma with ocular disorders that contribute to a
secondary elevation in IOP.

Prior to ocular examinations, trained social workers
conducted interviews to collect demographic and other
details using a structured questionnaire. Information
was collected regarding prior eye examinations, including
service provider visited, the duration since the last
examination, and the reason for an eye examination.
Information was collected on whether people did not visit
an eye doctor even though they had felt a need, and the
reason for not visiting an eye doctor.

Persons requiring eye care in this study population
were defined as persons with presenting vision in the
better eye worse than 6/18 and/or a diagnosed ocular
pathology after examination.

The study protocol was approved by the institutional
review board of Johns Hopkins Bloomberg school of
Public Health, Baltimore, Maryland, and Aravind Eye
Care System, Madurai, India. The tenets of the Treaty of
Helsinki were followed. Verbal informed consent was
obtained prior to the study because a significant
proportion of the population was illiterate.

Bivariate and multivariate logistic regression was
performed to explore for associations with utilization
patterns using STATA version 7.0 (College Station, Texas).
We considered P values <.05 to denote statistical
significance.

RESULTS

Of the eligible 5,539 persons aged 40 years or older, 5,150
were examined (a response rate of 93.0%). Three
thousand four hundred and seventy-six (72.7%) of 5,150
subjects examined required eye care examinations. One
thousand eight hundred and twenty-seven persons
(35.5%) gave a history of prior eye examinations, primarily
from a general hospital (n = 1,073, 58.7%). Table 1 shows
the prevalence (95% confidence interval [CI]) of
glaucoma in the study population. Sixty-four subjects had
POAG. The prevalence of exfoliation in this population
was 6.0% (95% CI: 5.3, 6.7). The median age of those with
any glaucoma was 60.0 years (mean, 60.8 ± 10.1 years;
range, 40-85 years), and 79 (60.0%) were males.

After best correction with refraction, 19 persons with
POAG were visually impaired, including one person who
was blind (best-corrected visual acuity <6/120 in either
eye). An additional 12 persons had unilateral blindness
due to glaucomatous optic neuropathy in that eye; thus 13
persons (20.3%) with POAG were blind in one or both
eyes due to POAG.

Only 1,827 subjects had ever received any prior eye
care. The most common reasons were lack of funds, time,
or an escort.

There were 912 persons who felt that they needed
eye care but did not seek it. Many reasons were given for
this lack of care (Figure 4). The most common reasons for
not obtaining care in this group were the lack of funds
(78.2%) and the lack of time to come for an examination
(70.0%).

Of the 132 persons with any glaucoma, 67 (50.1%)
had never had an eye examination. Nineteen (14.4%) of
the 132 persons with any glaucoma had their last eye
examination within the 2 years immediately preceding our
survey. Forty-five (34.1%) of the 132 persons with any
glaucoma had previously been to a hospital, and an
additional 21 (15.9%) persons had ever visited an eye
doctor. However, only 11 (16.9%) of the 65 persons with
glaucoma who previously had an ocular examination had
received any treatment for glaucoma at the time of the
survey. This included nine persons taking eye care
services increased significantly with age (Table 2). After
adjusting for age and sex, the odds for utilization

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increased with increasing education. Persons with unilateral or bilateral vision impairment or blindness were more likely to use services ($P < .01$).

Of the 64 subjects diagnosed with POAG, 32 (50.0%) had never had an eye examination in the past. Although 7 (21.9%) of the 32 subjects diagnosed with open-angle glaucoma who previously accessed eye care services had an eye examination within the 2 years immediately preceding our survey, none of them had an eye examination within the 1 year preceding the survey. Only 6 (18.75%) of these 32 subjects who accessed eye care services had received any antiglaucoma treatment at the time of the survey; thus 93.0% of those with open-angle glaucoma had not been diagnosed until the survey.

**DISCUSSION**

The results of National Eye Institute–sponsored multicentered studies have all shown that adequate IOP lowering has the potential to prevent both the development and the progression of visual field loss leading to visual disability and blindness.23-25 That is, glaucoma is a treatable disease. The skills required to detect glaucoma are relatively simple and include measurement of the IOP and examination of the visual acuity, pupils, visual fields, and optic nerve. One can lower eye pressure and thus treat glaucoma in one of several ways: medications, surgery, or lasers. Lasers are not commonly available in rural India. However, eye pressure–lowering medications such as beta-blockers, alpha-agonists, and carbonic anhydrase inhibitors are commonly available at reasonable prices in India (less than US $1.00). Surgical skills to perform glaucoma filtration surgery are also commonly taught.

Even though medications and surgical skills to treat glaucoma are available, 30% of those detected in our study who had glaucoma were blind or visually disabled in one eye and 34% had severe visual field loss in both eyes. Low utilization of glaucoma eye care services, in a region where first-rate services are available, may be responsible...
for this dismal state of affairs. Many steps are needed to remedy this situation.

First, ophthalmologists must begin to educate individuals at an early age about the role of health care resources and how to better utilize them. People should be taught that blindness is not a normal part of aging and an expected outcome of older age. Marketing of health care availability and utilization, through videos, the media, and local religious and service organizations could also help. Better education about prevention of blindness, in a nation where blindness is so evident, might well help to minimize it.

People also need to be educated about the types of eye care providers. In the population studied, 39% of those requiring eye care services had sought some help, and almost 60% of these had gone to a general hospital, not an eye care provider. Less than one quarter had visited an ophthalmologist. Because of the relative paucity of ophthalmologists in India compared to developing countries, we must educate patients about the proper place to go for ophthalmic care and also educate other health care practitioners about diseases such as glaucoma, glaucoma screening, the fact that glaucoma can be successfully treated, and the ability of glaucoma to cause blindness.

As in more developed nations, finances can definitely influence the utilization of ophthalmic health care in developing countries. This can be a complex issue. During the period of the study, the Aravind eye care hospital system, which prides itself on giving high-quality cost-efficient eye care, offered such care at no charge to over one half of patients with no questions asked and gave free surgery to almost two thirds of all patients. In those who felt that they needed eye care, but who did not obtain it, it is important to note that the reasons given may be typical of a relatively poor agrarian society. Money is an obvious reason for not seeking medical care, but it becomes a thought-provoking cause when one notes that the actual eye care would have been free. Subjects may or may not have known that they could have received such free care at Aravind. However, we did not ask about or account for monies lost by the subject or accompanying family member by not earning a day’s wage when visiting eye care facilities. Strategies to improve utilization should include education regarding the cost-efficient nature of obtaining appropriate health care relative to the costs of blindness. Many patients come to physicians with a family member accompanying them. This might be especially true of individuals with limited vision, needing guidance and help navigating the potentially dangerous southern Indian road and bus systems. The fact that escorts were not available for many suggests that improvements in transportation and support systems to obtain health care might be necessary to improve utilization.

We previously found that 3.14% of those individuals who are 60 years of age or older have glaucoma in this population. That this potentially preventable cause of visual impairment and blindness, which is relatively common in this older population, is grossly underdetected may suggest the need for better strategies to identify persons with glaucoma in rural India. However, there are several issues relating to screening for glaucoma to be considered. A major concern is the lack of a good method to screen for glaucoma in populations. The definition of glaucoma requires correlation with visual fields in all cases unless perimetry cannot be performed for any reason. Computerized threshold automated perimetry is costly and time-intensive, and it may not be feasible for eye care programs, particularly those catering to rural populations in developing countries. We reported earlier that a vertical cup-to-disk ratio of >2 SD may be an alternative measure to suspect glaucoma in this rural population. However, dilation would be required, and this may be difficult in this population, where clear media is not commonly present due to either cataract or corneal opacification. Intraocular pressure measurements are neither sensitive nor specific for glaucoma screening in this population.

Less than one fifth of those with glaucoma in our survey had been previously diagnosed as having the disease despite having had an eye examination in the past. The cross-sectional design of the survey does not allow us to determine if these subjects actually had glaucoma at the time of their examination, or if glaucoma had developed during the period since their last eye examination. However, the work of Wilson and others in St Lucia, West Indies, shows that if left untreated, glaucoma can frequently result in increased visual field loss and blindness. Our results suggest the potential need to revisit residency curricula and to ensure that every ophthalmologist becomes familiar with techniques and interpretation of perimetry, slit-lamp biomicroscopy, gonioscopy and classification of the anterior chamber angle, and applanation tonometry.

Training of human resources and development of infrastructure are currently the focus of initiatives against blindness, including VISION 2020 and the National Program for Control of Blindness in India. Results from our study, however, suggest the additional need to focus on developing strategies to improve utilization of existing services. The low utilization of eye care services, even in those who perceived a need in a region where free and good quality eye care is readily available, is quite disappointing. It is disconcerting to note that only one third actually had had an eye examination at any time in
their lives, yet three fourths of persons aged 40 years or older in this rural population required eye care services. The number of persons who had had an eye examination in the previous year was only 15.1%. The need for strategies to improve utilization is further emphasized when we consider that none of the subjects with a diagnosis of glaucoma prior to our survey had had an eye examination within the previous year. The need for improved utilization assumes greater importance because recent reports have proven the efficacy of early intervention in reducing the potential for blindness in persons with glaucoma.\textsuperscript{23-25}

The results reported here suggest that the focus of eye care programs has to broaden beyond identification of cases of glaucoma if the burden of blindness due to glaucoma is to be reduced. Strategies for better education of patients and physicians and for better marketing of available services to improve utilization are urgently required to ensure follow-up and compliance to advised therapy.

REFERENCES


DISCUSSION

Dr. Bruce E. Spivey. As one would anticipate, the work by Dr. Robin and co-workers was scrupulously planned, exquisitely implemented, and thoughtfully presented. I will comment on the study and its approach only to say it is based on well accepted, previously described, and appropriate statistical, geographical, and analytical evaluation. This report adds substantially to understanding the barriers to eye care services in South India, previously noted to be the cost of services and access problems due to transportation and the lack of accompanying persons.

When we hear of South India and the state of Tamil Nadu, those unfamiliar may assume that there is little opportunity for eye care in the region. This is far from reality. The Aravind Eye Care system based in Madurai is the largest private eye care system in the world. Last year, the system saw more patients (1,480,012) and operated on more eyes (202,066) than any other eye system. As noted, they provide a free service for those who cannot pay. This means their findings have more profound and daunting implications than it would initially appear. Superb care is readily available in this state in India. Thus, the barriers are not the obvious: availability or even cost of care. The barriers, however, are fundamental and deeply based on the society, patient education, effective marketing, and family travel support.

The paper has implications far beyond glaucoma in South India. As the quality and availability of eye care is slowly improved throughout the world, there clearly cannot be even a minor victory declared when good quality, free, and available eye care does not translate directly to the successful treatment of preventable blindness. The implications are enormous. Ophthalmologists have traditionally felt that if they are in place with commitment and infrastructure, functional vision success will naturally follow. The Baltimore Eye Study has shown the same problems in the United States.

Strategies for involvement of community leaders (formal and informal), existing community services, intense outreach through marketing in all possible forms, and supportive transportation services are absolute necessities and must be considered an integral part of any eye care service program. It must truly be a team effort—planned and implemented with the same approach we take with eye surgery itself.

I recently had the opportunity to visit multiple ophthalmology training programs in Nigeria. Here the capacity is far less than what is available from the Aravind Eye Care System. The gap between what is available in almost all of sub-Saharan Africa and that in Aravind is far greater than that between Aravind and the United States.

Even an experienced observer with very substantial credible time in the field such as Dr. Robin, has not yet created a series of definitive steps that, if taken, would be successful. We have learned to provide efficient, effective, and low cost (even free) eye care. We have not yet learned how best to ensure those who actually need that care will avail themselves of the availability.

Dr. George L. Spaeth. We have to think frequently about what we are here for. Presumably, one of the reasons is to try to prevent people from going blind, or, even more pertinently, to increase their quality of life. Dr. Robin did not mention some of the other studies that have been done recently by David Friedman of Wilmer and by Rohit Varma of Los Angeles. Dr. Varma has recently shown that in Hispanics, the increase in the prevalence of glaucoma is so great with increasing age that it gets to be 24 percent in individuals over 80. In blacks, the increase is almost, but not quite, as exponential. These are the two groups that are undergoing a significant worldwide increase in population. We literally are having an epidemic of glaucoma. Jeff Henderer at Wills has been interested in how to get people into care. He did a study recently where he went to church groups in Philadelphia, realizing that people are likely to have some kind of tie-in, and then screened people for glaucoma. The church people were addressed by a doctor and by a social worker, were given an appointment, and then were given a voucher for free travel to the hospital. Only 11% came for the appointment. When we consider the amount of resources that are being put into studying matrix metalloproteinases in comparison to preserving sight, how do we start all over?

Dr. Dennis M. Robertson. Aravind Eye Hospital, which can be regarded as a model of eye care for the world, has 1,000 beds for eye patients; it is an amazingly effective system of eye care in large part because the staff who work there are spiritually dedicated to the whole movement of saving sight in India. Most of the people who are working there have had an opportunity to practice in other environments; some have had successful practices in the United States but they returned to India to work for very little monetary reward. The success of Aravind is not only because of the spiritual dedication of the people on the staff but also because of the help from other people. For example, Suresh Chandra, who has been with Combat Blindness, has helped develop a manufacturing plant at
The Utilization of Eye Care Services by Persons With Glaucoma in Rural South India

The Aravind site that produces intraocular lenses for approximately $5. They now supply about one-third of the world's intraocular lenses; with the income from lenses that are sold and from the income from those patients who can pay, they have been able to provide care for many who have no means of paying for their care.

Dr. John T. Flynn. The best reason why there are starving human beings in the midst of plenty is because of poverty. About two months ago, there was an editorial in the New England Journal of Medicine where an internist had the job of telling a 24-year-old Hispanic woman, mother of two or three children, that she had AIDS. He went through a tremendous examination of his conscience and how terrible he felt about this. When he told her, she just sort of brushed it off. Why did she brush it off? Because she had family problems, economic problems, work problems, drug problems that were so staggering that the diagnosis of AIDS—which is essentially today a sentence of death, even though it's prolonged life—meant little or nothing to this woman. Unless we address the socioeconomic basis, which is poverty, we're never going to change the way they access the healthcare system.

Dr. Edward L. Raab. I recently had the privilege of doing two separate volunteer faculty tours for ORBIS in India, and I can tell you that what you describe is common in other areas of India as well. I was most recently in West Bengal, and your description matched with the situation there very well. One of the things that impressed me was the compliance issue, and it makes me now distinguish between the two types of non-compliance. One is willful non-compliance, or neglectful non-compliance, and the other is logistical non-compliance. People have to be in the fields working, they don't have money to get to where they have to go or to obtain the necessary treatments, and that is a much larger problem in the area where I worked than was willful non-compliance. Maybe the distinction between those two concepts would be useful in describing some of your data.

Dr. Alan L. Robin. I thank Dr. Spivey for his thought-provoking and excellent initial discussion and I'd like to agree with all the comments by the other discussants. The question of how do we go about changing behavior is really the most fundamental question that should engage us as leaders. Dr. Spaeth's comment about the allocation of research funding is a very important issue. We must re-examine our priorities. I believe that the most important question facing our current advanced medical system is the question of how to get patients who should be in the system, with real pathology, into facilities that can render good quality care. I think the last comment about the universality of the problem is true. If it happens in Baltimore, in West Bengal, and in Madurai, then it is a universal problem. The Gurwitz study (1993) found that the mean number of days of missed glaucoma therapy in 2,440 patients was 112 days per year. Mark Preslan found that parents, even when given free care, social workers' assistance, and free spectacles, did not utilize this care and allowed their children to become amblyopic. What about physicians' families and their compliance patterns? There have been compliance studies looking at physicians' wives and how they comply with amoxicillin therapy for their children with otitis media. In this group of potentially well-motivated and well-educated people, nearly 80 percent of the mothers did not fully comply with the antibiotic regimen. The AREDS data found that only 75 percent of patients in the AREDS study fully complied with the vitamin regimen. Following the study, less than one-half of this group of well-motivated individuals complied.

Debra Roter at our Johns Hopkins Bloomberg School of Public Health did a study that investigated how well patients heard what doctors told them when their medications were changed. During doctor patient interactions, a tape recorder actually recorded all instructions. Patients were then interviewed and approximately 60 percent of the patients did not remember that the doctor had changed their therapy.

How can we change this behavior and improve utilization of available resources, compliance and adherence? I think we have to market good quality care! India is the biggest user of videos in the whole world (Bollywood). If one could put a leader on every one of those videos that related the appropriate use of eye care facilities and this would train individuals that losing vision is not a normal part of growing old, it would be a tremendous step toward eradicating blindness. Efforts like those of Bruce Spivey, George Spaeth, and others in this room are needed to educate patients that their own efforts can prevent some of their own visual disability so that potentially they can maintain their independence and improve their quality of life.
CORNEAL ENDOTHELIUM AND POSTOPERATIVE OUTCOMES 15 YEARS AFTER PENETRATING KERATOPLASTY

BY Sanjay V. Patel BMBS,* David O. Hodge MS, and William M. Bourne MD

ABSTRACT

Purpose: To determine changes in the central endothelium and thickness of grafted corneas, and the cumulative probability of developing glaucoma, graft rejection, and graft failure 15 years after penetrating keratoplasty.

Methods: In a longitudinal cohort study of 500 consecutive penetrating keratoplasties by one surgeon, regrafted eyes, fellow eyes of bilateral cases, and patients not granting research authorization were excluded, leaving 388 grafts for analysis. At intervals after surgery, we photographed the endothelium and measured corneal thickness by using specular microscopy. The presence of glaucoma, graft rejection, and graft failure was recorded.

Results: The 67 patients examined at 15 years represented 30% of the available clear grafts (107 patients had died, 76 grafts had failed). Endothelial cell loss from preoperative donor levels was 71 ± 12% (mean ± SD, n = 67), endothelial cell density was 872 ± 348 cells/mm², and corneal thickness was 0.59 ± 0.06 mm. Endothelial cell density was unchanged between 10 and 15 years (minimum detectable difference was 96 cells/mm², α = .05, β = .20, n = 54), whereas corneal thickness increased (P = .001, n = 55). The mean annual rate of endothelial cell loss from 10 to 15 years after surgery was 0.2 ± 5.7% (n = 54). The cumulative probability of developing glaucoma, graft rejection, or graft failure was 20%, 23%, and 28%, respectively, and six of the eight graft failures after 10 years resulted from late endothelial failure.

Conclusions: From 10 to 15 years after penetrating keratoplasty, the annual rate of endothelial cell loss was similar to that of normal corneas, corneal thickness increased, and late endothelial failure was the major cause of graft failure.

INTRODUCTION

After the importance of the corneal endothelium in penetrating keratoplasty was established in the 1970s, we reported 5- and 10-year data on a cohort of patients who had undergone this procedure. The annual rate of endothelial cell loss from 3 to 5 years after penetrating keratoplasty was 7.8% per year, and from 5 to 10 years, was 4.2% per year. The 10-year cumulative risk of developing glaucoma, graft rejection, and graft failure was 21%, 21%, and 22%, respectively.

We report here data for the same cohort of patients, which has now been observed for 15 years after surgery, thus updating the status of the corneal endothelium, central corneal thickness, and the cumulative probability of developing glaucoma, graft rejection, and graft failure.

METHODS

The cohort consists of 500 consecutive patients who had penetrating keratoplasty performed by one surgeon (W.M.B.) between 1976 and 1986. Thirty-six repeated grafts and 70 fellow eyes were excluded from the study, leaving 394 grafts in 394 patients (ie, 394 independent observations) available for analysis to 10 years after surgery. For the 15-year data, six patients had withdrawn research authorization, leaving 388 grafts available for analysis. There were 141 males (36%) and 247 females (64%), and age at keratoplasty was 62 ± 20 years (mean ± SD; range, 3 to 93 years).

The surgical technique has been described in detail previously. Donor buttons, with mean diameter of 7.9 mm (range, 6.5 to 10.5 mm), were cut from the endothelial side and sutured into the recipient by using a double-running technique, except for high-risk grafts, in which interrupted sutures were used. Postoperatively, prednisolone acetate 1% was administered topically by the patient from the time of epithelial healing to 3 to 6 months, but rarely more than once per day after the second month.

The central donor corneal endothelium was

*Presenter.

Bold type indicates AOS member.
photographed by using a specular microscope before storage in either McCarey-Kaufman medium at 4°C, organ culture at 34°C, or K-Sol medium at 4°C. Follow-up examinations were scheduled for 2 months and for 1, 3, 5, 10, and 15 years after keratoplasty. At each visit, central corneal endothelium was photographed and corneal thickness was measured by contact specular microscopy. Patients were examined to detect complications, including glaucoma, graft rejection, and graft failure.

The outlines or apices of at least 50 endothelial cells were digitized from specular micrographs. We calculated mean endothelial cell area, mean endothelial cell density, coefficient of variation (SD/mean) of endothelial cell area, and the percentage of cells that were hexagonal. Endothelial cell loss was the decrease in cell density between the preoperative examination and the 15-year examination, expressed as a percentage of the preoperative cell density. The annual rate of endothelial cell loss was calculated by assuming that the endothelial cell loss between 10 and 15 years was exponential (first order):

\[ ECD_{15} = ECD_{10}e^{-rt} \]

where \( ECD \) is endothelial cell density, the subscripts 10 and 15 indicate the postoperative year, \( r \) is the annual rate of endothelial cell loss, and \( t \) is time in years. On the interval from 10 to 15 years, \( t = 5 \) years.

Endothelial cell density through 15 years was also fitted to a biexponential model to combine the rapid, early endothelial cell loss after keratoplasty, and the slow, chronic endothelial cell loss. This model was described by Armitage and associates:

\[ ECD_t = pe^{-at} + qe^{-bt} \]

where \( ECD_t \) is endothelial cell density at time \( t \), \( p \) and \( q \) are constants, the sum of which is equal to the initial endothelial cell density, and \( a \) and \( b \) are the fast and slow exponential rate constants, respectively. The half-lives of the fast and slow components of this model are \( 0.693/a \) and \( 0.693/b \), respectively.

Patients were classified as having glaucoma if they had required a surgical procedure to lower intraocular pressure or had used ocular hypotensive agents long-term (for 3 months or longer). Graft rejection was defined as the occurrence of epithelial or endothelial rejection lines, subepithelial infiltrates in the graft only, or a substantial number of new keratic precipitates usually accompanied by segmental ciliary injection, a mild anterior chamber reaction, and an increase in stromal thickness. Only initial rejection episodes were included in the database for computing statistics. Graft failure was defined as an irreversible loss of central graft clarity. Primary endothelial failure was defined as irreversible graft swelling without apparent cause and was classified as either primary donor failure or late endothelial failure. Primary donor failure was defined as unexplained failure of the graft to become thinner and clearer in the first few postoperative weeks. Late endothelial failure was defined as gradual graft decompensation without apparent cause, unresponsive to corticosteroids, and with no recent history of a rejection episode.

Differences in endothelial cell density, coefficient of variation of cell area, percentage of hexagonal cells, and corneal thickness were compared between groups by using a paired \( t \) test when data were normally distributed, or a Wilcoxon signed-rank test when the data were not distributed normally. Differences among diagnoses were tested by using the Kruskal-Wallis test for nonnormal data, and significant differences were investigated by using the Student-Newman-Keuls procedure. Correlations between continuous variables were examined by calculating Pearson’s correlation coefficient (\( r_p \)) for normal data and Spearman’s rank correlation coefficient (\( r_s \)) for nonnormal data. The cumulative probability of initial rejection episodes, graft failure, or glaucoma was estimated by using the Kaplan-Meier method. Log-rank tests and Cox proportional hazards models were used to evaluate possible risk factors for graft failure. Multivariate models were fit for the overall graft failure end point to determine if the effect of donor age could be explained by other potential risk factors. These models were fit using the Cox proportional hazards models. A two-tailed probability of 5% or less was considered statistically significant.

RESULTS

Of the 394 patients from the original analysis, 332 (84%) of the grafts were for Fuchs’ dystrophy, keratoconus, or corneal edema from aphakia or pseudophakia (Table 1). Sixty-seven patients, 30% of the clear grafts available for follow-up, returned for their 15-year postoperative examination. At 15 years, 76 grafts were known to have failed and 107 patients were known to have died, reducing the overall cohort by 164 because 19 graft failures and deaths occurred in the same patients (Table 2). A further six patients withdrew research authorization during the 10- to 15-year interval, leaving 224 patients who were presumed to be alive and without graft failure (Table 2).

There were 157 patients who were presumed to be alive and without graft failure who were not examined at 15 years. Of these, there were 58 males (37%) and 99 females (63%), age at keratoplasty was 61 ± 21 years.
Endothelial Cell Morphometry and Corneal Thickness

The results from all visits for all patients who were seen at 15 years (n = 67) are shown in Table 3. Endothelial cell loss from preoperative donor levels was 71 ± 12%. Endothelial cell density at 15 years after keratoplasty was 872 ± 348 cells/mm², which did not differ from cell density at 10 years (960 ± 470 cells/mm², P = .6). The minimum detectable difference was 96 cells/mm² (α = .05, β = .80, n = 54). From 10 to 15 years after keratoplasty, there were no differences in endothelial cell loss, coefficient of variation of cell area, or hexagonal cells, whereas central corneal thickness increased from 0.58 ± 0.06 mm to 0.60 ± 0.06 mm (P = .003, Table 4). The mean rate of endothelial cell loss from 10 to 15 years after surgery was −1.0 ± 5.4% (n = 27), consistent with a small increase in cell density.

Endothelial cell loss at 15 years compared to preoperative donor levels was 73 ± 7% for keratoconus (n = 36), 71 ± 8% for Fuchs’ dystrophy (n = 12), 67 ± 14% for pseudophakic corneal edema (n = 8), and 65 ± 24% for aphakic corneal edema (n = 2). The differences in cell loss at 15 years between these four most common preoperative diagnostic groups were not significant (P = .61, Kruskal-Wallis test). The minimum detectable difference between the keratoconus and Fuchs’ dystrophy groups was 9% (α = .05/6, β = .80).

Higher endothelial cell loss was strongly associated with higher preoperative donor endothelial cell density (rs = 0.43, P < .001, n = 66), whereas it was more weakly associated with younger donor age (rs = −0.28, P = .02, n = 66). Endothelial cell loss was not associated with recipient age (rs = −0.19, P = .13, n = 66).

### Table 2.

<table>
<thead>
<tr>
<th>EXAMINATION</th>
<th>NO. KNOWN TO HAVE FAILED DURING PREVIOUS INTERVAL</th>
<th>NO. KNOWN TO HAVE DIED DURING PREVIOUS INTERVAL</th>
<th>NO. PRESUMED ALIVE WITHOUT FAILURE</th>
<th>NO. EXAMINED (% OF NO. PRESUMED ALIVE WITHOUT FAILURE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preoperative</td>
<td>–</td>
<td>–</td>
<td>394</td>
<td>394 (100)</td>
</tr>
<tr>
<td>Postoperative</td>
<td>2 mo</td>
<td>19</td>
<td>373</td>
<td>355 (95)</td>
</tr>
<tr>
<td></td>
<td>1 yr</td>
<td>10</td>
<td>357</td>
<td>329 (92)</td>
</tr>
<tr>
<td></td>
<td>3 yr</td>
<td>17</td>
<td>331</td>
<td>231 (70)</td>
</tr>
<tr>
<td></td>
<td>5 yr</td>
<td>11</td>
<td>311</td>
<td>157 (60)</td>
</tr>
<tr>
<td></td>
<td>10 yr</td>
<td>11</td>
<td>257</td>
<td>119 (46)</td>
</tr>
<tr>
<td></td>
<td>15 yr</td>
<td>8</td>
<td>224#</td>
<td>67 (30)</td>
</tr>
</tbody>
</table>

*This table has been modified from that published at 10 years after surgery because patients with failed grafts who subsequently died had inadvertently been counted twice.
†Two deaths were also graft failures in a previous interval.
‡One patient with graft failure also died in the same interval.
§Two patients with graft failure also died in the same interval.
¶Six deaths were also failures in a previous interval.
#Six patients withdrew research authorization.
Biexponential Model of Endothelial Cell Loss

Figure 1 shows the mean endothelial cell densities in Table 3 (all subjects) fitted to the biexponential model (equation 2). The fast half-life was 8.2 months, and the slow half-life was 229.5 months. The residual standard deviation was 123.9 cells/mm². The rate of cell loss represented by the slow exponential was 3.6% per year.

The mean endothelial cell densities in Table 4 (the 27 patients who attended all examinations and had no graft rejection or surgery that could affect the endothelium) can also be fitted to equation 2 (Figure 2). The fast half-life was 13.0 months, and the slow half-life was 266.5 months. The residual standard deviation was 206.0 cells/mm². The rate of cell loss represented by the slow exponential was 3.1% per year.

Glaucoma

Of the 67 patients who returned for the 15-year examination, five developed glaucoma. The onset of glaucoma was within 2 months for one patient (grafted for pseudophakic bullous keratopathy), between 1 and 3 years after surgery for two patients (both were grafted for Fuchs' dystrophy), between 3 and 5 years after surgery for one patient (grafted for a corneal scar), and between 5 and 10 years after surgery for one patient (grafted for pseudophakic bullous keratopathy). No patient developed glaucoma between 10 and 15 years after surgery.

The 15-year cumulative probability for developing glaucoma was 20% (Figure 3). Corneas transplanted for a preoperative diagnosis of keratoconus had a significantly lower cumulative probability of developing glaucoma than corneas transplanted for aphakic or pseudophakic corneal edema ($P < .001$, Bonferroni-adjusted for 10 compar-

### Table 3. Corneal Endothelium and Thickness for All Subjects After Penetrating Keratoplasty (Mean ± SD)

<table>
<thead>
<tr>
<th>EXAMINATION</th>
<th>n</th>
<th>ENDOTHELIAL CELL DENSITY (CELLS/mm²)</th>
<th>ENDOTHELIAL CELL LOSS (%)</th>
<th>COEFFICIENT OF VARIATION OF CELL AREA</th>
<th>HEXAGONAL CELLS (%)</th>
<th>CENTRAL CORNEAL THICKNESS (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preoperative</td>
<td>393*</td>
<td>2973 ± 550</td>
<td>–</td>
<td>0.26 ± 0.06</td>
<td>68 ± 11</td>
<td>–</td>
</tr>
<tr>
<td>Postoperative</td>
<td>2 mo</td>
<td>355</td>
<td>2467 ± 675</td>
<td>17 ± 19</td>
<td>0.25 ± 0.06</td>
<td>60 ± 10</td>
</tr>
<tr>
<td></td>
<td>1 yr</td>
<td>329†</td>
<td>1958 ± 718</td>
<td>34 ± 22</td>
<td>0.26 ± 0.06</td>
<td>62 ± 9</td>
</tr>
<tr>
<td></td>
<td>3 yr</td>
<td>231</td>
<td>1376 ± 586</td>
<td>53 ± 19</td>
<td>0.26 ± 0.07</td>
<td>64 ± 11</td>
</tr>
<tr>
<td></td>
<td>5 yr</td>
<td>187</td>
<td>1191 ± 523</td>
<td>59 ± 17</td>
<td>0.29 ± 0.09</td>
<td>61 ± 13</td>
</tr>
<tr>
<td></td>
<td>10 yr</td>
<td>119</td>
<td>960 ± 470</td>
<td>67 ± 17</td>
<td>0.33 ± 0.11</td>
<td>56 ± 12</td>
</tr>
<tr>
<td></td>
<td>15 yr</td>
<td>67</td>
<td>872 ± 348‡</td>
<td>71 ± 12‡</td>
<td>0.34 ± 0.10‡</td>
<td>55 ± 11‡</td>
</tr>
</tbody>
</table>

*There was no significant difference between 10 and 15 years after surgery (paired t tests). Minimum detectable differences ($\alpha = 0.05$, $\beta = 0.80$, n = 27) were: endothelial cell density, 112 cells/mm²; endothelial cell loss, 3.5%; coefficient of variation of cell area, 0.047; hexagonal cells, 6.9%.

### Table 4. Corneal Endothelium and Thickness for All Subjects Who Returned for All Examinations, and Who Had No Rejection Episodes or Reoperations That Might Have Affected the Endothelium (Mean ± SD)

<table>
<thead>
<tr>
<th>EXAMINATION</th>
<th>n</th>
<th>ENDOTHELIAL CELL DENSITY (CELLS/mm²)</th>
<th>ENDOTHELIAL CELL LOSS (%)</th>
<th>COEFFICIENT OF VARIATION OF CELL AREA</th>
<th>HEXAGONAL CELLS (%)</th>
<th>CENTRAL CORNEAL THICKNESS (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preoperative</td>
<td>27</td>
<td>3196 ± 490</td>
<td>–</td>
<td>0.25 ± 0.05</td>
<td>70 ± 11</td>
<td>–</td>
</tr>
<tr>
<td>Postoperative</td>
<td>2 mo</td>
<td>27</td>
<td>2556 ± 913</td>
<td>20 ± 25</td>
<td>0.26 ± 0.07</td>
<td>59 ± 11</td>
</tr>
<tr>
<td></td>
<td>1 yr</td>
<td>27</td>
<td>2177 ± 698</td>
<td>31 ± 21</td>
<td>0.26 ± 0.05</td>
<td>62 ± 9</td>
</tr>
<tr>
<td></td>
<td>3 yr</td>
<td>27</td>
<td>1481 ± 599</td>
<td>54 ± 17</td>
<td>0.26 ± 0.05</td>
<td>62 ± 12</td>
</tr>
<tr>
<td></td>
<td>5 yr</td>
<td>27</td>
<td>1192 ± 455</td>
<td>62 ± 13</td>
<td>0.29 ± 0.08</td>
<td>64 ± 9</td>
</tr>
<tr>
<td></td>
<td>10 yr</td>
<td>27</td>
<td>844 ± 261</td>
<td>73 ± 10</td>
<td>0.34 ± 0.09</td>
<td>54 ± 10</td>
</tr>
<tr>
<td></td>
<td>15 yr</td>
<td>27</td>
<td>876 ± 232*</td>
<td>72 ± 9*</td>
<td>0.37 ± 0.10*</td>
<td>53 ± 9*</td>
</tr>
</tbody>
</table>

*There was no significant difference between 10 and 15 years after surgery (paired t tests). Minimum detectable differences ($\alpha = 0.05$, $\beta = 0.80$, n = 27) were: endothelial cell density, 112 cells/mm²; endothelial cell loss, 3.5%; coefficient of variation of cell area, 0.047; hexagonal cells, 6.9%.

†Central graft thickness was higher at 15 years after surgery than at 10 years for those patients who had both 10- and 15-year follow-up ($P = .003$).
Corneal Endothelium and Postoperative Outcomes 15 Years After Penetrating Keratoplasty

FIGURE 1
Endothelial cell data fitted to a biexponential decay model for all available data through 15 years. The dotted lines represent the 95% confidence interval. The coefficients are shown with their standard error (se) and corresponding \( P \) value. The fast half-life was 8.2 months, and the slow half-life was 229.5 months. The residual standard deviation was 123.9 cells/mm\(^2\). The rate of cell loss represented by the slow exponential was 3.6% per year.

FIGURE 2
Endothelial cell data fitted to a biexponential decay model for patients who attended every follow-up examination and who had no episode of rejection or surgery that could affect the endothelium (n = 27). The dotted lines represent the 95% confidence interval. The coefficients are shown with their standard error (se) and corresponding \( P \) value. The fast half-life was 13.0 months, and the slow half-life was 266.5 months. The residual standard deviation was 206.0 cells/mm\(^2\). The rate of cell loss represented by the slow exponential was 3.1% per year.

Graft Rejection

Eighteen patients who returned for the 15-year examination had at least one episode of graft rejection. The initial rejection episode was within 1 year of surgery in eight patients, between 1 and 3 years after surgery in five patients, and between 5 and 10 years after surgery in three patients. Only two patients had an initial episode of graft rejection between 10 and 15 years after surgery, and both were endothelial rejection. Of the 18 patients, eight had a preoperative diagnosis of keratoconus, six had Fuchs’ dystrophy, two had pseudophakic bullous keratopathy, and two had corneal scars (one was herpetic).

The 15-year cumulative probability for developing graft rejection was 23% (Figure 4). The cumulative probability of developing graft rejection did not differ among preoperative diagnoses (\( P > .05 \), Bonferroni-adjusted for 10 comparisons).

Graft Failure

Eight patients developed graft failure between 10 and 15 years after keratoplasty. Six had late endothelial failure (preoperative diagnosis was Fuchs’ dystrophy in four patients, keratoconus in one, and Chandler’s syndrome in
one), one failed from superficial scarring (grafted for keratoconus), and one failed because of glaucoma (grafted for aphakic bullous keratopathy). Late endothelial failure was the leading cause of graft failure by 15 years after keratoplasty, responsible for 29% of all known failures (Table 5).

The 15-year cumulative probability for developing overall graft failure was 28% (Figure 5). Corneas transplanted for keratoconus had a lower cumulative probability of failing compared with corneas transplanted for Fuchs’ dystrophy \( (P = .05, \text{Bonferroni-adjusted for 10 comparisons}) \) or for aphakic corneal edema \( (P = .006, \text{Bonferroni-adjusted for 10 comparisons}) \). Overall graft failure was not related to preoperative donor endothelial cell density \( (P = .82) \) or to recipient age \( (P = .09) \), but donor age was related to overall graft failure \( (P = .03, \text{univariate analysis}) \). Donor age was still related to overall graft failure after using multivariate models to adjust for the effect of preoperative donor endothelial cell density \( (P = .01) \) or for recipient age \( (P = .04) \). A 10-year increase in donor age increased the risk of failure by 1.2, after adjusting for either preoperative donor endothelial cell density \( (95\% \text{CI, 1.1 to 1.4}) \) or for recipient age \( (95\% \text{CI, 1.0 to 1.3}) \).

The 15-year cumulative probability for developing late endothelial failure was 12% (Figure 6). Corneas transplanted for keratoconus had a lower cumulative probability of developing late endothelial failure compared to corneas transplanted for Fuchs’ dystrophy \( (P = .03, \text{Bonferroni-adjusted for 10 comparisons}) \). However, a multivariate analysis showed that this difference was not significant after adjusting for the recipient age \( (P = .15) \). Late endothelial failure was not related to donor age \( (P = .20) \). Preoperative donor endothelial cell density was related to late endothelial failure \( (P = .03) \), with a decrease in preoperative donor endothelial cell density of 500 cells/mm\(^2\) increasing the risk of late endothelial failure by 1.6 \( (95\% \text{CI, 1.0 to 2.4}) \). Recipient age was also related to late endothelial failure \( (P = .02) \), with a 10-year increase in recipient age increasing the risk of late endothelial failure by 1.4 \( (95\% \text{CI, 1.1 to 1.9}) \).

**DISCUSSION**

From the original 394 patients in this cohort who underwent penetrating keratoplasty by the same surgeon,
67 attended for a 15-year follow-up examination. Although the 67 patients represent only 30% of the cohort presumed alive and without graft failure, these data are valuable in the long-term prospective evaluation of the corneal endothelium and outcomes after penetrating keratoplasty, and they represent the largest such cohort in the literature. Although the patients who did not return at 15 years had similar preoperative characteristics to the original cohort, this loss to follow-up may have introduced bias, and because our results and conclusions are based on only 30% of the surviving grafts, our data should be interpreted with caution. The complicated follow-up data published at 10 years after surgery\(^7\) (equivalent to Table 2 in the present study) were found to contain small inaccuracies because patients with graft failure who subsequently died were often counted twice instead of once when reducing the number of grafts available for analysis from the original cohort. These small inaccuracies were insignificant and have been corrected.

We did not detect differences in any of the endothelial cell variables between 10 and 15 years after surgery, suggesting relative stability of the corneal endothelium compared to the first 10 years after surgery. However, the wide variation in parameters between grafts and our limited sample size at 15 years after surgery are likely to prevent us from detecting small changes in these parameters.

Despite our endothelial data suggesting relative stability between 10 and 15 years after surgery, we were able to detect a continuing increase in central graft thickness. The increase in corneal thickness between 5 and 10 years after surgery can be attributed to the decrease in endothelial cell density and the decreased ability of the endothelium to dehydrate the corneal stroma.\(^9\) Although a small, undetected decrease in endothelial cell density could be responsible for the increase in corneal thickness between 10 and 15 years, other mechanisms could also contribute. For example, a loss of the number or function of pump sites in each endothelial cell or chronic changes in the glycosaminoglycan composition of the stroma could increase the hydration of the cornea. This increase in corneal thickness is unlikely to be related to endothelial permeability, which decreases in long-term grafts and would tend to thin the cornea.\(^9\)

Uncomplicated grafts lost endothelial cells at a rate of \(-1.0 \pm 5.4\%\) per year (\(n = 27\)) from 10 to 15 years after keratoplasty, similar to the rate for all grafts of \(0.2 \pm 5.7\%\) per year (\(n = 54\)). This compares to a rate of \(7.8\%\) per year from 3 to 5 years after keratoplasty\(^9\) and \(4.2\%\) per year from 5 to 10 years after keratoplasty.\(^9\) Although the present study suggests that the rate of endothelial cell loss from 10 to 15 years after surgery is similar to that of normal adult corneas that have not undergone surgery (\(0.6 \pm 0.5\%\) per year), this may not be a valid conclusion given the wide variation in the rate of cell loss between 10 and 15 years after surgery. Zacks and coworkers studied grafts between 15 and 45 years after surgery, and endothelial cell loss rate was \(2.6\%\) per year beyond 15 years.\(^9\) The large standard deviation in our data demonstrates that individual grafts vary widely from the mean.

High rates of endothelial cell loss have been demonstrated early after penetrating keratoplasty\(^2\) and cataract extraction.\(^11\) However, the rate of endothelial cell loss after cataract extraction plateaus at \(2.5\%\) per year within a year after surgery;\(^11\) whereas there is a continued higher rate of cell loss to 10 years after penetrating keratoplasty.\(^3\) Armitage and associates\(^5\) demonstrated that the changes in endothelial cell density after cataract surgery and penetrating keratoplasty were reasonably approximated by a biexponential model that yielded half-lives and cell loss rates of the rapid and slow components. The penetrating keratoplasty data in Armitage’s study was from the same cohort as the present study, but the 15- and 20-year data were incomplete because not all patients had reached that length of follow-up. We present the biexponential decay model fitted to all the data available from this cohort through 15 years, and the half-lives are similar to those calculated by Armitage and associates. We applied the same model to the data from the 27 patients who attended all examinations to 15 years and had no rejection episodes or further surgery affecting the endothelium. The cell loss rate of \(2.4\%\) per year for the late, slow component was similar to the \(2.5\%\) per year found after cataract surgery, supporting similar mechanisms of chronic endothelial cell loss after cataract surgery and penetrating keratoplasty.\(^3\)

In an earlier study, we proposed that the chronic cell loss rate of \(2.6\%\) per year found by Zacks and coworkers\(^9\) in long-term corneal transplants may represent a stable rate attained years after intraocular surgery.\(^13\) Although the slow component of the biexponential model (\(2.4\%\) per year) supports this hypothesis, the actual findings indicate that the cell loss rate in corneas transplanted more than 10 years ago is considerably lower and may be similar to that of normal corneas. In the 54 patients with examinations at both 10 and 15 years, the annual cell loss rate for the interval was \(0.2 \pm 5.7\%\) (95\% CI, \(-1.3\%\) to \(1.7\%\)). For the 27 grafts with no rejection episodes or further surgery affecting the endothelium, the annual cell loss rate from 10 to 15 years postoperative was \(-1.0 \pm 5.4\%\) (95\% CI, \(-3.1\%\) to \(1.1\%\)). The chronic annual cell loss rate of \(2.4\%\) to \(2.6\%\) hypothesized above is well outside the confidence intervals of our findings, whereas the normal cell loss rate of \(0.6\%\) per year\(^9\) is not.
Endothelial cell loss at 15 years appeared lower in the grafts transplanted for aphakic corneal edema than in those transplanted for keratoconus, Fuchs’ dystrophy, or pseudophakic corneal edema, when compared to preoperative levels. With our limited sample size, however, we were unable to demonstrate a significant difference at 15 years between diagnoses, although we did demonstrate that endothelial cell loss was lowest in the aphakic corneal edema group at 10 years after keratoplasty. The highest endothelial cell losses in our study were in grafts for keratoconus. This contradicts other studies, which have found that endothelial cell loss is least after penetrating keratoplasty for keratoconus, and higher for grafts for Fuchs’ dystrophy or corneal edema. These studies suggest that central endothelial cell density decreases because of peripheral migration of endothelial cells to areas of abnormal host endothelium. Our results do not support this hypothesis. We have previously shown that graft rejection was lowest for the keratoconus group, and the high endothelial cell loss for grafts in keratoconus patients cannot be explained by classic graft rejection. Chronic subclinical innate processes between the donor endothelium and the healthy host endothelium of young recipients might account for higher endothelial cell loss in keratoconus patients.

The 15-year cumulative rate of new glaucoma after penetrating keratoplasty was 20%, which is similar to other published rates of 15% to 18%. None of the patients returning for a 15-year evaluation had developed glucoma between 10 and 15 years after keratoplasty. The highest risk of developing glaucoma after keratoplasty is within the first 5 years.

The cumulative probability of graft rejection at 15 years after keratoplasty was 23% in our study and did not differ between preoperative diagnoses. Although most episodes of rejection were within the first few years, two patients had an initial episode of graft rejection between 10 and 15 years after keratoplasty. Our cumulative probability is lower than other published rates and may reflect the low proportion of high-risk grafts in our cohort.

The graft failure rate at 15 years after keratoplasty was 28% and was lowest for corneas transplanted for keratoconus. A multivariate analysis of our data at 15 years continues to show that increased donor age increases the risk of graft failure overall. Eight grafts failed between 10 and 15 years after surgery, and six of these were because of late endothelial failure. Late endothelial failure became the predominant cause of graft failure (16/19) after 5 postoperative years. Although late endothelial failure was not related to donor age, it was associated with lower preoperative donor endothelial cell densities and higher recipient ages. Grafts destined to develop late endothelial failure begin with a lower preoperative endothelial cell density and lose more endothelial cells during preservation and transplantation than grafts that do not develop late endothelial failure.

In summary, we have followed a cohort of patients to 15 years after penetrating keratoplasty and shown that the rate of endothelial cell loss from 10 to 15 years after surgery may be similar to that of normal corneas, although individual grafts vary widely from the mean. The major clinical complication between 10 and 15 years after keratoplasty is graft failure, which is predominantly caused by late endothelial failure.

**REFERENCES**

Corneal Endothelium and Postoperative Outcomes 15 Years After Penetrating Keratoplasty

DISCUSSION

Dr John D. Gottsch. From 1976 to 1986, Dr Bourne performed 500 consecutive corneal grafts and followed them for endothelial cell density, corneal pachymetry, graft rejection, graft failure and the development of glaucoma. Patients were evaluated at two months and at one, three, five, 10, and now remarkably 15 years later. A wealth of information on long-term graft survival has been obtained and a series of important papers have been published. We are much indebted to Dr Bourne for his foresight in initiating this study and his perseverance in shepherding this research of an important anterior segment procedure that has remained highly relevant through these many years.

The overall picture of endothelial cell density over the entire 15 years is one of a rapid rate of cell loss for the first five years, then slowing to about half that from five to 10 years. At 15 years, the rate of endothelial cell loss appears to slow further and approximates the normal rate of cell loss in eyes without surgery. The mathematical model proposed by Dr Bourne to best fit the 15-year data is a biexponential decay formula that describes a rapid initial rate of cell loss followed by a slower component that persists for years. It is assumed by this model that the slow component can be extrapolated and with the further decrease in the rate of endothelial cell density loss, there hopefully would be a better prognosis for graft survival over the next five years. Could these older grafts at last be stabilizing and are likely to last out the life expectancy of the recipient?

A look at another parameter used quantitatively to measure these grafts during the course of the study suggests that there are ominous storm clouds on the horizon. Pachymetry is an important measure of the health of a corneal graft. As elegantly described by Maurice, in normal eyes the ordered array of collagen fibrils provides for the passage of light rays through the cornea without backscatter. Thus there is corneal clarity and the potential for good vision. However, swelling disrupts the fibril lattice, and then increased light scatter will result, limiting vision. Dr Bourne reports that there was a significant increase in pachymetry measurements from 10 to 15 years. If pachymetry over the 15 years is graphed, corneal thickness does not plateau, as does endothelial cell density loss. It appears that the rate of increase in pachymetry from 10 to 15 years, if not accelerating, is at least remaining constant.

Thus in five years, if enough patients survive and are available for follow up, Dr Bourne could present us with another important paper on the natural history of grafts with marginal endothelial cell counts and increasing endothelial dysfunction. I am curious as to what Dr Bourne thinks is likely to happen over the next five years as he further studies these important corneal transplants for endothelial cell densities and pachymetry.

Dr Dan B. Jones. The Australian Graft Registry determined that the greatest predictor of corneal graft failure is prior grafting. Have you looked at that subset of patients who have prior grafting and can you tell us about their pattern of cell loss? In following the patients with graft failure, did you see any pattern of more accelerated cell loss prior to the event? Was contact lens wear a risk factor for endothelial cell loss and failure?

Dr Jules L. Baum. In the early ’70s, there was an anecdotal paper published looking at a few patients who had corneal transplant in keratoconus and then developed an acute corneal graft rejection. Over six months, in the absence of another transplant, there was spontaneous clearing of the graft in some. This observation suggested that, if you had a healthy recipient endothelium as you might expect in keratoconus, as opposed to someone with Fuchs’ dystrophy, the endothelial cells of the recipient would grow in and then deturgess the cornea. Your data, if you dissect out the keratoconus patients, suggests that’s not the case.

Dr Ivan R. Schwab. Over 25 percent of the patients died. That is to be expected in an elderly cohort, but I would wonder if, like cataract extraction, there is a more rapid rate of death for those patients who have corneal transplantation as compared to an age- and sex-matched control group.

Dr Woody S. Van Meter. Since specular microscopy was done presumably before preservation on the donor cornea, was there any change in your technique for doing specular microscopy that might account for any shift in numbers over the course of 15 years? There was a natural skew in the data if you look at the number of patients that
are represented by diagnosis in your final cohort that made it through for 15 years. The majority cohort had keratoconus, and the second most common cohort had Fuchs’ dystrophy, and the third most common cohort had aphakic and pseudophakic corneal edema. This of course would be expected because keratoconus patients tend to be younger and so that data may reflect the age difference in your patients at the time of graft. Do you think we need to do anything different for younger patients versus older patients based on your data?

DR JOHN T. FLYNN. When you compare the endothelial cell counts of normal control subjects with the endothelial cell counts of your graft at 10-15 years post graft, did the normal controls also have an increase in corneal thickness accompanying whatever changes they had in their corneal cell count? If you can’t find it in the numbers of endothelial cells, is there anything about the morphology of the endothelial cells that might be giving you a clue as to what’s happening to cause that thickening of the cornea?

DR JACOB T. WILENSKY. It’s been a classic teaching that high pressure is inimical to the health of corneal grafts. Was there anything in your data that suggests that the 28 percent of patients who developed glaucoma might have a higher rate of failure than the non-glaucomatous eyes?

DR SANJAY V. PATEL. I’d like to thank Dr Gottsch for reviewing the paper and discussing it. There are several questions to answer. Over the next five years, I think we will see stabilization of the endothelial cell count, although individual grafts vary quite widely from the mean of all the grafts. The overall rate of cell loss over a long period is now approaching that of normal corneas but will be different for individual grafts. Some will develop late endothelial failure.

We have not performed a subanalysis to determine whether prior grafting affected cell loss. The effects of contact lenses were not analyzed for this study. Concerning the question about keratoconus and acute prolonged rejection, a European study suggests there is greater cell loss in Fuchs’ dystrophy corneas than in keratoconus corneas. It’s proposed that there is a migration hypothesis of endothelial cells from the healthy donor onto the abnormal host rim in Fuchs’ dystrophy. Our data do not support that hypothesis. Rejection in the keratoconus group is actually low in our group, so we don’t think that rejection is why keratoconus grafts lose cells at a similar rate to Fuchs’ dystrophy grafts. It’s possible that there are some subclinical innate processes going on which we have not identified, possibly because these recipients in keratoconus are younger and actually have healthier tissues.

Twenty-five percent of our patients died during the 15-year period. Our study did not assess a control group for a comparison of risks of death. There have been some changes in the laboratory techniques of specular microscopy, but we have made the appropriate adjustments to the calculations. Grafts for younger recipients would be expected to have a longer survival than grafts for older recipients. Our data suggest that this could be achieved by using graft tissue from younger donors.

Why does corneal thickness continue to increase despite no evident decrease in cell density? I wonder if there are cellular or extracellular matrix changes in the stroma, such as changes in glycosaminoglycan composition, that may account for an increase in corneal thickness. We have not looked at endothelial cell or stromal morphology to determine a reason for increasing cell thickness at this time.
LIGHT-ADJUSTABLE LENS: DEVELOPMENT OF IN VITRO NOMOGRAMS

BY Daniel M. Schwartz MD,* Christian A. Sandstedt PhD, Shiao H. Chang PhD, Julie A. Kornfield PhD, AND Robert H. Grubbs PhD

ABSTRACT

Purpose: To determine whether digital spatial intensity patterns can be developed to effect precise in vitro correction of myopic, hyperopic, and astigmatic refractive errors in a silicone light-adjustable lens (LAL). Also, to determine whether a new spatial intensity pattern for “lock-in” is effective in vitro.

Methods: A digital interferometer/irradiation system was developed to irradiate LALs and measure the power change following irradiation. Light-adjustable lenses were mounted into a wet cell maintained at 35.0 ± 0.5°C (simulated ocular temperature) and allowed to equilibrate for a minimum of 2 hours. Ultraviolet light was then applied with spatial light intensity patterns to correct hyperopia, myopia, and astigmatism. Light-adjustable lenses were also treated to effect lock-in with a separate spatial light intensity pattern. Treated lenses were characterized for power change and optical quality. In the case of lock-in, exhaustive chemical extraction was also performed to determine the percentage of remaining macromer.

Results: Appropriate digital irradiation spatial intensity patterns were created to develop nomograms for in vitro correction of myopia, hyperopia, and astigmatism in approximate 0.25 D steps. Power changes were reproducible and did not alter optical quality of the LALs. Further, lock-in dosing of the LALs did not alter optical quality or significantly change LAL power.

Conclusions: In vitro nomograms have been developed for a silicone LAL that permit precise correction of myopia, hyperopia, and astigmatism. Furthermore, a spatial light intensity pattern has been devised that effects lock-in without significantly altering LAL power or optical quality.


INTRODUCTION

With current intraocular lens (IOL) designs and biometry, more than 95% of cataract surgery patients achieve best-corrected visual acuity of 20/40 or better.10 Residual postoperative refractive error in these patients often creates a gap between uncorrected visual acuity and best-corrected visual acuity, leaving approximately one third of patients in need of spectacles for optimized distance vision. The discrepancy between postoperative corrected and uncorrected distance vision is often due to inaccurate IOL power determination and preexisting astigmatism.12 Further difficulties in appropriate IOL power determination are encountered in patients who have undergone previous corneal refractive procedures.13-16 A means to postoperatively correct residual spherical and astigmatic refractive errors after cataract surgery would allow a greater number of IOL patients to achieve the desired refractive outcome.

Previous investigators have recognized the need for an IOL with the capacity of postoperative power adjustment.17-22 While potentially enabling adjustment of IOL power postoperatively, these lens designs require invasive adjustment procedures and/or do not allow correction of astigmatism and higher-order optical aberrations.

Recently, we reported on a silicone light-adjustable lens (LAL) that is adjusted using safe levels of ultraviolet light.21 The LAL formulation consists of four basic components: a silicone matrix polymer, macromer, photoinitiator, and ultraviolet light absorber. The LAL material is a clear and flexible elastomer capable of

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folding during implantation. After implantation, the lens power may be increased or decreased noninvasively by the application of the appropriate spatially resolved irradiation profile. Upon irradiation with 365-nm light, the macromer molecules in the irradiated region are photopolymerized to form an interpenetrating network. This produces a concentration gradient between the irradiated and unirradiated regions of the lens. Macromers from the unirradiated portion of the lens diffuse along this concentration gradient into the photopolymerized portion of the lens to reestablish thermodynamic equilibrium. Macromer diffusion produces a swelling in the irradiated region that effects a change in the lens curvature with a concomitant power change. When the central portion of the lens is irradiated preferentially and the periphery left nonirradiated, macromer migrates into the center of the lens, causing an increase in the lens power and a hyperopic shift (Figure 1). By irradiating preferentially the peripheral portion of the lens, macromer migrates outward, causing a decrease in lens power, producing a myopic correction. The refractive index of the macromer is designed to match the silicone matrix for optimal optical compatibility; therefore, the power change of the LAL upon irradiation is induced primarily by the shape (radius of curvature) change.

Once the appropriate power adjustment is achieved, approximately 12 to 18 hours after irradiation, the entire lens is irradiated to “lock in” and stabilize lens power by polymerizing the remaining reactive macromer. By irradiating the entire lens with the appropriate profile, there is no macromer diffusion and thus no further change in lens power.

In our previous report, we addressed issues of biocompatibility and efficacy of the LAL in a rabbit model. Preliminary efforts at developing a nomogram for myopic adjustments of LAL power were also presented. Herein we determine whether digital spatial intensity patterns can be developed to effect precise in vitro correction of myopic, hyperopic, and astigmatic refractive errors. We also test a new spatial intensity pattern for lock-in.

**METHODS**

Work was performed at Calhoun Vision, Inc, Pasadena, California. A digital interferometer/irradiation system was developed in the laboratory to irradiate the LALs and measure the power change following irradiation. There are two main components of this optical instrument (Figure 2). The first is the irradiation system, which is composed of a mercury (Hg) arc lamp filtered to 365 nm (±5 nm full width half maximum), a critical illumination/projection system, and a digital mirror device. This device is a pixelated, micromechanical spatial light modulator formed monolithically on a silicon substrate. Typical digital mirror device chips have dimensions of 15.1 mm × 12.7 mm. The individual micromirrors are 13 to 17 µm on an edge and are covered with an aluminum coating. The micromirrors are arranged in an xy array, and the chips contain row drivers, column drivers, and timing circuitry. The addressing circuitry under each mirrored pixel is a memory cell that drives two electrodes under the mirror with complementary voltages. Depending on the state of the memory cell (a “1” or “0”), each mirror is electrostatically attracted by a combination of the bias and address voltages to one of the other address electrodes. Physically the mirror can rotate ±10 degrees. A “1” in the memory causes the mirror to rotate +10 degrees, whereas a “0” in the memory causes the mirror to rotate –10 degrees. A mirror rotated to +10 degrees reflects incoming light into the projection lens and onto the LAL. When the mirror is rotated –10 degrees, the reflected light misses the projection lens and
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LAL. Thus, the great utility and advantage of the digital mirror device in its relation to the LAL is the ability to easily define a particular spatial intensity profile, program this into the device, and then irradiate the LAL. Because of the digital nature, the digital mirror device technology offers greater resolution of the spatial light profile, enabling the delivery of more precise, complex patterns to provide greater range and control to the LAL corrections.

The optical analysis portion of this instrument utilizes a phase-shifting Fizeau interferometer (Wyko model 400) operating in double-pass configuration fitted with a 4-inch transmission sphere. In practice, a set of LALs is first mounted into the wet cell maintained at 35.0 ± 0.5°C (simulated ocular temperature) and allowed to equilibrate for a minimum of 2 hours. The wet cell is adjusted along the optical axis of the interferometer until the power in the wavefront across the full test aperture is minimized (≤0.010 waves). A measurement of the wavefront in the exit pupil of the LAL and its position along the radius slide are recorded, followed by irradiation of the lens. The total time for macromer diffusion is between 12 and 18 hours and depends upon the applied intensity and time. At 24 hours after irradiation, the LALs are returned to their original position on the radius slide followed by measurement of the LALs adjusted wavefront. Analysis of the postirradiated wavefront, along with subtraction of the preirradiated and postirradiated wavefronts, gives direct information regarding the magnitude of the induced power change, the size of the affected area, and any changes in the other aberrations induced by the irradiation procedure (eg, spherical aberration, coma, astigmatism). Knowledge of the spatial intensity profile applied to the LAL, coupled with the analysis of the altered wavefront, allows guidance in the modification of the pattern to produce the desired changes.

The LALs before and after irradiation were characterized for power change and optical properties by measuring magnification (line pair separation method), resolution (US Air Force target method), and modulation transfer function. The spectral transmittance of the preirradiated and postirradiated LAL material was also assessed by ultraviolet-visible spectrophotometry.

To assess lock-in efficacy, exhaustive chemical analysis of extractables was used to determine the percentage of remaining macromer.

RESULTS

Figure 3 shows spatial intensity patterns for correction of hyperopia (a) and myopia (b) and a “flat-top” spatial intensity pattern used for lock-in irradiation (c).

A representative 2 D myopic adjustment is shown in Figure 4a, where the periphery of the LAL was irradiated, causing the diffusion of macromers from the central portion of the lens out to the lens periphery. The interference fringes are depicted 24 hours after irradiation at the preirradiation focus position. The most striking feature of this figure is the addition of approximately 12 fringes (in double pass) of defocus (optical path difference) added to the lens, which corresponds to –2.0 D of myopic correction.

Optical characterization of the LALs before and after irradiation by measuring resolution (US Air Force target method), modulation transfer function, and spectral transmittance shows no significant change following irradiation (Figure 4b, c, d).

Results from optical testing to generate nomograms for myopia and hyperopia are shown in Figure 5. Dose-response curves are generated with gradations of approximately 0.25 D from +0.75 D to +2.50 D for hyperopia and –0.5 D to –2.75 D for myopia. Adjustments are obtained with a precision of ≤0.25 D.

The flat-top lock-in spatial intensity patterns achieved
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Successful lock-in of the LALs. This was confirmed by exhaustive chemical extraction of explanted LALs from the in vivo study in a rabbit model, which showed less than 1% remaining macromer. Results of photolocking 48 LALs demonstrate a change in lens power of less than 0.25 D (±0.08 ± 0.19) without altering lens optical quality as determined by resolution efficiency (≥4-3) and modulation transfer function measurement (0.43 ± 0.04).

**FIGURE 4**
Optical testing of the light-adjustable lens (LAL) upon irradiation. (a) Fizeau interference fringes of an LAL (base power +20 D) immersed in water at 35°C before irradiation at best focus (double pass) and 24 hours after irradiation showing the addition of about 12 fringes (≈2 D) to the preirradiated lens, (b) resolution efficiency of an LAL before and after irradiation through a standard 1951 USAF target, (c) modulation transfer function curve before and after irradiation, and (d) light transmittance curve before and after irradiation.

**FIGURE 5**
Hyperopic (a) and myopic (b) nomograms demonstrating reproducible power adjustments with a precision of ±0.25 D. Each point on the nomogram consists of a minimum of 16 light-adjustable lenses per dose.

**FIGURE 6**
Digital light delivery device, developed in collaboration with Zeiss-Meditech.
An astigmatic nomogram was generated using the Zeiss digital light delivery device with an embedded digital mirror device (Figure 6). The digital pattern projected onto the LAL is shown in Figure 7a. Representative three-dimensional wavefronts of two irradiated LALs are shown in Figure 7b. The table in Figure 7 shows dose-response data demonstrating inducement of astigmatic power changes with approximate 0.25 D steps between 1.0 and 2.0 D.

**DISCUSSION**

We demonstrate that appropriate digital irradiation spatial intensity patterns could be devised to create nomograms for in vitro correction of myopia, hyperopia, and astigmatism in the LAL. Using the appropriate spatial intensity profile, nomograms with approximate 0.25 D steps were developed. Power changes were reproducible and did not alter optical quality of the LALs. Further, we demonstrate that lock-in dosing of the LALs did not alter optical quality nor significantly change LAL power. While our previously reported study of the LAL showed a good correlation between in vitro and in vivo adjustments in a rabbit model, translating the current work into nomograms for clinical use remains untested.

The human cornea has significant and important differences from the rabbit that may limit the use of nomograms presented above. The rabbit cornea is about two thirds as thick as the human cornea and lacks Bowman’s layer. Because the differences may affect light transmission as well as scattering, use of the spatial light intensity patterns and dosing may need to be altered as the LAL is used clinically.

With respect to corneal light transmission at 365 nm in humans, there is considerable variation reported in the literature. Values have ranged from 20% to 75%. This large discrepancy may be explained by studies measuring total (measurement of both the forward transmitted and scattered light) versus direct (light transmitted through a 1-degree cone) transmission, a much smaller value. Also, these measurements have been performed in vitro on eye bank corneas and are not necessarily predictive of what will be encountered in vivo.

Thus, although we have demonstrated the feasibility of these novel IOL materials to be adjusted precisely to correct hyperopia, myopia, and astigmatism, the practicalities of using this system in patients remains to be determined.

**REFERENCES**


**DISCUSSION**

**Dr Roger F. Steinert.** Dr Schwartz and his colleagues have described another key step in their development of a novel technology. The ability to adjust the power and possibly other optical characteristics of an IOL, such as modifying multifocality and reducing high-order aberrations, has captured the imagination of cataract and refractive surgeons worldwide. Rarely is pre-clinical technology so well known and repeatedly discussed by clinicians. From the point of view of an outside observer keenly intrigued by the potential for this device, the process of this development has been characterized by a rigorously designed and meticulously achieved series of milestones.

The current presentation is essentially a proof of concept. After conceiving the chemical principle of migrating macromolecules that could be polymerized after IOL implantation by light, and then developing the material in a form suitable for a lens, the inventors faced the challenge of precise control of the delivery of the light energy. This required the development of the digital light delivery system whose key component is the digital mirror.
device (DMD).

In an in-vitro laboratory setting, Dr. Schwartz and his coworkers have now developed nomograms for control of both hyperopic and myopic lens power changes presented here. The standard deviations are impressively tight, well within clinically acceptable levels. Details about methodology need to be expanded, however. The text indicates that “each point on the nomogram consists of a minimum of 16 LALs/dose,” implying that the number of lenses tested varied from group to group. Why? Were some results unexpected and the outliers discarded? If so, the standard deviation is artificially small. Similarly, we would like much more detail about the lenses that were photo locked and then tested for optical stability and resolution. Summary results are presented on 48 photo locked LALs, again with apparently tight variability, but the text is silent on the underlying data and method of statistical analysis as well as the possibility of discarded data points.

Beyond these technical points, however, the authors appropriately alert us to some of the future challenges. One key question is the consistency of transmission of the 365-nanometer light by corneas of different patients. Another major issue is safety. While safety concerns are beyond the scope of the current presentation, the inventors will need to demonstrate the short- and long-term safety of both the material and the ultraviolet light, whose wavelength is toxic to the retina and the corneal epithelium. I have been assured that these concerns are well known and being satisfactorily addressed by the researchers.

Other issues will arise as this technology moves into clinical testing. Some of these issues are practical, such as the need for patients to wear UV absorbing spectacles, at least outdoors, in the days or weeks prior to photo locking, and whether patients will comply with this.

Many other issues are socioeconomic. The technology of the digital light delivery system, the IOL material itself, and the considerable extra surgeon and technician time involved in the postoperative interventions all add considerable expense. As with some other new IOL technologies, such as the accommodating IOL, the restrictions on balance billing of cataract patients have forced a business model in the U.S. where these expensive technologies are marketed for refractive lens exchange in patients without lens opacities. How much extra is such a patient willing to pay for optical perfection?

Notwithstanding these challenges, known and unknown, Dr Schwartz and his coworkers are to be congratulated both for their innovative technology and for their methodical scientific development of its potential.

Dr. David L. Guyton. To do the lock-in process, you have to have a very widely dilated pupil to irradiate the whole lens. There are many cataract patients whose pupils do not dilate well. How critical do you view this problem and do you have a solution for those cases where the pupil just won’t dilate?

Dr. Michael Nork. As a retinal specialist, I was hoping that we had seen the end of silicone lenses since these hydrophobic lenses present a difficulty when performing retinal operations. The lenses develop condensation, making it difficult to see the retina once you perform an air-fluid exchange. Although it is true that most people with implants will never require a vitrectomy procedure, on the other hand, most people that need vitrectomy surgery are pseudophakic. Is there any way to coat the lens or technologies to prevent condensation from forming during air-fluid exchanges?

Dr. George L. Spaeth. From the point of view of quality of life, what would be the benefit to the patient? Resources are limited. Is the investment in this type of technology justified in terms of other issues that need to be solved?

Dr. Daniel M. Schwartz. First, I would like to address the issue of the number of lenses used for adjustment nomograms and lock-in. As Dr Steinert notes, there were variable numbers of lenses used to develop each point of the nomograms. Our in-vitro lens adjustments/lock-ins are performed in groups of eight lenses by one to three scientists. The nomogram data reported ranged from 16 to 48 lenses for each point generated by a minimum of two different operators and represents the total number of lenses tested under each condition. All data collected were reported with no data points rejected. In spite of the different operators, the data remained reproducible with small standard deviations.

Dr. Steinert also raises the important potential problem of variable corneal transmission of 365-nanometer light used to irradiate the lenses. This is an important issue because if there is variability from patient to patient, it’s going to make this technology very difficult to adopt by the practitioner. With the data to date, we don’t know how much variability there’s going to be from patient to patient. However, we have adjusted eight consecutive patients since the manuscript was submitted. Four were adjusted with an intended refractive change of +1.5 diopters, 2 for +1.0 diopters, and 2 for -1.0 diopters. After adjustment, all were within 0.25 diopters of the intended refractive outcome. One of the patients we adjusted for +1.5 diopters was 50 years old and one was 85, and yet we achieved the same dioptic change. We are using pachymetry measurements before and after surgery to confirm that patients recover to their pre-op
pachymetry levels, thus minimizing corneal edema as a variable. Fortunately, the technology is somewhat tolerant of these differences. We can get the same power changes with about a 10 percent difference in corneal light transmission.

There are concerns about irradiation safety, and these relate to potential damage to the cornea with photokeratitis or damage to the retina from these UV light sources. There is animal and human data on these potential toxicities. The threshold for toxicity for photokeratitis is approximately 70 joules per cm² at 365 nm. We have not encountered any cases of photokeratitis either in our rabbit studies for preclinical submission to the FDA or in the patients that we have treated.

We also are concerned about retinal toxicity, and the retinal threshold in primates at this wavelength is approximately 5.5 joules per cm². Dr David Sliney is widely published on the subject of the effects of UV radiation on the eye and serves as member, advisor and chairman of numerous committees and institutions which are active in the establishment of safety standards for protection against non-ionizing radiation (ANSI, ISO, ICNIRP, ACGIH, IEC, WHO, NCRP). He has advised that we not exceed 2 joules per cm² at the retina, and light treatments are within that guideline.

Dr Steinert raised some important issues about the expense of the technology and the time requirement for practitioners. I do not have detailed information about these issues, but I can say the lens material itself is surprisingly inexpensive. It costs just a few dollars more to make this lens than conventional silicone lenses. The sales price for the digital light delivery device is approximately $80,000, which compares favorably with the excimer laser.

As Dr Guyton notes, pupillary dilation is very important. For typical adjustment and lock-in, spatial intensity profiles are projected onto nearly the entire 6mm diameter of the IOL. We can customize the size of the adjustment profile to accommodate smaller pupils; however, the entire lens must be irradiated for lock-in. At this time we are limiting the technology to patients who dilate 7mm or more preoperatively. Undoubtedly there will be some patients who dilate less after surgery. For those patients, we could use a gonioscopic-type lens that would direct light around the edges of the pupil to achieve lock-in. We are also developing a second-generation lens formulation that will not require lock-in. Pupillary dilation would not be an issue with such a formulation since lock-in would not be necessary.

Dr Nork raises an important issue about condensations that can form on silicone IOLs during vitrectomy surgery, especially when silicone oil is used. The development of proliferative vitreoretinopathy (PVR) requiring vitrectomy among those patients who develop pseudophakic retinal detachments is probably on the order of 5 to 7 percent. With PVR, equal success is achieved using either gas or silicone oil, so silicone oil would be avoided in patients with a silicone light adjustable lens. For those patients in whom there is an increased risk of retinal detachment recognized prior to the cataract surgery, an adjustable silicone lens may not be indicated. We are not restricted to silicone lenses and we can adapt our technology to acrylic lenses, thereby avoiding these condensation issues. We have developed a prototype acrylic formulation that has been successfully adjusted.

Dr Spaeth asks how is this going to make our patients’ lives better, and is it really worth the cost? I do believe that there is a need for this technology since more patients would like to be spectacle-free both at near and distance. I have discussed a monofocal version of this lens, but we can also make a customized multi-focal version of the lens. Using the digital light delivery device, we can emmetropize the eye and then create a multi-focal optic in situ. Furthermore, if a patient will not tolerate multifocality, the multi-focal optic is potentially reversible. Because of the ability to modify lens power multiple times until it is locked in, we can also try a patient with monovision and then reverse it if the patient wishes. As discussed above, the technology is going to be a fairly insignificant increase in cost in terms of the IOL material. Whether patients themselves will want to undergo implantation with an adjustable IOL given the extra cost related to financing the light delivery device and physician time, only the market will provide the answer. You still have to perform a refraction, but the physician time for adjustment and lock-in is minimal. Treatments are on the order of 30 seconds to two minutes each for adjustment and lock-in.
ABSTRACT

Purpose: Von Hippel–Lindau (VHL) disease is a hereditary cancer syndrome expressed in multiple organs caused by germline alterations of the VHL gene. We have shown VHL deletion in the “stromal” cells of retinal angiomas. The VHL protein–associated complex is a primary ubiquitin ligase for the ubiquitination of hypoxia-inducible factor (HIF). This study examines VHL and ubiquitin expression in optic nerve hemangiomas and juxtapapillary angiomas.

Methods: Using microdissection and polymerase chain reaction, four optic nerve hemangiomas (one also had juxtapapillary angioma) associated with VHL disease were analyzed for loss of heterozygosity in the VHL gene. In addition, expression of HIF and ubiquitin was evaluated in these tumors by immunohistochemistry.

Results: All informative optic nerve and juxtapapillary lesions showed loss of heterozygosity in the VHL gene detected in vacuolated “stromal” cells. Both HIF and ubiquitin were highly expressed in the hemangiomas of all four VHL cases.

Conclusions: Like retinal angiomas and other VHL tumor lesions, VHL gene deletion is found in optic nerve hemangiomas and juxtapapillary angiomas. These tumor cells also express HIF and ubiquitin, the protein responsible for the negative regulation of HIF that results in the hypervascularization characteristic of VHL disease.

INTRODUCTION

Von Hippel–Lindau (VHL) disease occurs in roughly 1 in 36,000 live births and is inherited as a highly penetrant autosomal dominant trait (ie, with a high individual risk of manifesting the disease). Von Hippel–Lindau disease is caused by germline alterations of the VHL gene, which has been cloned and identified as a tumor suppressor gene on the short arm of chromosome 3, 3p25.5.1,2 The major lesions in VHL disease include hemangioblastomas in the central nervous system, retinal angiomas, clear cell renal cell carcinomas, pheochromocytomas, pancreatic tumors, epididymal cystadenomas, endolymphatic sac tumors, carcinoid tumors, and multiple cysts of the kidney, pancreas, and epididymis. Retinal angioma and cerebellar hemangioblastoma are the most frequent and earliest manifestations of VHL disease. Retinal angioma has been reported in nearly 60% of patients with VHL disease.3 VHL gene deletion and alteration are reported in VHL tumors in various systemic organs. In the eye, VHL gene deletion is found in the vacuolated clear, or “stromal,” cells of VHL retinal angiomas.4

Histologic features of VHL tumors are characterized by their high degree of vascularization and the presence of a clear cell component.5,6 Hypervascularization, angiogenesis, or both are induced by overexpression of vascular endothelial growth factor (VEGF). Since the principal function of VHL protein is the negative regulation of hypoxia-inducible mRNAs, including VEGF mRNA, inactivation of VHL gene plays a critical role in the angiogenesis of VHL tumors. We have detected expression of VEGF messenger and protein in retinal angiomas associated with VHL disease.4 The VHL protein–associated complex is a primary ubiquitin ligase for ubiquitination of the α subunits of the hypoxia-inducible factor (HIF).8 Loss of VHL protein function in VHL disease leads to the accumulation of ubiquitin and HIF-α during normoxic condition, which in turn causes constitutive induction of HIF-responsive genes, including VEGF.9

Optic nerve hemangiomas and juxtapapillary angiomas are relatively rare, and the prognosis is often poor.10 The present study examines VHL gene and ubiquitin expression in optic nerve hemangiomas obtained from patients with VHL disease.

METHODS

Three pathological specimens with intracranial optic nerve hemangiomas and one specimen with juxtapapillary
hemangiomas were collected from four patients with family histories and clinical diagnoses of VHL disease (Figure 1). All patients were examined clinically by one of the authors (E.Y.C.). This study has been approved by the National Cancer Institute and National Eye Institute institutional review boards for human subjects.

All pathological specimens were embedded in paraffin after fixation in 10% formalin. Sections were cut and thoroughly reviewed to identify each angiomatous lesion in the optic nerve. Manual microdissection was performed to obtain hemangiomas and normal cells separately. In each case, tumor cells were procured from areas with predominantly “stromal” cells, and normal cells were procured from areas without tumor cells and with normal morphology. Microdissected cells were immediately placed in DNA extraction buffer containing proteinase K and subjected to polymerase chain reaction as described previously. Briefly, all samples were examined for loss of heterozygosity (LOH) using the microsatellite markers D3S1038, D3S1110, and D3S2452 flanking the VHL gene (Research Genetics, Huntsville, Alabama). A case was considered informative for a polymorphic marker if normal tissue DNA showed two different alleles (heterozygosity). The criterion for LOH was a complete or near complete absence of one allele in the tumor DNA as defined by direct visualization.

Deparaffinized sections were then subjected to immunohistochemical analysis utilizing the avidin-biotin-peroxidase complex technique. The primary antibodies were mouse anti-HIF monoclonal antibody (Novus Biologicals, Littleton, Colorado) and rabbit anti-ubiquitin proteosome polyclonal antibody (Chemicon International, Temecula, California). The secondary antibodies were biotin conjugated horse anti-mouse IgG or goat anti-rabbit IgG, respectively.

RESULTS

Two specimens were optic nerve only, one specimen was orbital tissue with optic nerve, and one was an enucleated eye with optic nerve. The two optic nerve lesions were surgically removed intracranially from two female patients (15 and 36 years of age). These patients were asymptomatic, and these lesions were detected upon screening (Figure 1). The orbital tissue was obtained from a 44-year-old man who had had an enucleation and radiation treatment for the affected orbit 22 years previously. Magnetic resonance imaging showed that he had a progressive intracranial lesion that was extending from the optic chiasm along the affected optic nerve to the orbit. He had gradual proptosis in the affected orbit that precluded proper fitting of his eyeglasses, which prompted the surgical removal of the orbital tissue. The enucleated eye came from a 12-year-old girl who had exudative and tractional retinal detachment from the retinal angiomas found in the retinal periphery as well as on the optic nerve. With the exception of the youngest patient, who had a normal fellow eye, all remaining patients had retinal angiomas with good vision in their fellow eyes.

Histopathologically, all four specimens contained classic VHL hemangiomas that were either juxtapapillary angioma (one specimen) or optic nerve hemangioma (four specimens) in the neural tissues, which matched clinical observations (Figure 2). The hemangiomas were composed mainly of densely packed, small capillary-like vascular channels and small cells with prominent dark nuclei and little cytoplasm, intermixed with vacuolated “stromal” cells and glial cells (Figure 2A). Scatter hemorrhages were often present inside the VHL lesions.

Three of the four cases were informative and showed LOH in the cells of the hemangiomas. One case showed slightly lower intensity in one allele as compared to the other allele in autoradiography (Figure 3). Retention of heterozygosity was shown in the normal cells. The one noninformative case (the orbital tissue specimen) previously had had a decalcified procedure and did not yield polymerase chain reaction products.

HIF-1 and ubiquitin were stained intensely in all four hemangiomas located juxtapapillary and inside the optic nerve regions. The staining pattern was even throughout each entire VHL lesion (Figure 4).

DISCUSSION

Although optic nerve lesions associated with VHL are a rare cause of blindness, they may be associated with
Von Hippel-Lindau Gene Deletion and Expression of Hypoxia-Inducible Factor and Ubiquitin In Optic Nerve Hemangioma

**FIGURE 2**
Microphotographs showing optic nerve hemangiomas located inside the optic nerve (A) and above the lamina cribrosa (asterisk, B) in two patients with von Hippel–Lindau disease (hematoxylin-eosin, original magnification ×200).

**FIGURE 3**
Autoradiographs showing allelic imbalance indicative of loss of heterozygosity (T) after amplification with VHL gene flanking primers D3S1038, D3S1110, and D3S656. Matched adjacent normal cells (N) show preservation of both alleles of the VHL gene.

**FIGURE 4**
Microphotographs showing positive staining for hypoxia-inducible factor-α (A) and ubiquitin (B) in two von Hippel–Lindau hemangioblastomas (avidin-biotin-complex immunoperoxidase, original magnification ×200).
higher morbidity and reduced life expectancy. In a recent study of 60 eyes with juxtapapillary capillary hemangioma and VHL, McCabe and associates observed that these patients more often presented at a younger age, had tumors with an endophytic growth pattern, and had bilateral, multiple lesions. Furthermore, in long-term follow-up, visual acuity was generally worsened in spite of laser photocoagulation therapy. Our four patients presented similarly complicated clinical courses and poor outcome.

The morphology of the optic nerve hemangiomas in our patients more closely resembles central nervous system hemangiomas than retinal angiomas. Small vascular components with hemorrhages are prominent, and the vascular channels seem more closely bundled together, thus making it difficult to identify the typical vaculated “stromal” cells. These features may explain the aggressive nature of VHL lesions in the optic nerve.

Von Hippel–Lindau disease is a heritable multisystem cancer syndrome that is associated with a germline mutation of the VHL tumor suppressor gene. Similar to the “stromal” cells of the VHL tumors in various systemic organs, the present study has documented LOH of the VHL gene in the optic nerve hemangiomas. Again, the “stromal” cells of the hemangiomas are the true neoplastic component of the VHL lesions.

According to the two-hit hypothesis, dominantly inherited predisposition to cancer entails a germline mutation, whereas tumorigenesis requires a second somatic genetic alteration. Typically, patients with VHL disease have inherited an inactive VHL allele from an affected parent. In other words, they are VHL heterozygous. Some VHL patients without a positive family history have, upon further investigation, been found to have a parent who is mosaic for a VHL mutation. This event has been documented in very early, premalignant lesions in the kidneys of the patients with VHL disease. It is presumed that mutations that affect one or more other genes are required for conversion of these premalignant renal lesions to frank renal cell carcinoma. Tumor development in VHL disease is linked to inactivation or loss of the remaining wild-type VHL allele in a susceptible cell or LOH (VHL /-), which leads to loss of the VHL gene product, VHL protein. Indeed, the VHL gene is frequently inactivated, whether as a result of mutation or hypermethylation, in nonhereditary clear cell renal carcinomas and hemangioblastomas. In these settings, VHL gene alterations are not inherited but rather occur somatically. The mechanism of tumor development at different sites and organs after VHL gene inactivation is still unknown.

The VHL mRNA encodes a VHL protein that contains 213 amino acids. Tumor formation by VHL protein–defective renal cell carcinoma can be suppressed after restoration of wild-type VHL protein function. Therefore, VHL protein is a tumor suppressor protein based on both genetic and functional criteria. The VHL protein forms stable complexes in mammalian cells with other proteins, including elongin B, elongin C, Cul2, and Rbx. Elongin C and Cul2 regulate protein turnover. Many proteins that undergo regulated destruction are first covalently modified by the attachment of a polyubiquitin tail, which serves as a signal for degradation by a multiprotein complex called a proteasome. The VHL protein ubiquitin ligase complex polyubiquitinylates and targets proteasomal degradation and, when oxygen is present, HIF-α subunits. In the absence of oxygen, HIF-α subunits accumulate and activate hypoxia-inducible genes. During normoxia, translated HIF-α subunits are hydroxylated, which results in their subsequent ubiquitination and degradation. During hypoxia, the hydroxylation is inhibited. The HIF-α subunits are therefore not ubiquitinated and thus accumulate, regulating transcription of the HIF-responsive genes. High expression of HIF and ubiquitin in our patients’ optic nerve hemangiomas and juxtapapillary angiomas is most likely due to the failure of ubiquitination in the VHL lesions.

HIF-1 is a heterodimer consisting of a constitutively expressed HIF-1β subunit and an oxygen- and growth factor–regulated HIF-1α subunit. Increased HIF-1 activity provides a molecular basis for VEGF-induced angiogenesis and other cancer cell adaptations to hypoxia critical for the establishment of a primary tumor and the eventual progression to the lethal phenotype. Retinal angiomas, central nervous system hemangioblastomas, and renal cell carcinomas in VHL disease are highly vascular and frequently overproduce angiogenic factors such as VEGF. The production of HIF mRNA is uncoupled from changes in ambient oxygen in VHL protein–defective tumor cell lines. This defect can be corrected by restoration of wild-type VHL protein function. Furthermore, the products of some HIF-1–inducible genes might be suitable targets for therapeutic approaches to VHL tumors, including retinal angioma and optic nerve hemangioma. Recently, Aiello and colleagues reported successful treatment in a patient with VHL and optic nerve hemangioma after systemic therapy with VEGF receptor inhibitor SU5416. On the other hand, Girmens and associates have shown that SU5416 is more effective for VHL-associated macular edema than for hemangiomas. Further clinical studies with careful monitoring and long-term follow-up of such treatments are required.

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**DISCUSSION**

**Dr Hans E. Gossniklaus.** If one graphs the age at diagnosis of retinal von Hippel Lindau (VHL) hemangioma versus percent of undiagnosed cases, two curves emerge, similar to the two curves that Knudson found when he derived his two-hit hypothesis for retinoblastoma. Like the retinoblastoma gene, the VHL gene is a tumor suppressor gene. The VHL gene has been mapped to 3p25.5 and cloned. In heritable VHL disease, patients inherit an inactive VHL allele and the other allele is inactivated or there is loss of heterozygosity (LOH). The VHL protein ubiquitin ligase complex polyubiquitinylates hypoxia inducible factor (HIF-α) subunits during normoxia. During hypoxia, the hydroxylation is inhibited, HIF-α subunits are not ubiquitinated, and VEGF is produced. Abnormalities in the VHL protein result in absence of ubiquitination, increased HIF-α subunits during normoxia and hyperoxia, and VEGF production.
In this study, Chan and coworkers used laser-capture microdissection to extract cells from optic nerve VHL hemangiomas and an enucleated eye with a peripapillary VHL hemangioma. They demonstrated LOH for the VHL gene in 3 of 4 cases and found HIF-1 and ubiquitin overexpression in all four cases, thus indicating loss of function of the VHL protein. This LOH was present in the tumor stromal cells, but not in endothelial cells. This reaffirms Chan’s previous work demonstrating LOH in the stromal cells of retinal VHL hemangiomas. This work is an example of how utilization of modern technologies advances the field of ophthalmic pathology.

Pathologists had previously noted two components in VHL hemangiomas: endothelial-lined vascular channels and vacuolated stromal cells. The lesion in retinal VHL hemangioma is identical to optic nerve hemangioblastoma and cerebellar hemangioblastoma. The work of Chan and coworkers definitively shows that the retinal and optic nerve lesions in VHL are primary stromal cell tumors with secondary vascularization due to increased VEGF production in the tumor. Hence, “hemangioma” and “angioma” are misnomers and should be supplanted. A better term is retinal hemangioblastoma, since it is identical to cerebellar hemangioblastoma.

The clever work by Chan and coworkers offers the opportunity for further discussion. What is the cell of origin in optic nerve hemangioblastoma? Is it intrinsic in the optic nerve, in the meninges in cases of the hemangioblastoma variant of meningioma, or both? Did any of the patients in the current study develop VHL syndrome? Also, do the authors recommend intravitreal injection of VEGF inhibitors for optic nerve or peripapillary hemangioblastoma?

**Dr Robert N. Frank.** In von Hippel–Lindau syndrome and in all the other VEGF-related diseases such as diabetic retinopathy and age-related macular degeneration, it should be noted that the major source of the VEGF is not the vascular cells; it is other cells, for example neuronal and glial stromal cells of retinal tissue, which then act secondarily on the vascular endothelium. Dr Chan’s presentation also emphasizes that it’s not just VEGF, but it’s what up-regulates VEGF, hypoxia-inducible factor, which is a transcription factor that hooks onto the DNA and stimulates it to make more mRNA and hence VEGF protein. Hence, the injection of a VEGF inhibitor alone may not be sufficient for therapy. Perhaps an approachable goal may be to develop technologies to down-regulate these transcription factors.

**Dr Robert B. Welch.** Von Hippel–Lindau disease and the AOS have a long history of association. Almost three-quarters of a century ago, Dr Harvey Cushing, who was at Peter Brigham Hospital, presented a fascinating case at this meeting. This was shortly after Dr Lindau had defined the syndrome in 1926. At that meeting, Dr Wilner discussed this paper and presented a beautiful fundus painting by Annette Burgess, our wonderful artist for so many years at Wilmer. Thirty-four years ago, my thesis for the AOS was on von Hippel–Lindau disease, in which I discussed the early lesions and treatment. Dr Chan is now advancing this disease another step forward on the molecular level. I hope this will provide us with new means of therapy, because this is a potentially blinding disease, especially for those with hemangioblastomas of the optic nerve.

**Dr John T. Flynn.** It is the hypoxia insult that produces VEGF that produces the tumor. What if you could treat these patients with hyperbaric oxygen so that their blood-oxygen was always saturated at a PAO2 of 120? Would that damage them vascularly, or could they survive and could that suppress the tumor?

**Dr Chi-Chao Chan.** I appreciate the thoughtful discussion by Dr Grossniklaus. There are debates on the cell of origin of optic nerve hemangioblastoma and retinal hemangioblastoma. Recent publications by Vortmyler and colleagues have demonstrated that the “stromal” or VHL cells may be developmentally arrested angioblasts that can co-express erythropoietin (Epo) and Epo receptor (EpoR). These hemangioblastoma cells have the potential to develop into primitive blood vessels and erythrocytes (extramedullary hematopoiesis). The hypothesis is that deletion of the VHL gene appears to be primarily responsible for the arrest of differentiation and possible up-regulation of Epo in EpoR expressing angiomesenchyme during embryonic development. In the preliminary study, we also found co-expression of Epo and EpoR messengers and protein in VHL retinal and optic nerve hemangioblastoma. Furthermore, we even detected many “stromal” cells bearing hematogenous and vascular stem cell markers such as CD31, CD34, and CD133 in the eyes with VHL disease. So, I agree with the proposal of Dr Grossniklaus to rename the retinal angioma or hemangioma associated with von Hippel-Lindau disease to retinal hemangioblastoma.

All four patients in the current study had VHL syndrome. Two presented with VHL lesions in the eye, CNS, and pancreas; one with VHL lesions in the eye, CNS, head, kidney, pancreas, and adrenal gland; and one in the eye, head, and kidney. Macugen (intravitreal EYE001, 3 mg, q 6 wks, x6) had no effect on retinal hemangioblastoma in five VHL patients. However, the therapy may decrease retinal hard exudates and macular edema, and thus may increase...
visual acuity. We suggest that Macugen may be useful as an adjunct therapy in patients with retinal hemangioblas-
toma to decrease macular thickening and exudates.³

I agree with Dr Frank that we should think about targeting other cell products in addition to VEGF since there are other transcription factors that are targeted by VHL and/or HIF. We have a proposal awaiting NIH approval where we plan to target Epo and EpoR. The problem with systemic treatment is that it may affect erythroid progenitor cells in the bone marrow. This will have problems of erythrocyte regeneration. We should consider locally targeted drug treatment or drug delivery.

Dr Welch is the person who defined von Hippel–Lindau disease for the ophthalmologists; I am very thankful for his remarks and kind words. Concerning Dr John Flynn’s comment, hyperbaric oxygen might be tried and proposed for a new therapeutic protocol.

REFERENCES

A MODEL OF SPECTRAL FILTERING TO REDUCE PHOTOCHEMICAL DAMAGE IN AGE-RELATED MACULAR DEGENERATION

BY Sanford M. Meyers MD,* Mikhail A. Ostrovsky PhD DSc, AND Robert F. Bonner PhD

ABSTRACT

Background/Purpose: Cumulative sunlight exposure and cataract surgery are reported risk factors for advanced age-related macular degeneration (AMD). Laboratory studies suggest that accumulation and photochemical reactions of A2E (N-retinylidene-N-retinylethanolamine) and its epoxides, components of lipofuscin, are important in AMD. To relate this data to the clinical setting, we modeled the effects of macular irradiance and spectral filtering on production of A2E and reactive oxygen intermediates (ROIs) in pseudophakic eyes with a clear or "yellow" intraocular lens (IOL) and in phakic eyes.

Methods: We calculated relative changes of macular irradiance as a function of light (390 to 700 nm) intensity, pupil size, age, and lens status, and modeled resulting all-trans-retinal concentration and rates of production of A2E-related photochemicals and photon-induced ROIs in rods and retinal pigment epithelium (RPE). We compared these photoproducts following cataract surgery and IOL implantation with and without spectral sunglasses to normal age-related nuclear sclerotic lens changes.

Results: Following cataract and IOL surgery, all-trans-retinal and lipofuscin photochemistry would theoretically increase average generation of 1) A2E-related photochemicals, 2) ROI in rods and 3) ROI in RPE, respectively, 2.6-, 15- and 6.6-fold with a clear IOL, and 2.1-, 4.1- and 2.6 fold with a yellow IOL, but decrease approximately 30-, approximately 20- and 4-fold with a vermillion filter sunglass and clear IOL compared to an average 70 year old phakic eye.

Conclusion: Sunglasses that strongly decrease both deep blue light and rod photobleaching, while preserving photopic sensitivity and color perception, would provide upstream protection from potential photochemical damage in subjects at risk for AMD progression after cataract surgery.


INTRODUCTION

The late stages of age-related macular degeneration (AMD), neovascularization and geographic atrophy, are important causes of severe visual loss and legal blindness in persons over 60 years of age in the United States.1-3 Clinical and epidemiological studies that report a significant association between both prior cataract surgery and cumulative exposure to sunlight and late-stage AMD lend support to the hypothetical role of photochemical reactions in the pathogenesis of AMD.4-10

In 1920, van der Hoeve11 observed that AMD was less common in eyes with cataracts and proposed that opacity of the lens diminished the severity of AMD, in 1925, Gjessing12 reported an inverse relation between lens opacity and AMD. Recent epidemiological studies have reported conflicting data on AMD and lens opacities but consistently suggest that prior cataract surgery, aphakia, and pseudophakia are risk factors for late-stage AMD.5,6,11,14

The Chesapeake Bay Waterman Study and the Beaver Dam Eye Study reported a significant association between AMD and cumulative sunlight exposure, 400 to 700 nm, the former with late-stage AMD and the latter with early AMD.15 Neither of these studies reported an association between AMD and exposure to ultraviolet A (320 to 400 nm) or B (290 to 320 nm) light.

Laboratory studies on acute photochemical injury to mammalian rod photoreceptors and retinal pigment epithelium (RPE) cells document two well-defined action spectra: (1) Ham-type photochemical injury to the RPE caused by intense blue and ultraviolet light with peak sensitivity at approximately 350 nm in aphakic monkeys and (2) rod damage associated with the rhodopsin absorption spectrum and enhanced by blue light.15,16 Recent in
vitro studies have demonstrated that RPE lipofuscin granules generate oxygen free radicals, singlet oxygen, and other reactive oxygen intermediates (ROIs), with an action spectrum very similar to that in the Ham-type in vivo study.17-20 Additionally, lipofuscin granules contain a number of different fluorescent species that (1) originate largely from photochemical reactions involving all-trans-retinal and include A2E (N-retinylidene-N-retinylethanolamine) and its epoxides and (2) are capable of acting as photosensitizers of singlet oxygen.19-20 A2E-derived fluorophores in lipofuscin appear to be produced during periods of photopic vision associated with significant rhodopsin bleaching and high levels of all-trans-retinal in the rod outer segment (ROS) disks. All-trans-retinal in the ROS disks can absorb short-wavelength light with a 350-nm peak and known wavelength dependence (or action spectrum) leading to a long-lived triplet state that acts as a photosensitizer of oxidative damage.20 A2E-derived fluorophores that accumulate in the RPE lipofuscin are potent photosensitizers of oxidative damage with a known aggregate action spectrum.20

To relate the epidemiological and laboratory data to the clinical setting, we modeled the effects of retinal irradiance and spectral filtering on the relative rates of all-trans-retinal photosensitization, production of A2E and A2E-derived lipofuscin fluorophores, and \( O_2 \) generation due to RPE lipofuscin photosensitization in phakic eyes and in eyes with a clear or “yellow” intraocular lens (IOL) with and without external spectrally selective filters. We discuss this model in relation to hypothetical pathogenic pathways in AMD and describe a possible preventive strategy for decreasing the potential risk of photochemical damage in AMD.

METHODS

We have developed a model relating retinal spectral irradiance as a function of age and lens status to the relative rates of rod bleaching, steady-state concentration of all-trans-retinal, its photosensitization of oxidative damage in the rod outer segments, or alternatively its reactions to form A2E-related species. In RPE cell culture experiments, A2E is rapidly ingested and concentrated through lysosomal processing into prototypical nascent lipofuscin granules.19 On irradiation, these granules form a complex mixture of oxidized A2E-related fluorophores that are potent photosensitizers of singlet oxygen generation and spectroscopically comparable to mature lipofuscin granules harvested from aged human retinas (R. Bonner, unpublished data, 2004). In our model, we assume that averaged over long time periods (1 year), the relative rate of “mature” lipofuscin accumulation within the RPE is proportional to the rate of production of A2E precursors in the rods. From the literature, we applied a variety of values describing the normal age dependence of critical ocular parameters to standard formulas in order to determine average retinal spectral irradiance as a function of age. We then used our model and published action spectra to estimate the normal average age dependence of these potential causes of chronic light injury at 7 to 11 degrees from the center of the fovea, a region of high lipofuscin accumulation and rod loss.22 Finally, we compared the estimates for the normally aging phakic subject following replacement of the “yellowed” aged lens with two different commercial IOLs (Alcon clear AcrySoft MA60BM UV-absorbing and light yellow AcrySoft Natural) with or without rod-sparing or blue-blocking spectrally selective sunglasses.

We calculated relative changes of macular irradiance as a function of light spectral (390 to 700 nm) intensity, pupil size, age, and lens status. The solar irradiance has direct, diffuse (scattered light from the atmosphere), and ground-reflected components and varies with the day of year, time of day, altitude, and latitude. For our calculations we used the following formula and daylight radiiances at the cornea between 9 and 4,400 candelas\(\text{cd}/m^2\) for the solar spectrum through air mass 1.2:

\[
H_R = \left(A n^{4/3} f^2\right) \int_{390}^{700} H_R(\lambda) t(\lambda) \, d\lambda.
\]

where \( H_R \) is the retinal irradiance where the specific photochemical reaction occurs; \( H_{\text{pupil}} = H_{\text{cornea}} \) is the “effective” solar irradiance at anterior corneal surface, \( H_{\text{cornea}} \); \( A \) is the area of pupil; \( f \) is the distance from pupil to macula; \( n \) is the index of refraction of ocular media; \( t \) is the transmission of the ocular media; and \( \lambda \) is the wavelength of light.

For diffuse sources, the solid angle of the retinal image determined by the pupil area \( (A n^{4/3} f^2) \) greatly diminishes retinal irradiance. For diffusely reflected sunlight, the surface reflectance or albedo of viewed objects further diminishes corneal irradiance so that at 500 nm the spectral irradiance of the sun hitting the ground might be approximately 1 mW/cm²/10 nm, but the retinal irradiance only a few microwatts per cm²/10 nm. Acute phototoxicity experiments in animals or cell cultures capable of inducing apoptosis generally use light intensities hundreds or thousands times greater than normal retinal irradiance in daylight. We assumed \( n \) was 1.33 and \( f \) was 21.5 mm. This formula and the assumptions we made are similar to those used in studies to calculate retinal irradiance at the surface of the retina from indirect ophthalmoscopes, slit lamps, and surgical lamps.21

In the visible spectrum, ocular spectral transmission \( t(\lambda) \) is determined mainly by absorption of light by the
A Model of Spectral Filtering to Reduce Photochemical Damage in Age-Related Macular Degeneration

The selected vermilion filter allows (1) little attenuation of pass, and a deep red or 570-nm long-pass; see Figure 1). The effects of external spectrally selective sunglasses after cataract surgery (a vermilion, a yellow or 480-nm long-pass filter, (5) 70-year-old lens, (6) vermilion filter, and (7) Russian Spectrum (light yellow) IOL, (3) Acrysoft light yellow IOL, (4) Acrysoft clear UV-absorbing IOL, (2) the pupil diameter, and (3) the transmission of the lens (and cornea). The cornea absorbs virtually all light below 300 nm. The lens transmits virtually 100% of light over 660 nm. Because the lens and UV-absorbing IOLs transmit less than 1% light below 390 nm, we considered light below 390 nm to have an insignificant effect on our calculations. We are principally interested in the role of protection of the lens as it ages ("yellows") compared to IOLs used to replace the lens following cataract surgery. We used published data on lens transmittance as a function of age in normals (due to nuclear sclerosis) and data on the transmittance of the Alcon clear AcrySoft UV-absorbing and light yellow AcrySoft Natural IOLs and a similar yellow Spectrum IOL developed and used in Russia beginning in the mid-1980s.24-26 We also examined the effects of external spectrally selective sunglasses after cataract surgery (a vermilion, a yellow or 480-nm long-pass, and a deep red or 570-nm long-pass; see Figure 1). The selected vermilion filter allows (1) little attenuation of long wavelengths and photopic sensitivity, (2) sufficient midrange blue transmission to provide good color perception, and (3) blockage of wavelengths efficiently absorbed by rod rhodopsin and short-wavelength blue light.

Retinal irradiance is a strong function of pupil diameter and corneal irradiance. We used published average normal data on pupil size as a function of age and after cataract removal and IOL implantation.27,28 The light-filtering effects of macular and photoreceptor pigments and RPE melanin are complicated functions of their radial and depth (z) distributions.29-30 Although we have used literature values for the radial (r) and depth (z) dependence, we present here only our model predictions for the photochemistry and spectral irradiance at 7 to 11 degrees from the center of the fovea.

The effect of the macular pigment on the spectral irradiance of the underlying macular photoreceptors and RPE is highest in the central 1 degree of the fovea, rapidly decreases from 1 to 5 degrees from the center of the fovea, and is considered negligible beyond approximately 7 degrees.29 Rod loss and lipofuscin accumulation occur predominantly outside the region of significant macular pigment absorption, and therefore its possible photoprotective role does not affect our calculations for 7 to 11 degrees. Our calculations of the "internal filter" photoprotection of rhodopsin and melanin absorption have been estimated assuming age-independent concentrations of rhodopsin and melanin at 7 to 11 degrees from the fovea.29 We modeled only the average age-dependent effects of macular spectral irradiance in a typical subject before and after cataract surgery. In eyes with early AMD, pigmentary and drusenoid changes may critically affect the potential for local photodamage and not be reflected in the reported average values (such as rod dark-adaptation time constant) that we used. Such complex interactions with spectral irradiance could not be reliably predicted and are beyond the scope of our model.

In order to estimate the average age-dependent rates of all-trans-retinal photosensitization of ROI production in the rods or of its reactions to form A2E and related molecules, we related the macular irradiance to the corresponding steady-state concentration of all-trans-retinal, [all-trans-retinal]ro, produced in the rods. We used the formula of Thomas and Lamb to calculate the steady-state bleaching of the rods, \( B(I, \text{age}) \), using the age-dependent average values of the rod visual cycle time constant \( t_\text{rh} \) (age) in seconds reported for a normal population:

\[
B(I, \text{age}) = I_c \left/ \left[ I + L_{rh}/t_{rh}(\text{age}) \right] \right. = a \left[ \text{all-trans-retinal}_{\text{ROS}} \right]
\]

Where \( I_c \) is the retinal irradiance in scotopic trolands, \( L_{rh} \) is an empirical constant reported to be \( 10^5 \), [all-trans-retinal]ros is the concentration of all-trans-retinal in the rod.
disks, and \( a \) is an age-independent constant.\(^{31} \) For different ambient corneal light levels, the scotopic trolands were calculated using the reported age-dependence of average pupil diameters as a function of corneal irradiance and the altered scotopic sensitivity of the solar spectrum transmitted through the aging lens (or IOLs). The addition of external filters (eg, spectrally selective sunglasses) merely adds another factor \( t_{SSG}(\lambda) \) in equation 2.

Following rod bleaching of the normal mammalian retina, all-trans-retinal in the rod outer segments is the predominant transiently increased species in the retina. Therefore, we assume in our model that [all-trans-retinal] is linearly with the steady-state rod bleaching (equation 3). All-trans-retinal, which reaches high concentrations in partially bleached rods (ie, for daylight \( \geq 200 \text{ cd/m}^2 \)), is a potent photosensitizer of oxidative damage. After absorbing a short-wavelength photon, the excited singlet-state all-trans-retinal undergoes intersystem crossing to a long-lived (approximately 10 nsec) triplet state that can efficiently transfer its energy to ground state \( O_2 \), creating highly reactive singlet oxygen \( O_2^* \). The action spectrum \( AS(\lambda)_{x_0} \) of this process is the absorption spectrum of all-trans-retinal with a maximum at 380 nm and rapidly diminishing with increasing wavelength. We assumed that the photochemical creation of reactive oxygen intermediates in the rods \( ROI_{rod} \) is predominantly driven by the creation of excited states of all-trans-retinal and is given by:

\[
ROI_{rod} = b \int_{300}^{700} [\text{all-trans-retinal}]_{RPE} \times H_{rod}(\lambda) \times AS(\lambda)_{x_0} \ d\lambda \ d\lambda (4)
\]

where [all-trans-retinal] is obtained from equation 3, \( H_{rod}(\lambda) \) from equation 1, and \( b \) is a constant (affected by \( O_2 \) and quantum efficiency of the photosensitizer reaction but not age). In this and all the subsequent calculations, we were interested only in long-time averages over many days in which daily or even seasonal variations in light exposure can be neglected. Since equation 3 is nonlinear with normal photopic corneal irradiances, the integral over time allows calculation of the average ROI production over any standardized temporal distribution of ambient corneal irradiances that reflect the range of typical daily light exposures. We used 5\% at 4,400 cd/m\(^2\), 20\% at 1,100 cd/m\(^2\), 30\% at 220 cd/m\(^2\), 25\% at 44 cd/m\(^2\), and 20\% at 9 cd/m\(^2\); even lower values would not contribute significantly to modeled photodamage. We modeled the effects due to spectral transmission changes in the lens assuming environmental light exposures do not vary significantly with age in order to evaluate the specific effects of aging on lens yellowing and pupil diameter changes. Epidemiological literature suggests that increased environmental light exposure is a risk factor for AMD, and higher or lower average ambient light distributions than the one we used would have roughly proportional changes in the computed rates of photochemistry at a given age.

Periods of rod bleaching and high [all-trans-retinal]_{RPE} also lead to the formation of A2E and related fluorophores. After disk shedding, these fluorescent molecules are concentrated by lysosomal processing within lipofuscin granules in the RPE. A2E and related molecules avidly partition into cellular membranes and appear to be potent cytotoxic agents capable of inducing DNA damage and apoptosis in the dark, which is enhanced by blue light.\(^{18} \) Since A2E and its phosphorylated precursor require the reaction of two all-trans-retinal molecules, its reaction rate is second order in [all-trans-retinal] and therefore should be proportional to [all-trans-retinal]\(^2 \) in the rods. An estimate of the average rate of A2E production over long periods of different ambient light levels is given by the time average of [all-trans-retinal]\(^2 \) determined by retinal scotopic irradiance \( (I_s) \) determined environmental light exposure:

\[
A2E \text{ production rate (age)} = \int_0^x k \times [\text{all-trans-retinal}]_{RPE}^2 \ d\tau \ (5)
\]

where \( x \) is the age in years and \( k \) is a rate constant assumed to be independent of age, or at least in the case of IOL implantation, independent of whether the aged lens has been replaced with an IOL.

A2E does not appear to be substantially broken down by lysosomal enzymes but rather accumulates in RPE lipofuscin granules where its photo-oxidation on irradiation with short-wavelength light results in a complex mixture of fluorophores.\(^{35} \) A2E avidly reacts with \( O_2^* \) to form epoxides of increasingly higher order, and this oxidation within the lipofuscin granules appears to be the principal route by which A2E concentration (as a distinct molecular species) is limited to approximately 1 pg per RPE cell (approximately 200 \( \mu \)M) in the human retina.\(^{30,35} \) We have modeled the accumulated A2E-derived lipofuscin fluorophores in the RPE (\( LF_{RPE}(x) \)) over many years to be proportional to the time integral of the rate of A2E precursor formation in the rods during periods of significant bleaching.

\[
LF_{RPE}(x) = \int_0^x (A2E \text{ production rate}) \ d\tau \ (6)
\]

where \( x \) is the age. The action spectrum of RPE lipofuscin \( AS(\lambda)_{x_0} \) has been determined by direct detection of \( O_2^* \) phosphorescence and falls exponentially with wavelength (approximately 20-fold from 360 to 460 nm).\(^{31} \) This action spectrum is very similar to that observed for macular RPE injury induced by 1,000-sec focal monochromatic irradiances in rhesus monkeys.\(^{31,34} \) In our model, we assumed
that the potent photosensitizers in lipofuscin granules are derived from A2E by oxidation (predominantly photolysis). We can describe the age dependence of generation of \( \text{O}_2 \) by lipofuscin granule photochemistry by:

\[
\text{ROI}_{\text{RPE}}(\text{age}) = b \int \left[ \int_{390}^{700} \text{LF}_{\text{RPE}}(x) \text{HRPE}(\lambda) \text{AS}(\lambda) \, d\lambda \right] \, dt \quad (7)
\]

where \( \text{ROI}_{\text{RPE}}(\text{age}) \) is the age dependence of lipofuscin \( \text{O}_2 \) generation within a typical RPE cell in the macula at 7 to 11 degrees from center of the fovea.

To predict all of the above processes after cataract surgery, we substituted the spectral transmission of the IOL, \( t_{\text{IOL}}(\lambda) \), for \( t_{\text{L}}(\lambda) \) in equation 2 at the specified time of IOL implantation and recalculated equations 1 and 3 through 7 for the years after implantation. Similarly, spectral transmission of spectrally selective external sunglasses was added to equation 2, and the results recalculated.

**RESULTS**

We are primarily interested in estimating the effects of lens aging and lens replacement with a clear or yellow IOL on the retinal spectral irradiance and the rate of modeled macular photochemistries. Variations among aging individuals in degree of lens yellowing, pupil diameter, rod dark adaptation rate \( t_{\text{d}}(\text{age}) \), and environmental light exposures might result in substantial differences in the amount of A2E-related compounds produced and modeled photo-oxidative damage in both the rods and RPE cells. In modeling macular photosensitization of ROI and production of related photochemicals, we did not evaluate the effects of antioxidant and molecular repair mechanisms. These ameliorating mechanisms may decrease with age but would not be expected to change due to cataract and IOL surgery.

Under our modeled age-independent mixed light exposures, described above, the time average rate of rod bleaching and average [all-\text{trans-retinal}]_{\text{RPE}} decreases only slightly from 0 to 60 years owing to a balance of the normal aging trend to smaller pupils (ie, less light) and slower \( t_{\text{d}}(\text{age}) \), whereas the scotopic transmission of the lens is reduced only slightly. During this period, the modeled average relative rate of rod oxidative damage via all-\text{trans-retinal} photosensitization (Figure 2) is affected largely by changes in the fraction of short wavelength (<450 nm) reaching the rods, which decreases most rapidly between the ages of 0 and 20. Beyond age 60, the progressive yellowing of the lens further reduces macular irradiance in scotopic trolands and consequently the average rod bleaching and [all-\text{trans-retinal}]_{\text{RPE}}. By age 70, the modeled average rate for rod ROI generation is reduced 20-fold and fourfold compared to a 1-year-old and 20-year-old, respectively. Removing the 70-year lens and replacing it with a clear UV-absorbing IOL (roughly equivalent to a 1-year-old lens) increases the average rate of rod ROI photochemical formation by a factor of 15 (Figure 2 and Table 1). Implanting an Alcon yellow IOL, roughly equivalent to a 20-year-old lens, limits the increase in rod ROI after cataract surgery approximately fourfold. Addition of spectrally selective sunglasses such as rod-sparing vermilion lens or long-wavelength-pass filters (yellow 480-nm long-pass or red 570-nm long-pass) would be expected to reduce dramatically rod ROI formation to levels well below those of the presurgery phakic eye as long as they are reliably worn during periods of moderate to high environmental lighting (>200 cd/m²; Table 1). A 480-nm long-pass yellow sunglass following clear IOL implantation is roughly equivalent to a 60-year lens.

Small monotonic decreases in the time average rate of rod bleaching and average [all-\text{trans-retinal}] with increasing age induce larger age-dependent decreases in the annual average rate of A2E formation and build-up within the RPE, according to our model parameters (Figure 3). The modeled average relative rate of A2E formation in RPE cells 7 to 11 degrees from the center of the fovea (Figure 3) is affected by age-dependent decreases in scotopic retinal irradiance balanced against increases in \( t_{\text{d}}(\text{age}) \) with age. Lens yellowing more significantly reduces scotopic transmission for ages greater than 60. By
age 70, the modeled average rate for A2E formation is reduced 2.2-fold and 1.9-fold compared to an infant and 20-year-old, respectively (Figure 3). Removing the 70-year lens and replacing it with a clear UV-absorbing IOL increases the average rate of A2E formation by a factor of 2.6 versus 2.1 for an AcrySoft Natural IOL (Figure 3 and Table 1). Addition of spectrally selective sunglasses such as rod-sparing vermilion lens or long-wave-pass filters (570-nm long-pass) would be expected to reduce dramatically average rod bleaching and A2E production to levels less than 1/30th of those for the presurgery phakic eye as long as they are reliably worn during periods of moderate to high environmental lighting (>200 cd/m²).

Assuming A2E and related photochemicals are largely concentrated by RPE lysosomal processing into lipofuscin granules rather than being enzymatically degraded or removed by transport to the choroidal microcirculation, the model predicts that the accumulation rate of lipofuscin fluorophores would be nearly linear for the first 60 years with a noticeable rate of slowing of accumulation beyond 60 years (Figure 4). After cataract and IOL surgery, the modeled rate of A2E-related fluorophore accumulation increases approximately linearly at about the same rate as in early childhood (with its clear lens). The vermilion and 570-nm long-pass sunglasses prevent significant rod bleaching and A2E formation and thus prevent accumulation of further A2E-related fluorophores in the RPE. Although A2E-derived lipofuscin photosensitizer concentration increases consistently with age, the aging lens filters out increasing fractions of the short-wavelength photons capable of allowing lipofuscin to photosensitize \( \text{O}_2 \) formation (macular-weighted spectral irradiance in Figure 4). This balance of aging effects leads to a predicted increase in RPE \( \text{O}_2 \) generation between age 10 and age 60 followed by a progressive decrease beyond age 60 (Figure 5). By dramatically increasing the transmission of short-wavelength photons to the retina and increasing the rate of A2E-related lipofuscin fluorophore accumulation, cataract and clear IOL surgery at 70 years would be expected to increase RPE \( \text{O}_2 \) generation approximately sevenfold relative to the phakic eye (Table 1). The Alcon yellow IOL (or Russian

![FIGURE 3](image)

Modeled average annual rate of formation of A2E as a function of age and lens status: for the phakic eye (○), for the addition of vermilion sunglasses after implantation of clear IOL at 70 (●), for clear IOL implanted at age 70 (△), and for the yellow IOL (•) implanted at age 70.

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**TABLE 1. FRACTIONAL CHANGES IN PHOTOCHEMISTRY VALUES 7 TO 11 DEGREES FROM THE CENTER OF THE FOVEA AFTER CATARACT SURGERY AT AGE 70 RELATIVE TO VALUES FOR TYPICAL PHAKIC SUBJECT AT AGE 70**

<table>
<thead>
<tr>
<th>Lens Status</th>
<th>&lt;rod bleach&gt;</th>
<th>&lt;rod ROI&gt;</th>
<th>&lt;A2E production&gt;</th>
<th>&lt;RPE ( \text{O}_2 )&gt;</th>
<th>&lt;[A2E\text{RPE}]&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native lens at age 70</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Clear IOL</td>
<td>1.8</td>
<td>15</td>
<td>2.6</td>
<td>6.6</td>
<td>0.44</td>
</tr>
<tr>
<td>Natural yellow IOL</td>
<td>1.6</td>
<td>4.1</td>
<td>2.1</td>
<td>2.6</td>
<td>0.9</td>
</tr>
<tr>
<td>Spectrum yellow IOL</td>
<td>1.6</td>
<td>4.9</td>
<td>2.2</td>
<td>2.9</td>
<td>0.8</td>
</tr>
<tr>
<td>Clear IOL + 450-nm LP</td>
<td>1.1</td>
<td>0.20</td>
<td>1.2</td>
<td>0.7</td>
<td>1.8</td>
</tr>
<tr>
<td>Clear IOL + vermilion</td>
<td>0.14</td>
<td>0.05</td>
<td>0.03</td>
<td>0.24</td>
<td>0.09</td>
</tr>
<tr>
<td>Clear IOL + 570-nm LP</td>
<td>0.05</td>
<td>&lt;0.01†</td>
<td>&lt;0.01†</td>
<td>&lt;0.01†</td>
<td>No estimate†</td>
</tr>
</tbody>
</table>

*All quantities in angular brackets ( < ) denote time averages over 1 year and our standardized ambient lighting changes. <Rod bleach>, cumulative rod bleaching; <Rod ROI>, cumulative reactive oxygen intermediates produced in rods via all-trans-retinal photosensitization; <A2E production>, cumulative A2E produced by rod bleaching; <RPE \( \text{O}_2 \)> , cumulative production of singlet oxygen via lipofuscin photosensitization in the retinal pigment epithelium; <[A2E\text{RPE}]>, average steady-state concentration of A2E within the RPE.†The small amount of light capable of bleaching rods or driving photosensitizer production of ROI when wearing the 570-nm long-pass filter suggests that these photochemical products will be determined entirely by periods during which the external filters are not worn, and consequently the [A2E\text{RPE}] could not be reliably estimated.
Spectrum yellow IOL) would result in approximately a threefold increase. The use of the vermilion sunglass with a clear IOL reduces RPE 1O₂ generation greater than 20-fold compared to the clear IOL alone and fourfold with respect to the phakic eye. A 570-nm long-pass filter almost completely prevents RPE 1O₂ generation during its use. A 480-nm long-pass filter combined with a clear IOL is roughly comparable to the aging phakic eye at 70 years (see Figure 1).

At ≥10 µM, A2E has been shown to be cytotoxic to RPE cells in culture even though its average extractable concentration found in the aged human RPE is approximately 1 pg/cell or approximately 200 µM. Therefore, RPE viability appears to require A2E segregation within the lipofuscin granules and some means to alter chemically A2E as it accumulates within the RPE with age. We modeled the removal of accumulated A2E through its oxidation by 1O₂ generated in turn by short-wavelength light absorption by oxidized A2E molecules within the same lipofuscin granule. This oxidation process within a lipofuscin granule requires a cumulative exposure equivalent to 80 J/cm² at 488 nm (less at shorter wavelengths), which for our modeled ambient exposures would be expected to require approximately 1 month in a subject under 20 years of age. We calculated our estimate of the relative steady-state A2E concentration in the RPE, [A2Eₜₘ]ₐₚₑ, as the average A2E production rate divided by its photo-oxidation rate within a lipofuscin granule as a function of age and ocular status (Figure 6). This calculation predicts that [A2Eₜₘ]ₐₚₑ should increase almost linearly with age in a normal phakic individual, even though the rate of modeled A2E production falls significantly with age, particularly after 60 (Figure 3). Following lens removal and IOL implantation, both the modeled...
production of A2E and the rate of its photo-oxidation increase. As shown in Figure 4, the total accumulated A2E-related fluorophores (largely oxidized A2E) in lipofuscin increase after IOL implantation more for the clear IOL than for the yellow IOL. In contrast, [A2E]_{RPE} (Figure 6) falls 2.3-fold following clear IOL implantation at 70 years but only 10% for yellow IOL implantation (Table 1).

**DISCUSSION**

The model described in this study is based on the long-held hypothesis that cumulative photochemical damage (eg, generation of toxic photochemicals or ROIs that result in oxidative damage) is an important factor in the pathogenesis of AMD. It has been long proposed that the reduced short-wavelength (<500 nm) transmission of the aged lens should photoprotect from macular photochemical damage and this protection would be substantially removed following cataract surgery. In the early IOLs, UV transmission was a significant factor, shortly avoided by introduction of UV absorbents cross-linked to the IOL polymers. Subsequently (first in Russia in the mid-1980s and then more recently in the West), in response to early criticisms of the removal of natural photoprotection of the aged lens, deep blue (420-nm) absorbents have been similarly formulated to create a light yellow IOL approximately as photoprotective against blue light photodamage as a 20-year-old lens.

In the model, we calculated the relative effects of macular irradiance, lens aging (nuclear sclerosis), a clear or yellow IOL, and additional selective spectral glasses on the production of possibly cytotoxic photoproducts. The modeled retinal area, 7 to 11 degrees from the center of the fovea, is a region of high rod loss and lipofuscin accumulation with age. Consequently, possible changes in photochemical photosensitization of rod and RPE ROIs within this region are likely to show the greatest changes on aging and following cataract and IOL surgery.

Shortly after cataract and IOL surgery, our model predicts that the average production of A2E-related photochemicals and ROIs in rods and RPE due to all-trans-retinal and lipofuscin photochemistry would theoretically increase, respectively, 2.6-, 15-, and 6.6-fold with a clear UV-absorbing AcrySoft IOL and 2.1-, 4.1-, and 2.6-fold with a “yellow” IOL (Table 1). An external vermilion filter sunglass in ambient lighting of ≥200 cd/m² would decrease the above values for a clear IOL approximately 30-, approximately 20-, and 4-fold compared to an average 70-year-old phakic eye. We would expect an increase in these photochemistries in eyes with larger pupil diameters, in phakic eyes with a higher lens transmittance, and in subjects with greater environmental light exposure compared to the age-dependent average values and light exposures we used in our calculations.

Our model is based in part on selected laboratory studies that have identified specific photo-oxidative damage mechanisms in rods and RPE cells and their action spectra. However, it is important to note that the short-wavelength thresholds for acute injury found in these studies (eg, >1 mW/cm² and >1 J/cm² at 430 nm) are greater than 100-fold higher than macular irradiances achieved under normal daylight conditions (Figure 4: inverted triangles, righthand axis). Although the empirical damage thresholds in animal experiments demonstrate an expected reciprocal relation between exposure intensity and duration for exposures up to a few thousand seconds, for much lower rates of ROI production over days or months, antioxidant protection and the rate of repair of oxidative damage can markedly increase damage thresholds (eg, J/cm² at 430 nm) and determine whether significant photo-oxidative stress or cellular damage occurs.

Direct photochemical injury to the rods mediated by photo-activation of rhodopsin has been hypothesized to result from excited-state photochemistry of all-trans-retinal in the rod outer segments. On absorbing short-wavelength light (380-nm peak absorbance), an all-trans-retinal molecule can be driven into a long-lived triplet excited state that can initiate photo-oxidative damage to ROS membrane lipids and rim proteins. The reported photo-aggregation of ABCA4, a photoreceptor transporter enzyme of the visual cycle, would theoretically decrease the rate of transport of all-trans-retinal from the ROS disks as they age. Under moderate daylight conditions, the older, more distal disks would increase local all-trans-retinal concentration under steady-state illumination, which in turn would increase the production of A2PE, A2E, and related species requiring the reaction of two all-trans-retinal molecules and one phosphatidylethanolamine. These reactions would be driven by significant rod photobleaching and associated high steady-state [all-trans-retinal]_{RPE} in moderate to bright daylight. Changes in our modeled rates of rod ROI production with increasing age are largely determined by the lower lens transmission of blue light, smaller pupil diameter, and slower rod dark adaptation time constant $t_{AD}$. The resulting modeled rate of rod ROI production via all-trans-retinal photochemistry monotonically decreases, on average, approximately 40% per decade throughout life (Figure 2). All-trans-retinal photo-induced ROI created within the rods should create much more damage when macular short-wavelength irradiances are high. Consequently, the model predicts approximately 20-fold higher rod ROI production in a young child than in a 70-year-old phakic person. An immediate 15-fold increase in ROI production is
predicted on cataract removal and clear UV-absorbing IOL implantation in a 70-year-old; this suggests a possible mechanism for accelerated rod loss following cataract and IOL surgery. The yellow IOL limits the postoperative increase to fourfold. In contrast, wearing the vermilion glasses (during ambient conditions ≥200 cd/m²) following clear IOL implantation reduces modeled rod ROI production approximately 20-fold compared to the preoperative 70-year-old phakic eye (Table 1). In the cones, the more rapid visual cycle kinetics would markedly reduce steady-state all-trans-retinal concentrations under intense light conditions compared to rods. Consequently, the cones may be less vulnerable to all-trans-retinal–mediated photo-oxidative injury and generate less A2E and related species. This may be due in part to the recently described alternative pathway for recycling all-trans-retinal in cones involving Mueller cells independent of the RPE.35

The accumulated A2E precursors in the shed distal disks are phagocytosed by the RPE cells and concentrated as A2E by lysosomal processing into the lipofuscin granules.36 Retinal pigment epithelial lipofuscin granules accumulate with age and contain a heterogeneous mixture containing at least 12 distinct extractable fluorophores, three of which exhibit fluorescence spectra similar to A2E.37 A2E epoxides formed by the reaction of 1O₂ with A2E are much more potent photosensitizers than A2E with a blue-shifted spectra and are likely to correspond to A2E by lysosomal processing into the lipofuscin granule. In our model, we assumed that lipofuscin photosensitization within the RPE results in the complex curve of RPE ROI (age) via lipofuscin photoactivation plotted in Figure 5. The modeled ROI increases with age over the first 60 years. However, due to the reduction in short-wavelength macular irradiance, it falls progressively (32% per decade) after 60. The large increase in short-wavelength macular irradiance on removal of the lens and replacement with an IOL leads to modeled increases in RPE ROI photosensitization (6.6-fold for clear IOL and 2.6-fold for the yellow IOL at 70 years), which continue to increase in successive years as the rate of lipofuscin photosensitizer accumulation increases. Wearing the vermilion spectral glasses following clear IOL implantation should reduce the RPE ROI photosensitization to modeled levels roughly one fourth of those for preoperative phakic eye.

Cytoplasmic A2E concentrations of ≥10 µM have been shown to damage mitochondrial membranes in vitro (in the dark), and approximately 1 pg of A2E per RPE cell (which is approximately 200 µM A2E on average in situ) has been extracted from human eyes.38 A2E molecules are avidly taken up by RPE cell cultures, concentrated within phagosomes, and within a few days appear as highly fluorescent lipofuscin-like granules. This efficient mechanism appears necessary to limit the cytotoxic (dark reaction) damaging effects of the detergent-like A2E molecules. The amount of chloroform-insoluble photosensitizers within each lipofuscin granule appears to increase with age.39 The high content of unsaturated carbon bonds in lipofuscin granules and the granule size (much greater than the distance O₂ diffusion distances in its 4 µsec lifetime) suggest that a large fraction of cellular oxidation induced by lipofuscin photogenerated O₂ may be confined to the granule in which it was generated. This process might have two positive attributes: (1) limit the concentration of O₂ escaping from the lipofuscin granules and thereby limit cell oxidative damage and (2) photodegrade A2E into A2E-related insoluble fluorophores that are incapable of redistributing into critical cellular membranes (thereby limiting A2E cytotoxic damage in the dark). We suggest this is reflected in a balance between the rate of A2E production and the rate at which A2E molecules would be photo-oxidized within a lipofuscin granule, both of which are light-driven processes. The relative steady-state [A2E₄(age)]RPE in Figure 6 is

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predicted from the ratio of the modeled rate of A2E production (Figure 3) and the modeled rate of its photodegradation within a lipofuscin granule (equation 7 and plotted as ▲ in Figure 4). Interestingly, the modeled [A2E_{(age)}]_{RPE} shows a continual increase beyond the age of 60 even though the A2E production rate falls. The age-related changes in lens transmission decrease short-wavelength photodegradation of A2E by lipofuscin generated O_2 more than they reduce rod bleaching and A2E production. Delori and associates have made in vivo fluorescence measurements in the human macula that are specific and proportional to [A2E] and show a similar age dependence in normals to our modeled [A2E_{(age)}]_{RPE} (Figure 6). If A2E is cytotoxic at the levels found in the aging RPE, then its increasing average steady-state levels in the RPE with advancing age might be a factor in the pathogenesis of AMD. Unlike our analysis of increases in ROI generation following cataract surgery at 70, the modeled [A2E_{(age)}]_{RPE} shows a 2.3-fold reduction within a few months following lens removal and clear IOL implantation (Table 1). The yellow IOL increases both the modeled light-driven A2E production and its photodegradation by roughly the same factor so that the resulting [A2E_{(age)}]_{RPE} is within 20% of the phakic preoperative eye. The vermilion sunglasses, worn after clear IOL implantation, reduce modeled A2E production much more than its rate of photodegradation and further reduce the [A2E_{(age)}]_{RPE} to approximately 10% of the preoperative levels. In contrast, a bright yellow 480-nm long-pass filter worn after clear IOL implantation increases the modeled [A2E_{(age)}]_{RPE} 1.8-fold over the preoperative levels. The technique of Delori and associates may be suitable to measure some of the larger predicted changes following cataract and IOL surgery. Comparisons with and without external filters that might decrease macular photo-oxidation but exhibit diverging effects on modeled [A2E_{(age)}]_{RPE} in patients following cataract and IOL surgery might test the assumptions and predictions of our model.

Overall after cataract removal and clear IOL implantation, the model predicts a marked increase in ROI photoproduction in both the rods and RPE but an associated decrease in the [A2E_{(age)}]_{RPE} cytotoxicity compared with the preoperative phakic eye. The total lipofuscin fluorophores, which are largely oxidized forms of A2E, are predicted to increase at a greater rate following lens removal and IOL implantation, but the yearly fractional rate of change is small given the lifetime accumulation of lipofuscin prior to cataract surgery. These modeled effects were for a typical aging eye without preoperative early AMD changes that may critically affect the potential for photo-oxidative damage or stress. The interaction between photo-oxidation and antioxidants or macromolecular repair mechanisms is presently not well understood in relation to macular aging changes or to AMD and thus is beyond the scope of our simplified model. Vermilion filters are widely used in commercial glasses and are well suited to acceptance by patients after cataract surgery. In a pilot study, patients who had late AMD in one eye and early AMD changes in the other eye adapted well subjectively to wearing the red 570-nm long-pass filters outside during bright daylight (S. Meyers and R. Bonner, unpublished data, 2003).

The pooled data of the Beaver Dam and Blue Mountain Eye Studies indicate a substantially higher risk for developing late-stage AMD in nonphakic compared with phakic eyes during a 5-year period (odds ratio equals 5.7 after adjustment for confounding variables). Short-wavelength macular irradiance is expected to have larger fractional increases on removal of nuclear cataracts than for the normally aged lens that we modeled. The epidemiological data coupled with our model predictions suggest that patients immediately after cataract removal and IOL implantation appear to be a particularly good population to evaluate the possible role of chronic photochemical injury in AMD. A randomized clinical trial to test the potential protective effects of an optimized “rod-sparing” external filter (eg, vermilion) would be a timely and important study in AMD. Furthermore, it would be of interest to evaluate such a filter in young patients with Stargardt’s disease and ABCA4 mutations, who would be of greater theoretical risk for photochemical injury associated with rod bleaching. Such studies might also discriminate among possible mechanisms for potential chronic photochemical injury.

REFERENCES


A Model of Spectral Filtering to Reduce Photochemical Damage in Age-Related Macular Degeneration


DISCUSSION

DR ROBERT N. FRANK. Meyers and his colleagues have presented a very elegant, theoretical model of irradiance of the macula over the visible spectrum as a function of age, age-related changes in the lens, and following cataract surgery in the presence of several types of ultraviolet-absorbing intraocular lenses (IOLs). The hypothesis underlying this analysis is that age-related maculopathy (ARM) and in particular its most severe forms (geographic atrophy and choroidal neovascularization, which we term age-related macular degeneration [AMD]) are in large part due to a lifetime of irradiance of the retinal rods with light in the short wavelength portion of the visible spectrum. Following the bleaching of the rod visual pigment, rhodopsin (cone visual pigments are less suscep-
tible to damage), the photoisomerized rhodopsin chromophore, all-trans-retinal, can both act as a photosensitizer of ROI and can undergo dark reactions leading to creation of the fluorescent molecule known as A2E, which accumulates in the retinal pigment epithelium (RPE) where it is incorporated into lipofuscin. Short-wavelength photo-excitation of A2E and its oxidized products in lipofuscin granules generate reactive oxygen intermediates (ROIs). Either the increasing concentrations of A2E and related molecules or the ROIs produced might damage the RPE, ultimately leading to the lesions of ARM/AMD. The model of Meyers, Ostrovsky, and Bonner predicts that though A2E production rates generally decrease with advanced age, the steady-state levels of A2E in the RPE rise as a result of larger decreases in the rate of its photo-oxidation within lipofuscin granules due to increasing short-wavelength light absorption as the lens yellows. Cataract extraction and implantation of a UV-absorbing IOL increases production of A2E and even more markedly of ROIs, but these processes can both be profoundly inhibited by the wearing of specific red or vermilion sunglasses.

I won't go into the details of this model in my discussion, since much of the photochemistry and the calculations are beyond my limited expertise. But it is important to note that this model is entirely a theoretical one. Its validity is based on the hypothesis I have stated above and, because it is a generalized model, it does not consider biological variability that might influence whether a particular individual is more or less susceptible to the development of AMD, even given the same lifetime history of short wavelength visible light exposure as another individual of differing genetic background or other characteristics. Evidence that sunlight exposure itself is a risk factor has been somewhat controversial, with different studies reaching different conclusions. However, there is very strong evidence from the combined Beaver Dam and Blue Mountains Eye Study results that cataract surgery substantially increases the five-year incidence of AMD (odds ratio 5.7, with 95 percent confidence interval 2.4-13.6 after multiple adjustments).

With this result in mind, and given the predictive and, above all, testable hypothesis of Meyers, et al., it seems to be time to initiate a controlled clinical trial of protective sunglass wearing for individuals who undergo cataract surgery. The major problem of such a trial, as it is with many clinical trials, is the duration (at least five years) and the sample size. The combined Beaver Dam/Blue Mountains study populations included 6,019 participants from whom 11,391 eyes were evaluated over a five-year follow-up. Of these, only 315 (fewer than 3 percent) were non-phakic, i.e. would qualify for the proposed clinical trial (and because of the requirement to wear sunglasses for the proposed study, it is subjects and not eyes that must be counted). Of these eyes, fewer than 7 percent (a total of 21) developed advanced AMD over the five-year follow-up. With these figures-and using a five-year relative risk (sunglass wearers vs. controls) of 0.8, approximately equal to the results of the Age-Related Eye Disease Study, with an alpha of 0.05, a power of 0.8, and approximately equal numbers of sunglass wearers and controls-using the uncorrected chi-square test to calculate results, I calculate a sample size of 4,700 in the treated group and an equal number of controls. If the relative risk is decreased to 0.5, the sample size decreases to 640 subjects in each group. Of course, this calculation will differ if, as in the AREDS, the entry criteria are modified to include only subjects who have early ARM at the outset. Given the frequency of cataract surgery and of ARM/AMD and the detailed hypothesis that Meyers and his colleagues have presented, I urge that such a trial be given strong consideration.

REFERENCES


DR DOUGLAS D. KOCH. I wonder if some of the experimental work could be done in vitro. Janet Sparrow has done a lot of work with various filters protecting against generation of A2E. You mentioned that you need some blue light coming in. If you could model that experimentally and then in an in vitro model, you might be able to confirm your hypothesis.

DR JOHN T. FLYNN. I might suggest another experimental approach. Take patients who need a cataract extraction but also have a very strong family history of macular degeneration. Consider entering those patients in a study. They receive a clear lens in one eye and they receive a vermilion lens in the other eye, or they receive two clear lenses but they wear a vermilion contact lens. It seems to me the sample size to get to a P value of <0.05 is six, and you may not have to study a large number of patients to prove your hypothesis. You have to have cooperative patients who are
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willing to subject their eyes to be their own control, so to speak, to answer this question.

DR RICK FERRIS. I applaud the authors for trying to add some science to the understanding of what’s accumulating in the RPE and how it accumulates. With regard to light toxicity, I spent many years in various studies looking at this question with a number of sunlight questionnaires and other approaches. If it is a risk factor, it is hard to demonstrate it, and it appears not to be an overwhelmingly strong risk factor. However, it does point out that this may be a complex issue with a number of other important factors leading to AMD.

It is important to discuss the issue of post-cataract surgery and the development of AMD. Unfortunately, the two epidemiologic studies that have recently been reported suffer from a major flaw. The flaw is that patients with early AMD suffer slight decreased vision from the AMD, and wind up getting a cataract removed, not because the cataract caused the decreased vision, but because the AMD caused the decreased vision.

So, in this case, the AMD causes the cataract surgery. When you do the epidemiologic and statistical studies, it could appear that the cataract surgery caused the AMD, but it is actually the other way around. Both sets of authors of those studies suggest that the ideal way of evaluating this would be to carefully examine these patients’ retinas before cataract surgery. We have been doing that in the age-related eye disease study for the last eight years or 10 years, and we have been looking at the development of AMD after cataract surgery in our study. At present, we cannot show an increased risk of the development of late AMD or advanced AMD following cataract surgery. The weakness of our study is that we have modest follow-up of five to six years after cataract surgery. We are doing additional analyses because it may take you many years of increased exposure before you are likely to see the increased risk develop.

DR SANFORD M. MEYERS. Our model probably has its greatest relevance in very early AMD or in patients without overt disease but who have a strong family history or hereditary risk. A trial of spectral filters comparing one eye with the other in patients after bilateral cataract and IOL implant surgery, as suggested by Dr Flynn, would avoid the genetic and non-genetic variables of interindividual comparisons. In such a study, compliance would be an important issue. Over the past few years, we studied about 25 unilateral late-stage AMD patients, phakic and pseudophakic, who wore reddish glasses with a 570-nm-long pass filter outside during bright daylight. We did not observe a dramatic effect. However, one of the patients, a physician who was bilaterally pseudophakic, fell in love with the reddish glasses and was 100 percent compliant. He had one photodynamic laser treatment after which he began wearing the reddish glasses. In the subsequent three years, he has not had a recurrent choroidal neovascular membrane in that eye (vision 20/60) and has not developed late AMD in the other eye. Overall, the compliance was good, but some of the patients were more compliant than others on subjective questioning. The vermilion filter may increase compliance (due to improved color perception and photopic sensitivity) and provides similar protection as the red 570 nm filter. A vermilion contact lens is a possibility but would create logistical issues on its use in subjects over the age of 70. A vermilion IOL would create problems for night vision unless suitable photochromic materials were developed.

Dr Ferris raises critical questions about some of the epidemiological studies. Patient responses on the history of sunlight exposure 30 years in the past are very subjective, especially in relation to our model with sophisticated mathematical analysis. Additionally, differences between individuals in regard to genetic factors, levels of antioxidants, and cellular damage repair mechanisms are confounding variables in epidemiological studies.

To address these issues and the large size of a clinical trial needed to test the efficacy of spectral filters, a small pilot study could be done in patients after cataract and IOL implant surgery to determine the feasibility of using in vivo techniques for retinal spectral fluorescent measurements that quantify levels of A2E and its epoxides and the effects of different ocular filters. If this pilot study verifies the model’s predictions, it would support a randomized clinical trial. We also suggest that the vermilion filter be considered in a study of Stargardt’s patients with mutations in the ABCA4 enzyme, which is critical in the processing of all-trans-retinal as stated in our manuscript. Dr Koch’s comments are addressed in the manuscript.
VITRECTOMY FOR EPIRETINAL MEMBRANES WITH GOOD VISUAL ACUITY

BY John T. Thompson MD*

ABSTRACT

Purpose: To evaluate the visual results of vitrectomy for epiretinal membrane in eyes with a preoperative visual acuity of 20/50 or better.

Methods: The visual results and complications were analyzed following vitrectomy for idiopathic epiretinal membranes and epiretinal membranes secondary to retinal tears. This study was a retrospective, consecutive case series of 40 eyes of 40 patients treated by a single surgeon.

Results: The mean preoperative visual acuity was 20/50 +2 (range, 20/30 +1 to 20/50 –3). The mean visual acuity improved to 20/40 +2 ($P = .02$) by the final examination at a mean of 2.4 years following surgery. The status of the lens at the final examination was correlated with the visual results of surgery. Twenty-one eyes were phakic preoperatively, and 14 of these eyes had cataracts removed by the final examination. The mean preoperative visual acuity in seven eyes that were still phakic at the final examination was 20/50, and this decreased to 20/50 –2 ($P = .82$). The mean preoperative visual acuity was 20/50 +2 in 33 eyes that were pseudophakic by the final examination, and this improved to 20/32 –2 ($P = .005$). The visual acuity improved by 2 or more Snellen lines in only one in seven eyes (14%) that were still phakic on the final examination and in 14 of 33 eyes (42.4%) that were pseudophakic by the final examination. There were no serious surgical complications.

Conclusions: Vitrectomy for epiretinal membranes is beneficial in eyes with relatively good preoperative visual acuities, but cataract surgery is necessary in phakic eyes to achieve long-term visual acuity improvement.


INTRODUCTION

Vitrectomy has become a common procedure for the treatment of visual loss due to epiretinal membranes (macular pucker) over the past two decades. Epiretinal membranes result from fibroglial proliferation on the surface of the retina.1,2 The prevalence of epiretinal membranes in the macula detectable by fundus photographs was 7% in a study of 3,654 persons who were aged 49 years or more.3 Multiple studies have reported visual acuity improvement following this surgery; but most published series have reported eyes with preoperative visual acuities of 20/60 or worse. The decision to recommend vitrectomy is largely based on patients’ symptoms and the preoperative visual acuity. Vitrectomy is sometimes recommended and performed in eyes with better visual acuities in patients who need excellent visual acuity or a high degree of stereopsis for occupational reasons. Some patients with good visual acuity who have severe metamorphopsia are also offered surgery. Performing surgery in patients with good visual acuity also carries some increased risks. Vitreous surgery in an eye with 20/40 visual acuity is much less likely to achieve large improvements in visual acuity. A 2-line improvement would require an increase in visual acuity from 20/40 to 20/25, and a 3-line improvement would require a postoperative visual acuity of 20/20. The purpose of this study was to evaluate the visual acuity results and complications of vitrectomy for epiretinal membrane removal in eyes with a preoperative visual acuity better than 20/60 to determine if the risks of surgery are justified in most eyes.

METHODS

Vitrectomy was performed in a retrospective case series of 40 consecutive eyes of 40 patients with symptomatic visual acuity loss or distortion due to epiretinal membranes. The surgeries were performed from December 1992 to November 2002 by a single surgeon (J.T.T.). All patients had symptomatic visual loss and desired vitrectomy despite relatively good visual acuities. No institutional
nuclear sclerosis was graded as 1.5 if it was between the 1.0 and 2.0 standards. This grading scale is very similar to the clinical lens standard photographs used in the Age-Related Eye Disease Study (AREDS).10 Cortical spoking was not routinely recorded for patients treated between 1992 and 1995, so this cataract variable was not analyzed in this manuscript. Prior studies have shown minimal effect of vitrectomy on cortical spoking, though.11 Follow-up of at least 1 year was attempted, but some patients were discharged from care because of transportation difficulties or were lost to follow-up before 1 year. Attempts were made to contact referring ophthalmologists in patients who were lost to follow-up or to obtain visual acuity data subsequent to cataract extraction if patients had not returned to the author for examination after cataract surgery. Paired sample t tests were performed when comparing numerical data such as a patient’s preoperative to postoperative visual acuity.

RESULTS

The baseline characteristics for the 40 eyes in the study are summarized in Table 1. The mean preoperative visual acuity was 20/50 +2 (logMAR = .367) with a range of 20/30 +1 to 20/50 –3. The mean duration of follow-up was 2.4 years with a range of 3 months to 11 years. Figures 1 through 3 report the visual results based on the preoperative lens status. Figure 1 compares the visual acuity data for all eyes, eyes that were phakic preoperatively, and eyes that were pseudophakic preoperatively. Overall, the mean visual acuity improved from 20/50 +2 to 20/40 +2 (P = .02) in the group of 40 eyes. This represents an improvement of 26.9% when the visual acuity improvement is compared against a benchmark “perfect” visual acuity of 20/20. This percentage improvement is calculated by the following equation:

\[
%I = \frac{-(\log MAR_{preop} - \log MAR_{final})}{\log MAR_{preop} - \log MAR_{20/20\, acuity}} - \frac{-(\log MAR_{preop} - \log MAR_{final})}{\log MAR_{preop}}
\]

where %I is % improvement; logMARpreop is logMAR of preoperative visual acuity; logMARfinal is logMAR of final visual acuity; and logMAR 20/20 acuity is 0.

Visual acuity showed consistent improvements in pseudophakic eyes following removal of the epiretinal membrane, whereas visual acuity actually decreased at 3 months in phakic eyes, presumably due to early cataract formation. All three groups showed improved mean visual acuity at the final examination, although the improvement did not reach statistical significance in eyes that were phakic preoperatively, primarily because of some eyes with substantial cataracts at the final examination.

Most patients were seen at 1 day, 1.5 weeks, 6 weeks, 3 months, and at variable times thereafter. Patients were monitored for complications. Cataracts were measured preoperatively and following surgery using the lens opacity classification system II (LOCS II) grading scale developed by Chylack.9 This scale grades nuclear sclerosis, posterior subcapsular cataract, and cortical spoking on a scale of 0 to 4 using reference photographs. Intermediate numerical values were assigned if a patient’s lens was judged between two standard photographs. The

The surgical technique consisted of a standard three-port pars plana vitrectomy using 20-gauge instrumentation. Most eyes had a posterior vitreous detachment at the time of surgery. A posterior vitreous detachment was created if none was present. Then a blunt vitreoretinal pick was used to lift the edge of the epiretinal membrane if an edge was identified intraoperatively. The elevated edge was then grasped with diamond dusted forceps and removed from the eye. If the edge of the epiretinal membrane could not be identified, then a sharp vitreoretinal pick or bent microvitreoretinal blade was used to create an edge of the epiretinal membrane, which was then grasped with the forceps. Indocyanine green was not used to help identify the epiretinal membrane or internal limiting membrane in any eyes, and the internal limiting membrane was not intentionally removed. Staining of the internal limiting membrane in eyes with epiretinal membranes using indocyanine green or trypan blue has the theoretical advantage of ensuring more complete removal of epiretinal membrane and is advocated by some surgeons.14 Other surgeons prefer to avoid removal of the internal limiting membrane, if possible, because the presence of internal limiting membrane in epiretinal membrane histology specimens was associated with poorer visual outcomes in one study.7 Intentional removal of internal limiting membrane in eyes with epiretinal membranes was associated with poorer visual results and higher visual field loss in another study.8

review board approval was obtained, but all patients consented to surgery after a discussion of the risks and benefits. Most epiretinal membranes were idiopathic, but some were secondary to retinal tears. Eyes with epiretinal membranes following retinal detachment repair were excluded, because most of the membranes developed in eyes with prior macular detachment, which may have decreased the best potential visual acuity. Eyes with intrinsic macular diseases that may have decreased visual acuity, such as diabetic retinopathy, branch retinal vein occlusion, or pars planitis, were excluded. All eyes had a visual acuity of 20/50–3 or better. Visual acuities were measured preoperatively and postoperatively using a projected-light Snellen chart with best current correction, but without refraction.

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Figure 2 shows the mean visual acuity change in Snellen lines (1 Snellen line = 0.1 logMAR unit). The visual acuity improved the most in eyes that were pseudophakic preoperatively with a mean gain of 1.4 Snellen lines. This represents a 38% improvement in visual acuity. Eyes that were phakic preoperatively gained only a mean of .61 line, which is a 16.9% improvement in visual acuity. Figure 3 shows the percentage of eyes that gained 2 or more lines, remained unchanged (final visual acuity within 0.2 logMAR of initial visual acuity), and lost 2 or more Snellen lines of acuity. Eyes that were pseudophakic preoperatively were more likely to gain 2 or more lines of acuity and less likely to lose 2 or more lines between the preoperative and final examination.

Visual acuity results based on preoperative lens status. Eyes that were phakic on the preoperative examination were more likely to lose 2 or more lines of acuity (0.2 logMAR units), and eyes that were pseudophakic were somewhat more likely to gain 2 or more lines between the preoperative and final examination.

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Thompson

This improvement represents an 11% reduction in visual acuity in phakic eyes and a 35% improvement in visual acuity in pseudophakic eyes on the final examination. Figure 6 shows the percentage of eyes that gained 2 or more lines, lost 2 or more lines, or remained unchanged based on the postoperative lens status. Twenty-nine percent of eyes that were phakic on the final examination lost 2 or more lines, and only 14% gained 2 or more lines despite the relatively good preoperative visual acuities in this group of eyes with epiretinal membranes.

Figures

Mean visual acuity based on postoperative status of lens. The mean visual acuity remained essentially unchanged between the preoperative and 3-month examination in all eyes and eyes that were pseudophakic by the final examination. The visual acuity improved a little at 3 months in eyes that were phakic by the final examination. Visual acuities improved significantly in all eyes (P = .02) and eyes that were pseudophakic by the final examination (P = .005). Visual acuities deteriorated slightly in eyes that remained phakic at the final examination (P = .82), primarily because of visually significant cataracts.

Causes of Decreased Acuity

Four of 40 eyes (10%) lost 2 or more lines of visual acuity. The causes of decreased acuity were evaluated in each patient. The first patient was pseudophakic preoperatively and had a history of chronic cystoid macular edema. The visual acuity decreased from 20/50 –1 prior to epiretinal membrane removal to 20/400 on the final examination, primarily on account of worsening cystoid macular edema. The eye also had a 2+ posterior capsule opacity. The visual potential of the eye is likely no better than 20/200 based on severe cystoid macular edema and the recorded visual acuity prior to development of the posterior capsular opacity. The second eye was 20/40 –2 preoperatively, and the visual acuity decreased to 20/200 due to a 2+ nuclear sclerotic cataract. This eye had been 20/40 at 3 months following epiretinal membrane removal, so the visual potential is believed to be favorable. The third eye was 20/40 +2 prior to epiretinal membrane removal. The visual acuity decreased to 20/80 –2 after 2 years for unknown reasons, but had been 20/40 at 17 months with a similar examination. No other ophthalmic abnormalities were seen to explain the decrease in acuity. The fourth eye had a preoperative visual acuity of 20/50 –2, which decreased to 20/80 –2 from a 2+ nuclear sclerotic cataract. Hence, of this group of 40 eyes, only two eyes (5%) had substantial loss of visual acuity independent of cataract formation. This emphasizes the relatively low risk of substantial visual loss following vitrectomy for epiretinal membranes.
membranes in eyes with relatively good preoperative visual acuities.

Complications

All phakic eyes developed increased nuclear sclerotic cataracts, and cataract surgery had been performed in 14 eyes by the final examination. The mean nuclear score increased from 0.68 (range, 0.25 to 1.50) preoperatively to 1.93 (range, 1.0 to 2.50) in eyes that were still phakic at the final examination. The mean posterior subcapsular cataract score remained essentially unchanged. This emphasizes the importance of nuclear sclerosis progression in causing decreased acuity in phakic eyes of patients over age 50 following epiretinal membrane removal.12 One eye (2.5%) developed a recurrent epiretinal membrane and required a second vitrectomy. Another eye developed decreased acuity associated with a macular hole 3 years following epiretinal membrane removal. This macular hole was closed with one additional surgery. There were no serious surgical complications such as infectious endophthalmitis or retinal detachment.

DISCUSSION

Indications for vitrectomy are strongly influenced by the potential benefits of surgery weighed against the risk of surgical complications. Most published reports of vitrectomy for epiretinal membranes consisted of eyes primarily with visual acuities of 20/60 or worse.13-18 Eyes with visual acuities of 20/40 to 20/60 were lumped together with many eyes having worse preoperative acuities. The results of surgery for eyes with epiretinal membranes and better levels of visual acuity have not been reported as a separate group with sufficient follow-up to allow most phakic eyes to have visual acuity measured following cataract surgery. The results of vitrectomy in eyes with visual acuities of 20/50 or better can be extracted from some manuscripts that have grouped eyes by preoperative visual acuity. A study of 264 eyes with epiretinal membranes reported by Rice and coworkers19 included six eyes with a visual acuity of 20/50. The mean acuity improved to 20/40. A report of vitrectomy in 70 eyes with idiopathic epiretinal membranes included three eyes with visual acuity of 20/50.20 The visual acuity improved to 20/25 in two eyes and 20/40 in the third eye. Another study of 119 eyes with epiretinal membranes following treatment of a retinal tear or detachment included one eye that improved from 20/50 to 20/50.21 A fourth study of epiretinal membranes in 11 eyes of persons younger than 30 years old had one eye with a preoperative visual acuity of 20/50, which improved to 20/25 following the surgery.22

Nuclear sclerosis progression was an important confounding factor, which decreased visual acuity postoperatively. Nuclear sclerosis develops following vitrectomy in virtually all patients over the age of 50 years.14,23-26 Nuclear sclerosis progression caused decreasing visual acuities following vitrectomy, with a mean decrease in visual acuity of —.74 Snellen lines per year in 288 phakic eyes treated for macular hole or epiretinal membrane (J.T. Thompson, unpublished data). The effect of nuclear sclerosis progression on visual acuity was especially important in this study, since the eyes had relatively good visual acuities preoperatively, so these eyes could potentially lose much more visual acuity as a result of cataract progression. Nuclear sclerosis progression caused almost all phakic eyes to show decreased acuity compared to the preoperative visual acuity by 3 to 12 months following epiretinal membrane removal. The current study emphasized that cataract surgery is essential in phakic eyes to achieve long-term improvement in visual acuities in eyes with epiretinal membranes and good preoperative acuities. Pseudophakic eyes do not have this confounding factor, so the postoperative course usually shows gradual improvement following epiretinal membrane removal until the postoperative visual acuity reaches a plateau.

This dichotomy in postoperative visual acuity between phakic and pseudophakic eyes may influence the timing of vitrectomy and whether it is recommended in eyes with epiretinal membranes when the visual acuity is relatively good. It is reasonable to recommend epiretinal membrane removal in pseudophakic eyes with better levels of visual acuity. The visual indications for surgery should be tempered in phakic eyes by the reality that visual acuity will decrease postoperatively until cataract surgery is performed. Some surgeons have advocated combined cataract surgery and vitrectomy with epiretinal membrane removal to avoid this problem.27

This study had several limitations: It is likely that eyes with better visual prognoses were selected for surgery in that surgery was not routinely recommended in eyes with long-standing epiretinal membranes with no recent deterioration in visual acuity. Hence, this study does not imply that all eyes with epiretinal membranes and visual acuity between 20/30 and 20/50 should receive surgery. Instead, the study shows that good results can be obtained with surgery if similar surgical indications and techniques are used. A second limitation is that none of the eyes in this study had a preoperative visual acuity of 20/20 to 20/25, so the results do not evaluate the visual results in eyes with metamorphopsia and essentially normal visual acuity. The third limitation is that long-term postoperative follow-up could not be obtained in all eyes and, specifically, that visual acuity could not be measured in seven eyes that had not had cataract surgery by the final
examination. The fourth limitation is that the visual acuities measured preoperatively and postoperatively were Snellen visual acuity with current correction. The use of protocol refraction using ETDRS visual acuity charts is a more accurate method of measuring visual acuity. Standardized visual acuity testing may have compensated for some visual loss seen prior to cataract surgery in phakic eyes, which usually causes a myopic refraction shift induced by progressive nuclear sclerosis. The method of visual acuity determination used in this study does reflect the methods of testing commonly used in a community setting outside of a multicenter clinical trial. The author does not believe that any of these limitations would have changed the primary outcomes reported in this study, though.

Visual acuity and patient symptoms are the most important factors to consider in deciding whether to recommend vitrectomy in an eye with an epiretinal membrane. The decision to recommend surgery is relatively straightforward in eyes with rapidly progressing epiretinal membranes causing decreased visual acuity. These eyes usually show substantial visual acuity improvements, because macular damage from the epiretinal membrane is short-lived and macular function recovers well. The greater clinical dilemma is to determine the appropriate timing of surgery in eyes with epiretinal membranes and a visual acuity of 20/30 with slow progression of the epiretinal membrane associated with decreasing acuity. Is it appropriate to operate at that point, or should the surgeon wait for the visual acuity to decrease to 20/60? If the surgeon waits until the visual acuity decreases from 20/30 to 20/60 over a period of 3 years, the final mean visual results will not be as favorable as if the patient would have received surgery when the visual acuity was 20/30. The current study showed that the risk of substantial decreases in visual acuity is small (about 5%), so it is at least reasonable to consider vitrectomy sooner (with better visual acuities) in eyes with greater anatomic abnormalities on the fluorescein angiogram or OCT compared with eyes with minimal findings on these diagnostic tests. It is also appropriate to recommend vitrectomy sooner in pseudophakic eyes that do very well following vitrectomy. Improvements in techniques and reduced complications have lowered the threshold for cataract surgery over the past decade, and the data in the current study suggests that the threshold for epiretinal membrane surgery may also be reevaluated in light of good surgical results.

REFERENCES

Vitrectomy for Epiretinal Membranes With Good Visual Acuity

Dr George W. Blankenship. Decisions to recommend treatment are based on several factors. Potential side effects, complications, and benefits of the treatment being considered, as well as those of alternative treatments, must be compared to the probable natural history of the patient's condition. The patient's present and future needs, the cost of treatment, and the impact on the patient's quality of life must all be considered.

Dr Thompson has improved visual acuity and presumably reduced distortion by performing pars plana vitrectomies and removing idiopathic epimacular membranes and traction that had produced macular distortion and puckers in a large number of cases. This series differs from others because the eyes had better preoperative visual acuities. Vision improved from 20/50-2 to 20/32-1 for 19 eyes that were pseudophakic before vitrectomy and for 14 eyes that were phakic before vitrectomy but subsequently had cataract surgery following vitrectomy. However, vision remained basically unchanged (20/50 pre-op to 20/50-2 post-op) for seven phakic eyes, which presumably could have had better postoperative vision if cataract surgery had been performed.

Accurate measurement of visual acuity is especially important in this series with the relatively small differences (1 Snellen line) between pre- and post-op acuities. As Dr Thompson discusses, visual acuity measurements were made with a projected light Snellen chart with best current correction, which may not have been as accurate as those obtained by more refined techniques.

Nuclear sclerotic cataracts occurred in all phakic eyes and were a major factor in postoperative vision. Other undesired side effects and complications were very rare with only one eye having a recurrent epiretinal membrane, one eye having a macular hole develop several years following vitrectomy, one eye having worsening of preoperative cystoid macular edema, and one eye having additional loss of vision for unknown reasons.

The natural history of idiopathic epimacular membranes is to cause moderate loss of visual acuity and distortion that tends to gradually stabilize. This is in marked contrast to more severe epiretinal membranes associated with retinal detachments and other intraocular diseases that typically cause much greater loss of vision. Michels demonstrated good visual and anatomical results with vitrectomy and epimacular membrane removal of these more severe secondary epimacular membranes.

Dr Thompson's manuscript does not include information on the visual acuity and condition of the fellow eyes. Obviously, 20/40+2 is better and preferable to 20/50+2 vision, but it is difficult to speculate on the impact this improvement of one Snellen line of acuity would have on an individual's overall visual function and the quality of his or her life.

The purpose of this study was to evaluate the visual acuity results and complications of vitrectomy for epiretinal membrane removal in eyes with a preoperative visual acuity of better than 20/60. Dr Thompson has nicely done this. The purpose was to also determine if the risks of surgery are justified in most eyes, and his results suggest so, especially in pseudophakic eyes. But, it is difficult to know if this operation is justified in most...
people without knowing the function and status of the fellow eye.

REFERENCE


DR DAVID L. GUYTON. As an adult strabismus surgeon, the most common thing I see in these patients with fairly good vision and an epiretinal membrane is central double vision from a little dragging of the central fovea in the presence of peripheral fusion. I have a poster at this meeting about the dragged fovea diplopia syndrome. Yet diplopia was not mentioned in this study. What is the denominator in these cases? How often does diplopia occur? We see the diplopia in some patients where the vision is pretty good in the eye to before surgery. Sometimes we see it arise in patients with worse epiretinal membranes when surgery is done to strip the membrane: the vision improved and now they have central diplopia.

DR WILLIAM S. TASMAN. Do you have information on the fellow eye on these patients? How many had posterior vitreous detachments and normal maculas in the fellow eye and, in light of that, declined the surgery because of the perceived likelihood they wouldn’t develop the same thing in the fellow eye. As a corollary to that, did any patients have surgery on both eyes?

DR CAROL L. SHIELDS. Did you find that the OCT of patients with CME predicted worse visual outcome? Can you comment on the influence of OCT in deciding about epiretinal membrane peeling in general?

DR GARY C. BROWN. One particularly important aspect is the vision in the second eye. We’ve reviewed many of the clinical trials in ophthalmology in our study of quality of life and cost effectiveness. One issue that is critically important to patients is how is the other eye doing in these studies? If you ask the patient, they probably care more about the second eye than whether the laser is bringing the treated eye from 20/50 to 20/40. If one eye already has poor vision and you improve the second eye, then the patient does gain some quality of life. If one eye has 20/20 vision and you bring the other eye from 20/50 to 20/30, the patient might not even notice a difference in his lifestyle. However, it does become important if they lose vision in the second eye.

DR ALAN H. FRIEDMAN. Did you measure binocular vision acuity? Did you do Amsler grids? Did you measure reading vision in either eye or both eyes?

DR JOHN T. THOMPSON. Dr Blankenship raises the important question of how much value a patient derives from a mean visual acuity improvement of one Snellen line and the cost to society for that improvement. I do not want the healthcare bureaucrats to determine which surgeries we can do as doctors just based on what the quality-adjusted life-year values are for a particular surgery, although this information is valuable in helping to guide our recommendations to patients.

Dr Blankenship mentioned the visual acuity measurements. I would emphasize that these are the real-world visual acuities that patients walk around with. We’re involved in a number of studies with ETDRS refractions and most of our patients don’t walk around with correction that gives them their best ETDRS visual acuity for various reasons. For example, very few phakic patients have multiple glasses changes starting 3 months after surgery due to progressive myopia induced by increasing nuclear sclerosis.

Concerning Dr Guyton’s questions, there should be a simpler way of determining this diplopia. People may get diplopia after this surgery because their vision is better. Diplopia was not measured in our group so I cannot tell you how many patients had diplopia. Some patients complained of distorted vision or blurred vision, and they might not have been able to articulate the diplopia issue.

In terms of Dr Tasman’s comments, most patients in this study had good vision in the other eye. I reviewed the visual acuities in fellow eyes subsequent to this presentation. The eye with epiretinal membrane surgery had a better visual acuity than the fellow eye on final examination in 35% of eyes. I didn’t tabulate the percentage of patients who declined surgery because of good visual acuity in their fellow eye but it is relatively common. In terms of Dr Shields’ comment, I think the OCT is very useful, although I did not have enough numbers to really make a comment about the prognoses with angiographic cystoid macular edema. There is a previous study authored by Dr Thomas Rice that demonstrated that eyes with cystoid macular edema preoperatively along with their epiretinal membranes had a worse visual outcome. I believe it is best to offer surgery before patients develop severe cystoid macular edema since it represents a poor prognostic factor.

I agree with Dr Brown’s comment about the importance of the fellow eye status. By the final examination, about one-third of the surgery eyes had better visual acuity than the fellow eye due to pre-existing conditions in the fellow eye or subsequent visual loss.

Dr Friedman asked about binocular vision. We do not
measure the binocular visual acuity or near visual acuity in
our office. Many of the patients had abnormal Amsler
grids, but it's very hard to quantify the distortion using an
Amsler grid.
AUTISM WITH OPHTHALMOLOGIC MALFORMATIONS: THE PLOT THICKENS

BY Marilyn T. Miller MD,* Kerstin Strömland MD, Liana Ventura MD, Maria Johansson MD, Jose M. Bandim MD, AND Christopher Gillberg MD

ABSTRACT

Purpose: To review the association of autism spectrum disorder (ASD) in individuals manifesting thalidomide embryopathy and Möbius sequence and compare them with three new studies in which ASD was also associated with ocular and systemic malformations: (1) a Swedish study of individuals with CHARGE association (Coloboma, Heart, choanal Atresia, developmental or growth Retardation, Genital anomaly, and Ear involvement); (2) a Swedish study of Goldenhar syndrome; and (3) Brazilian Möbius syndrome (sequence) study.

Methods: In the Swedish CHARGE study, 31 patients met the inclusion criteria (3+ or 4 of the common characteristics of the CHARGE syndrome). The same team of investigators also evaluated 20 Swedish patients with Goldenhar syndrome. In the Brazilian Möbius study, 28 children with a diagnosis of Möbius sequence were studied; some children had a history of exposure during their mother’s pregnancy to the abortifacient drug misoprostol in an unsuccessful abortion attempt.

Results: In the CHARGE study, five patients had the more severe autism disorder and five had autistic-like condition. In the Goldenhar study, two had autism disorder and one had autistic-like condition. In the Brazilian Möbius study, the systemic findings of the misoprostol-exposed and misoprostol-unexposed patients were almost undistinguishable, and ASD was present in both groups (autism disorder in five and autistic-like condition in three).

Conclusion: Autism spectrum disorder has been reported in two conditions with known early pregnancy exposure to the teratogenic agents thalidomide and misoprostol. In the Brazilian Möbius study, autism also occurred in both the misoprostol-exposed and misoprostol-unexposed groups. Autism also was present in patients with both CHARGE association and Goldenhar syndrome.


INTRODUCTION

The beginning of the story was an unanticipated finding of autism while the investigators were engaged in the study of 86 Swedes who had been identified in the early 1960s as having the typical findings of thalidomide embryopathy and a maternal history of thalidomide intake during early pregnancy. Because of the large number of affected individuals described in the literature, including many informative cases in which the time of drug intake was known, it has been previously determined that the teratogenic-sensitive period extended from day 20 to day 36 after fertilization. The literature also indicated that early exposure to the drug (days 20 to 25) resulted in involvement of the cranial nerves (especially VI and VII) and external ear, abnormal ocular movement such as Duane syndrome, aberrant lacrimation, and thumb anomalies. The four cases of autism in the Swedish study manifested the characteristic effects of early exposure.

Autism spectrum disorders (ASDs) are neurodevelopmental disorders characterized by impairment in social interaction and communication and associated with repetitive behaviors and interests. There are several clinical ASD phenotypes, including autistic disorder, childhood autism, Asperger syndrome, and atypical autism (also referred to as autistic-like condition and pervasive devel-
opment disorders not otherwise specified). The pathophysiology of ASD remains elusive, despite clues from genetic studies, neurochemistry, autopsy reports, functional research, radiologic imaging, research on environmental influences, and many other approaches.\(^{11-14}\)

A subset of individuals with ASD have associated medical conditions (eg, syndromes, chromosomal anomalies).\(^{15}\) Insight into these comorbid conditions may give some insight into the pathophysiology of autism, and the thalidomide association has provided unique information into the timing factors of the developmental insult in some cases.\(^{11,12}\)

Intrigued by the association of autism with an uncommon type of strabismus (Duane syndrome) and facial nerve palsy in the thalidomide-exposed individuals, and by a few case reports of a connection between Möbius syndrome with its involvement of cranial nerves VI and VII and autism,\(^{15-17}\) a multidisciplinary team further explored these associations in another Swedish study.\(^{18}\) Möbius syndrome has more recently been designated “Möbius sequence,” since the term “sequence” defines a cascade of secondary events that occur after a single embryonic insult from heterogeneous causes. Although many functional anomalies may coexist in Möbius sequence, the most accepted clinical criterion is evidence of congenital sixth and seventh cranial nerve involvement. The systemic and ocular findings of the 25 Swedish patients in the 1995-1998 study were similar to those described in the Möbius literature, except for the remarkable finding that 7 of 22 (32%) had autistic disorder or autistic-like condition.\(^{13,15}\) As in the thalidomide study, the neuropsychiatric evaluations were performed by child psychiatrists. No consistent etiologic event could be ascertained through history of other tests, except that there appeared to be more than the usual adverse pregnancy events, such as bleeding (8 patients), a chronic villi sampling procedure (1), and history of drug use (1).\(^{13}\)

After the Swedish Möbius study, the multidisciplinary team decided to study other conditions in which ASD had been noted with craniofacial syndrome. They selected Goldenhar syndrome (oculoauriculovertebral dysplasia syndrome) and the CHARGE association because of case reports of autism in these conditions\(^{26-22}\) and the similarity of some of the craniofacial malformations. The findings of these two studies will be described here.

Another related chapter to the ophthalmology-autism connection was reported in the early 1990s in the literature from South America, especially from Brazil. A number of papers described a group of children who had limb anomalies and whose mothers had taken an abortifacient drug, misoprostol (Cytotec), early in pregnancy.\(^{23-26}\) Alone, misoprostol appears to be a poor abortifacient drug; it often only causes some bleeding or uterine contraction but with the pregnancy continuing to term. In addition to limb anomalies, many of these affected individuals had cranial nerve malformations and manifested the characteristic findings of Möbius sequence.\(^{20-32}\) Most of these cases were from Brazil, where abortion is illegal, except for a few reasons, but where there are a large number of self-induced attempts at abortion, often with the use of misoprostol early in pregnancy. To further investigate this misoprostol-Möbius connection, a prospective study of patients with the diagnosis of Möbius syndrome/sequence was undertaken by Ventura\(^{21}\) in Pernambuco, Brazil. The purpose of her study was to describe systemic, neuropsychiatric, and ophthalmic findings of these patients with Möbius sequence. The study included children with Möbius sequence from pregnancies both with and without misoprostol exposure. The results will be described.

**METHODS**

**Swedish CHARGE Study (1998-2002)**

A multidisciplinary study in Sweden evaluated patients referred by the medical profession with a diagnosis of CHARGE association or with a registered diagnosis of CHARGE. Although the literature is not consistent as to what clinical characteristics are necessary to make the diagnosis, the original description of CHARGE association by Pagon and associates\(^ {14}\) (Coloboma, Heart, choanal Atresia, Retarded growth and/or development, Genital hypoplasia, and Ear anomalies and/or deafness) was used to establish the key characteristics. Most patients had four characteristics, but a few had three with other frequently associated systemic anomalies. Thirty-one patients were considered to meet the minimal requirements for CHARGE association. The ophthalmic and systemic evaluations were performed with predetermined protocols similar to those of the Swedish Möbius study, and by essentially the same multidisciplinary team. In the CHARGE and Goldenhar studies, the criteria for autistic disorder from the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) were used, in addition to the Childhood Autism Rating Scale (CARS), the Autistic Behavior Checklist (ABC), and the Autism Diagnostic Interview—Revised (ADI-R).\(^ {21,26}\)


The Goldenhar/hemifacial microsomia (HFM) study of 20 patients was done concurrently with the CHARGE study, and by the same team. Certain modifications were necessary because of the differences in clinical characteristics of these two entities. Vertebral anomalies, ocular dermoids, and mandibular hypoplasia are characteristic of the
Goldenhar syndrome but not as frequent in the CHARGE association. In contrast, ocular coloboma and genitourinary and cardiovascular malformations are more frequently noted in the CHARGE syndrome. However, a comprehensive physical examination was done on all patients. In the neuropsychiatric evaluation, the methodology used was the same as in the CHARGE study.

In the neuropsychiatric evaluations of all individuals reported here, care was taken to remove items and criteria in diagnostic instruments when scoring for autism spectrum disorders might be affected by cranial nerve palsy and severe visual and hearing impairment (eg, items and criteria concerned with facial mimicry, eye contact, and intonation of speech).

The relevant ethics committees approved all Swedish studies.

**Brazilian Möbius Study (2000-2001)**
A prospective multidiscipline study was performed in Pernambuco, Brazil, an area in northeastern Brazil. Detailed pregnancy and social history was collected, with particular attention to the timing, dosage, and method of taking misoprostol or other abortifacient drugs. The inclusion criterion of Möbius sequence was evidence of involvement of the sixth and seventh cranial nerves.

The study was initiated in August 2000, and most of the patients were recruited in the first 6 months. Of the 31 patients presented with a possible diagnosis of Möbius, 28 met the criteria of the study. The patients were divided into two groups. Group 1 was composed of children without known exposure to misoprostol, and group 2 consisted of children with a history of misoprostol exposure by their mothers in early pregnancy. Detailed social, demographic, and psychological data were obtained on 26 patients and ophthalmologic data on 28 patients. Initially, the mother was interviewed and a database of information about the pregnancy, including medical history, genetic background, and drug exposure, was created. The importance of truth and confidentiality and the purpose and methods of the study were explained to the mother, and an informed consent was signed. The investigators had some concern that there might be reluctance on the part of the mother to admit to taking the drugs. However, the observation of the team was that most mothers were very desirous of discussing their problems with nonjudgmental medical staff. On separate days the children were examined by a multidisciplinary team representing the specialties of pediatrics, ophthalmology, neurology, cardiology, otolaryngology, dentistry, genetics, psychiatry, speech and language, and radiology. All examinations were performed using a constant database similar to that of the Swedish Möbius study.

Psychiatric and intellectual evaluations were performed on children old enough to be formally evaluated. These included an interview utilizing the DSM IV and CARS.

The study met the requirements established by the Brazilian National Health Council for Research in Humans.

**RESULTS**

**Swedish CHARGE Study**
Table 1 indicates the systemic and functional abnormalities in 31 study patients with CHARGE association and compares them to estimates reported in the literature. Colobomas, often with microphthalmia, were prominent and observed in about 90% of the patients, which is slightly higher than reports in the literature and may represent some study ascertainment bias. In 19 patients the colobomas were bilateral but showed great variation in type, ranging from an isolated iris or disk coloboma to complex colobomas involving all uveal tissues (Table 2). Severe visual impairment was common. Sixteen patients had cardiovascular anomalies, with persistent ductal arterious being the most frequent type. Vestibular symptoms were surprisingly frequent. The ear anomalies and hearing loss included a “characteristic CHARGE ear,” other external ear malformation, and involvement of the inner ear structures. Although not part of the diagnostic criteria, the fairly frequent findings of facial nerve palsy, cleft lip or palate, and short stature have been documented also in the literature.38-41

Five individuals manifested characteristics of the full autistic disorder, and five had fewer characteristics and thus a diagnosis of autistic-like condition. Other patients showed only autistic traits but are not reported here. A comprehensive neuropsychiatric evaluation will be reported elsewhere (Johansson M, et al., “Autism spectrum disorders and underlying brain pathology in CHARGE association,” unpublished data, 2004). Table 3 summarizes the diagnostic characteristics and other findings in these 10 patients. They do not appear to have a unique set of systemic malformations compared to the other study patients, and more detailed evaluations will be reported elsewhere.42

**Goldenhar/HFM Syndrome Study**
Table 4 summarizes the systemic findings of the 20 study patients. The observed malformations and functional findings were as expected in the diagnostic characteristics, such as ear tags, microtia, lipodermoids, epibulbar dermoids, and vertebral malformations. The many associated malformations underline that this is a multisystem and not only a craniofacial syndrome.

Autistic disorder was present in two cases and autis-
tic-like condition in one case (Table 5). The associated anomalies did not seem to distinguish this subgroup from the rest of the study patients. More details of the psychiatric evaluations will be reported elsewhere.

Brazilian Möbius Study
Table 6 summarizes the major systemic findings of the 28 patients; findings are not separated by groups because there was minimal difference between groups 1 and 2. In the 17 patients with misoprostol exposure (group 2), 13 mothers had taken misoprostol only and four had taken misoprostol plus tea, which was a culturally popular drug felt (but not proven) to induce abortion. Three patients took misoprostol plus injection of an unidentified medication. One patient in the unexposed group (group 1) also had taken tea. A few patients in each group took other unidentifiable medications, which may have been misoprostol. Misoprostol was taken both orally and vaginally alone or together. The average number of pills ingested was 4.8 (each pill was 200 mg). In the group with misoprostol exposure, 15 patients had a history of bleeding early in pregnancy, compared to four in the unexposed group. The average duration of bleeding was approximately 9 to 10 days in both groups. Not surprisingly, bleeding was more frequent in the “attempted abortion” group than the “etiology unknown” group.

Common associated anomalies were micrognathia and posterior rotated ear; there was no difference in prevalence between the two groups. Limb anomalies were present in 22 of the 28 patients in the study, with clubfoot and clinodactyly the most frequent. Abnormal tearing was present in both groups. Many patients had oral or dental malformations, including cleft palate, abnormal tongue anatomy, altered tongue tone, and poor sucking. There were only slight differences in a few malformations. A detailed analysis of this study is reported elsewhere by Ventura.33

Radiologic imaging was done on 25 of the 28 patients. The main findings were brain-stem calcification in six patients, Dandy-Walker or variant in two, arachnoid cyst in two, hydrocephalus in three, cerebral atrophy in four, and a variety of other single anomalies. There did not seem to be a significant difference between the misoprostol-exposed and the unexposed groups.
Of the 28 patients, 23 had an evaluation for ASD (Table 7). In 23 patients examined, five met the diagnostic criteria for autistic disorder according to DSM-IV and two had autistic-like condition. There was a positive history of misoprostol in three of the five with autism disorder and in one of the two with autistic-like condition. In the “etiology unknown” group, two had autistic disorder and one had autistic-like condition. Because the misoprostol cohort had more cases than the group with unknown diagnosis (17 versus 11), the percentage of ASD seems to be comparable in the two groups. Bandim and associates have reported the detailed psychiatric evaluation.

Although the number of patients with ASD in groups with and without exposure to misoprostol is not sufficient for accurate statistical comparison of subgroups, clinodactyly, equinovarus, mitral prolapse, and involvement of cranial nerves IX and X occurred in each group. This finding provides support for a final common pathophysiology that produces the manifestations of the condition designated as Möbius sequence.

Table 2. Swedish CHARGE Study: Description of Types of Coloboma and Microphthalmos

<table>
<thead>
<tr>
<th>Condition</th>
<th>No. of Eyes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coloboma</td>
<td>46</td>
</tr>
<tr>
<td>(27 patients, 19 bilateral)</td>
<td></td>
</tr>
<tr>
<td>Iris</td>
<td>4</td>
</tr>
<tr>
<td>Iris + uvea</td>
<td>2</td>
</tr>
<tr>
<td>Iris + uvea + optic nerve</td>
<td>15</td>
</tr>
<tr>
<td>Uvea</td>
<td>5</td>
</tr>
<tr>
<td>Uvea + optic nerve</td>
<td>10</td>
</tr>
<tr>
<td>Optic nerve</td>
<td>10</td>
</tr>
<tr>
<td>Microphthalmos</td>
<td>21</td>
</tr>
<tr>
<td>(13 patients, 8 bilateral)</td>
<td></td>
</tr>
<tr>
<td>Microphthalmos + coloboma</td>
<td>15</td>
</tr>
<tr>
<td>Iris + uvea</td>
<td>2</td>
</tr>
<tr>
<td>Iris + uvea + optic nerve</td>
<td>10</td>
</tr>
<tr>
<td>Uvea + optic nerve</td>
<td>1</td>
</tr>
<tr>
<td>Optic nerve</td>
<td>2</td>
</tr>
<tr>
<td>Microphthalmos</td>
<td>2</td>
</tr>
<tr>
<td>(coloboma indeterminable)</td>
<td>4</td>
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</tbody>
</table>

Autism With Ophthalmologic Malformations: The Plot Thickens

Of the 28 patients, 23 had an evaluation for ASD (Table 7). In 23 patients examined, five met the diagnostic criteria for autistic disorder according to DSM-IV and two had autistic-like condition. There was a positive history of misoprostol in three of the five with autism disorder and in one of the two with autistic-like condition. Because the misoprostol cohort had more cases than the group with unknown diagnosis (17 versus 11), the percentage of ASD seems to be comparable in the two groups. Bandim and associates have reported the detailed psychiatric evaluation.

Although the number of patients with ASD in groups with and without exposure to misoprostol is not sufficient for accurate statistical comparison of subgroups, clinodactyly, equinovarus, mitral prolapse, and involvement of cranial nerves IX and X occurred in each group. This finding provides support for a final common pathophysiology that produces the manifestations of the condition designated as Möbius sequence.

Other malformations and functional disorders also showed a fairly similar percentage in both the misoprostol and the “etiology unknown” group. Psychosocial data, economic information, and more detailed clinical reports are found in Ventura’s thesis.

DISCUSSION

Autistic disorder is not a rare condition; estimated prevalence is about 1 to 2 individuals per 1,000. If one expands the diagnosis to include all variants of ASDs, the estimate increases to about 0.5% to 1% of the general population.

Even if we consider only the patients with the full autism syndrome (autistic disorder), the rates of autism in thalidomide exposure, Möbius sequence, CHARGE association, and Goldenhar syndrome are unquestionably significant.

The diagnosis of ASD is based on clinical characteristics and an established interview questionnaire with parents. The clinical behavioral characteristics include difficulties in social interaction, often with impaired verbal and nonverbal communication. Often individuals with ASD show very restrictive habits of behavior and interests and a need for routine schedules. There is often a lack in cognitive play and poor interpersonal sensitivity. Mental retardation is common, but a subset of those with ASD have normal or about average intelligence (Asperger syndrome).

There is no recognized biomarker, no consistent radiologic imaging abnormalities, and no consistent evidence of a time or location of developmental disturbance of the brain responsible for ASD. The evidence for a genetic component is high but complex, with many genes implicated as risk factors. Whereas most individuals with ASD do not have obvious systemic malformations, there is a subset of individuals with autism that have recognizable associated conditions.

This report summarizes two previous studies in the literature and three newer studies in which there is a group of individuals with the characteristic findings of ASD and also the systemic and functional findings indicating an early insult in embryonic development. This does not imply or mean to suggest that most cases of ASD result from a similar developmental disturbance, but the observations add a few more pieces of the puzzle of this complex neuropsychiatric disorder.

The research interest in this area was initiated by a somewhat serendipitous observation of four individuals with thalidomide embryopathy who demonstrated the classic behavior of severe autistic disorder. Although this Swedish study of 86 individuals with thalidomide embryopathy was prospective, it was ophthalmologically oriented and psychiatric data were not collected in an organized manner; psychiatric consultation was required to confirm autism in these four individuals. A search of the literature on thalidomide embryology indicated that teratogenicity occurred only in days 20 to 36 ± after fertilization and that the cranial nerve involvement and autism by association were in the early sensitive period of about days 20 to 25 after fertilization. Some literature evidence of ASD in a condition with similar findings, Möbius sequence, prompted a follow-up prospective
multidisciplinary study, which revealed a surprisingly high presence of ASDs.

The three studies reported here are a natural extension of interest in syndromes/sequences with characteristic evidence of early developmental errors of ocular structures, cranial nerves, and systemic organs and more than chance presence of autism syndrome disorders. The Swedish team involved in the Möbius study decided to continue and selected CHARGE association and Goldenhar syndrome because of a few case reports of ASD in these conditions.

An “association” is a characteristic group of anomalies observed in patients seemingly more than by chance, but usually without a definite etiologic diagnosis. One example is the CHARGE association initially described by Pagon and associates. The acronym “CHARGE” be used to describe the characteristic findings (Coloboma, Heart, choanal Atresia, Retardation,

<table>
<thead>
<tr>
<th>CASE</th>
<th>AGE (YR)/SEX</th>
<th>AUTISM</th>
<th>OCULAR FINDINGS</th>
<th>CHOLENAL ATRESIA</th>
<th>DEVELOPMENT DELAY</th>
<th>GENITAL</th>
<th>EAR</th>
<th>OTHER ANOMALIES/ FUNCTIONAL PROBLEMS</th>
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<tr>
<td>1</td>
<td>5M AD</td>
<td>++</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>++</td>
<td>+</td>
<td>Short stature, spine, limb, dysphagia</td>
</tr>
<tr>
<td>2</td>
<td>6F AD</td>
<td>++</td>
<td>+</td>
<td>PDA, Fallot</td>
<td>++</td>
<td>Labial</td>
<td>+</td>
<td>Short stature, spine, dysphagia</td>
</tr>
<tr>
<td>3</td>
<td>7F AD</td>
<td>++</td>
<td>+</td>
<td>ASD, VSD, PDA</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>Cleft palate, anal atresia, renal, spine, dental, dysphagia</td>
</tr>
<tr>
<td>4</td>
<td>13M AD</td>
<td>++</td>
<td>0</td>
<td>PDA</td>
<td>++</td>
<td>Cryptorchism</td>
<td>+</td>
<td>Cleft palate, tracheoesophageal fistula, dental, short stature, dysphagia</td>
</tr>
<tr>
<td>5</td>
<td>16F AD</td>
<td>++</td>
<td>+</td>
<td>PDA, ASD</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>Cleft lip/palate, short stature, dysphagia</td>
</tr>
<tr>
<td>6</td>
<td>4M ALC</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>Short stature, dysphagia, facial nerve</td>
</tr>
<tr>
<td>7</td>
<td>5F ALC</td>
<td>++</td>
<td>0</td>
<td>PDA, VSD, PS</td>
<td>++</td>
<td>0</td>
<td>+</td>
<td>Facial nerve, TE fistula, anal atresia, limb, dysphagia</td>
</tr>
<tr>
<td>8</td>
<td>14M ALC</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>Facial nerve palsy, delayed puberty, balance, short stature, spine</td>
</tr>
<tr>
<td>9</td>
<td>17F ALC</td>
<td>++</td>
<td>+</td>
<td>0</td>
<td>++</td>
<td>0</td>
<td>+</td>
<td>Craniosynostosis, balance</td>
</tr>
<tr>
<td>10</td>
<td>18F ALC</td>
<td>++</td>
<td>0</td>
<td>0</td>
<td>++</td>
<td>Labial</td>
<td>+</td>
<td>Delayed puberty, limb, dysphagia</td>
</tr>
</tbody>
</table>

AD, autistic disorder; ALC, autistic-like condition; ASD, atrial septal defect; EXT, external; PDA, patent ductus arteriosus; PS, pulmonary stenosis; TE, tracheoesophageal; VSD, ventricular septal defect.

*++ = bilateral; + = unilateral; 0 = absent.
†+ = present; 0 = absent; - =
‡++ = severe; + = mild; 0 = not present.
§+ = malformation or functional deficit present; 0 = no malformation or functional deficit present.
that caveat that the most frequent malformations reported in this association are colobomas, choanal atresia, and heart defects, there is a strong suggestion from a recent review of cases by Källén and associates that microphthalmia/anophthalmia be included in the “C”; that penis hypoplasia was frequently associated; that “R” includes other brain malformations; and that growth retardation or developmental delay was common and heart defects were frequent but nonspecific. Also, cleft lip or palate occurred in 15% to 20% of CHARGE patients. Blake and associates estimated the prevalence of CHARGE at about 1 in 10,000. Byerly and Pauli brought attention to the many children with CHARGE association who have facial nerve palsies and feeding and swallowing difficulties and reported a case of CHARGE association with Möbius sequence. Blake and associates proposed that cranial nerve dysfunction (anosmia, facial nerve palsy, sensorineural deafness and vestibular problems, swallowing difficulties) be considered a major criterion. The involvement of cranial nerves may be a thread that exists with CHARGE association, thalidomide embryopathy, and Möbius sequence. A common pathway for CHARGE and oculoauriculovertebral spectrum has been suggested by Van Meter and Weaver.

Whereas most cases of CHARGE are sporadic with unknown etiology, there are some familial cases and some associated with chromosomal anomalies. Many features suggest defects in neural crest cell development or migration, which led some investigators to suggest that CHARGE association should be considered in the group of neurocristopathies. Why ASD exists in a significant number of cases is still a mystery. However, the time of initial embryonic insult is necessarily early, because ocular colobomas, a significant characteristic, are caused by failure of closure of the embryonic fetal fissure by about the sixth week, although according to the thalidomide study, an earlier insult (25 to 27 days ±) can result in an ocular coloboma.

Genital and Ear anomalies). The diagnostic criteria in this CHARGE study were the presence of four of these six characteristics, or three characteristics plus other malformations that have been frequently reported by other investigators. Subsequent reports in the CHARGE literature have suggested some modifications of the core diagnostic group. Since the findings cover multiple disciplines of medicine, there is often a bias of ascertainment in any series reflecting the population evaluated, the interest of the investigators, or the sophistication of the examination of any given organ or structure. Even with the caveat that the most frequent malformations reported in this association are colobomas, choanal atresia, and heart defects, there is a strong suggestion from a recent review of cases by Källén and associates that microphthalmia/anophthalmia be included in the “C”; that penis hypoplasia was frequently associated; that “R” includes other brain malformations; and that growth retardation or developmental delay was common and heart defects were frequent but nonspecific. Also, cleft lip or palate occurred in 15% to 20% of CHARGE patients. Blake and associates estimated the prevalence of CHARGE at about 1 in 10,000. Byerly and Pauli brought attention to the many children with CHARGE association who have facial nerve palsies and feeding and swallowing difficulties and reported a case of CHARGE association with Möbius sequence. Blake and associates proposed that cranial nerve dysfunction (anosmia, facial nerve palsy, sensorineural deafness and vestibular problems, swallowing difficulties) be considered a major criterion. The involvement of cranial nerves may be a thread that exists with CHARGE association, thalidomide embryopathy, and Möbius sequence. A common pathway for CHARGE and oculoauriculovertebral spectrum has been suggested by Van Meter and Weaver.

Whereas most cases of CHARGE are sporadic with unknown etiology, there are some familial cases and some associated with chromosomal anomalies. Many features suggest defects in neural crest cell development or migration, which led some investigators to suggest that

### TABLE 4. GOLDENHAR/HFM STUDY: SUMMARY OF MOST FREQUENT MALFORMATIONS AND FUNCTIONAL PROBLEMS (N = 20)

<table>
<thead>
<tr>
<th>PROBLEM</th>
<th>NO. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cranial nerve involvement</td>
<td></td>
</tr>
<tr>
<td>Facial nerve palsy</td>
<td>8/20 (40)</td>
</tr>
<tr>
<td>Neurosensory deafness</td>
<td>3/20 (15)</td>
</tr>
<tr>
<td>Systemic/ocular malformations</td>
<td></td>
</tr>
<tr>
<td>Microsomia</td>
<td>15/20 (75)</td>
</tr>
<tr>
<td>Ear tags</td>
<td>14/20 (70)</td>
</tr>
<tr>
<td>Ocular dermoids</td>
<td>13/20 (65)</td>
</tr>
<tr>
<td>Lipodermoid</td>
<td>13/20 (65)</td>
</tr>
<tr>
<td>Epibulbar dermoids</td>
<td>10/20 (50)</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>11/20 (55)</td>
</tr>
<tr>
<td>Microtia</td>
<td>10/20 (50)</td>
</tr>
<tr>
<td>Vertebal anomaly</td>
<td>10/20 (50)</td>
</tr>
<tr>
<td>Developmental delay</td>
<td>7/20 (35)</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>6/20 (30)</td>
</tr>
<tr>
<td>Fistula</td>
<td>4/20 (20)</td>
</tr>
<tr>
<td>Functional problems</td>
<td></td>
</tr>
<tr>
<td>Hearing</td>
<td>14/20 (70)</td>
</tr>
<tr>
<td>Severe developmental delay</td>
<td>4/19 (21)</td>
</tr>
<tr>
<td>Autism spectrum disorder</td>
<td>3/18 (17)</td>
</tr>
</tbody>
</table>

HFM, hemifacial microsomia.

### TABLE 5. GOLDENHAR/HFM STUDY: FINDINGS ASSOCIATED WITH PATIENTS WITH AUTISM SPECTRUM DISORDER

<table>
<thead>
<tr>
<th>CASE</th>
<th>AGE(YR)/SEX</th>
<th>ABNORMAL EAR (EXTERNAL)</th>
<th>DECREASED HEARING</th>
<th>DERMOID</th>
<th>AUTISM TYPE</th>
<th>OTHER MALFUNCTIONS/SYMPTOMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4/M</td>
<td>0</td>
<td>+</td>
<td>Epibulbar lipodermoid</td>
<td>AD</td>
<td>Cardiovascular, gastrointestinal anomalies, mandibular hypoplasia, microphthalmos</td>
</tr>
<tr>
<td>2</td>
<td>16/F</td>
<td>+</td>
<td>+</td>
<td>Epibulbar lipodermoid</td>
<td>AD</td>
<td>Limb, gastrointestinal, genitourinary, vertebral</td>
</tr>
<tr>
<td>3</td>
<td>6/M (Microtia, ear tags, fistula)</td>
<td>+</td>
<td>Epibulbar lipodermoid</td>
<td>ALC</td>
<td>Vertebral, facial palsy, gastrointestinal</td>
<td></td>
</tr>
</tbody>
</table>

AD, autistic disorder; ALC, autistic-like condition; OAV, oculoauriculovertebral dysplasia syndrome; symbols: + = present, 0 = normal.
who manifest a spectrum of malformations involving the ear, mandible, mouth, eye, and, often, the cervical spine. The findings occur unilaterally in most, but not all, patients. It is usually sporadic, but family occurrences, especially with only a few anomalies, have been reported. Goldenhar syndrome has been proposed to represent a variant of this entity. Initially, Goldenhar described a number of patients with a combination of epibulbar dermoids, lipodermoids, and preauricular skin tags and fistula. Later, upper lid coloboma and facial and vertebral anomalies became appreciated as part of the syndrome.

Duane syndrome has been reported in a number of patients with Goldenhar syndrome but is not a common characteristic. Although no consistent evidence of pathophysiology or etiology is accepted, disruption of embryonic vasculature has been suggested as one mechanism to explain the observed findings. The time of embryonic insult is more difficult to pinpoint in Möbius sequence than it is in thalidomide embryopathy, but best estimates are early in pregnancy, probably around 4 to 6 weeks of development. Lam proposed that ectodermal nondisjunction involving the otic placode could produce the malformations seen in Goldenhar syndrome. If correct, this would explain the multisystem findings and also place the time early in the fourth week. Another suggestion is that Goldenhar syndrome can be a result of “reproductive wastage” in high-risk conceptions, based on one case of possible monozygotic twins conceived by in vitro fertilization and embryo transfer. There were a few in vitro fertilization cases in our series also. These observations support the concept of a nonspecific early embryonic event resulting in a malformation complex. If correct, this makes the association with ASD even more intriguing. Although the number of cases of autistic disorder or autistic-like condition in our Goldenhar group (3 in 18) was not as high as in the Möbius studies, it certainly is more than chance, with the estimated prevalence of autism being 1 to 2 per 1,000.

In the early 1990s there were a number of case reports in the Brazilian literature of infants born with malformations involving limbs, cranial nerves, and other anomalies following self-induced but failed abortions. The abortifacient drug used was misoprostol, a prostaglandin type E analogue. In some of these reports, the children exhibited the typical findings of Möbius syndrome with and without limb anomalies. Misoprostol as a drug for self-induced abortions has gained much popularity in South America, especially Brazil, where abortions are not legal except in a few situations. It was estimated to be used in more than 50% of attempted abortions in some areas of Brazil. Misoprostol was cheap and readily available because of its accepted use in medical conditions such as gastric ulcers and arthritis. Medically induced abortions have advantages over clandestine abortion from unlicensed “professionals.” They avoid risk of anesthesia and surgical complications in unclean environments and, perhaps most important, can be done in privacy. Misoprostol is also utilized for planned abortions, conducted by medical professionals in many countries, but almost always combined with another drug, such as mifepristone. However, misoprostol alone is a poor abortifacient drug, and many pregnancies continue to term. It appears to be a fairly weak teratogen, since the reported percentage of malformation is low, but because of its tremendous popularity as an abortifacient drug for self-induced abortions, even low-incidence complications such as Möbius sequence occurred in sizable numbers.

The Swedish study of Möbius, along with reports in the Brazilian literature of the association of Möbius sequence and misoprostol, prompted a prospective multidisciplinary study in Brazil by Ventura and associates. It was designed to be descriptive of malformations and functional problems.

### Table 6. Brazilian Möbius Study: Summary of Most Frequent Systemic Malformations and Functional Problems (n = 28)

<table>
<thead>
<tr>
<th>Problem</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cranial nerve involvement</td>
<td></td>
</tr>
<tr>
<td>Abducens (sixth)</td>
<td>28/28 (100)†</td>
</tr>
<tr>
<td>Facial (seventh)</td>
<td>28/28 (100)†</td>
</tr>
<tr>
<td>Trigeminal (fifth)</td>
<td>5/28 (18)</td>
</tr>
<tr>
<td>Hypoglossal (twelfth)</td>
<td>5/28 (18)</td>
</tr>
<tr>
<td>Tearing symptoms‡</td>
<td>21/28 (75)</td>
</tr>
<tr>
<td>Systemic malformations</td>
<td></td>
</tr>
<tr>
<td>Cleft lip/palate/uvula</td>
<td>13/28 (46)</td>
</tr>
<tr>
<td>Micrognathia</td>
<td>18/28 (64)</td>
</tr>
<tr>
<td>Limb</td>
<td>22/28 (70)</td>
</tr>
<tr>
<td>Tongue (microglossia/asymmetry/function)</td>
<td>14/25 (56)</td>
</tr>
<tr>
<td>Poland syndrome</td>
<td>1/28 (4)</td>
</tr>
<tr>
<td>Functional problems</td>
<td></td>
</tr>
<tr>
<td>Mental retardation</td>
<td>14/23 (61)</td>
</tr>
<tr>
<td>Hearing</td>
<td>8/28 (29)</td>
</tr>
<tr>
<td>Sucking in infancy</td>
<td>18/28 (64)</td>
</tr>
<tr>
<td>Dysphagia</td>
<td>9/28 (32)</td>
</tr>
<tr>
<td>Autism spectrum disorder (AD, ALC)</td>
<td>7/23 (30)</td>
</tr>
<tr>
<td>Seizures</td>
<td>12/27 (44)</td>
</tr>
</tbody>
</table>

AD, autistic disorder; ALC, autistic-like condition.

*Some patients did fail appointments for some examinations, and some were too young or difficult to examine.
†Inclusion criteria.
‡A few were suggestive of abnormal innervation of the lacrimal system, and some may have been secondary to facial nerve palsy.

AD, autistic disorder; ALC, autistic-like condition.
tional disorders in all study patients and also to compare the findings of those whose mothers took misoprostol early in pregnancy with the findings of those with no exposure history. Additionally, there was interest to see if ASD occurred in some patients.

The Brazil study is the pièce de résistance with new, key pieces to the puzzle of autism, reaffirming observations in the other four studies of ASD associated with cranial nerve, ophthalmic, and craniofacial malformations. In addition to thalidomide, it introduced a second teratogen, misoprostol, associated with ASD. It supports the conclusions of the Swedish Möbius study that ASD has a significant association with Möbius sequence, but in another ethnic group with another research team.18 And perhaps the most exciting observation in the South America study was that ASD occurred in individuals with and without known exposure to misoprostol during their mother’s pregnancy.

Another very interesting finding is the marked increase in cases of Möbius sequence in some areas of South America after misoprostol became a commonly used abortifacient drug. The fact that Ventura33 and her multidisciplinary team were able to recruit 28 cases of Möbius sequence in 6 months from only one surrounding region is remarkable. It is probably explained by the 17 cases that had known misoprostol exposure.

A number of questions come to mind when looking at these three present studies and the previous thalidomide and Swedish Möbius study. The first might be whether the cases with ASD in these conditions were chance occurrences or represented some study bias. To answer this concern, the prevalence of autism must be analyzed along with the methodology of all studies. In the thalidomide study, the rate of ASD was the lowest, with four of 86 proven by formal neuropsychiatric examinations to have the full-blown autistic disorder.2 Since only five individuals with severe neuropsychiatric behavior noted by the pediatric ophthalmologists doing the study were referred for psychiatric evaluation, these numbers could easily be an underestimation of what might be present if the study would have included neuropsychiatric behavior data in all study patients. Even so, this rate of 46 per 1,000 is significant. The Swedish Möbius study in the literature was more dramatic, reporting six of 23 study patients showing the full autistic disorder and one with autistic-like condition (a prevalence of 260 in 1,000). The Brazilian Möbius/misoprostol study had five of 23 patients meet the criteria for the full autistic disorder and two for autistic-like condition. The Swedish CHARGE association indicated that in the individuals old enough to be evaluated, five had autistic disorder and five autistic-like condition. In the 18 patients able to be tested in the

<table>
<thead>
<tr>
<th>CASE</th>
<th>AGE (YR)</th>
<th>MISOPROSTOL EXPOSURE</th>
<th>STRABISMUS PRIMARY POSITION</th>
<th>FACIAL NERVE PALSY</th>
<th>OTHER CRANIAL NERVES</th>
<th>AUTISM TYPE</th>
<th>CARS SCORE*</th>
<th>MENTAL RETARDATION†</th>
<th>OTHER ANOMALIES/ FUNCTIONAL PROBLEMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4F</td>
<td>+</td>
<td>Straight</td>
<td>9th, 10th</td>
<td>AD</td>
<td>39</td>
<td>+</td>
<td>Cleft palate, micrognathia, clindactyly, calcification of brain stem</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2F</td>
<td>0</td>
<td>ET</td>
<td>9th, 10th</td>
<td>ALC</td>
<td>38</td>
<td>+</td>
<td>Arthrogryposis, micrognathia, club foot, clindactyly, arachnoid cyst, hydrocephaly, polymicrogyria, cerebral atrophy, cleft uvula</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>11M</td>
<td>+</td>
<td>ET</td>
<td>9th - 12th</td>
<td>AD</td>
<td>45</td>
<td>+</td>
<td>Cleft palate, club foot, arthrogryposis, normal MRI</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>9F</td>
<td>0</td>
<td>XT</td>
<td>9th, 10th</td>
<td>AD</td>
<td>46.5</td>
<td>+</td>
<td>Mitral valve prolapse, clindactyly, club foot, Dandy-Walker anomaly</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>3M</td>
<td>0</td>
<td>ET</td>
<td>9th, 10th</td>
<td>AD</td>
<td>47.5</td>
<td>+</td>
<td>Cerebral atrophy, calcification of brain stem, cleft uvula</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>2M</td>
<td>+</td>
<td>ET</td>
<td>9th, 10th</td>
<td>ALC</td>
<td>29</td>
<td>+</td>
<td>Cleft uvula, micrognathia, club foot, normal MRI</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>2F</td>
<td>+</td>
<td>Straight</td>
<td>9th, 10th</td>
<td>AD</td>
<td>38</td>
<td>+</td>
<td>Clindactyly, stenosis of aqueduct of Sylvius, hydrocephalus</td>
<td></td>
</tr>
</tbody>
</table>

AD, autism disorder (DSM IV criteria); ALC, autistic-like condition; CARS, Childhood Autism Rating Scale; ET, esotropia; MRI, magnetic resonance imaging; XT, exotropia; symbols: + = anomaly present, 0 = absent.

*Median score for Möbius cases without autism, 18.4.
†Per Wechsler Intelligence Scale for Children.
Goldenhar/HFM study, two had autistic disorder and one had autistic-like condition. The diagnosis in all studies was made by experienced child psychiatrists utilizing more than one of the accepted diagnostic tools of the DSM-III-R and DSM-IV criteria and the International Statistical Classification of Diseases and Related Health Problems, tenth revision (ICD-10) criteria as operationalized in the ADI-R, CARS, and ABC. The recruitment was based on the diagnostic characteristics of the condition and not autism, so there seems little bias of ascertainment.

Why is the coexistence of ASD and systemic conditions not more widespread in a large body of autism literature? The thalidomide observation may be explained by the fact that most of the literature involved infants or young children in whom the extensive limb and other systemic anomalies were more pertinent issues for the individual. There were a few multidiscipline studies of adults, but although there were psychological symptoms, the diagnosis of autism was not made.

Autism coexisting in individuals with Möbius sequence, CHARGE association, and Goldenhar syndrome is reported in the literature, but not in the degree noted in these four studies. However, after attention had been drawn to the possibility of this association, we have been informed of a number of unreported cases, especially with Möbius sequence. For example, there is a Möbius support group, and requests have been made to address the subject of autism at their meeting because of a number of parents with concerns about the behavior of their children.

Another question might be, Is there a common thread or pathway that ties the patients with Möbius sequence, CHARGE association, and Goldenhar syndrome together? The most common characteristic of the anomalies associated with autism in these studies is that they result from an early adverse embryonic event. The literature can be confusing and must be read carefully when it relates to embryonic timing issues. “Gestational age,” used by obstetricians and many others, is calculated from the last menstrual period. A gestational age of 1 month of an embryo is actually 2 weeks postconception/fertilization. These 2 weeks are certainly not trivial when looking at early embryonic events. The final studies described events by the actual age of development of the fetus. The most reliable timing is developmental insult, as in the thalidomide data, in which the four individuals with autism were exposed to thalidomide 20 to 25 days postfertilization because of their associated malformations (Duane syndrome, facial nerve palsy, ear anomalies). The next evidence of embryonic timing is from the misoprostol group. Although less precise, it appears that exposure was at 4 to 6 weeks (6 to 8 weeks from the last menstrual cycle). In Möbius patients with unknown etiology, there is no definite information except that it seems consistent with the misoprostol group to be early (eg, 4 to 6 weeks). There are cases reported with later adverse pregnancy events associated with Möbius sequence, so one has to be careful not to make absolute conclusions. The least established timing is in the CHARGE group, although we know the ocular embryonic fissure is closed in around the sixth week of embryogenesis, so the insult must be some time before that date. From the thalidomide timetable it could be as early as the late fourth week. The Goldenhar group seems to be at 4 to 6 weeks of embryogenesis, based on associated anomalies.

Although the coexistence of ASD with these reported conditions is quite convincing, the proposed pathophysiology mechanisms are not clear. Comprehensive reviews of possible pathophysiology in thalidomide have not resolved the issue. Stephens and associates68 noted that although there were 2,000 papers published in the last 40 years concerning thalidomide teratogenicity, the mechanism of action still remains elusive.

In the sporadic cases of Möbius sequence, there is some agreement in the literature as to possible causal mechanisms. A popular theory is that it belongs in a group of “disruption syndromes,” although there is disagreement as to the causes of the embryonic disruption. The most frequently stated cause is that of a vascular disruption in the early embryonic period. Some investigators refer to it as the “subclavian disruption syndrome.”79,80 They postulate a primary vascular disruption causing hypoxia, ischemia, edema, and hemorrhage, followed by secondary events that may affect other organs. The timing extent of this hypoxic event will determine the ultimate malformations based on the sensitive tissues at the time of the hypoxia.

There are clinical case reports that support the vascular disruption concept. For example, malformations suggestive of Möbius sequence have been reported in fetuses exposed to cocaine, presumably causing vasoconstriction of the uterine vessels.74,75 Also, chorionic villi sampling has been suggested as an occasional cause of limb anomalies and of Möbius sequence, although there are reports both supporting and refuting this association.76-79 Möbius cases have been associated with hypovolemia in a splenic bleed during pregnancy and inadvertent exposure to ergotamine with apparent uterine constriction.90-92 A few children with Möbius sequence have a history of polyhydramnios in pregnancy.72 In the Swedish Möbius study and other studies, there was an apparent increase of bleeding in early pregnancy reported without known precipitating causes, and also one case with a history of chorionic villi sampling procedure. Courtens and associates89 reported a case associated with a history of exposure
to benzodiazepines. The apparent common characteristic of all these cases in the literature is an early adverse pregnancy event resulting in a possible short period of hypoxia brought on by disturbance in the blood supply from uterine constriction. This line of reasoning received support by the association of misoprostol taken early in pregnancy in failed abortion attempts resulting in infants with characteristic findings of Möbius syndrome.

Another type of disruption was proposed by Bamforth, who described a process termed “organizational disruption” (blastogenic disruption) as an explanation for some of the observed malformations, suggesting that it is a better explanation for some phenotypes. He proposes that there are a group of organizational molecules (morphogenes), highly conserved and determined by chromosomes in a sequential manner, that are important in the early stage of organization. This organization is imposed on embryonic cells by activation determined by homebox genes. If something interferes with the organization of these morphogenes, higher or lower concentrations may cause activation of genes at inappropriate times, which could result in malformation of organs or histologic development. This theory is perhaps compatible with the observations that some of the HOX gene defects in the animal models result in the same brain-stem malformations that are sometimes associated with autism in humans.

How do we get from early-onset insult that seems to affect multiple brain-stem structures to autism disorders that involve higher centers not yet formed? Is there a group of unidentified cells that are even at this time programmed for a higher brain center, which are damaged, or is there an interruption in a series of connections ultimately crucial for higher centers to develop correctly? These are key questions, but we may only be able to speculate about answers at this time on some evidence from other studies of the associated conditions or malformations.

Rodier and associates noted almost complete absence of facial nuclei and shortening of brain stem in a patient with autism. In magnetic resonance imaging (MRI) studies by Hashimoto and associates and Cody and associates, the MRI findings in individuals with autism are noted. Radiologic abnormalities, albeit not consistent or conclusive, have also been reported in Möbius syndrome. Some include abnormalities of the brain stem. There are a number of literature reports of central hypoventilation, brain-stem changes, and, in some, Möbius sequence. Marques-Dias and associates reported neuropsychologic findings in three cases of Möbius syndrome related to misoprostol, finding calcification of the brain stem involving some of the cranial nerve nuclei. They felt this was due to vascular disruption. In the Brazilian Möbius study, there was a variety of MRI abnormalities, the most common being brain-stem calcification.

Aberrant innervation does not occur frequently in nature, and yet examples of aberrant innervation of brain-stem structures appear throughout these studies; the most striking is in thalidomide embryopathy with Duane syndrome and paradoxical lacrimation. We note aberrant lacrimation in patients with Möbius sequence. Abnormal tearing symptoms were also present in many in the Brazilian study, in both the misoprostol related and “etiology unknown” groups. Amaya and associates noted excessive lacrimation in 11 of 18 cases in a series of Möbius cases, with three patients also manifesting tearing when eating. It is interesting that early in embryogenesis the sixth and seventh cranial nerve nuclei and lacrimal nuclei are in close proximity. Destruction or failure of development of these structures might result in aberrant repair processes with inappropriate innervation. There is also a literature report of another type of paradoxical innervation, Marcus Gunn jaw winking, with CHARGE association. Local vulnerability and critical time may be the necessary factors for these neurologic mismatches to occur.

In conclusion, although autism may result from a variety of mechanisms and causes, evidence from the thalidomide, Möbius, CHARGE, and Goldenhar studies seems to establish quite firmly that early insults in embryogenesis, often involving brain-stem structures, may be associated with ASD.

REFERENCES

Autism With Ophthalmologic Malformations: The Plot Thickens


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**DISCUSSION**

Dr Gerhard W. Cibis. The lead authors of this presentation, Drs Miller and Strömland, were the first to report the association of autism in 5 percent of Thalidomide victims. Most importantly, they were able to pinpoint the timing of toxicity to days 20-24 after conception based on their autism patients having external ear anomalies but no involvement of the limbs, which occurs days 25-35. In this study, the relationship of autism with ophthalmic disorders involving cranial nerves, specifically CHARGE syndrome, MOEBEUS sequence GOLDENHAR syndrome and MISOPROSTOL exposure, are added. The Brazilian cases show autism in a racially different population than the more homogenous Swedish population.

The cause of autism is unknown. Very few neurons form as early as the fourth week of gestation; most that do are cranial. The brainstem in autism is shorter than normal. The facial nucleus and trapezoid body are closer to the hypoglossal nucleus and inferior olive. The superior olive is missing and the facial nucleus is smaller than normal. Such changes can occur only in early gestation. Behaviors disturbed in autism such as language and planning and interpretation of social cues are of course not brainstem but higher brain functions of the cerebral cortex and hippocampus.

This study links autism to cranial nerve disruption from early embryologic events before the higher regions of the brain are even formed. As the authors point out, presumably the evolution of unidentified cells into higher brain structures is somehow damaged or altered. It need not be the cells themselves directly that are damaged but the evolutionarily highly conserved homeobox control genes that determine embryogenesis. Rodier has found variants in HOXA1 allele to be twice as common in autism patients as in their non-autistic family members. HOXA1 is thought to be only one of many genes involved in the spectrum of autism disorders. Patients with autism have a reduction in the number of neurons in the cerebellum that controls muscle movement. Parts of the cerebellum are activated during certain tasks requiring high-level cognitive processing. Some symptoms common in autism, such as lack of facial expression, hypersensitivity to touch, sound and sleep disturbances, could be associated with the more primitive brain stem functions. Our understanding of the neurologic sources of autism is still poor.

Fetal alcohol mouse model confirms a similar disruption of later brain and eye formation. In ETOH patients, the electrotretinogram shows abnormalities reflecting abnormal retinal circuitry. An ERG study of autism patients might be of interest in that regard.

**REFERENCES**


Dr Irene H. Ludwig. A number of years ago, I reported on a group of patients who were somewhat similar: They had congenital, central hypoventilation syndrome, which is a brain stem disorder affecting respiratory control, and they also had similar eye findings to your thalidomide groups. In the studies of those families, the pulmonologists were never able to identify a genetic cause. But, they did find geographic clustering, suggesting that there may be an environmental influence. Have you looked for environmental teratogens in your groups? The group of hypoventilation syndrome patients also had very high incidence of autism and ADD-type disorders.

Dr John T. Flynn. Marilyn Miller is like Archibald Garad, the great English teratologist, who by minute observation of these abnormalities began the whole study of teratology...
in the 19th century. Marilyn has taken us on a tour of the brain stem and the malformations that occur by exposure to different kinds of teratogens. I think the central thread of your studies is similar to a generalization that John Opitz, the American teratologist, made: the organism reacts to a whole series of insults in very stereotyped ways. The insults can be many, but it is the time when the insults are delivered that causes these different anomalies.

Dr Marilyn T. Miller. Dr Ludwig brings up something interesting. A number of the Möbius patients also have difficulty with ventilation and there’s no question there is a spectrum of problems that are somehow related to the time of insult. We probably did not look sufficiently for environmental factors; we queried extensively the pregnancy histories in both the Swedish study and the Brazilian study. In the Swedish study, there did not seem to be any factors except that there were more histories of bleeding early in pregnancy. The Brazilian study was also not fruitful in the pregnancy history, except in that case we were dealing with the misoprostol exposure, so obviously these women had histories of bleeding because they attempted abortions. Most of them had about 12 or 24 hours of uterine contractions and some bleeding, but they didn’t abort and they went on to term.

Dr Flynn, that is my favorite quote from Opitz. There’s no question that we’re dealing with time sensitivity and structures that are developing. There is a lot of speculation in the literature what these reasons are, but the brain stem structures seem to be very sensitive to hypoxia at that time. That is also the time that the embryo is folding, and some people have speculated that makes it even more sensitive. It is probably a matter of the sensitivity of the area and the timing. Whether autism is related to other structures or to the brainstem is not known. In the Brazilian study, we performed MRIs and CTs on 25 of the 28 with 15 demonstrating abnormalities, mostly with signs of necrosis or calcification, but also a variety of non-specific findings too.
RETINAL PIGMENT EPITHELIUM RESURFACING OF AGED SUBMACULAR HUMAN BRUCH’S MEMBRANE

BY Vamsi K. Gallapalli MD, Ilene K. Sugino MA, Yancy Van Patten MS, Sumit Shah BS, AND Marco A. Zarbin MD PhD*

ABSTRACT

Purpose: To determine whether cultured fetal human retinal pigment epithelium (RPE) cells can attach and differentiate on submacular Bruch’s membrane from donors over age 55.

Methods: Differential debridements of Bruch’s membrane were performed to expose three different surfaces: the RPE basement membrane, the superficial inner collagenous layer (ICL) directly below the RPE basement membrane, and the deeper ICL. Approximately 3,146 cells/mm² were seeded onto these Bruch’s membrane explants and cultured for 1 or 7 days. Explants were bisected and examined histologically or analyzed with scanning electron microscopy. Nuclear density counts were performed on stained sections. Morphology and cell density were compared to those of cells seeded onto bovine corneal endothelial cell–extracellular matrix (BCE-ECM).

Results: Compared to cells seeded onto BCE-ECM at similar density, cell coverage and cellular morphology were poor at both time points. Unlike cells on BCE-ECM, cell density remained the same or decreased with time. In general, cell morphology on all surfaces worsened by day 7 compared to day 1. Although cells were more pigmented on RPE basement membrane and deep ICL at day 7, poor cellular morphology indicated the remaining cells were not well differentiated. An explant from a donor with large soft drusen showed the poorest resurfacing at day 7 in organ culture.

Conclusions: These data indicate that aged submacular human Bruch’s membrane does not support transplanted RPE survival and differentiation. The formation of localized RPE defects, cell death, and worsening cellular morphology on aged Bruch’s membrane suggest that modification of Bruch’s membrane may be necessary in patients with age-related macular degeneration receiving RPE transplants to prevent graft failure.


INTRODUCTION

Choroidal new vessel (CNV) excision has been proposed as a treatment for choroidal neovascularization¹ and, in patients with age-related macular degeneration (AMD), is associated with iatrogenic retinal pigment epithelium (RPE) defects due to the intimate association of RPE cells and the CNVs.²⁻⁵ Combined RPE transplantation and CNV excision has been attempted in eyes with AMD, but it has not led to significant visual improvement in most patients.⁶⁻⁸ In contrast, RPE transplantation in animal models of retinal degeneration has been proved to rescue photoreceptors and preserve visual acuity.⁹⁻¹² Possible causes of RPE transplant failure in human patients include immune rejection, which can be overcome with immune suppressive therapy, and inability of transplanted RPE cells to survive and differentiate on aged submacular Bruch’s membrane. An important distinction between humans with AMD and laboratory animals in which RPE transplantation has been successful is the age-related modification of Bruch’s membrane in human eyes, which may have a significant effect on RPE graft survival.

With normal aging, human Bruch’s membrane, especially in the submacular region, undergoes numerous changes (eg, increased thickness, deposition of extracellular matrix and lipids, cross-linking of protein, nonenzymatic formation of advanced glycation end products).¹³⁻¹⁵ The impact of these changes on RPE survival on Bruch’s membrane has not been elucidated thoroughly. In vitro
experiments, however, indicate that some of these changes can adversely affect cell attachment and survival. In addition to age-related changes in Bruch’s membrane, CNV excision can disrupt Bruch’s membrane with exposure of the inner collagenous layer and its lipids and, occasionally, exposure of the elastic layer. Thus, it is important to know whether aged submacular Bruch’s membrane supports RPE graft survival and differentiation, independent of immune rejection.

Because aging changes occur more prominently in submacular Bruch’s membrane and because AMD-related changes predominate in the macular region, we sought to examine the ability of submacular Bruch’s membrane to support initial and long-term survival of RPE cells in order to improve results of RPE transplantation in humans with AMD. We chose to address this issue by comparing the ability of cultured fetal human RPE cells to attach and grow on an “ideal” surface (ie, bovine corneal endothelial cell–extracellular matrix [BCE-ECM]) with their ability to grow on different sublaminae of aged submacular Bruch’s membrane, which are the surfaces that transplanted RPE cells will encounter in AMD eyes that have undergone CNV excision. We chose to work with cultured fetal RPE cells (vs adult RPE cells) for two reasons. First, a cultured fetal RPE cell line is fairly homogenous (vs cultured adult RPE cultures), and we did not want variability in cell behavior to alter cell survival and differentiation on a given surface. Second, cultured human fetal RPE cells are robust and can adhere to various sublaminae of Bruch’s membrane (vs adult RPE cells, which do not adhere well to the inner collagenous layer of aged submacular Bruch’s membrane). We examined cultured, passaged human fetal RPE cell survival on (1) aged submacular human RPE basement membrane, (2) the lipid-rich superficial inner collagenous layer (ICL), and (3) the deeper ICL. Despite the ability of cultured fetal RPE cells to initially resurface all three layers, we demonstrate decreasing cell survival and worsening morphology with time on all three surfaces.

METHODS

Human Donor Tissue
Fetal eyes were obtained from the Central Laboratory for Human Embryology (University of Washington, Seattle, Washington). Eyes used for Bruch’s membrane explants were received from numerous eye banks placed through the National Disease Research Interchange (Philadelphia, Pennsylvania) or from the North Carolina Eye Bank, a Vision Share member (Apex, North Carolina). Donor criteria for tissue acceptance included (1) no history of chemotherapy or radiation to the head, (2) up to 8 hours from death to enucleation, (3) up to 48 hours from death to experimentation, and (4) intact Bruch’s membrane under the macula as visualized through a dissecting microscope. This research followed the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board of the New Jersey Medical School, University of Medicine and Dentistry of New Jersey.

RPE Isolation and Culture
Human fetal RPE cells were isolated using 0.8 mg/mL collagenase type IV (Sigma, St Louis, Missouri), as previously described. Isolated RPE sheets were plated on BCE-ECM–coated dishes and cultured in supplemented Dulbecco’s modified Eagle’s medium (DMEM). A single fetal RPE cell line (gestation age, 17.4 weeks) of passage 2 to 4 was used in all the attachment studies to avoid complicating factors arising from differences in cell lines.

Bruch’s Membrane Explant Preparation
Globes were immersed briefly in povidone-iodine solution followed by two 10-minute washes in DMEM containing 2.5 mg/mL amphotericin B and prepared as previously described. To reproduce different surfaces to which RPE cells must attach in patients following CNV excision, debridements were created to expose the RPE basement membrane, the superficial ICL directly below the RPE basement membrane, or deeper layers of the ICL. A modification of a previously published method was used to create differential debridements of Bruch’s membrane from older donors. Differential debridements were created by wiping with a moistened surgical sponge to expose the desired Bruch’s membrane sublamina. Specimens (27 Bruch’s membrane explant specimens; donor age, 65.6 ± 10 years) were prepared prior to beginning the RPE attachment studies to determine reproducibility of debridements. These specimens were analyzed with scanning electron microscopy (SEM). Identification of the depth of debridements was based on the surface morphology of the explant with SEM and further confirmed with light microscopic analysis.

Attachment Studies
Differential debridements were performed on submacular Bruch’s membrane explants as described above. Each submacular explant of a donor pair was prepared to expose a different layer of Bruch’s membrane (eg, RPE basement membrane in one eye and superficial ICL in the fellow eye). Explants were situated within 7-mm-diameter trephines (Storz Ophthalmics, St Louis, Missouri), seeded with cultured fetal human RPE (3,146 viable cells/mm²), and incubated for 1 day (RPE basement membrane, n = 7 [74.86 ± 9.3 years]; superficial ICL, n = 7 [66.3 ± 6.8 years]; deep ICL, n = 6 [66.5 ± 8.1 years]) or
Comparisons between two groups were performed by the data was confirmed before using analysis of variance. For statistical analysis, normal distribution of confirm morphological observations following embed-glutaraldehyde in 0.1 M phosphate buffer, pH 7.4) to modified Karnovsky fixative (2% paraformaldehyde, 2.5% mens, one fourth of the explant was fixed in half-strength freehand line tool using NIH Image J. In some speci- of Bruch's membrane in the analyzed areas were obtained. Linear measurements of Bruch's membrane in the analyzed areas were obtained by digital image acquisition and measurement with the freehand line tool using NIH Image J. In some speci-mens, one fourth of the explant was fixed in half-strength modified Karnovsky fixative (2% paraformaldehyde, 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4) to confirm morphological observations following embed-ment in JB4. For statistical analysis, normal distribution of the data was confirmed before using analysis of variance. Comparisons between two groups were performed by Student’s t test, and if there were more than two groups, the Tukey-Kramer honestly significant difference test was used. A P value of <.05 was considered statistically signif-icant.

Scanning Electron Microscopic Analysis
Explant halves were further fixed in half-strength modi-fied Karnovsky fixative. Following rinsing in phosphate-buffered saline, explants were dehydrated in a graded series of ethanol, critical point dried, and sputter-coated with 20 nm gold/palladium. Samples were examined in a JEOL 35U (JEOL, Peabody, Massachusetts) and were analyzed for debridement surface, cell coverage, and cellular morphology.

RESULTS

Day 1
Bovine Corneal Endothelial Cell–Extracellular Matrix
Cells on BCE-ECM were large, elongate, and very flat. Nuclei were distributed unevenly and were oval or rectan-gular in shape. Cell thickness appeared uniform even though some cells were extremely elongated (Figure 1A). Cisternae were infrequent.

By SEM, cells appeared fairly uniform in shape and showed smooth, flattened surfaces. Short cell extensions over adjacent cells were common (Figure 1B).

RPE Basement Membrane
Cells were present on Bruch’s membrane predominantly as a monolayer and, unlike cells on BCE-ECM, had highly variable morphology. Bruch’s membrane was totally or almost totally resurfaced except for a few small defects in coverage by RPE cells. Cell shape and thickness were highly variable between explants. Cellular morphology ranged from round, to flattened or extremely flattened, to spindle-shaped, or rectangular. Cellular morphology within a single explant could be variable or predominantly one cell shape and/or thickness (Figure 2A). Defects in RPE coverage of Bruch’s membrane could be seen even in explants exhibiting high RPE cell density in some areas. The ability of the cells to resurface the explant at this time did not appear to depend on Bruch’s membrane donor age. The explant showing the most compact cells was that of a 90-year-old donor. In the areas where the cell density was high, cells were often on top of basal laminar deposit (Figure 2B). In areas showing defects in RPE cell coverage, basal laminar deposit was not evident (Figure 2C). Subcellular or intercellular spaces (cisternae) were present in all explants to varying degrees (Figure 2A, arrowhead). Condensed, darkly staining nuclei were present on all specimens (Figures 2A and 2B, large arrows).

Scanning electron microscopy of cells seeded onto
RPE basement membrane confirmed light microscopic observations and showed variable cell size and shape and, except for one explant (donor age, 90 years), total or almost total Bruch’s membrane coverage with small RPE defects (<1 cell diameter in size). Compared with cells seeded onto BCE-ECM, cells could be similar, larger or smaller, or rounder or flatter, and in some explants a mixture of cell sizes was present (Figure 2D). In general, cell extensions onto neighboring cells were more numerous, of variable size, and often larger than those found on RPE basement membrane. The cells covering the explants were variable in morphology: flat, extremely flat, or spindle-shaped (Figure 3B). Densely staining cells and cells with misshapen and/or densely staining nuclei were observed in five of seven explants.

Scanning electron microscopy confirmed the presence of smooth, flattened cells of highly variable size and shape in the three of four explants with defects in RPE coverage of Bruch’s membrane (Figure 3C). The fourth explant was incompletely resurfaced with enlarged, thick cells. Long cell extensions were sometimes observed with lamellipodia and filopodia extending over several cells. One explant showing almost complete coverage by light microscopy (Figure 3B) was covered with cell debris, which obscured the underlying cells. The remaining two explants showed almost complete Bruch’s membrane coverage by flattened cells with small defects in the resurfacing (Figure 3D). Varying amounts of supernumerary cells or cell debris were present on the surface of attached cells or directly on the superficial ICL in Bruch’s membrane explants.

Deep Inner Collagenous Layer
Cell resurfacing and morphology were similar to that of cells seeded onto RPE basement membrane in five of six explants, except that defects in Bruch’s membrane coverage were more common and often larger. Cells seeded onto the deep ICL were predominantly in a monolayer with highly variable morphology (Figure 4A). As with cells on the RPE basement membrane, cell density within a single explant was highly variable. The sixth explant was incompletely resurfaced by enlarged cells with enlarged nuclei (Figure 4B).

Similar to cells seeded onto RPE basement membrane, SEM showed that predominantly a monolayer of cells of variable cell size and shape resurfaced the deep ICL. As with cells on RPE basement membrane, cell thickness and shape were variable between Bruch’s membrane explants (Figure 4C). Some multilayering was
evident in three of six explants in localized areas, with enlarged or elongate cells present on top of the monolayer. Cell extensions were common. Small to large RPE defects were evident in five of the six samples (Figure 4D).

**Age-Related Macular Degeneration Eye**

This donor eye showed large, soft drusen in one eye only. Light microscopy (Figures 5A and 5B) showed both eyes resurfaced by a monolayer of large, flattened cells with large nuclei. No cisternae were present.

Scanning electron microscopy of the eye with drusen showed areas of Bruch's membrane lacking RPE cells in the center of the explant with complete coverage by RPE cells in the subperimacular area by large, flattened polymorphic cells (Figure 5C). The areas lacking cells were not as large as the defects seen in 90-year-old donor explant with a similar debridement (Figure 2E). The fellow eye, which did not have drusen, was resurfaced by cells of similar size and morphology with infrequent small defects (Figure 5D).
Day 7
*Bovine Corneal Endothelial Cell–Extracellular Matrix*

Cells uniformly resurfaced the culture dish with predominantly a monolayer of flattened cells (Figure 6A). Flattened nuclei were distributed fairly uniformly. Some localized bilayers were seen with cells exhibiting very flat nuclei in the top layer (Figure 6B). Cell cytoplasm was stained uniformly. Cisternae were abundant and occasionally large. The majority of cells were unpigmented. Occasional large, rounded pigmented cells were seen. Cells were much flatter than they were at day 1.

Scanning electron microscopy showed a monolayer of small, hexagonal-shaped cells on BCE-ECM. Larger, flat cells were present within the monolayer interspersed among the hexagonal cells (Figure 6C).

**RPE Basement Membrane**

Cells were present on RPE basement membrane in incomplete monolayers with some multilayering in four of six explants. In three explants, cells were highly variable in morphology. Many RPE cells in the monolayer showed variable loss of cytoplasm with a remaining (sometimes condensed) nucleus. Many cells were spindle-shaped and flattened to varying degrees, and other cells were very elongate, extending over subcellular cisternae, cell fragments, or cell ghosts (Figure 7A). Cisternae were common. Cells were more pigmented than at day 1, but even in areas with highly pigmented cells, cells were irregularly shaped. The fourth explant with incomplete resurfacing showed a monolayer of unpigmented cells that were healthier (more intact) and often on residual basal laminar deposits (Figure 7B). Cells on bare RPE base-
ment membrane without deposits were of more variable morphology, ranging from similar to those on basal laminar deposit to elongate and spindle-shaped. Few small drusen were located on the surface with overlying cells (Figure 7C). The unresurfaced areas appeared to be free of basal laminar deposit. Of the two explants showing the best resurfacing, both explants had cells that were predominantly attached onto basal laminar deposit as a monolayer or a monolayer with localized bilayers. When a bilayer was present on basal laminar deposit, the cells directly on top of the deposit were often large and rounded with lightly staining cytoplasm (Figure 7D). Cells on top of this layer were either similarly enlarged or very flat with flat nuclei. Some cells rested on cell fragments and ghosts. Cells directly on basal laminar deposit were flat with flat nuclei, most closely resembling cells on BCE-ECM (Figure 7E). Most RPE cells on these resurfaced explants were not pigmented.

Scanning electron microscopy revealed that the RPE basement membrane was resurfaced predominantly by a monolayer of RPE cells that appeared more uniform than day 1 owing to the lack of numerous cell extensions (frequently seen at day 1) and the uniform thickness of adjacent cells. Cell shape within an explant could transition from extremely flat and smooth polygons to thick and elongate cells, or small and compact cells (but larger than those on BCE-ECM). Large defects several cell diameters wide were present in four of seven explants (Figure 7F). Cells bordering the RPE defects were either thickened and elongated, forming a distinct border around the

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**FIGURE 4**

Cultured fetal RPE seeded onto the deep inner collagenous layer (ICL) of aged submacular Bruch’s membrane after 1 day in culture. A. Light micrograph of cells on a 79-year-old explant. Spindle-shaped cells resurfaced this area of the explant with small defects in coverage. Arrowhead points to an intercellular cisterna. Condensed nuclei are present (toluidine blue; bar = 20 μm). B. Light micrograph of cells on a 61-year-old explant. Cells resurfacing the deep ICL on this donor were very large with enlarged nuclei and lightly staining cytoplasm. This explant showed the least resurfacing of the deep ICL explants at day 1 (toluidine blue; bar = 20 μm). C. Scanning electron micrograph of cells seeded onto a 79-year-old explant (same donor as A). This explant was partially resurfaced by a multilayer of cells (entire field except for the top left corner). Cells resurfaced the explant incompletely where the cells are monolayered (asterisk) (bar = 50 μm). Inset shows small defects in Bruch’s membrane coverage by RPE cells (arrowheads) (original magnification ×1,000). D. Scanning electron micrograph of cells seeded onto a 61-year-old explant (same donor as B). Cells resurfaced the deep ICL with areas of predominantly large, flat cells (asterisks). Rounded cells are located within the monolayer of flat cells (arrows). Localized areas of incomplete resurfacing are seen in areas where the cells are not well attached (white cells). Cell extensions are common. Arrowhead points to thickened cell extension on both low- and high-magnification images (bar = 50 μm). Inset (original magnification ×1,000) shows one area of incomplete resurfacing (asterisk, deep ICL surface). Republished with permission of Elsevier from “Impaired RPE Survival on Aged Submacular Human Bruch’s Membrane” by Gullapalli, et al., Exp Eye Res, 2004 (In Press).
defect, or extended very flat processes into the defect. The explant showing the best resurfacing (cells on top of basal laminar deposit) showed no defects in Bruch’s membrane coverage with cells of highly variable thickness and shape (Figure 7G). This explant did not have the very large, flattened cells seen in the explants with RPE defects. The second explant with cells on basal laminar deposit showed the smallest cells on RPE basement membrane (Figure 7H). Supernumerary cells were not as common as at day 1 on all explants.

Superficial Inner Collagenous Layer
Cells resurfacing the superficial ICL had variable morphology, sometimes with large RPE defects (two explants). Four of six explants were almost completely resurfaced with small RPE defects. In explants with large defects plus one explant that was almost completely resurfaced, cells were in a monolayer of variably flattened to extremely flattened cells. Many cells were spindle-shaped (more than seen on RPE basement membrane). Some cells were poorly attached (lifted off Bruch’s membrane), and some were fragmented (Figure 8A). Some areas were resurfaced by flat cells that appeared to be lying on top of cell debris. RPE cells were nonpigmented or variably pigmented, but they never were as pigmented as on RPE basement membrane. Cisternae were abundant. Of the remaining two explants, one was resurfaced with large cells with large nuclei (similar to that seen in Figure 4B). The remaining explant was resurfaced with a monolayer and bilayer of cells that were similar to cells seeded onto basal laminar deposit at day 7 in some areas (Figure 7D and 7E), whereas other areas were resurfaced with spindle-shaped cells. Cells were not pigmented in these explants.

Scanning electron microscopy of RPE cells growing on the superficial ICL showed sparse to incomplete coverage in two of six explants, with single cells or patches consisting of flat cells. The remaining four explants showed almost complete coverage by large flat cells with small defects (Figure 8B). Some multilayering was seen in two of these explants, with large, flat, elongated cells on
top of the monolayer. Varying amounts of cell debris were found on three of six explants.

Deep Inner Collagenous Layer
Five of the six explants were resurfaced predominantly by a monolayer of very flat, spindle-shaped cells with abundant cisternae (Figure 9A). Cells were variably pigmented. Many cells appeared to be fragmented. The remaining explant was resurfaced by healthier-appearing cells (more intact) that were of variable morphology but mostly unpigmented. This explant also contained areas where the cells were extremely flat and spindle-shaped.

Scanning electron microscopy of cells on deep ICL shows large defects in Bruch’s membrane in only one explant. Resurfacing in all explants tended to be by large, polymorphic, and flattened cells (Figure 9B). Two of the explants had varying amounts of cell debris.

Age-Related Macular Degeneration Eye
Cells resurfacing the central portion of the explant (ie, submacular Bruch’s membrane) were present on RPE basement membrane and the superficial ICL as patches (sometimes clumps) or single cells that were predominantly flattened and spindle-shaped or rounded (Figure 10A). Many cisternae were present under and between cells. Large RPE defects were present. A few clumps contained enlarged, pigmented cells, but most of the cells were unpigmented. In the subperimacular area, the explant was resurfaced with RPE cells that were of more uniform morphology; cells covered the surface with a monolayer and, occasionally, a localized multilayer of flattened, spindle-shaped cells (Figure 10B).

Scanning electron microscopy showed large defects in the center of the explant with resurfacing in the subperimacular area by a complete monolayer of cells (Figures 10C and 10D). RPE defects showed distinct borders with few lamellipodia or filopodia extending onto the bare surface (Figure 10B). High magnification revealed that the surface was superficial ICL in the areas not resurfaced.

Summary of Morphological Observations
All donor eyes showed basal linear deposit throughout the ICL and in the intercapillary pillars. These studies showed no correlation between attached RPE cell density and the extent of Bruch’s membrane deposits as visualized by toluidine blue staining. There did not appear to be a clear correlation between cellular morphology and the age of the explant. Cells seeded onto the superficial ICL appeared to have the poorest cellular morphology and the least amount of Bruch’s membrane surface coverage at day 1. By day 7, cellular morphology was poor with apparent cell death on all three surfaces and cell defects in almost all explants, regardless of the surface. Cells seeded on basal laminar deposit or on top of other cells (which were sometimes dead [ghosts] or fragmented) had better cellular morphology than those directly in contact with Bruch’s membrane at day 7. Flattened, spindle-shaped cells were the most common cellular morphology on all three surfaces.

Nuclear Density Measurements
At day 1, the nuclear density was similar on all three surfaces of Bruch’s membrane examined as well as on

![Figure 6](image-url)
By day 7 in culture, there was a statistically significant increase in the nuclear density of RPE cells growing on BCE-ECM ($P = .007$). However, the RPE cell nuclear density declined on Bruch's membrane on all three surfaces. Only the decrease on the deep ICL was statistically significant ($P = .004$).

**DISCUSSION**

The lack of significant visual improvement in human patients undergoing RPE transplantation has been attributed to immune rejection, inability of cells to resurface an aged and/or damaged Bruch's membrane, or atrophy of BCE-ECM (Figure 11). By day 7 in culture, there was a statistically significant increase in the nuclear density of RPE cells growing on BCE-ECM ($P = .007$). However, the RPE cell nuclear density declined on Bruch's membrane on all three surfaces. Only the decrease on the deep ICL was statistically significant ($P = .004$).
Aging and AMD are characterized by numerous changes in Bruch’s membrane that may have an adverse effect on survival and function of both in situ and transplanted RPE cells. One way to assess the effect of aged submacular Bruch’s membrane on transplanted RPE cell survival is to grow RPE cells on Bruch’s membrane explants from human donor eyes. Several studies have used this explant model to examine the ability of RPE to attach to and resurface Bruch’s membrane. Limitations of this system include absence of overlying photoreceptors and underlying choroidal circulation that may influence RPE behavior in the subretinal space following transplantation. Similarly, culture conditions, including levels of serum and growth factors, do not reflect conditions in the subretinal space. Nevertheless, the human Bruch’s membrane explant system allows the study of RPE interaction with Bruch’s membrane independent of other factors (with the possible exception that surviving choroidal cells in the explant may affect the RPE).

Using cultured adult human RPE and peripheral human Bruch’s membrane, Tezel and coworkers showed that the attachment rate was highest on RPE basement membrane and was lower on the outer layers of Bruch’s membrane. Unlike the findings from the present study, cultured adult RPE resurfaced peripheral Bruch’s membrane almost completely by 14 days when native adult human RPE and peripheral human Bruch’s membrane, Tezel and coworkers showed that the attachment rate was highest on RPE basement membrane and was lower on the outer layers of Bruch’s membrane. Unlike the findings from the present study, cultured adult RPE resurfaced peripheral Bruch’s membrane almost completely by 14 days when native adult human RPE and peripheral human Bruch’s membrane, Tezel and coworkers showed that the attachment rate was highest on RPE basement membrane and was lower on the outer layers of Bruch’s membrane. Unlike the findings from the present study, cultured adult RPE resurfaced peripheral Bruch’s membrane almost completely by 14 days when native
RPE basement membrane was present. Because light microscopic examination of the tissue was not performed and because the appearance of the cell surface by SEM does not correlate with the actual condition of the cells (as observed by light microscopy in the present study), it is difficult to know whether the morphology of cells declined on RPE basement membrane at day 14 or day 21 in the previous study. Whereas the current study demonstrates resurfacing of the explant at day 7, there is no increase in nuclear density with time. Additionally, there are several differences between the explant system used in the current study versus previous studies: (1) cultured fetal RPE cells were used in our study (vs primary or passaged cultured adult RPE); (2) submacular Bruch’s membrane specimens were used in this study (vs peripheral Bruch’s membrane specimens); (3) RPE cells were removed mechanically in this study (vs RPE removal with ammonium hydroxide); (4) RPE cell density at initial seeding (3,146 cells/mm²) was high in this study (vs low [530.59 cells/mm²]); (5) serum-containing medium was used in this study (vs serum-free medium for a 24-hour incubation); and (6) live choroidal and vascular endothelial cells were present in the explant system used in this study (vs no live cells in explant due to ammonium hydroxide removal of RPE cells). Still, the results from previous and current experiments might mean that submacular and peripheral Bruch’s membrane differs in the ability to support RPE survival following initial attachment. Additionally, in the present study, the existence of large RPE defects in the center of the submacular Bruch’s membrane explant of the eye with AMD, where the drusen were present, may indicate that submacular Bruch’s membrane versus subperimacular Bruch’s membrane also differ in their ability to support RPE cell survival and differentiation.

Our published studies show that the initial attachment of cultured human fetal RPE onto submacular RPE basement membrane was higher than attachment onto the deep ICL 1 hour after seeding. However, the results of the current study indicate that although initial attachment may be lower on the deep ICL, by 24 hours after seeding, RPE nuclear density and cellular morphology on the RPE basement membrane and the deep ICL are similar (29.22 ± 5.4 nuclei/mm and 30.09 ± 3.1 nuclei/mm,
with aging, and the collagen fibers themselves can accumulate particulate material onto this layer. Additionally, the ICL external to this layer material may lead to less effective initial RPE attachment.

FIGURE 11

Nuclear density of cultured fetal RPE seeded on aged submacular human Bruch’s membrane sublaminae or bovine corneal endothelial cell–extracellular matrix (BCE-ECM) at day 1 and day 7. The number of RPE nuclei was counted, and the length of Bruch’s membrane was measured in four to five sections 40 to 50 µm apart in each sample, and expressed as number of nuclei/mm. Average nuclear densities of samples and standard deviation are shown. Numbers above the graphs represent P values. The increase in nuclear density on BCE-ECM and the decrease on deep inner collagenous layer (DICL) are statistically significant. SICL, superficial inner collagenous layer. BM, RPE basement membrane. Republished with permission of Elsevier from “Impaired RPE Survival on Aged Submacular Human Bruch’s Membrane” by Gullapalli, et al., Exp Eye Res, 2004 (In Press).

respectively). Whereas RPE cells attached onto the superficial ICL showed a slightly lower nuclear density (27.93 ± 4.4 nuclei/mm), the presence of cell debris, rounded cells, and incompletely or sparsely resurfaced explants (four of seven explants) indicates that early resurfacing of the superficial ICL may not be as effective as on RPE basement membrane or the deep ICL. Because the ICL immediately below the RPE basement membrane (ie, the superficial ICL) has been shown to accumulate dense lipid deposits with aging and can appear as a spherical carpet of cholesterol-rich particles, the presence of this material may lead to less effective initial RPE attachment onto this layer. Additionally, the ICL external to this layer (ie, the deep ICL) can accumulate particulate material with aging, and the collagen fibers themselves can become thickened (V. K. Gullapalli, MD, and H. Wang, MD, unpublished data, 2003). Perhaps these changes underlie the poor survival of cells on the deep ICL at 7 days. Thus, the differences in cell behavior within explants and between donors may reflect differences in composition or accumulation of aging changes in Bruch’s membrane.

By using cultured fetal RPE that grows robustly in culture, the explant system eliminates RPE-related factors such as cell variability seen in cultured aged adult RPE that might lead to poor growth. In contrast to the nearly homogenous appearance of the cells on BCE-ECM, defects in RPE coverage can be present on all three of the surfaces of aged submacular Bruch’s membrane explants studied. The presence of RPE defects on explants with viable surrounding cells indicates that the defects are directly related to RPE–Bruch’s membrane interactions. Additionally, published studies using a similar organ explant culture system show that the Bruch’s membrane explant can support in situ aged RPE survival, migration, and proliferation. Thus, it is reasonable to attribute poor RPE survival observed in the present study to the Bruch’s membrane surface on which the cells grew. We noted that cell morphology appeared to be best if the RPE cells were attached onto basal laminar deposit rather than directly to RPE basement membrane or the ICL.

In the AMD donor explants, the areas not covered by RPE cells were mostly limited to the submacular area, where the drusen were located (Figure 10). The cells surrounding the defects in RPE coverage of Bruch’s membrane were similar in appearance to aged adult RPE cells resurfacing mechanically debrided aged submacular Bruch’s membrane explants. The cells at the edge of the RPE defects were either thickened and oriented parallel to the defect perimeter or extended very flat lamellipodia into the uncovered region of Bruch’s membrane. The inability of fetal RPE to attach and survive on or resurface the defects (the latter having been created from cells either not attaching or dying after attachment) indicates that RPE resurfacing of AMD eyes via transplantation or wound healing will be impaired. The inability of even cultured fetal RPE to resurface portions of aged submacular Bruch’s membrane implies that even if the aged adult RPE cells were robust (as the fetal cells are), they would not be able to resurface iatrogenic RPE defects created in patients with AMD after CNV excision.

CONCLUSIONS

The inability of aged submacular human Bruch’s membrane to support transplanted RPE survival in organ culture is consistent with the histologic findings from a patient who received a transplant of uncultured aged adult RPE. In this immune suppressed patient, transplanted RPE did not fully resurface the localized RPE defect, and the RPE cells remaining in the subretinal space were not directly in contact with Bruch’s membrane but were resting on the residual portions of the CNV. Within the time course of the current organ culture study (7 days), we observed RPE cell death; RPE defects formed with surrounding RPE unable to resurface the defects, and remaining RPE appeared relatively undifferentiated compared to their behavior on BCE-ECM. Resurfacing of two donor eyes with AMD was the poorest we observed. These results indicate that modification of Bruch’s membrane may be necessary to support RPE.
survival and differentiation in the context of RPE transplantation in AMD patients undergoing CNV excision.

REFERENCES


DISCUSSION

Dr Susan G. Elner. Dr Zarbin and co-workers present their study evaluating the ability of fetal human RPE cells to attach and differentiate on aged Bruch's membrane explants from donors aged 55-75 years. Mechanical debridement was used to expose varied layers of Bruch's membrane to simulate the substrate that may result following surgical excision of choroidal neovascular membranes and upon which RPE cells might be transplanted. The RPE nuclear density, cell morphology, and cell coverage were evaluated at one- and seven-day time points. Bovine corneal endothelial-extracellular matrix was used as the positive control substrate. In this study, the authors demonstrated that transplanted fetal RPE could resurface debrided RPE-basement membrane or the inner collagenous layer of Bruch's membrane, but that the transplanted fetal RPE failed to grow by Day 7, with worsening cell morphology and decreased nuclear counts compared to control. The authors conclude that aged Bruch's membrane does not support transplanted RPE survival.

The design of this study, however, still leaves some question whether it is the "aged" Bruch's membrane that is solely responsible for the failure of the RPE to survive and thrive. Inclusion of Bruch's membrane from young donors as a control in this study rather than bovine corneal endothelial cell-extracellular matrix as control would strengthen the authors' conclusions. The issue of young versus aged Bruch's membrane has previously been investigated by Ho and co-workers, who studied RPE attachment to Bruch's membrane obtained from young (average age 32 years) compared to older (average age 76 years) donors. Higher RPE reattachment rates after 6 hours were found on younger Bruch's membrane compared to older donors (64±2.5% vs. 52.4±3.6%). Subsequent studies by Del Priore and co-workers failed to demonstrate a significant difference in RPE attachment rates to intact Bruch's membrane between young and old donors. A significant reduction in RPE attachment/resurfacing to older Bruch's membrane that underwent debridement to deeper collagenous and elastin layers, however, was seen. This later finding correlates with the findings of poor RPE resurfacing on debrided Bruch's membrane as reported by Dr Zarbin in this study.

With age, many changes occur in Bruch's membrane, including the deposition of peroxidized lipids, esterified cholesterol, triglycerides and phospholipids. Increased advanced glycation end products (AGEs), collagen type I, matrix metalloproteinases -2 and -9 have also been noted with age. However, collagen type IV, laminin, and fibronectin have all been noted to decrease in the RPE basement membrane with aging, particularly in areas of drusen formation. The question remains: what are the changes in aged Bruch's membrane that reduce RPE attachment and survival? Is it the deposition of extraneous materials or the reduction of possible important RPE attachment factors that reduces RPE resurfacing and survival?

This study continues the ongoing work of Dr Zarbin and colleagues toward advancing our knowledge of RPE-Bruch's membrane interactions with the anticipated application of this knowledge eventually to successful RPE transplantation in age-related macular degeneration. I would like to thank the authors for providing their manuscript with ample time for review and congratulate them for their ongoing research, which may eventually benefit many patients afflicted with age-related macular degeneration.

REFERENCES


Dr Marco A. Zarbin. I thank Dr Elner for her careful evaluation of our work. We agree that it is essential to carry out comparative experiments using young versus old submacular Bruch's membrane to determine whether aging changes in Bruch's membrane or some other property of Bruch's membrane underlies poor cultured fetal
human RPE survival on aged submacular human Bruch’s membrane. It has been difficult for us to obtain young tissue that is in good condition, but we are undertaking these studies currently. I would like to emphasize that our studies focus not on RPE cell attachment, but on RPE survival and differentiation following initial attachment. The conditions of our experiments are such that there is 100 percent coverage of Bruch’s membrane by cultured fetal RPE cells 24 hours after seeding the cells in organ culture, even on aged submacular human Bruch’s membrane. Over time (e.g., seven or 14 days after seeding), however, these cells die, which leads to a decline in RPE nuclear density (in contrast to results seen on bovine corneal endothelial cell extracellular matrix-coated dishes). We are now trying to delineate (and reverse) the sequence of events in which initial successful attachment to Bruch’s membrane is followed by the unexpected adverse outcome of RPE cell oncosis.
AGGRESSIVE RETINAL ASTROCYTOMAS IN FOUR PATIENTS WITH TUBEROUS SCLEROSIS COMPLEX

BY JERRY A. SHIELDS MD,* RALPH C. EAGLE JR MD, CAROL L. SHIELDS MD, AND BRIAN P. MARR MD

ABSTRACT

Objective: To report the clinical and histopathologic findings of retinal astrocytic tumors that showed progressive growth in four patients with tuberous sclerosis complex (TSC).

Methods: Four young children each developed an enlarging retinal neoplasm that eventually necessitated enucleation of the affected eye. The systemic findings, clinical course, and histopathologic findings were reviewed.

Results: Each patient had a progressively enlarging retinal mass associated with a total exudative retinal detachment and neovascular glaucoma. Enucleation was necessary in each case because the affected eye became blind and painful. The mean patient age at enucleation was 7 years, and the median age was 3 years. At the time of enucleation the tumors ranged from 10 to 20 mm in basal diameter and from 10 to 25 mm in thickness. Histopathologic studies of each eye revealed a giant cell astrocytoma that had produced a total exudative retinal detachment. The tumor cells showed positive immunoreactivity to neuron-specific enolase and glial fibrillary acidic protein. The retinal neoplasms in these cases were identical histopathologically to the subependymal giant cell astrocytoma that typifies TSC in the brain. One tumor filled the entire eye and perforated the globe. Although the lesions simulated retinoblastoma clinically, each patient had ocular and systemic findings of TSC, supporting the diagnosis of astrocytic hamartoma.

Conclusions: Although retinal astrocytic lesions of TSC generally are stationary, they can sometimes grow relentlessly and cause severe ocular complications. Patients with retinal astrocytic hamartomas should have serial ophthalmic evaluations because of this possibility.


INTRODUCTION

Retinal astrocytic hamartoma is the best-known ocular manifestation of tuberous sclerosis complex (TSC).1,2 It is generally a sessile or slightly elevated lesion in the nerve fiber layer of the retina, but it can have several clinical variations. It can be unilateral, bilateral, solitary, multifocal, transparent, opaque, noncalcified, or calcified.1-2 Retinal astrocytic hamartoma in association with TSC generally is considered to be a relatively stationary lesion that has little potential for aggressive behavior.1-5 In rare instances, however, a retinal astrocytic hamartoma can show progressive growth and cause severe local complications. We report the clinical course and histopathologic findings in four patients with TSC, each of whom developed progressive growth of a juxtapapillary astrocytic hamartoma that caused secondary retinal detachment and neovascular glaucoma, necessitating enucleation of the affected eye.

METHODS

The clinical records and histopathologic findings were reviewed and summarized for four patients with TSC who underwent enucleation of one eye because of tumor growth and neovascular glaucoma. Clinical findings evaluated included patient age at enucleation, patient's sex, tumor dimensions, and frequency of retinal exudation, retinal detachment, neovascular glaucoma, and extraocular extension. Assessment of pathologic findings included review of grossly sectioned eyes, histopathologic sections, and immunohistochemical preparations. The literature on aggressive retinal astrocytic neoplasms that came to enucleation was reviewed, and a comparison was made between those associated with TSC and those unassociated with TSC.
**RESULTS**

In the computerized files of the Ocular Oncology Service at Wills Eye Hospital, we identified four cases of aggressive astrocytic retinal tumors associated with TSC that required enucleation. The TSC in each patient was characterized by hypopigmented cutaneous macules, facial angiofibromas, and subependymal or cortical lesions typical of TSC seen with computed tomography or magnetic resonance imaging. One patient had renal cysts and none had cardiac, lung, or other lesions of TSC.

Concerning ocular manifestations, each patient had a similar clinical course, characterized by progressive enlargement of a previously recognized yellow retinal juxtapapillary mass (Figure 1) that ultimately caused a blind, painful eye and necessitated enucleation. In addition, three of the four patients had multiple retinal astrocytic tumors in both eyes and only one patient had a solitary retinal tumor.

The pertinent clinical information on our four cases is summarized in Table 1. There were two boys and two girls. Each tumor was surrounded by yellow intraretinal exudation that was documented to slowly progress to a total exudative retinal detachment and neovascular glaucoma. This progressive exudation and retinal detachment appeared to be directly related to gradual enlargement of the tumor. The intervals from the initial diagnosis of retinal tumor to enucleation ranged from 6 months to 13 years. In the patient with the 13-year interval, enucleation had been advised at age 7 years, but the parents had refused and only consented to enucleation when the tumor caused perforation of the globe 6 years later. Based on clinical estimation and ultrasonography, the tumor sizes at the time of enucleation ranged from 12×8×9 mm to 20×20×25 mm, with the latter (case 4) being the tumor that filled the entire globe and perforated the cornea. Seven years earlier that tumor measured 8×8×4 mm, attesting to the relentless, slow growth that characterized all of the tumors.

It is of interest that the smaller, more peripheral astrocytomas in the ipsilateral and contralateral eyes of three patients did not exhibit growth during the course of follow-up, ranging from 3 to 12 years. In every case, only one larger tumor near the optic disk showed progressive growth and retinal detachment, whereas all other tumors remained stationary.

Two of our patients (cases 2 and 3) had surgical attempts elsewhere to control the growing tumor and the retinal detachment by laser, vitrectomy, and retinal reattachment. In each case, the attempts failed and there was slow, relentless growth of the tumor.

**Pathological Findings**

The macroscopic appearance of the sectioned enucleated eyes is illustrated in Figures 2 through 5. In cases 1 through 3, yellow-white tumors arose from the posterior retina in the region of the optic disk. These tumors measured 7, 10, and 14 mm in largest diameter and were associated with total secondary retinal detachments. Two were predominantly exophytic, and the third had a combined exophytic-endophytic growth pattern. In case 4, the neoplasm filled the entire globe and perforated the sclera.

Pertinent histopathologic features are summarized in Table 2. All four eyes had florid iris neovascularization and secondary angle closure (Figure 6), and all had total nonrhegmatogenous exudative retinal detachments. The retina in case 4 was largely destroyed. All four neoplasms contained extensive areas of necrosis, comprising 50% to 95% of the tumor (Figure 7). Each of the retinal neoplasms was composed of two types of cells that were present in varying proportions. One group of cells had copious quantities of pale eosinophilic cytoplasm and resembled those found in subependymal giant cell astrocytoma (SEGA) of the brain in TSC (Figure 8). These giant cells typically were large and round or oval in profile, and the periphery of their cytoplasm occasionally was vacuolated. Many of the giant cells had large round or oval nuclei with prominent nucleoli. The cells in the second group were more elongated and fusiform in shape and had more intensely eosinophilic cytoplasm and smaller, darker nuclei (Figure 9). In several cases the retina and the parenchyma of the optic nerve contained large numbers of these plump spindle cells. The large, aggressive neoplasm in case 4 had invaded the choroid and optic nerve; it had a moderate degree of nuclear pleomorphism and atypia and occasional mitotic figures. Glial cells with abundant cytoplasm were found in the retrolaminar part of the atrophic optic nerve in all cases. All of the astrocytic tumors contained foci of basophilic, multilaminated calciospherites (Figure 10), and two contained metaplastic bone. Hamartomas of the iris and ciliary epithelia were present in two eyes. The latter finding has been reported previously.

The results of special immunohistochemical studies are summarized in Table 3. All tumors coexpressed neuronal marker neuron-specific enolase (NSE) and glial marker glial fibrillary acidic protein (GFAP). In general, the giant cells were immunoreactive for NSE but were negative or only minimally reactive for GFAP (Figure 11). In contrast, the spindle cells expressed both NSE and GFAP (Figure 12). The tumor cells also were strongly immunoreactive for S-100 protein and vimentin. Melanoma marker HMB-45 was uniformly negative.

 Reported cases of aggressive retinal astrocytomas without clinical evidence of TSC are summarized in Table 4. We were able to identify 12 reported cases that we believe are acceptable based on a review of the clinical description and the illustrations. 

Reported cases of
Aggressive Retinal Astrocytomas in Four Patients With Tuberous Sclerosis Complex

FIGURE 1
Case 2. Fundus photograph showing bilobed nodular mass arising from the retina and overlying the optic nerve.

FIGURE 2
Case 2. Gross appearance of sectioned globe showing bilobed epipapillary retinal mass and total retinal detachment.

FIGURE 3
Case 1. Gross appearance of sectioned globe showing exophytic retinal mass and total retinal detachment.

FIGURE 4
Case 3. Gross appearance of sectioned globe showing yellowish white epipapillary mass and total retinal detachment.

FIGURE 5
Case 4. Gross appearance of sectioned globe showing neoplasm totally filling interior of eye and extending anteriorly through corneoscleral perforation.

FIGURE 6
Case 2. Neovascular glaucoma. Florid fibrovascular membrane flattens anterior iridic surface central to wide peripheral anterior synechia. Ectropion iridis is present (hematoxylin-eosin, original magnification ×50). N indicates area of necrosis.
Case 3. Low magnification photomicrograph showing largely endophytic epipapillary tumor with extensive sheets of basophilic necrosis. The neighboring retina is detached by densely proteinaceous subretinal fluid (hematoxylin-eosin, original magnification ×10).

Case 2. Giant cells. Round or oval cells have abundant pale eosinophilic cytoplasm with peripheral vacuolization and round nuclei with nucleoli. They resemble cells found in subependymal giant cell astrocytoma (hematoxylin-eosin, original magnification ×100).

Case 1. Plump spindle cells in retinal stalk. Plump fusiform cells have intensely eosinophilic cytoplasm and oval nuclei that are smaller and darker (hematoxylin-eosin, original magnification ×100).

Case 2. Calcospherites. Tumor contains multilaminated, basophilic calcium deposits (hematoxylin-eosin, original magnification ×100).

Case 2. Immunoreactivity of giant cells for neuron-specific enolase (NSE) and glial fibrillary acidic protein (GFAP). Giant cells are immunoreactive for NSE, but do not stain for GFAP. Neighboring spindle cells are strongly GFAP-positive (original magnification ×100).

Case 1. Immunoreactivity of giant cells for neuron-specific enolase (NSE) and glial fibrillary acidic protein (GFAP). Spindle cells show intense immunoreactivity for both NSE and GFAP (original magnification ×100).
Aggressive Retinal Astrocytomas in Four Patients With Tuberous Sclerosis Complex

TABLE 1. CLINICAL INFORMATION ON FOUR PATIENTS WITH TUBEROUS SCLEROSIS COMPLEX AND AGGRESSIVE RETINAL ASTROCYTOMA

<table>
<thead>
<tr>
<th>CASE</th>
<th>AGE AT ENUC</th>
<th>SEX</th>
<th>RETINAL EXUDATION</th>
<th>RD</th>
<th>NVG</th>
<th>MONTHS FROM INITIAL DIAGNOSIS TO ENUC</th>
<th>TUMOR SIZE AT ENUC (MM)</th>
<th>EOE</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>M</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>20</td>
<td>12x8x9</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>M</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>24</td>
<td>14x14x7</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>F</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>6</td>
<td>10x10x10</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>14</td>
<td>F</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>156 (13 yr)</td>
<td>20x20x25</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Enuc, enucleation; EOE, extraocular extension; NVG, neovascular glaucoma; RD, retinal detachment.

TABLE 2. HISTOPATHOLOGIC FINDINGS IN FOUR PATIENTS WITH TUBEROUS SCLEROSIS COMPLEX AND AGGRESSIVE RETINAL ASTROCYTOMA

<table>
<thead>
<tr>
<th>CASE</th>
<th>GROWTH PATTERN</th>
<th>% NECROSIS</th>
<th>GIANT CELLS</th>
<th>SPINDLE CELLS</th>
<th>CALCOSPHERITES</th>
<th>BONE</th>
<th>RD</th>
<th>NVI</th>
<th>EOE</th>
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<tr>
<td>1</td>
<td>Exo</td>
<td>95%</td>
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<td>Yes</td>
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<td>2</td>
<td>Exo</td>
<td>70%</td>
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<td>Yes</td>
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<td>No</td>
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<tr>
<td>3</td>
<td>End-exo</td>
<td>50%</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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<td>Yes</td>
<td>Yes</td>
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<tr>
<td>4</td>
<td>End-exo</td>
<td>60%</td>
<td>Yes</td>
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End, endophytic; EOE, extraocular extension; Exo, exophytic; NVI, iris neovascularization; RD, retinal detachment.

TABLE 3. SUMMARY OF IMMUNOHISTOCHEMICAL REACTIVITY* 

<table>
<thead>
<tr>
<th>CASE</th>
<th>NSE-GC</th>
<th>NSE-SP</th>
<th>GFAP-GC</th>
<th>GFAP-SP</th>
<th>S-100</th>
<th>VIMENTIN</th>
<th>HMB-45</th>
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<tr>
<td>1</td>
<td>+2</td>
<td>+3</td>
<td>–</td>
<td>+3</td>
<td>+3</td>
<td>+4</td>
<td>Not done</td>
</tr>
<tr>
<td>2</td>
<td>+2</td>
<td>+2</td>
<td>Trace or</td>
<td>+2</td>
<td>+2</td>
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<td>+2 focal</td>
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</table>

GC, giant cell component; GFAP, glial fibrillary acidic protein; HMB-45, melanoma-specific antigen; NSE, neuron-specific enolase; S-100, S-100 protein; SP, spindle cell component.

*Staining was interpreted as trace to intense (+4) as graded on a scale of +1 to +4. Lack of staining was indicated with a minus (-).

aggressive retinal astrocytomas in patients with TSC, including our four cases, are summarized in Table 5. Our literature search revealed five previously reported acceptable cases of aggressive retinal astrocytic hamartomas that were clearly associated with TSC, one of which is included in our series of four cases.

A comparison of Tables 3 and 4 suggests that there are many similarities between aggressive retinal astrocytomas unassociated with overt manifestations of TSC and those associated with TSC. However, those unassociated with TSC tended to require enucleation at an older age, and choroidal melanoma was often a diagnostic consideration prior to enucleation. All were solitary lesions that occurred in patients who had no other fundus tumors or other abnormalities. There appeared to be a greater component of spindle cells in tumors unassociated with TSC, but this was difficult to assess accurately in our literature review. Otherwise, there were no important clinical or histopathologic differences between the two groups. Histopathologically, the tumors in each group were similar to the subependymal giant cell astrocytoma that characterizes TSC.

DISCUSSION

Tuberous sclerosis complex is a heritable disorder characterized by a variety of hamartias and hamartomas, including congenital hypopigmented cutaneous macules (“ash-leaf sign”), facial angiofibromas (“adenoma sebaceum”), intracranial paraventricular or subependymal astrocytomas, cardiac rhabdomyoma, renal angiomyolipoma, and retinal astrocytic hamartomas. About 60% of cases are
### TABLE 4. REPORTS OF ENLARGING RETINAL ASTROCYTOMAS WITH NO EVIDENCE OF TUBEROUS SCLEROSIS COMPLEX, MANAGED BY ENUCLEATION *

<table>
<thead>
<tr>
<th>First author</th>
<th>Year</th>
<th>Age (yr) at enuc</th>
<th>Sex</th>
<th>Solitary</th>
<th>Multifocal</th>
<th>Unilateral</th>
<th>Bilateral</th>
<th>Tumor size (base x thickness)</th>
<th>Yellow exudation</th>
<th>Necrosis</th>
<th>Calcification</th>
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<td>Boles7</td>
<td>1958</td>
<td>6</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
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<td>8 x 6</td>
<td>BPE</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>Fono8</td>
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<td>31</td>
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BPE, blind, painful eye; ENUC, enucleation; R/O MM, rule out melanoma; R/O RB, rule out retinoblastoma.

*Incomplete list of reported cases. A few reported cases were not included because of poor documentation.
†Largest basal diameter and thickness. Some sizes are estimated, based on review of published illustrations.

### TABLE 5. REPORTS OF ENLARGING RETINAL ASTROCYTOMAS WITH EVIDENCE OF TUBEROUS SCLEROSIS COMPLEX, MANAGED BY ENUCLEATION

<table>
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<tr>
<th>First author</th>
<th>Year</th>
<th>Age (yr) at enuc</th>
<th>Sex</th>
<th>Solitary</th>
<th>Multifocal</th>
<th>Unilateral</th>
<th>Bilateral</th>
<th>Tumor size (base x thickness)</th>
<th>Yellow exudation</th>
<th>Necrosis</th>
<th>Calcification</th>
<th>Optic nerve extension</th>
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</table>

BPE, blind, painful eye; Enuc, enucleation; R/O MM, rule out melanoma; R/O RB, rule out retinoblastoma.

*This is same case reported by Gunduz et al.22
†Largest basal diameter and thickness. Some sizes are estimated, based on review of published illustrations.
sporadic, and 40% are familial and appear to be inherited in an autosomal dominant fashion. However, the disorder is believed to be recessive at a molecular level, similar to retinoblastoma. About half of the cases with TSC show linkage to chromosome 9q34 and about half to chromosome 16p13.23.26 The clinical manifestations of both types seem to be very similar.

Each of our patients had clinical findings compatible with TSC. The main ocular finding of TSC is the astrocytic hamartoma of the retina. Less frequent ocular manifestations include patches of iris hypopigmentation and atypical inferior iris colobomas. Another recently described intraocular lesion in patients with TSC is hamartoma of the iris and ciliary body epithelium. This lesion usually is not appreciated clinically but has been found on histopathologic examination of enucleated eyes. It was present in two of our patients and possibly is more common, but it is overlooked clinically and histopathologically.

Retinal astrocytic hamartomas occur in about 50% of patients with TSC, about 50% of whom have bilateral retinal involvement.6 This tumor can manifest as a small, sessile noncalcified lesion in the nerve fiber layer, as a multinodular yellow-white calcified lesion, or as a combination of the two.6 Most lesions are relatively small, ranging from 0.5 to 5.0 mm in diameter, and larger lesions are exceptional.

There have been a number of reports in the literature on retinal astrocytomas associated with TSC that remained fairly stable or, rarely, produced mild vitreous tumor seeding or vitreous hemorrhage, but did not require enucleation. Those reports were not cited in this review, which addressed only lesions that were aggressive enough to require enucleation and undergo histopathologic studies.

It has been stressed that astrocytic hamartoma of TSC is generally a fairly stationary lesion that shows little or no tendency to grow. The four cases reported here are clearly exceptions to that rule. Each showed slowly progressive enlargement that eventually caused total retinal detachment and neovascular glaucoma that required enucleation of the affected blind, painful eye.

The retinal tumors described in this report are similar to those that occur in the brain in patients with TSC. The characteristic brain lesion of TSC is the subependymal astrocytoma.21,24 In both the brain and the retina, the astrocytic hamartoma can be an isolated finding without other clinical evidence of TSC. Those in the brain, like those in the retina, are generally asymptomatic and are detected on radiographic evaluation. However, the subependymal astrocytoma of TSC sometimes can behave aggressively and cause a sudden increase in intracranial pressure, visual loss, and death. 17 However, the prognosis for life is good when tumors are surgically removed or decompressed.27 It is of interest that such aggressive behavior in both the brain and the retina is more likely to occur in very young children, often before the age of 5 years. In our review, the retinal astrocytic neoplasm appeared to require enucleation at a somewhat earlier age in children with TSC (mean, 9 years; Table 5) than those patients without TSC (mean, 20 years; Table 1).

The histopathologic findings of retinal astrocytic hamartoma can vary from case to case. The typical small, stationary astrocytic hamartoma usually is confined to the nerve fiber layer of the retina and is composed of elongated fibrous astrocytes with interlacing cytoplasmic processes. The more aggressive tumors described in this report are composed predominantly of large, plump cells with abundant eosinophilic cytoplasm, nuclear pleomorphism, and some mitotic activity. The tumors characteristically show extensive necrosis and calcospherites. These findings, present in our four cases, are virtually identical to those that characterize the subependymal giant cell astrocytoma of TSC.

Immunohistochemical analysis of the retinal giant cell astrocytoma characteristically shows a mixed glioneuronal phenotype. Despite the fact that the tumor has an overtly astrocytic appearance, the giant cells are often nonreactive or weakly positive for GFAP, but typically stain strongly for NSE. Most tumors are also immunoreactive for S-100 protein. This mixed immunophenotype suggests that the tumor is heterogeneous or hybrid in nature, expressing proteins of both glial and neuronal cells. Our cases tended to show a biphasic pattern of immunoreactivity; the larger giant cells were positive for NSE and negative or only weakly positive for GFAP, whereas the neighboring plump spindle cells were positive for NSE and GFAP. These immunohistochemical properties are also similar to those of the giant cell astrocytomas of the central nervous system.

An intriguing aspect of our cases was that three patients had multiple, bilateral retinal astrocytomas, but only one of the tumors demonstrated progressive growth and complications. Each tumor that showed growth was located adjacent to the optic disk, and all four tumors invaded the optic nerve. It is of interest that neuropathologists still classify the giant cell astrocytoma of TSC as a benign neoplasm, despite the fact that it can slowly enlarge and demonstrate local invasion. However, the more peripheral tumors in the ipsilateral and contralateral eyes remained stable and had no tendency to proliferate.

There have been several case reports of tumors that seem identical histopathologically to the cases reported here but occurred as unilateral solitary lesions in somewhat older patients who had no clinical findings of TSC. We have chosen to call such lesions acquired retinal astrocytomas in order to differentiate them from the.
congenital astrocytic lesions that classically are associated with TSC. However, it is not possible to categorically state that the acquired retinal astrocytoma is not a forme fruste of TSC in which the disease is expressed only as a solitary retinal lesion that may cause ocular complications somewhat later in life. It was not possible to perform genetic studies on our patients, but in the future, genetic studies may answer the question as to whether the solitary acquired retinal astrocytoma is related to TSC.

The clinical differential diagnostic considerations in the progressive retinal astrocytic hamartoma include retinoblastoma, choroidal melanoma, and several other fundus conditions. In an infant or young child, systemic and ocular manifestations of TSC should suggest astrocytic hamartoma rather than retinoblastoma. Ophthalmoscopically, retinal astrocytic hamartoma characteristically shows an exudative retinal detachment with yellow, lipoproteinaceous exudation in the sensory retina and subretinal space. Such exudation is not seen in untreated retinoblastoma. Even though the retinal blood vessels that supply retinal astrocytic tumors are slightly dilated, they typically are not markedly dilated and tortuous, as seen in a comparable-sized retinoblastoma. In rare instances, a carefully planned fine-needle aspiration biopsy has been employed to differentiate retinal astrocytic hamartoma from retinoblastoma. However, that technique should be reserved for exceptional cases where the diagnosis has not been established by other methods.

In contrast to choroidal melanoma, progressive retinal astrocytic hamartoma is entirely amelanotic, produces yellow exudation, and is located in the sensory retina rather than the choroid. In one reported case, the eye was enucleated with the diagnosis of melanoma. In retrospect, the typical fundus features and the finding of subependymal astrocytomas should have suggested the diagnosis of astrocytic hamartoma. A review of other reported cases that clinically simulated melanoma also shows features more suggestive of retinal astrocytoma.

Other fundus tumors such as choroidal hemangioma, choroidal osteoma, and choroidal metastasis should not be confused with astrocytoma, because they have distinguishing features and lack appreciable yellow retinal and subretinal exudation. In addition, conditions that produce yellow exudation, like retinal capillary hemangioma and Coats’ disease, have typical features that should differentiate them from retinal astrocytoma.

In summary, we report here on four patients with TSC who developed progressive growth of a retinal astrocytic hamartoma, each of whom eventually required enucleation because of complications of neovascular glaucoma. The histopathologic features of these unusual tumors are identical to those seen with the subependymal giant cell astrocytoma of TSC. Similar lesions have been reported in patients without clinical manifestations of TSC and may represent a forme fruste of that syndrome. Progressive retinal astrocytic hamartoma has rather distinctive features that serve to differentiate it from retinoblastoma, choroidal melanoma, and other related fundus conditions.

ACKNOWLEDGMENT

The authors thank Drs Stephen Sinclair, Abdel Hadj, Marwan Zeidan, David Doka, and Mark Wood for referral of the patients in this study.

REFERENCES

The paper by Shields reports four cases of singularly unusual giant cell astrocytomas that developed in the retinas of patients who had features of tuberous sclerosis; in each case, the tumors demonstrated aggressive growth with destruction of the eye, and in each case enucleation was required. The tumors in these cases are unusual in that they do not represent the type of spindle cell astrocytic retinal tumor usually seen in cases with tuberous sclerosis. The cell type in the four cases reported by the authors has two major cell components: one a spindle cell and the other a larger neuronal cell. These retinal tumors appear similar to the subependymal giant cell astrocytomas that occur in the brain in patients with tuberous sclerosis. The question arises as to whether or not these retinal tumors can actually become malignant and metastasize. The issue of malignancy in the giant cell astrocytomas in the brain has largely been settled. In a large, systematically studied series of giant cell astrocytomas reviewed at the Mayo Clinic among patients with tuberous sclerosis, although cytologic atypia was common occurring in approximately 80 percent of the cases and mitotic figures were recognized in one to slightly more than five mitotic cells per high-power field, these histologic findings appeared not to affect the prognosis and none of the tumors have been known to metastasize.1,2

This is a very rare tumor. The incidence of tuberous sclerosis in population studies has been estimated to be between 1 in 15,0003 and 1 in 150,000.4 The likelihood of finding retinal tumors in patients with tuberous sclerosis is approximately 50 percent. I have been fortunate to work with Dr. Manfred Gomez at the Mayo Clinic, who developed an in-depth interest in tuberous sclerosis and who attracted a large referral practice of patients with tuberous sclerosis. In 1976, I published a study based on 116 patients that we had seen at the Mayo Clinic with tuberous sclerosis. In 1979, I published a series of tumors of the Central Nervous System. Atlas of Tumor Pathology, Third series, Fascicle 10. Washington, DC: Armed Forces Institute of Pathology; 1994:383-385.

The authors acknowledge that the retinal lesions in tuberous sclerosis generally do not grow. This well-known fact has been supported by numerous reports, including a study published by Zimmer-Galler and Robertson in 1995.6 Doctor Zimmer-Galler and I published a series of cases with long-term follow-up showing the relative stability of these tumors over intervals beyond two decades. Although the development of intratumoral calcifications over follow-up of one to two decades was illustrated in that publication, we also showed that a typical translucent velvety hamartoma could remain unchanged without developing calcification over follow-up of nearly two decades.

It appears that the diagnosis of the retinal tumors, such as reported by the authors, even in the setting of tuberous sclerosis, may be very difficult. To absolutely...
differentiate these from retinoblastoma may not be possible without histology. In all four cases reported by the authors, the tumors were first seen in infancy, were para-papillary, contained calcium, and developed exudative components coincident with their growth. Are the authors aware of any patients who have developed co-existing retinoblastoma in the setting of tuberous sclerosis?

Finally, when faced with another similar case, have the authors formulated a strategy for some form of therapeutic intervention that might conceivably save the eye. If these tumors were recognized at an early age when their growth was just beginning and if histology confirmed the nature of the lesion, and if the optic nerves were not invaded, would the authors consider transvitreal endoresection with vitrectomy, removal of the tumor, and retinal repair? The latter is a formidable procedure, but since the tumors themselves appear not to be comprised of malignant cells, it is not likely that the surgical procedure itself would be associated with a systemic problem. Also, since enucleation appears otherwise inevitable, such a procedure might be a reasonable consideration.

REFERENCES


Dr Victor M. Elner. Did any of the other hamartomas in the brain grow in these patients? In other words, is this only something that was isolated to the retina or did the same event occur within the CNS lesions? How is this distinguished histopathologically from massive gliosis or could this be a massive gliotic response to tumor?

Dr Jerry A. Shields. Concerning Dr Robertson’s comments, I can make the following comments and responses. From our literature review and personal experience, we are not aware of metastasis from retinal astrocytoma. This parallels the lack of metastasis from the similar neoplasm in the brain. The co-existence of retinoblastoma and tuberous sclerosis must be extremely rare. We are aware of one report of presumed retinoblastoma and astrocytoma in the same eye of a patient who apparently did not have tuberous sclerosis.

I believe there is a strong argument for early management of an aggressive retinal astrocytic hamartoma. If we detect a small astrocytic hamartoma that shows either progressive growth or exudation and retinal detachment, early treatment would seem justified. However, transvitreal endoresection would probably not be my first choice. If the tumor were still small, one could consider laser treatment. If it were medium-sized, one could consider plaque brachytherapy. If it were a large tumor with extensive retinal detachment, one might consider vitrectomy, intraocular gas, and endolaser or cryotherapy or subsequent plaque brachytherapy. If the patient has total retinal detachment and neovascular glaucoma, as in our cases, then enucleation is justified.

With regard to the questions of Dr Elner, the similar tumor in the brain can also rarely show aggressive local behavior and produce hydrocephalus or other complications. To our knowledge, none of the brain tumors have progressed in the patients reported in our series or in other reported cases of aggressive retinal astrocytoma. The solitary tumor reported here can be clinically similar to some cases of massive gliosis of the retina, which can occasionally present as a distinct mass. However, massive gliosis generally occurs as extensive, diffuse gliosis in eyes that have had trauma or chronic retinal detachment from Coats disease, retinal capillary hemangiomatosis and other such insults.

REFERENCE

HUMAN INTRAOCULAR PENETRATION PHARMACOKINETICS OF MOXIFLOXACIN 0.5% VIA TOPICAL AND COLLAGEN SHIELD ROUTES OF ADMINISTRATION

BY Seenu M. Hariprasad MD,* William F. Mieler MD, Gaurav K. Shah MD, Kevin J. Blinder MD, Rajendra S. Apte MD, Nancy M. Holekamp MD, Matthew A. Thomas MD, Jingluan Chi PhD, and Randall A. Prince PharmD

ABSTRACT

Purpose: To determine penetration of moxifloxacin 0.5% into human aqueous and vitreous via topical and collagen shield routes of administration.

Methods: Moxifloxacin 0.5% was administered prior to vitrectomy surgery through one of three routes: topical drops every 2 hours for 3 days, versus topical drops every 6 hours for 3 days, versus delivery using a 24-hour dissolvable cross-linked corneal collagen shield. Aqueous and vitreous moxifloxacin concentrations were assayed using high-performance liquid chromatography.

Results: Mean moxifloxacin concentrations in the every-2-hour group for aqueous (n = 9) and vitreous (n = 10) were 2.28 ± 1.23 µg/mL and 0.11 ± 0.05 µg/mL, respectively. Mean moxifloxacin concentrations in the every-6-hour group for aqueous (n = 10) and vitreous (n = 9) were 0.88 ± 0.88 µg/mL and 0.06 ± 0.06 µg/mL, respectively. Levels of minimum inhibitory concentration at which 90% of isolates are inhibited (MIC90) were far exceeded in the aqueous for a wide spectrum of pathogens that most commonly cause postoperative endophthalmitis. Moxifloxacin concentration in the vitreous did not exceed the MIC90 for several key organisms. Delivery of moxifloxacin via a collagen shield revealed a mean aqueous concentration of 0.30 ± 0.17 µg/mL 4 hours after placement (n = 5). Vitreous levels at 4 hours, as well as aqueous and vitreous levels at 24 hours, were negligible using this route of administration.

Conclusions: The findings of this investigation reveal that topically administered moxifloxacin 0.5% can achieve relatively high aqueous concentrations. Although aqueous moxifloxacin levels achieved through the use of a collagen shield delivery device are lower, there are several advantages to this route of delivery that make it appealing in the immediate postoperative period. Future studies will be needed to precisely define the role of fourth-generation fluoroquinolones and presoaked collagen shields in the prophylaxis or management of intraocular infections.

INTRODUCTION

Bacterial endophthalmitis is one of the most serious complications after intraocular surgery. The microbiologic spectrum of infecting organisms in postoperative endophthalmitis was investigated in the Endophthalmitis Vitrectomy Study. This study represents the largest number of postoperative endophthalmitis cases from which bacteriologic data were prospectively obtained. The vast majority (94.2%) of confirmed growth isolates were gram-positive pathogens, most commonly Staphylococcus epidermidis and Staphylococcus aureus. Gram-negative pathogens, the most common being Proteus mirabilis, accounted for only 5.9% of confirmed growth isolates.1 The spectrum of infecting organisms in post-traumatic endophthalmitis differs from those of postoperative endophthalmitis, with Bacillus species playing a more prominent role.2 Numerous strategies have been described to try to decrease the incidence of postoperative endophthalmitis.3 Unfortunately, it is difficult to demonstrate superiority of one prophylactic strategy over another owing to the low occurrence rate of postoperative infection. The fluoroquinolones have been commonly used for prophylaxis during the perioperative period, typically through the
topical route of administration. The choice of antibiotic can be difficult because there are many different aspects by which the efficacy of an antibiotic is determined. One of these aspects is bioavailability. The bioavailability of an antibiotic determines its ability to penetrate into the tissues of concern and reach bacteria. In order to be bioavailable, a topical ophthalmic antibiotic must have a high rate of penetration and good solubility. The purpose of this investigation is to determine the penetration pharmacokinetics of moxifloxacin 0.5% ophthalmic solution into the human aqueous and vitreous via topical and collagen shield routes of administration.

Moxifloxacin 0.5% (Vigamox; Alcon Laboratories, Inc, Fort Worth, Texas) and gatifloxacin 0.3% (Zymar; Allergan, Inc, Irvine, California) are two newly released fourth-generation fluoroquinolones. They have a spectrum of activity encompassing gram-positive and gram-negative bacteria, including S. epidermidis, S. aureus, S. pneumoniae, S. pyogenes, H. influenzae, E. coli, Bacillus cereus, N. gonorrhoeae, and P. mirabilis. Additionally, the fourth-generation fluoroquinolones have good activity against atypical pathogens such as Mycoplasma, Legionella, and Chlamydia species, as well as the anaerobic organism P. acnes. New-generation fluoroquinolones, such as moxifloxacin, gatifloxacin, grepafloxacin, and trovafloxacin, represent advances in the evolution of this antibiotic class. The more favorable pharmacokinetic properties of the previously mentioned agents are due to alterations of the original fluoroquinolone moiety. For example, moxifloxacin and gatifloxacin possess an 8-methoxy side-chain (Figure 1), which may be responsible for their enhanced activity against gram-positive organisms, atypical pathogens, and anaerobes while retaining potencies and broad-spectrum coverage against gram-negative organisms comparable to older-generation fluoroquinolones. Each of the fourth-generation fluoroquinolones has its own subtle strengths by in vitro testing, however, further studies will reveal if these in vitro differences are clinically relevant.

We chose to study the penetration pharmacokinetics of topically applied moxifloxacin 0.5% ophthalmic solution into the human aqueous and vitreous for two reasons. First, older-generation fluoroquinolones, such as ofloxacin 0.3%, ciprofloxacin 0.3%, and levofloxacin 0.5%, have been shown to achieve effective levels in the aqueous, but not the vitreous, after topical administration in the noninflamed eye. Second, the MICs values of moxifloxacin against the pathogens most commonly responsible for postoperative, post-traumatic, and bleb-associated endophthalmitis were generally lower than those of the other fluoroquinolone antibiotics we surveyed (Table 1).

The corneal collagen shield was originally developed as a bandage lens for the treatment of corneal epithelial damage. Prior investigations have demonstrated that the collagen shield may be well suited for drug delivery in the perioperative setting and has several advantages over topical and subconjunctival routes of antibiotic administration. For these reasons, we chose to determine the intraocular penetration of moxifloxacin 0.5% using a 24-hour dissolvable cross-linked corneal collagen shield device.

**METHODS**

The study was carried out with the approval of the Washington University School of Medicine Institutional Review Board. Thirty adult patients, age range 55 to 86 years (68.2 ± 7.9 years), undergoing elective pars plana vitrectomy surgery between September 2003 and February 2004 at the Barnes Retina Institute were included in the study. Exclusion criteria included the following: known sensitivity to fluoroquinolones, renal disease (creatinine level >1.8 mg/dL), use of any other antibiotic(s) in the preceding 3 weeks, pregnancy or currently breast-feeding, current use of a class IA or III antiarrhythmic agent, previously vitrectomized eyes, fresh vitreous hemorrhage as indication for vitrectomy (less than 1 month old), or active endophthalmitis.

After informed consent was obtained, the first 20 patients were asked to self-administer topical moxifloxacin 0.5% ophthalmic solution for 3 days prior to surgery in the eye scheduled for operation. The first 10 patients received one drop of moxifloxacin every 2 hours (Q2H), and the second 10 patients received one drop of moxifloxacin every 6 hours (Q6H). On the day of surgery, the patients continued dosing as they had on the 3 preceding days. Additionally, topical 0.5% moxifloxacin was administered to all eyes 5 to 10 minutes preoperatively as a single drop. Patients were asked to return their bottle of moxifloxacin on the day of surgery to determine compliance to their assigned dosing regimen.

The last 10 patients enrolled received a 24-hour dissolvable cross-linked corneal collagen shield (Oasis Medical, Glendora, California) presoaked in moxifloxacin...
0.5% for 10 minutes prior to insertion into the eye scheduled for surgery. After placement, the eye was patched with a soft shield. The collagen shield was placed in the eye for approximately 4 hours (4H) in the first five patients and 24 hours (24H) in the second five patients prior to surgery.

Aqueous and vitreous samples were obtained before infusion of any intraocular irrigating solution in order to obtain pure samples. In the operative suite, approximately 0.1 mL of aqueous fluid was aspirated through a paracentesis site using a 30-gauge needle attached to a syringe. Within 10 minutes, 0.2 to 0.3 mL of vitreous fluid was obtained using a vitreous cutting device attached to a syringe via a short length of tubing. Aqueous and vitreous samples were immediately frozen at –83°C. These samples were shipped with dry ice in appropriate packaging material to the University of Houston College of Pharmacy, Houston, Texas. Moxifloxacin concentrations were determined in each of the samples using a previously described high-performance liquid chromatography technique.14 Aqueous and vitreous moxifloxacin concentrations were compared with already established in vitro MIC90 data.4,5 Student’s t test was performed to determine if any significant differences existed between various subsets of patients.

**RESULTS**

Indications for operation in the 30 patients were as follows (Tables 2 and 3): macular hole (10 patients), epiretinal membrane (10), branch retinal vein occlusion (3), central retinal vein occlusion (2), diabetic macular edema (2), chronic cystoid macular edema (1), vitreomacular traction syndrome (1), and intraocular lens exchange (1).

Mean moxifloxacin concentrations in the topical Q2H group for aqueous (n = 9) and vitreous (n = 10) were 2.28 ± 1.23 µg/mL and 0.11 ± 0.05 µg/mL, respectively. Mean moxifloxacin concentrations in the topical Q6H group for aqueous (n = 10) and vitreous (n = 9) were 0.88 ± 0.88 µg/mL and 0.06 ± 0.06 µg/mL, respectively. Although the mean aqueous concentration of moxifloxacin was significantly different between the Q2H and the Q6H groups, this was not the case for the vitreous (P = .01 and P = .08, respectively) (Table 2 and Figure 2).

Compliance to assigned topical dosing regimens was determined by counting the number of drops remaining in each patient’s moxifloxacin 0.5% bottle on the day of surgery. To determine the number of drops administered,
this number was subtracted from 78, because this is the number of drops in an average 3-mL moxifloxacin 0.5% bottle (on file, Alcon Laboratories, Inc). Only one patient (No. 17, Table 2) did not return a bottle. The mean number of moxifloxacin drops administered in the Q2H and the Q6H groups was 42.90 ± 9.86 and 21.67 ± 4.72 drops, respectively.

Aqueous topical data from patient 1 and vitreous topical data from patient 20 were removed from the study because either there was insufficient sample volume to perform high-performance liquid chromatography (HPLC) or concentrations were too low to be detected by HPLC. In both the Q2H and the Q6H groups, there appeared to be several values that were considered outliers. For example, patient 2 had aqueous levels approximately 13 times below the mean of the rest of the Q2H group. We chose to include all data obtained in the study, because the investigators could not explain these high or low concentrations and attributed them to variability of moxifloxacin pharmacokinetics in individual patients (Table 2).

Four of the 10 patients in the Q2H group and five of the 10 patients in the Q6H group were phakic. In the Q2H group, aqueous and vitreous moxifloxacin concentrations were not significantly different when comparing phakic versus pseudophakic eyes (P = .25 and P = .10, respectively). The same was found in the Q6H group, where aqueous and vitreous moxifloxacin concentrations were not significantly different when comparing phakic versus pseudophakic eyes (P = .08 and P = .12, respectively).

Collagen shields were placed for 3.75 ± 1.41 hours prior to surgery in the 4H group and 24.80 ± 0.84 hours in the 24H group. Mean moxifloxacin aqueous concentrations in the 4H group (n = 5) were 0.30 ± 0.17 µg/mL. Two of five patients in the 4H group had detectable vitreous moxifloxacin levels of 0.03 µg/mL. In the 24H collagen shield group, two of five patients had detectable aque-

<table>
<thead>
<tr>
<th>PATIENT NO.</th>
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<th>DOSING REGIMEN</th>
<th>NO. OF DROPS ADMINISTERED</th>
<th>AQUEOUS µg/mL</th>
<th>VITREOUS µg/mL</th>
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<td>Q6H</td>
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BRVO, branch retinal vein occlusion; CME, chronic cystoid macular edema; CRVO, central retinal vein occlusion; ERM, epiretinal membrane; IOL, intraocular lens; MH, macular hole.

*Not detected by high-performance liquid chromatography, presumably due to low concentration or insufficient sample volume.
†Moxifloxacin 0.5% bottle not returned.
ous moxifloxacin levels of 0.04 ± 0.01 µg/mL. Vitreous levels of moxifloxacin were undetectable in all five patients in the 24H group (HPLC assay for moxifloxacin can detect levels >0.025 µg/mL) (Table 3).

No serious adverse reactions were attributed to the antibiotic agent or the collagen shield. In our series, only one patient from the Q2H topical group complained of mild ocular discomfort. No patients in our series complained of nonocular adverse events. Corneal clarity in all patients included in this study was described as excellent by the contributing surgeons.

**DISCUSSION**

After cataract extraction, bacterial endophthalmitis is most commonly caused by *S epidermidis* (70% of isolates in the Endophthalmitis Vitrectomy Study). This typically presents as a moderately severe infection 5 to 7 days after surgery. Less commonly, two other forms of endophthalmitis can take place after cataract extraction. The first is a chronic, indolent endophthalmitis that presents several months after surgery and is usually caused by *P acnes*. A second, less common form of postoperative endophthalmitis is an early, fulminant type usually presenting 2 to 4 days after surgery, which is caused by *Streptococcus* or *Staphylococcus* species as well as gram-negative organisms (most commonly *P mirabilis*). One reason we chose to study the intraocular penetration of moxifloxacin is that the MIC₉₀ values of moxifloxacin against the pathogens most commonly responsible for postoperative, post-traumatic, and bleb-associated endophthalmitis were generally lower than those of the other fluoroquinolone antibiotics we surveyed (Table 1). In our study, MIC₉₀ levels were far exceeded in the aqueous for a wide spectrum of pathogens in both the topical Q2H and Q6H groups, including *S epidermidis*, *S aureus*, *S pneumoniae*, *S pyogenes*, *P acnes*, *H influenzae*, *E coli*, *B cereus*, *N gonorrhoeae*, *P mirabilis*, and other organisms. Concentration of moxifloxacin in the vitreous after topical administration did not exceed the MIC₉₀ for several organisms; however, in the Q2H group, the MIC at which 50% of isolates are inhibited (MIC₅₀) was exceeded for *S epidermidis*, *S aureus*, *S pneumoniae*, *H influenzae*, *B cereus*, and other gram-negative organisms.

Topically administered moxifloxacin was unable to achieve intraocular levels effective against *Pseudomonas*; furthermore, the MIC₉₀ for *Enterococcus* was only exceeded in the Q2H aqueous group. Although *Pseudomonas* and *Enterococcus* are only very rarely encountered in postoperative endophthalmitis, moxifloxacin 0.5% may not be a suitable treatment choice for intraocular infections known to be caused by these organisms.

Another reason we chose to study the intraocular penetration of topically administered moxifloxacin 0.5% is that older-generation fluoroquinolones, such as levofloxacin 0.5%, ofloxacin 0.3%, and ciprofloxacin 0.3%, have been shown to achieve effective levels in the aqueous, but not the vitreous, after topical administration in the noninflamed human eye. Table 1 compares the mean intraocular concentrations achieved with several other fluoroquinolones agents, as well as their corresponding MIC₉₀ values, against the pathogens most commonly responsible for bacterial endophthalmitis. The intent of this table is not to directly compare the intraocular penetration of the different agents, since the dosing frequency of each investigated fluoroquinolone was different. Additionally, given the study design of these types of investigations, it is difficult to precisely determine if samples are being obtained during drug peak or trough levels. Given these limitations of Table 1, several important findings are apparent. First,

### TABLE 3. PATIENT CHARACTERISTICS AND INTRAOCULAR MOXIFLOXACIN CONCENTRATIONS AFTER COLLAGEN SHIELD PLACEMENT

<table>
<thead>
<tr>
<th>PATIENT NO.</th>
<th>AGE (YR)</th>
<th>INDICATION FOR SURGERY</th>
<th>PHAKIC STATUS</th>
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<td>7</td>
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<td>Pseudo</td>
<td>24H</td>
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<tr>
<td>9</td>
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<td>24H</td>
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</table>

BRVO, branch retinal vein occlusion; CRVO, central retinal vein occlusion; DME, diabetic macular edema; ERM, epiretinal membrane; MH, macular hole; VMTx, vitreomacular traction syndrome.

*Dash indicates not detected by high-performance liquid chromatography, presumably due to low concentration or insufficient sample volume.
no topically administered fluoroquinolone investigated achieves intravitreal levels sufficient to exceed the MIC$_{90}$ for the organisms that most commonly cause bacterial endophthalmitis. Intravitreal concentration of moxifloxacin 0.5% Q2H comes very close to the MIC$_{90}$ for *S epidermidis* (the most common causative organism in bacterial endophthalmitis). This concentration may be sufficient for prophylaxis, but is not sufficient for treatment of active infection.

Previous studies suggest that intraocular penetration of systemic antibiotics may be higher in an eye that has sustained trauma, is infected, or is inflamed (ie, the postoperative eye). This may be due to disruption of the blood-ocular barrier, and it is conceivable that the intravitreal penetration of topically administered moxifloxacin may be high enough to exceed the MIC$_{90}$ level for *S epidermidis* and several other organisms of concern in the postoperative setting. Another finding that becomes apparent upon reviewing Table 1 is that compared to older-generation fluoroquinolones, moxifloxacin concentration achieved in the aqueous has fewer gaps in coverage for the organisms most commonly implicated in bacterial endophthalmitis.

Previous studies have demonstrated that orally administered fourth-generation fluoroquinolones can achieve therapeutic levels in the noninflamed human eye. Garcia-Saenz and associates$^{16,17}$ investigated the penetration of orally administered moxifloxacin into the human aqueous humor for potential use as a prophylactic agent in cataract surgery. They found that moxifloxacin achieved a mean aqueous concentration of $2.33 \pm 0.85$ µg/mL. Unfortunately, penetration of moxifloxacin into the vitreous was not investigated in this study. Gatifloxacin, another fourth-generation fluoroquinolone, has been shown to achieve levels as high as $1.34 \pm 0.34$ µg/mL and $1.08 \pm 0.54$ µg/mL in the human vitreous and aqueous after oral administration, respectively.$^{18}$ Although oral administration of a fourth-generation fluoroquinolone results in intravitreal concentrations several times higher than after topical administration, an interesting finding is that topical administration Q2H of moxifloxacin 0.5% can achieve aqueous levels comparable to those after oral administration. Therefore, topically administered moxifloxacin 0.5% may be useful in the management of infections limited to the anterior segment. One example of such an infection is localized conjunctival filtering bleb infection, or "blebitis." The most common causative organisms in delayed-onset bleb-associated endophthalmitis are *Streptococcus* and *Staphylococcus* species.$^{19}$ *H influenzae* is also commonly encountered in this condition. The concentration of moxifloxacin achieved after topical administration in the aqueous is several times higher than the MIC$_{90}$ for these organisms. If blebitis progresses to bleb-associated endophthalmitis, one may consider the addition of an orally administered fourth-generation fluoroquinolone as an adjunct to the current management of bleb-associated endophthalmitis.

The collagen shield data obtained from this study reveal that peak aqueous levels of moxifloxacin occur soon after surgery. This is when a high level of antibiotic is most needed to clear the anterior chamber of bacteria remaining in the eye. In the 4H collagen shield group, the MIC$_{90}$ and MIC$_{50}$ for several organisms that most commonly cause postoperative endophthalmitis were exceeded.$^{20}$ By 24 hours, negligible levels of moxifloxacin were found in the eye. This is consistent with other studies investigating drug delivery from collagen shields, which show that peak intraocular drug levels occur in the first 4 hours of collagen shield application.$^{21}$ Therapeutic moxifloxacin levels in the vitreous cannot be achieved with this method of drug delivery, and the clinical significance of this is yet to be determined.

There are several advantages to using collagen shields for moxifloxacin delivery in the immediate postoperative period. One such advantage is the ability to leave the eye patch undisturbed after surgery while the collagen shield releases antibiotic. Additionally, there is evidence that collagen shields have a beneficial effect on the corneal epithelium and promote healing.$^{22}$ Collagen shields have advantages over subconjunctival injections as well; these include avoiding inadvertent globe perforation and subconjunctival hemorrhage. Additionally, pain associated with subconjunctival antibiotic injection can be avoided with the use of a collagen shield when cataract surgery is performed using topical anesthesia. Lastly, with the advent of sutureless 25-gauge vitrectomy surgery, the vitreoretinal specialist should consider the theoretical risk of serious retinal toxicity if a subconjunctivally administered antibiotic such as gentamicin were to enter an air-filled eye through an unsutured sclerotomy site.

Moxifloxacin 0.5% is unique in that it is free of preservatives, specifically benzalkonium chloride. The lack of this preservative is valuable when using a collagen shield delivery device, because there is a theoretical risk of preservatives causing corneal damage after sustained drug delivery. Corneal clarity was rated as excellent by the contributing surgeons for all 30 patients participating in this study; however, no formal fluorescein staining was performed to evaluate subtle corneal epithelial changes.

Moxifloxacin 0.5% is very well tolerated; the majority of adverse reactions are described as mild. These most commonly include dry eye, ocular hyperemia, ocular discomfort, and ocular itching. In our series, only one patient from the Q2H topical group complained of mild ocular discomfort. No patients in our series complained of nonocular adverse events. The dosage of moxifloxacin 0.5% recommended by Alcon Laboratories, Inc, is one drop three times a day (bacterial conjunctivitis indication). In our study design, we chose to use a regimen of Q2H and Q6H. Our rationale for dosing at Q2H was to
determine if intensive topical therapy could be used to obtain therapeutic levels in the vitreous. The Q6H dosing schedule was included in the study because this is a commonly used dosing regimen for cataract surgery prophylaxis. After calculation of the number of drops that were self-administered, patient compliance in both groups was considered excellent (Table 2).

The authors would like to emphasize that the purpose of this research is to provide proof-of-principle that moxifloxacin 0.5% can attain therapeutic intraocular concentrations. Moxifloxacin 0.5% may be beneficial for prophylaxis against the risk of infection after eye surgery or intravitreal injections; however, it should be noted that antibiotics are only one component of a thorough prophylactic regimen.

In summary, moxifloxacin has a spectrum of coverage that appropriately encompasses the most common causative organisms in endophthalmitis. The pharmacokinetic findings of this investigation reveal that topically administered moxifloxacin 0.5% can achieve relatively high aqueous concentrations. Although aqueous moxifloxacin levels achieved through the use of a collagen shield delivery device are lower, it is conceivable that intraocular levels of moxifloxacin may be higher in an eye that has undergone surgery. Additionally, there are several advantages to the collagen shield route of delivery that make it appealing in the immediate postoperative period. Future studies will be needed to precisely define the role of fourth-generation fluoroquinolones and presoaked collagen shields in the prophylaxis or management of intraocular infections.

REFERENCES


DISCUSSION

Dr M. Gilbert Grand. Endophthalmitis is among the most feared complications of intraocular surgery. While the incidence is low, because of the potential for cata-
The MIC90 of organisms that most commonly cause acute concentrations of moxifloxacin in the aqueous that exceed aqueous of non-inflamed eyes. Their data indicate bioavailability of topically applied moxifloxacin in the growth of more virulent organisms.

and alterations of the normal ocular flora to allow the acceleration of development of resistance, ocular toxicity, and alterations of the normal ocular flora to allow the growth of more virulent organisms.

The authors have presented data showing the bioavailability of topically applied moxifloxacin in the aqueous of non-inflamed eyes. Their data indicate concentrations of moxifloxacin in the aqueous that exceed the MIC90 of organisms that most commonly cause acute postoperative bacterial endophthalmitis, blebitis, and filter bleb-associated endophthalmitis.

In reviewing this manuscript, it is apparent that the study population was, in fact, small and was further divided into multiple smaller treatment groups. The data points collected show a wide range of values, sometimes as wide as the mean value itself. Furthermore, compliance, as measured by drop count, appeared to be inconsistent. Yet despite these concerns, the study strongly suggests that topical moxifloxacin may be of great value as a prophylactic antibiotic to reduce the risk of acute postoperative bacterial endophthalmitis. The authors, however, prudently remind us that the use of antibiotics, whether preoperatively, intraoperatively or postoperatively, is only one aspect in an overall scheme to prevent endophthalmitis. Perhaps the most significant finding of the study is the potential value of moxifloxacin in the treatment of H. influenzae or Streptococcus-induced blebitis and as an adjunct to intravitreal or systemic therapy in the treatment of filter bleb-associated endophthalmitis.

The data presented stimulate a number of questions: what is the ideal timing and frequency of administration of moxifloxacin preoperatively to achieve a significant reduction in viable microorganisms in the ocular flora? Will topical moxifloxacin administered for two hours prior to surgery achieve the same reduction in ocular flora and the same concentration in the aqueous as treatment administered over three days preoperatively? Can modifications in the design and construction of the matrix of collagen shields be achieved that would allow a more prolonged administration of moxifloxacin or similar drugs to achieve adequate antibiotic concentrations in the aqueous and vitreous postoperatively without associated ocular toxicity? Finally, despite the potential broad spectrum coverage and bioavailability of moxifloxacin, in the hopes of preventing the induction of resistance, would it be prudent to avoid the use of fourth generation fluoroquinolones as prophylactic agents and reserve them only for treatment of infections such as blebitis or filter bleb endophthalmitis?

**REFERENCE**


**Dr Seenu M. Hariprasad.** Dr Grand's first question is astute. He asks if there is a difference in the ocular flora if moxifloxacin is dosed for three days versus for only two hours preoperatively? A very similar question was answered by Dr Ta and colleagues two years ago at Stanford; they dosed ofloxacin for three days in one group of patients and dosed ofloxacin in a second group one hour preoperatively. They found that positive conjunctival cultures were found prior to surgery in about 20 percent of eyes which received three days of ofloxacin versus 40 percent of eyes that received ofloxacin only right before surgery. Similarly, postoperatively, those eyes which had received ofloxacin for three days had less than 50 percent ocular surface contamination compared to the group that received ofloxacin only right before surgery (it should be noted that all patients received a povidone-iodine scrub). Therefore, this data strongly suggests that a longer preoperative antibiotic dosing regimen is more effective in eliminating bacteria from the ocular surface.

To address Dr Grand's second question, the effectiveness of a corneal collagen shield as a drug delivery device depends on its drug uptake and its subsequent rate of release. The factors that determine this include collagen shield cross-linking versus non-cross linking, dissolution time of the collagen shield, and water-solubility of the drug. A cross-linked shield (such as the one we used in this study) can provide more desirable drug delivery than non-cross-linked shields because drug levels can be sustained for longer periods of time. Likewise, longer dissolution times are also preferable, and that is why we used a 24-hour collagen shield rather than a 12-hour shield. So to answer your question, Dr Grand, the design of a “better” collagen shield may be achieved by altering the molecular structure of the shield and possibly the
physicochemical properties of the drug.

Finally, the proper use of ophthalmic topical fluoroquinolones represents an insignificant selection pressure for promoting resistant bacteria. I use the term “proper” to mean the use of a topical antibiotic at therapeutic levels for a short period of time. Approximately 200,000 kilograms of fluoroquinolones are used annually of which only 24 kilograms constitute ocular use. Therefore, my impression is that agriculture, veterinary, general medicine, and surgical uses of fluoroquinolones have a much greater selection pressure for the development of resistant organisms compared to ophthalmology.

I would like to convey my gratitude to the program committee for allowing me to present our research today and once again I would like to thank Dr Grand for his meticulous review of our paper.
PRESUMED SINUS-RELATED STRABISMUS

BY Irene H. Ludwig MD,* AND Joe Frank Smith MD

ABSTRACT

Purpose: To determine whether sinus disease may cause acquired strabismus.

Methods: Patients with idiopathic acquired (nonaccommodative) esotropia and/or hypotropia were questioned in detail about possible contributing factors (trauma; family history of strabismus; thyroid, neurologic, or rheumatologic disorders). Acute versus chronic onset was ascertained. Those without obvious cause of strabismus were investigated for possible sinus disease with sinus computed tomographic scan and otolaryngologic consultation.

Results: Over a period of 5 years, 59 patients were identified with sinus disease that correlated to their strabismus pattern(s). Twenty-three had “possible” sinus-related strabismus. They had sinus findings that correlated with the strabismus pattern (eg, hypotropia and adjacent maxillary sinus disease). Twenty-six had “likely” sinus-related strabismus. These patients had additional features, such as their own recognition that strabismus worsened along with sinus symptoms, or unusually severe sinus disease. Ten were diagnosed with “very likely” sinus-related strabismus. They had strong correlation between treatment of sinus disease and strabismus improvement. Eighteen patients required sinus surgery owing to failure of medical control. Age at onset of strabismus ranged from 6 months to 81 years. Forty patients required strabismus surgery. All had restriction of motility on forced duction testing under anesthesia. Control of sinus disease combined with range-of-motion eye exercise improved symptoms in 19 who did not require strabismus surgery.

Conclusions: Occult sinus disease may cause acquired strabismus. Perhaps sinusitis leads to inflammation and secondary contracture in adjacent extraocular muscles. Although difficult to prove owing to the high frequencies of both strabismus and sinus disease, the association between the two may prove significant to strabismus treatment and long-term control.

INTRODUCTION

Despite complete evaluation of patients in a strabismus practice, there exists a subset of cases in which etiology remains unclear. This group includes acquired nonaccommodative esotropia in children and adults, gradually progressive vertical strabismus, and combinations of the two. These patients may have evidence of muscle fibrosis, but workups for thyroid ophthalmopathy, rheumatologic disorders, or other predisposing systemic diseases are negative.

It is the clinical impression of this author (I.H.L.) that the inferior and medial recti are the extraocular muscles most prone to develop fibrosis, excepting cases with prior muscle surgery or trauma. This led to the postulate that fibrosis of the medial and inferior recti could be related to their proximity to the adjacent ethmoid and maxillary sinuses, respectively.

METHODS

Patients with atypical acquired strabismus (nonaccommodative acquired esotropia, gradually progressive vertical strabismus) suggesting fibrosis of the medial and/or inferior rectus muscles were evaluated for sinus disease. Others with fibrotic muscles and/or thickened, fibrotic orbital fat pad in the inferior fornix observed at surgery also underwent sinus investigation.

Patients were treated in several locations but, when possible, were referred to the same otolaryngologist (J.F.S.). For the patients seen in this center, the otolaryngology Clinical Indicators Compendium for rhinosinusitis was used to evaluate symptomatology. Major clinical indicators were facial pain, facial congestion, nasal obstruction, nasal discharge, and unpleasant odor. Minor clinical indicators were headache, fever, bad breath, fatigue, dental pain, cough, and ear pain. Each indicator was given...
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a scale of 0 to 10, for a maximum total score of 120. Of the 59 cases in this study, 26 were evaluated by J.F.S. These 26 cases were analyzed separately to grossly estimate incidence of sinus disease in acquired strabismus.

In other locations, patients were referred to another otolaryngologist or pediatrician for sinus evaluation and treatment. Sometimes, to expedite evaluation, sinus computed tomographic (CT) scans were obtained first, to rule out sinusitis before referring the patient.

RESULTS

Fifty-nine patients were identified with strabismus and sinus disease. They were subdivided into three groups based upon the strength of the association between the two conditions in each patient. “Very likely” sinus-related strabismus was defined as those patients whose alignment improved with sinus disease treatment. Ten patients fell into this category. “Likely” sinus-related strabismus was defined as strabismus with unusually severe adjacent sinus involvement, or moderate sinus disease plus additional suggestive features, such as the patient’s realization that strabismus symptoms worsened simultaneously with upper respiratory tract illness. This group included 26 patients. “Possible” sinus-related strabismus was the finding of sinus disease on CT scan without other supporting evidence. Twenty-three patients were classified in the “possible” group. Age at onset ranged from 6 months to 81 years, with a mean of 34 years.

Most of the 59 patients had resolution of sinus disease with medical control, but sinus surgery was required in 18.

Of 26 strabismus patients seen by J.F.S. for otolaryngologic evaluation, 24 (92.3%) had positive findings of sinus disease on limited paranasal sinus CT scan. Fifteen (57.6%) had active sinusitis, and nine (34.6%) had significant sinus anatomic abnormalities. For major clinical indicators, 15 (57.6%) had mild symptoms of sinus disease, 9 (34.6%) had moderate symptoms, and 2 (7.6%) had severe symptoms. Six cases (23%) had a total score of ≤10 out of 120, which is classified as no clinical history of sinusitis.

Of the 59 cases in this series, 40 required strabismus surgery. All those who underwent strabismus repair had positive forced duction testing suggesting fibrosis of the extraocular muscles. The most commonly involved muscles were the inferior and medial recti. The resistance to forced duction by the oblique muscles was assessed in 21 patients by the measurement of passive resistance to intorsion and extorsion of the eye. Normal was defined as 60 degrees or more torsion before resistance was met. The exaggerated forced duction test of the obliques was also used to confirm oblique tightness. Tight superior obliques were found in 16 patients, and tight inferior obliques were detected in 13. Resistance to depression or adduction, which would suggest fibrosis of the superior or lateral recti, was not detected. Eleven had resolution of symptoms with sinus treatment and motility exercise alone, and eight had persistent diplopia controlled by prism without requiring strabismus surgery.

Thirty-seven patients had acquired esotropia. Twelve of these had been previously diagnosed with sixth cranial nerve palsy. Force generation testing confirmed normal lateral rectus strength when the diagnosis was in doubt. Twenty-five had comitant esotropia, and 12 had a high accommodative-convergence relationship. Intermittent esotropia was present in 16, and they underwent preoperative prism adaptation to uncover their full deviations.

Nineteen patients had vertical strabismus with measurements suggesting fourth cranial nerve palsy. The diagnostic clue to fibrosis of the contralateral inferior rectus was usually mild extorsion of the hypotropic eye as opposed to the extorsion of the hyperdeviating eye expected in fourth cranial nerve palsy. At surgery, these patients had tightness of the involved inferior rectus and no laxity of the superior obliques by forced duction testing. They each had a thickened, fibrotic orbital fat pad in the inferior fornix (Figure 1). Most actually had tightness of the superior obliques on forced duction testing.

One patient had severe upgaze restriction with ptosis due to ipsilateral maxillary sinus infection, which mimicked partial third cranial nerve palsy.

Seven patients developed strabismus recurrence despite good alignment after initial strabismus repair. The unexplained recurrences led to sinus investigations in this group.

Stereopsis testing was recorded in the 52 patients who were old enough to perform the test. After alignment was restored, only one showed no stereopsis to the near Titmus test. This patient had more than 30 years of

FIGURE 1

Inferior fornix fibrosis seen during surgery for fibrotic inferior rectus in a patient with acquired vertical strabismus, presumably due to chronic sinus disease
constant esotropia. Fifteen patients had 40 seconds(s) of stereopsis or better, 17 had 50 to 80 seconds, 10 had 100 to 200 seconds, and 9 had 400 to 800 seconds.

A mother of a child with recurrent esotropia volunteered that she can now predict a recurrence of sinusitis when the child’s esotropia worsens. His otolaryngologist has confirmed each episode. Prompt antibiotics then improve his alignment control. One patient had amblyopia in an eye, which had nonetheless been aligned. She developed hypotropia of that eye without diplopia. She had complete opacification of the ipsilateral maxillary sinus.

Four additional patients were not included in this series because they had preexisting strabismus due to nonsinus etiology. They all had undergone previous successful strabismus repairs, but then developed strabismus recurrences following sinusitis. All had known orbital bony wall defects. Two had thyroid ophthalmopathy with previous orbital decompressions, and two had prior orbital trauma. The thyroid patients responded to prompt antibiotics plus motility exercise, and the post-traumatic patients needed small additional strabismus repairs.

Case Reports

Case 1. Pseudo Sixth Nerve Palsy
A 5-year-old boy was brought for a sixth opinion regarding esotropia. He had developed acute esotropia [LE(T) = 30] at age 3 coincident with otitis media. A diagnosis of acute viral left sixth cranial nerve palsy was made at that time. Computed tomographic scan of the head was normal and esotropia persisted. He underwent botulinum toxin injection to the left medial rectus 3 months later, with no effect. Glasses for a +2.00 hyperopia, with +2.50 add, were prescribed. Seven months after onset of acute esotropia, he underwent recession of the left medial rectus, which reduced the esodeviation in the glasses, but uncorrected esotropia of 25 PD persisted.

The patient’s parents were not accepting of a diagnosis of accommodative esotropia, because his identical twin brother had no strabismus and there was no family history of strabismus or glasses wear (Figure 2).

By age 5, at our visit, he had accommodative esotropia with a high accommodation convergence/accommodation (AC/A) relationship, which was controlled with full cycloplegic correction with bifocals except for manifest esotropia of 14 in downgaze. Version testing showed full abduction in both eyes and apparent superior oblique underaction. Visual acuity was 20/25 in each eye, stereopsis was 100 seconds of arc, and fundi were mildly extorted. He was healthy, other than occasional nasal congestion. Computed tomography of the sinuses showed severe pansinusitis, including the sphenoids (Figure 3). The sinus disease failed to respond to medical treatment, and he underwent sinus surgery. V-pattern esotropia persisted, and he then underwent bilateral medial rectus recession together with multiple myotomy lengthening procedures to the inferior recti. Medial and inferior recti were found to be stiff and fibrotic, and there was no laxity of the superior obliques to forced duction. One year postoperatively, V-pattern is resolved, as is the high AC/A relationship. Cycloplegic
spherical equivalent is +3.25, but he can now maintain alignment with +2.25 and no bifocals. Stereopsis is 40 seconds.

Both parents and his identical brother were tested for stereopsis, which was 40 seconds in each. The brother underwent complete examination, which showed esophoria of 4 PD and hyperopia of +1.25 diopters but was otherwise normal. The boy had had a long history of sinus disease, allergies, and asthma, which had been regularly treated. Perhaps early sinus treatment protected him from esotropia.

Case 2. Pseudo Sixth Nerve Palsy
A 15-year-old girl presented with a 1-month history of diplopia. She had already undergone extensive ophthalmologic and neurologic evaluations at a major university medical center for idiopathic esotropia, which was diagnosed as sixth cranial nerve palsy. Past medical history was negative.

She had esotropia of 14 PD, greater on right gaze, decreased on left gaze. There was minimal limitation of abduction in both eyes and mild elevation limitation in the left eye. Examination was otherwise normal. Magnetic resonance imaging scan of the head, which had already been performed at the hospital, was neurologically normal, but showed incidental maxillary and ethmoid sinus disease. After several days of an oral antibiotic and decongestant, diplopia resolved. Several months later a follow-up motility examination was normal.

Case 3. Pseudo Fourth Nerve Palsy
A 43-year-old woman reported having vertical diplopia since childhood. It was improved with left head tilt and had become increasingly symptomatic with age. She had right hypertropia of 12 PD, increasing to 30 PD on left gaze, 40 PD on right gaze, and 18 PD on right head tilt. There was 3+ overaction of the right inferior oblique, 2–underaction of the right superior oblique, and mild elevation deficit of the left eye. Subjective torsion measurement was 7 degrees exocyclotorsion OD, 5 degrees extorsion OS. She was diagnosed with right superior oblique palsy and underwent 12-mm recession of the right inferior oblique and 4-mm advancement of the superior oblique under local anesthesia. The use of local anesthesia prevented adequate forced duction testing.

Postoperatively, alignment measured orthotropic, but the patient complained of diplopia. Two months later she had a left hypertropia of 4, increasing to 14 on downgaze. She had 7 degrees of extorsion OS. Diagnosis of unmasked left superior oblique palsy was made, and reoperation was undertaken, this time under general anesthesia. Forced duction test surprisingly showed marked fibrosis of both inferior recti and tightness of the superior obliques and left medial rectus. Both inferior recti were recessed with nonabsorbable suture, the left 4 mm, and the right 5 mm. The right inferior oblique was also maximally recessed posteriorly. The inferior recti were found to be fibrosed to surrounding orbital fat, which was also fibrosed and thickened, creating a bulge in the inferior fornix.

Postoperatively, she was again orthotropic, but uncomfortable, and with vague symptoms of blurred vision, although vision measured 20/20 OD, 20/15 OS. Two months later, left hypertropia of 8 PD had recurred, and she underwent further recession of the right inferior rectus, and recession of the right superior oblique to its original insertion. Again, she was orthotropic for 2 months before the left hypertropia recurred. The right eye showed restriction to upgaze. There was no limitation to downgaze OS.

Because of presumably increasing inferior rectus fibrosis, the patient was referred for otolaryngologic examination and sinus CT scan. Otolaryngologic history was normal with a total score of 2/120. Her otolaryngologic examination was normal, as was her nasal endoscopy. CT scan showed paranasal sinus disease in both maxillary sinuses, both ethmoids, and the left frontal, rating 4 and 5 on the Lund-Kennedy staging system. Two months of continuous medical treatment failed to clear the sinusitis, and she underwent fiberoptic endoscopic sinus surgery, with intraoperative findings of ethmoid polyposis and fungal-appearing mucus. After sinus surgery, she felt marked improvement in her discomfort and well-being and resolution of “blurred vision,” but diplopia persisted owing to left hypertropia of 10 PD. She underwent a fourth strabismus procedure with 2-mm additional recession of the right inferior rectus and inferior conjunctival recession. She has maintained orthotropia with complete resolution of all visual symptoms for 4 years. Her sinus disease is controlled with topical corticosteroid spray.

Case 4. Pseudo High AC/A Ratio
A pediatrician brought her 6-year-old daughter for examination because the child's teacher had noticed esotropia. The child's mother then began to notice intermittent esotropia when she was tired. Alignment was normal at distance in all directions of gaze, but intermittent esotropia of 12 PD was seen at near. Versions showed mild underaction of the inferior obliques, and mild elevation deficiency in one eye. Her mother was counseled about possible sinus infection but declined to investigate the sinuses because of the child's lack of sinus symptoms.

Two months later, the child was hospitalized for treatment of bacterial meningitis, felt to be due to spread from bilateral maxillary sinusitis. She was successfully treated medically. Esotropia did not recur.
DISCUSSIONS

Isolated strabismus cases due to acute sinusitis have been reported in the literature. Two cases of acute-onset Brown's syndrome occurred due to pansinusitis, and superior oblique palsy attributed to sinusitis has also been described.

Ophthalmic complications of adjacent sinusitis have long been known. These include cellulitis, orbital abscess, orbital myositis, cavernous sinus thrombosis, and blindness. Strabismus due to sinusitis was usually attributed to cranial nerve palsies owing to cavernous sinus involvement. Most reported cases of ophthalmic complications of sinusitis were related to symptomatic, acute, fulminant sinus disease. Vertical diplopia due to chronic asymptomatic sinusitis has been described due to orbital floor collapse, which leads to enophthalmos and hypoglobus.

Sinus lesions such as mucoceles, osteomas, and malignancies are known to cause diplopia due to direct orbital extension. Damage to extraocular muscles with secondary strabismus is a reported complication of sinus surgery.

The strabismus cases in this study were different from previously reported cases in that most of the patients were unaware of sinus disease. Few had clinical evidence of sinus disease. Vertical diplopia due to chronic rhinorrhea, but before treatment, neither she nor his pediatrician had been concerned about his mild symptoms. Office otolaryngologic examination was not adequate to diagnose sinusitis in this group. Sinus CT scans were required to diagnose and monitor sinusitis.

In a population-based study of childhood esotropia, idiopathic acquired nonaccommodative esotropia was reported in 10.4%. During data analysis of a large series of accommodative esotropia, a number of cases did not fit into the accepted definitions of congenital or accommodative esotropia. These may also represent acquired nonaccommodative esotropia. The children with sinus-related esotropia in this series may be similar to the same subsets of childhood esotropia as the other studies.

Superior oblique palsy appearing in late childhood without neurologic abnormality or trauma was felt to be due to decompensation of a previously asymptomatic but congenital defect. Nineteen of the cases in this study could have been classified as fourth cranial nerve palsy based upon alignment testing. The findings of contralateral inferior rectus fibrosis described above differentiated these cases from true fourth cranial nerve palsy.

Gradual-onset adult strabismus is also often attributed to decompensation of congenital strabismus, unless neurologic or myopathic etiology is diagnosed. Some of the patients in this series had been wearing gradually increasing spectacle-mounted prisms for many years, and one had noticed the temporal coincidence between his yearly sinus infection and the need for a yearly increase in prism strength.

Some of the patients in this study had acute or subacute onset of diplopia. Cranial nerve palsy was the clinical diagnosis until imaging disclosed sinusitis (Figure 4). Force generation testing of the suspected palsied muscle was a useful diagnostic test in the older children and adults. Motility limitation was less marked in these cases than would be expected in cranial nerve palsy, and saccades were not slowed.

Since the advent of broad-spectrum antibiotics, the serious complications of acute sinusitis of the past have become rare. The serious ophthalmic complications of sinusitis that appeared in the early literature included blindness and severe palsy of multiple cranial nerves. These are not found in the modern literature but were familiar to clinicians prior to 1950. Sinus disease itself remains common, however. One study showed positive CT scan findings of sinus disease in 15% of asymptomatic individuals. Another recent study found mucosal thickening in 17% of a control group of CT scans for unspecified orbital disease, but only 2% had radiologically significant sinus disease. A chronic, smoldering sinusitis could be predicted to cause a milder inflammation with secondary fibrosis of adjacent orbital tissues and extraocular muscles rather than palsy of the cranial nerves. A 1950 study ascribed strabismus to sinusitis in 10 cases. Diplopia was attributed to cranial nerve palsy in all 10, and most had severe acute sinusitis with multiple symptoms. Sinus treatment improved motility without strabis-

![FIGURE 4](image-url) Magnetic resonance imaging scan of a 73-year-old man with acute onset of esotropia, showing bilateral ethmoid sinusitis. Esotropia fully resolved after 1 month of antibiotic treatment.
prior strabismus could be predisposed to worsening or recurrence of strabismus if the extraocular muscles become inflamed by sinusitis. They often have less fusional reserve than normal and may be less able to adapt to changes in muscle tension.

When the strabismus patient presents with an atypical history and examination, which could represent extraocular muscle fibrosis, it is worthwhile to investigate for possible sinus disease. Sinus treatment may improve alignment if the strabismus is of recent onset. Sinus disease management may also reduce the risk of strabismus recurrence after successful surgery. Regular stretching of the extraocular muscles to prevent shortening seemed to assist some patients when they began to notice recurrent diplopia. If unexpected fibrosis of the extraocular muscles is detected at surgery, sinus evaluation may still be of value to prevent strabismus recurrence.

**REFERENCES**

Presumed Sinus-Related Strabismus


**DISCUSSION**

**DR DAVID R. STAGER, Sr.** Atypical or unexplained acquired strabismus poses a challenge to the strabismus specialist and it is therefore commendable that the authors have undertaken this attempt to relate such cases with possible sinus-related abnormalities. However, as a survey supported by the American Academy of Otolaryngology has found, 42 percent of people surveyed reported having at least one sinus infection in the last 12 months. It has been estimated that 37 million people in this country have sinus disease. This certainly creates a distinct possibility of a causal relationship between strabismus and sinus disease. From an epidemiologic standpoint, it would be important to identify the incidence of strabismus problems in patients who have sinus disease. How many patients in this study with acquired strabismus have a normal CT scan (and could serve as a control group)? The answers to these questions would help us determine whether there is a cause-and-effect relationship between sinus disease and atypical acquired strabismus or whether this relationship is coincidental. I found some of the clinical descriptions confusing. Do the measurements change as the fibrotic muscle is placed on stretch? Would that help distinguish the 25 patients with comitant esotropia or the 19 patients with suspected fourth nerve palsy versus fibrosis of the inferior rectus muscle? How does muscle fibrosis cause a high AC/A ratio?

Secondly, is there a way of documenting an inflammatory basis of eye muscle involvement such as high resolution MRI or histopathology of adjacent tissue or experiments with an animal model? Could one demonstrate an improvement of length-tension curves of the inflamed and fibrotic muscles before and after treatment of sinus disease?

Dr Ludwig has alerted us to a potential cause of acquired incomitant strabismus. Historically, she has proven herself to be a keen observer. If further investigation confirms the association of sinus disease and strabismus, we will owe her our gratitude. This will provide us with a non-surgical treatment that may be more effective than what is currently available. However, a great deal of investigative work needs to be done. What level of sinus disease can cause strabismus? What types of strabismus may be due to this syndrome, as opposed to a coincidental relationship?

Although this concept is in its infancy, and quite tenuous, Dr Ludwig’s paper is innovative and thought provoking. She does think “outside the box,” a talent that often leads to great progress.

**DR MALCOLM L. MAZOW.** You might try to determine the frequency or incidence of strabismus that occurs after significant orbital cellulitis. In my practice, it does not seem to be very common.

**DR ALLAN J. FLACH.** You seem to have converted a surgical disease into a medical disease and should be congratulated. In my adult practice, my most troublesome patients are thyroid patients. Could you comment about what you think might occur in thyroid patients when they have an orbital decompression? Does the decompression do something to the sinus and might it have some impact on your study? I do not see anything wrong with a therapeutic trial of antibiotics in your patients with this condition since it might help someone avoid surgery.

**DR ROBERT RITCH.** I came back from Germany in January with bad sinusitis, losing all sense of olfaction and taste. A couple of days after that, I developed diplopia, which turned out to be a comitant partial sixth nerve palsy. I was scheduled to get an MRI when, as Chair of this AOS program, your abstract came in the mail. A couple of days later, I was in a conference call with Dr Marilyn Miller and she mentioned she had similar cases. I performed a literature search but really could not find very much outside of severe complications, like orbital cellulitis and orbital pseudotumor. I urge you to continue to delve.
Dr. Edward L. Raab. Many of us in pediatric ophthalmology see children, especially in the hospital, who might have orbital cellulitis and actually turn out to have periorbital cellulitis. In most children old enough to have sinuses, they have a lot of evidence of sinusitis, but hardly ever any restriction to motility. Any reason to think there’s leakage into the orbit? Where there is leakage, more often than not it would result in a subperiosteal abscess, and many of those do not result in any limitation of motility. Any reason to think there’s relationship with that kind of evidence.

Dr. John T. Flynn. What muscle-stretching exercises should be performed in this context?

Dr. George B. Bartley. The frequency in this cohort of sinus surgeries seems pretty high. Did the culture results from these cases differ from what is typically seen in age-related controls that have sinusitis but yet do not require surgery?

Dr. Edward L. Raab. Many of us in pediatric ophthalmology see children, especially in the hospital, who might have orbital cellulitis and actually turn out to have periorbital cellulitis. In most children old enough to have sinuses, they have a lot of evidence of sinusitis, but hardly ever any restriction to motility. Any reason to think there’s leakage into the orbit? Where there is leakage, more often than not it would result in a subperiosteal abscess, and many of those do not result in any limitation of motility. So, it is hard to put this together as a cause-and-effect relationship with that kind of evidence.

Dr. Irene H. Ludwig. With regard to the questions of Dr. Stager, I don’t have good incidence figures on normal CT scans in this series. This was a gradual evolution of thinking, and I kept records on patients who had what I suspected to be positive sinus-related strabismus. I have now started to collect more data. My impression is that when I do suspect possible sinus-related strabismus, I find severe sinus disease about 50 percent of the time. A number of ENT colleagues have related that I have sent some of the most challenging sinus cases they have seen. There are other patients who have evidence of extraocular muscular fibrosis without evidence of thyroid disease and with negative CT scans. They may have other causes of acquired muscle fibrosis, such as rheumatologic disorders. I have some patients with strabismus and rheumatologic disorders, which may be related. Their findings are often similar to the findings in sinus-related strabismus.

It is difficult to measure young children for incomitance. The adults in this series often demonstrated mild incomitance, such as an esotropia of 25 diopters in the primary position and 35 diopters on side gaze, suggesting tightness of the medial recti or mild weakness of the lateral recti. When I perform force generation testing on these patients, they have strong muscles with no evidence of muscle palsy.

Alignment testing with prism and alternate cover for fourth cranial nerve palsy is inadequate without evaluation of torsion. The three-step test in a patient with acquired vertical strabismus due to inferior rectus fibrosis is identical to the measurements in a contralateral fourth cranial nerve palsy. The fundus exam, however, demonstrates extorsion in the lower eye when inferior rectus fibrosis is present. The forced duction test is very helpful, although the force generation test for the superior oblique is very subtle and very weak.

How might you possibly cause a high AC/A ratio with this problem? It might occur with a slight shortening of the extraocular muscles, not enough to affect the primary position or the distance alignment, but just enough that the patient can’t control alignment at near. We know that medial rectus recession will decrease a high AC/A relationship, and we know that a low AC/A relationship can be collapsed with a slight resection of the medial rectus. Therefore, it is certainly possible to develop a high AC/A relationship from a slight shortening of the medial rectus.

I do not have a good animal model or imaging method yet to look at inflammation in the eye muscles. I have consulted with several investigators at LSU but still have not been able to develop an animal model of sinusitis and strabismus. We have been developing a convenient muscle hook with a built-in strain gauge to measure length-tension curves on all surgical strabismus patients. Sinus-related strabismus patients have stiffness in their muscles, but this is a subjective finding. It would be better to have objective documentation of muscle stiffness. Sinus treatment probably does not improve muscle compliance in patients unless the process is caught early. Most of these people have had prolonged sinus disease and the best I hope for is arrest of progression. There are a number of people in this series who had strabismus recurrences. I straightened their eyes, but six months later, the strabismus recurred due to unrecognized preoperative sinusitis. It was not until the sinus disease was controlled that the strabismus stabilized.

The extraocular muscle may not be the only tissue affected by the adjacent sinusitis. The motility restrictions may be created more from the orbital tissues, the orbital septae, and the connections to the eye muscles. The patients that develop sinusitis and then diplopia probably have different orbital defects. I have a few anecdotal cases not in this series where patients have known orbital trauma with good alignment after surgery but then develop a sinus infection and strabismus.
Presumed Sinus-Related Strabismus

To answer Dr Mazow’s question about orbital cellulitis and strabismus incidence, I have seen only a few cellulitis cases, and they did not have strabismus. The patients who develop strabismus have chronic, long-term inflammation that may recur periodically over years before manifestation of strabismus. I have the impression that the patients who develop the most problems are the ones with posterior sinus disease. Their sinuses don’t drain, and they are unaware of their sinus disease. As cellulitis is treated promptly, chronic extra-ocular muscle fibrosis probably does not have time to develop.

In answer to Dr Flach’s question, I have one patient who had thyroid ophthalmopathy with an orbital decompression and extraocular muscle surgery. He indicates that every time he has a sinus infection, his strabismus returns. With prompt use of oral antibiotics and extensive exercise of his extraocular muscles, the strabismus resolves. This is just one anecdotal case, but it is an interesting one.

In response to Dr Ritch’s discussion, there is one patient in my series who developed what was diagnosed a fourth cranial nerve palsy. Being a physician, he knew that the maxillary sinus was adjacent to the eye muscles, so he treated himself with antibiotics. Within a day, the strabismus resolved. I would welcome any references you can add to my search.

To answer Dr Friedman’s question, I did not find obvious extraocular muscle changes on imaging study in my patients. I do not have access to EMGs in my practice. I do not obtain tissue for histopathologic evaluation on these patients since I have been taught to avoid disrupting the extraocular muscle tissue. Obtaining a biopsy of an extra-ocular muscle could cause increased fibrosis and adhesion.

Dr Flynn, the eye muscle stretching exercises in a young child are performed by having the parents use a target, like a toy, and encouraging the child to rotate the eyes into side gaze, upgaze, and up and obliquely to the corners since the superior obliques are often tight in these cases. For adults, it’s easier to teach them to fixate on a target, such as a television, and then have them rotate the head and hold the eyes in extreme side and upgaze for prolonged periods of time.

Dr Bartley, we did not perform cultures on these patients. Their otolaryngologists, following prolonged antibiotic use, performed the sinus surgeries.

Dr Raab commented on the number of sinusitis patients who don’t have motility restrictions. The ones that do not develop problems with strabismus are the ones who are draining or who are being treated promptly. It is the patient who is unaware of his sinus disease who seems to present with strabismus.
GRADED PARTIAL TENOTOMY OF VERTICAL RECTUS MUSCLES FOR TREATMENT OF HYPERTROPIA

BY Hye Bin Yim MD PhD, Albert W. Biglan MD,* AND Tara H. Cronin MD

ABSTRACT

Purpose: To evaluate the effectiveness of graded (adjustable intraoperatively) partial vertical rectus muscle tenotomy at the insertion in correcting small degrees of hypertropia.

Methods: All patients with best-corrected visual acuity of better than 6/30 in both eyes who over a 30-month period underwent partial tenotomy of vertical rectus muscle(s) only (no concurrent oblique muscles) were included. Improvement was evaluated 6 weeks postoperatively as change in alignment in prism diopters (PD) in primary gaze and in the field of action of the affected rectus muscle(s). Binocular function was evaluated by Titmus stereoacuity and the Worth 4-light tests.

Results: All 24 patients who met criteria for inclusion had diplopia preoperatively versus seven patients (29%) postoperatively ($P < .005$, Student's paired $t$ test). Prisms were used by six preoperatively versus two postoperatively ($P < .05$, Student’s paired $t$ test). The average vertical deviation in primary gaze decreased from 8 PD to 2 PD ($P < .005$, Student’s paired $t$ test). In the field of action of the treated rectus muscle, hypertropia decreased from an average of 8 PD to 3 PD ($P < .005$, Student’s paired $t$ test). For the preoperative and the postoperative assessments available, stereoacuity improved after 10 (56%) of the 18 procedures and Worth 4-light testing showed improvement or maintenance of fusion after 15 (79%) of 19 procedures.

Conclusions: Graded vertical rectus partial tenotomy can effectively reduce small degrees of hypertropia and associated diplopia, improve binocular function, and reduce or eliminate the need for prism correction.


INTRODUCTION

Vertical strabismus may occur as a result of decompensated congenital strabismus, or it may be acquired as the result of trauma or a disease process that affects the orbit or its contents. Although small degrees of horizontal misalignment can be corrected by horizontal vergence movements, small amounts of acquired vertical misalignment of the visual axes often cannot, and thus they will produce diplopia.

Comitant hypertropias that are small in size and symptomatic are often treated successfully with prisms. However, if the prism requirement increases, patients will complain of image distortion, glasses will become heavy, and patients will object to wearing spectacles. Deviations that are incomitant respond poorly to treatment with prisms. In these situations, surgical correction of the vertical deviation becomes a consideration.

In 2000, Alan B. Scott, MD, described a rectus muscle weakening procedure called “graded rectus muscle tenotomy,” which he performed with the patient under local anesthesia, to treat small degrees of vertical strabismus. The procedure consists of making successive small cuts in the tendon of a rectus muscle at the insertion until the desired effect is achieved.

Tenotomy of a rectus muscle is not a new procedure. During the middle of the 19th century, use of the procedure to correct strabismus evolved as an extension of its use in orthopedic surgery. The first procedures to correct strabismus were tenotomies, partial or complete. Dieffenbach, Albrecht and Ferdinand von Graefe, Stromeyer, Cunier, Gibson, and many others performed complete tenotomy of one or more rectus muscles to correct large degrees of strabismus.

Lucien Howe, in his 1908 summary of surgical techniques of the day, devoted a chapter to partial tenotomy of a rectus muscle for correction of strabismus. Partial tenotomy has also been called “graded tenotomy.”
Modifications of this technique followed, but as instrumentation improved and finer suture materials became available, tenotomy was replaced by surgical recession. After Scott's description of the usefulness of partial tenotomy for treating small degrees of vertical strabismus, we evaluated this procedure in our own patients with diplopia, to improve binocular function and to eliminate or reduce the power of prisms in those patients with prisms in their spectacles.

**METHODS**

This study was presented to the University of Pittsburgh Medical Center Institutional Review Board, which determined that this study was outside of their jurisdiction. In conformity with US Health Insurance Portability and Accountability Act (HIPAA) guidelines for maintaining patient confidentiality, our surgical coordinator identified all patients in our private practice surgical log who had undergone partial tenotomy of a vertical rectus muscle during the 30-month study period after we started to perform the procedure and who had been followed for at least 3 months postoperatively.

Patients were excluded from the study if they had concomitant oblique muscle (but not horizontal rectus muscle) surgery or if they had best-corrected visual acuity in one eye of less than 6/30. The remaining patients gave permission to include their pooled data in the study and in study publications. Each patient's clinical record was then assigned a study reference number, and data were collected under that number, without inclusion of personally identifying information.

All patients had undergone comprehensive ophthalmologic evaluation, and most had undergone measurement of their ocular alignment on at least two occasions prior to the procedure.

**Partial Tenotomy Procedure**

We performed each partial tenotomy procedure in a surgicenter with the patient under intravenous sedation and monitoring supervised by an anesthesiologist. The partial tenotomy procedure is performed as follows. After the patient has been sedated and the surgical field prepared, 0.10 mL of mepivacaine 2% is injected under the conjunctiva adjacent to the lateral border of the muscle. An incision is made down to the sclera just lateral and posterior to the muscle’s insertion. The muscle is secured with a Stevens muscle hook that is then replaced with a Jameson hook. A Stevens muscle hook is then used to reflect the conjunctiva and anterior Tenon’s capsule overlying the insertion of the muscle to expose the insertion (Figure 1). Wet-field cautery is used to blanch the anterior ciliary vessels, and then a Westcott scissors is used to cut through approximately 60% of the width of the tendon.

After partial tenotomy, sedation is suspended, the instruments and drapes are removed, and the patient is helped to a sitting position. The patient's eyeglasses or, for patients with prisms, eyeglasses created for this step without prisms, are positioned to avoid contaminating the operative area, and cover testing at distance and near is performed using targets that stimulate accommodation.

After testing, the eyeglasses are removed and the surgeon redrapes the field with sterile towels and changes gloves. If the results of cover testing indicate lack of fusion, an additional cut is made in the tendon and then the alignment is tested again. This is repeated until the patient has single binocular vision in the desired gaze positions. An antibiotic-corticosteroid drop is placed on the eye, and the patient is sent home with instructions to instill one drop of the solution daily for the next 3 days.

Patients were scheduled for follow-up visits 3 to 7 days after surgery, 6 weeks after surgery, and thereafter as considered necessary.

**Data Collection and Analysis**

Data collected from the medical records and analyzed for this study included the patient’s age at surgery, best-corrected visual acuity, presence of diplopia, cause of hypertropia, muscle(s) operated upon, use of prisms, the results of Maddox double-rod testing in patients with symptoms of cyclotropia, and whether the patient and surgeon considered the procedure to have been “successful” or “unsuccessful” in resolving the patient’s diplopia.

**FIGURE 1**

Drawing showing procedure for graded partial tenotomy of vertical rectus muscle for treatment of hypertropia. With the patient under moderate sedation, the superior rectus is exposed, the tendon is cauterized near its insertion, and a Westcott scissors is used to make a cut in the tendon. The patient is tested with eyeglasses (without prisms), and if correction of vertical deviation is inadequate, the tenotomy is extended successively until the desired effect is achieved.
Student’s paired t test was used to identify statistically significant differences between group means for preoperative and 6-week postoperative values for proportion of patients complaining of diplopia; use of prisms; deviation measured in prism diop ters (PDs) in primary, left, and right gaze and in the reading position; results of the Titmus stereoacuity test; and results of the Worth 4-light test at near (1/3 meter) and far (6 meters).

RESULTS

During the study period, a total of 30 patients underwent tenotomies of vertical rectus muscles. Five patients were excluded from the study either because they had decreased visual acuity in one eye or because surgery on an oblique muscle was performed in conjunction with rectus muscle tenotomy. A sixth patient was excluded because tenotomy was planned but at the time of the procedure it was converted to muscle recession using a suture on account of insufficient effect of the partial tenotomy. The remaining 24 patients who met study inclusion criteria had undergone a total of 26 tenotomy procedures.

The mean age of the 24 patients was 62 years (range, 19 to 83 years), and 16 (67%) of the subjects were male. Twenty-one patients had best-corrected visual acuity between 6/6 and 6/12, one patient had visual acuity of 6/15 in both eyes, one had visual acuity of 6/21 in one eye, and the final patient had best-corrected visual acuity of 6/24 in one eye. Eleven patients had a history of strabismus surgery. The mean follow-up period was 6 months (range, 1 to 29 months).

Effect of Tenotomy on Vertical Alignment
The primary outcome measure was the effect of the tenotomy procedure on vertical alignment in the primary gaze position (Table 1). Compared with the mean preoperative value of 8 PD, hypertropia at the 6-week postoperative visit was a mean of 2 PD. This mean improvement of 6 PD in primary gaze was statistically significant (P < .005, Student’s paired t test).

Among the 21 patients in whom vertical alignment in the primary gaze position was measured both early (at 1 week) and at 6 weeks after the procedure, the average improvement was 7 PD at 1 week compared with 6 PD at 6 weeks. Nine patients who were followed for longer than 6 weeks postoperatively had a mean improvement from the preoperative visit of 5 PD in primary gaze.

We also measured the effect of the tenotomy procedure on deviation in the field of action of the treated rectus muscle by comparing preoperative to 6-week postoperative values (Table 2). Of the 22 patients who underwent testing 6 weeks postoperatively, 18 had undergone unilateral tenotomy and four had vertical rectus tenotomies performed on both eyes. The average preoperative hypertropia in these patients was 8 PD, and the average postoperative hypertropia was 3 PD. The mean improvement of 5 PD was statistically significant (P < .005, Student’s paired t test).

No patient in this study had an overcorrection in primary gaze. One patient did have a transient overcorrection of 1 PD in the field of action gaze position (Table 2).

No patient has demonstrated an increase in correction with longer follow-up after tenotomy on an inferior rectus muscle.

Stereoaucity and Fusion
Stereoaucity was measured both preoperatively and 6 weeks postoperatively in 17 patients (18 procedures). Stereoaucity improved or remained the same after 10 (56%) of the 18 procedures (Table 3).

Worth fusion improved or remained the same in 15 (79%) of 19 patients who were tested both preoperatively and postoperatively (Table 3).

Diplopia
Before surgery, all 24 patients had subjective diplopia. The reasons for diplopia included cataract surgery in nine patients, cranial nerve IV palsy in six patients (one of whom had metastatic breast cancer), surgery to repair a detached retina in two patients, and thyroid eye disease or trauma in one patient each. The remaining five patients had long-standing unexplained hypertropia (Table 1).

At the most recent postoperative visit, 7 (29%) of the 24 patients had persistent diplopia. By Student’s paired t test, the postoperative reduction in proportion of patients with diplopia was statistically significant (P < .005).

Of the seven patients with persistent diplopia, two (one with restrictive strabismus after retinal detachment repair and one after trauma to the orbit) had persistent diplopia in downgaze only and were treated with prisms. Three of the seven patients had intermittent diplopia and elected no additional treatment. The remaining two patients (No. 8 and No. 23 in Table 1) had two separate intraoperative adjustable tenotomy procedures, and both procedures in both patients were considered to be failures. One of the patients had a history of four previous operations for strabismus and was unable to adjust his head position to relieve diplopia. The second patient had diplopia following cataract surgery complicated by endophthalmitis and repair of a retinal detachment. Both of these patients were subsequently, and successfully, treated by incisional surgery combined with adjustable suture techniques.

Prisms
Six (25%) of the 24 patients had prisms in their spectacles
before surgery and only two (8%) of the 24 patients required prisms at a reduced power at the most recent visit. By Student’s paired t test, the postoperative reduction in proportion of patients using prisms was statistically significant (P < .05).

**DISCUSSION**

This pilot study affirms the effectiveness of an intraoperative adjustable partial vertical rectus muscle tenotomy with associated diplopia. In our consecutive series of 24 patients with small vertical deviations and diplopia from a variety of causes, graded partial tenotomy effected mean improvement in vertical alignment in the primary gaze position of 6 PD and mean improvement in lateral gaze in the field of action of the treated muscle of 5 PD. Furthermore, about half of patients had improved binocular function. Finally, diplopia was relieved by surgery in 17 (71%) of the 24 patients, four of six who used prisms preoperatively did not need prisms after surgery, and both the surgeon and the patient rated the surgery “successful” in the majority of cases.
The effects of partial tenotomy seem to stabilize by 6 weeks after surgery, although this should be confirmed by longer follow-up. The insufficient numbers of patients in this series precluded analysis of the causes of vertical misalignment for which the procedure may be most or least successful.

Advantages Over Other Procedures for Hypertropia
Graded partial tenotomy has several advantages over other procedures for managing mild hypertropia. First, compared to the recession procedure, a partial tenotomy is easier to perform under intravenous sedation with subconjunctival anesthesia. Second, adjustments are made to the degree of correction intraoperatively, presumably giving a greater chance for success with the first operation. Third, partial tenotomy requires no suturing, thus avoiding a possible foreign body reaction, and because the sclera is not penetrated, poses less risk of penetration of the globe. Fourth, bleeding is prophylactically controlled with wet-field cautery. Fifth, by placing the incision lateral to the insertion, the conjunctiva covers the cut segment of tendon, thereby reducing the risk for infection compared to incisions placed directly over the insertion. Sixth, in our series, partial tenotomy led to only one case of overcorrection, which was only transient, in the field of action of the treated muscle. In our experience, patients with overcorrected hypertropia are displeased until the overcorrection is remedied.

<table>
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<tr>
<th>PATIENT NO.</th>
<th>PREOP DEVIATION (PD)</th>
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<th>POSTOP DEVIATION (PD)</th>
<th>POSTOP CHANGE (PD)</th>
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<td>ORTHO</td>
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<td>ORTHO</td>
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Average 8 PD 3 PD −5 PD
95% CI 7–10 PD 1–4 PD −3 to −7 PD
SD 4 PD 4 PD 5 PD

CI, confidence interval; LHT, left hypertropia; LIR, left inferior rectus; LSR, left superior rectus; N/A, not assessed (patient unable to return for 6-week visit); ORTHO, orthotropia; PD, prism diopters; Preop, preoperative; Postop, postoperative; RHT, right hypertropia; RIR, right inferior rectus; RSR, right superior rectus; SD, standard deviation.

*Second procedure.
†Combined with a recession of LIR, and resection of the LSR.
‡Patient could tolerate measurement only in primary gaze.
tendency of this procedure to not overcorrect hypertropia is desirable.

Other Applications
Graded partial tenotomy of vertical rectus muscles may have applications beyond that described in this study. For example, one of us (A.W.B.) combined intraoperative adjustable tenotomy of vertical rectus muscles with surgery on oblique muscles to treat a contracted superior rectus muscle in the presence of a long-standing superior oblique palsy. The procedure has also been performed on the contralateral yoke muscle in combination with surgery on an inferior oblique muscle. We excluded these cases from this study so that we could measure the effect of the procedure solely in the field of action of the vertical rectus muscle.

Limitations of a Graded Partial Tenotomy
Partial graded tenotomy has some limitations. It will not correct large deviations. A graded tenotomy may not be sufficient to correct hypertropia in all cases. One patient was excluded from this study because of insufficient correction with tenotomy; although improvement was observed, it was not sufficient to correct the diplopia. The tenotomy was abandoned; a suture was placed through the tendon, and the muscle was recessed using an intraoperative adjustable suture technique.

A second limitation is suggested. The further reduction of 1 PD of effect in the nine patients followed more than 3 months, when compared to the 3-month follow-up period, suggests a potential diminution in the effect with time.

Limitations of the Study
This study has some limitations. The study was conducted in a retrospective manner. Some measurements were missing in some patients. The presence of constant diplopia in some patients obviated collection of preoperative binocular function data. This would not have been an issue in a prospective study.

The fact that one of the investigators in this study was also involved in caring for the patients could have added...
bias, especially with regard to subjective results (whether the surgery was considered “successful”). However, the primary outcome measure (vertical alignment) was objective, as was testing for binocular function. Several of the measurements were obtained by staff and residents and fellows.

There is a learning curve in performing any surgical procedure. Thus, the success rate would be expected to be higher for patients operated on later in the series, although we did not analyze this factor.

**Future Research**

Extended follow-up needs to be completed to determine the long-term results of graded partial tenotomy.

Another area for further investigation is cyclotorsion that might result if a cut in the tendon shifts the vector of force that the muscle exerts on the globe either temporally or nasally, depending on where the partial tenotomy is made. In fact, two patients in this study volunteered during the intraoperative adjustment that there was torsion of the fixation target. As a result, the Maddox double-rod test was performed in several subsequent patients before and after the procedure. No cyclotorsion was detected, although this could have been due to inadequate test sensitivity.

**REFERENCES**


**DISCUSSION**

Dr Malcolm L. Mazow. Small-angle, vertical deviations of less than 10 diopters become significantly important when they present with uncontrollable, uncomfortable, intermittent or constant diplopia. This problem generally does not present with congenital or infantile strabismus unless decompensation occurs after visual maturation.

However, as mentioned by the authors, this problem frequently occurs in acquired incomitant deviations resulting from mechanical or restrictive problems as well as in paralytic strabismus. In addition, in the initial phase after correction, a patient may be left with residual debilitating diplopia.

Conventional surgery, with or without an adjustable suture, frequently will result in over- or under-correction, leaving as the only alternative glasses with prisms, which often are unacceptable to the patient. Using the adjustable myotomy technique as described by Scott, the authors have shown that small angles of vertical strabismus can be successfully treated to eliminate bothersome diplopia.

Realizing that this was a retrospective study and could not necessarily adhere to principals assessing diplopia, I would offer some suggestions. In evaluating a patient with diplopia, certain further testing would have added to the significance of the results: 1) Repeated measurements at different times of the day and on different visits might reveal a change in the angle of deviation, as would unilateral patching in the office to eliminate fusion, uncovering the maximal deviation that is present. 2) In those patients who had an area of single binocular vision in the preoperative period, binocular fields should have been done and compared to postoperative binocular fields, which would allow the observer to determine whether improvement and enlargement of the binocular fields occurred in the primary and reading positions. These points aside, the results reported are excellent, with a 71 percent elimination of diplopia, binocular improvement in 41 percent and stereoscopic improvement in 50 percent.

In general, statistical analysis may cement the results of any patient population study. With such small angles of deviation, this method of analysis becomes much less significant. Rather, we see a trend to successful elimination of diplopia by the technique utilized here.

The authors state that doing a temporal fornix incision rather than a nasal incision provides better coverage of the muscle and wound, lessening the problems associated with infection. In my experience, infection is a very rare entity in strabismus surgery and coverage seems to be adequate regardless of where the incision is made. It would be interesting to know if there was a difference in results whether the marginal myotomy was done temporally or nasally. One would wonder when the 60 percent marginal myotomy was accomplished initially and adjustment was required, what increment of increase was achieved before it was decided that no further myotomies
should be performed. Dr Biglan and his associates have shown that of the 24 patients, 15 (63 percent) were orthotropic in the primary position of gaze and 13 (54 percent) were also ortho in the field of action of the offending muscle. When looking at this analysis in more detail, 12 (80 percent) of the 15 who were ortho in the primary position were also ortho in the field of gaze. Three (20 percent) of the 15 who were ortho in the primary position were not ortho in the field of gaze. One (8 percent) of the 13 who were ortho in the field of gaze was not ortho in the primary position and one (4 percent) of the 24 was overcorrected in the field of gaze. This speaks very well for the technique in avoiding complications. The objective of any strabismus surgery is to make the patient symptom-free in the primary position and in functional fields of gaze, and these results corroborate the goal of this particular strabismus operation.

I applaud their ingenuity in trying to find a technique to correct significant vertical deviations that are seen in acquired strabismus.

DR EDWARD L. RAAB. Does it matter if you’re going from the nasal to the temporal side? This procedure seems to be analogous to one that has been reported for convergence insufficiency, which was a slanted recession of the medial recti. But, that was not sutureless. Can you, from grading the percentage of transsection, predict where the receded tendon will knock down, because what you have in effect is a hang-back from that portion of the tendon? Can you, by any triangulation or trigonometry, predict where that will reattach? Do you still need an adjustable technique to play with that?

DR ALBERT W. BIGLAN. This is a pilot study. Obtaining diplopia fields, both pre-op and post-op, are constructive suggestions to consider in proceeding with this study and binocular fields should be considered in the evaluation of additional patients.

As part of my evaluation of adults, and some children, I perform prolonged occlusion of the non-dominant eye. I will have the patient (family) place a patch on an hour or more before the patient arrives at the office. When I see the patient and complete the history, I then proceed directly to the measurement. I then remove the patch in doing so, not allowing the patient to become binocular, and perform the cover testing.

As for the infection question: I make the incision either temporal or nasal, as opposed to right over the tendon. I reflect or slide the conjunctiva over the insertion to achieve exposure of the tendon insertion. At the end of the procedure, I replace the conjunctiva so that the insertion is covered. I am attempting to protect the area of surgical procedure by covering it with conjunctiva.

How much can I cut? When you get up to about 90 percent, this gets a little risky since you have the potential of having a “lost muscle.” Another issue is torsion. I performed the Maddox double rod test pre- and post-op on several patients, but the test wasn’t sensitive enough for me to pick up a change in torsion. Two patients volunteered during the intraoperative adjustment that they actually noticed that the “E” was tilting while I was making the adjustments. These observations have prompted me to look for torsion before and after the procedure.

The slanting procedures that Nemet\textsuperscript{1,2} has advocated and that I have also written about,\textsuperscript{3} were used to reduce AC/A and V patterns. This accomplishes this by slanting the muscle. If you look at the force vectors on the superior and inferior recti, you should be inducing some torsion by laterally translating the vector of pull of the muscles.

Dr Raab asks if this procedure can be done by estimation without the intraoperative adjustment. I believe that the input of the patient is most important in obtaining the best result. I adjust, and then ask the patient what they see in the fields of gaze tested. When the patient says, “That’s single.” I then stop. In the patients that I have had to re-op, the tendon is attached to the sclera with the cut tendon adhering in a slanted position.

REFERENCES

VALUE-BASED MEDICINE AND OPHTHALMOLOGY: AN APPRAISAL OF COST-UTILITY ANALYSES

BY Gary C. Brown MD MBA,* Melissa M. Brown MD MN MBA, Sanjay Sharma MD MSc(епид) MBA, Heidi Brown BS, Lindsay Smithen MD, David B. Leeser MD, and George Beauchamp MD

ABSTRACT

Purpose: To ascertain the extent to which ophthalmologic interventions have been evaluated in value-based medicine format.

Methods: Retrospective literature review. Papers in the healthcare literature utilizing cost-utility analysis were reviewed by researchers at the Center for Value-Based Medicine, Flourtown, Pennsylvania. A literature review of papers addressing the cost-utility analysis of ophthalmologic procedures in the United States over a 12-year period from 1992 to 2003 was undertaken using the National Library of Medicine and EMBASE databases. The cost-utility of ophthalmologic interventions in inflation-adjusted (real) year 2003 US dollars expended per quality-adjusted life-year ($/QALY) was ascertained in all instances.

Results: A total of 19 papers were found, including a total of 25 interventions. The median cost-utility of ophthalmologic interventions was $5,219/QALY, with a range from $746/QALY to $6.5 million/QALY.

Conclusions: The majority of ophthalmologic interventions are especially cost-effective by conventional standards. This is because of the substantial value that ophthalmologic interventions confer to patients with eye diseases for the resources expended.


INTRODUCTION

Evidence-based medicine is the practice of medicine incorporating the highest level of scientific evidence available.1,2 Since the inception of the term in 1992,1 it has gained widespread notoriety.

Value-based medicine is the practice of medicine incorporating the highest level of evidence-based data2 with the patient-perceived value conferred by healthcare interventions for the resources (dollars) expended.4,8 Value-based medicine takes the best evidence-based data from clinical trials, then converts these data to value-based form using the preferences of patients who have lived with the disease or health state under study. The patient-perceived value of virtually any intervention in healthcare can then be compared to that of any other intervention using the quality-adjusted life-year (QALY) as a common outcome measure. When the associated costs are added, the dollars expended for the value ($/QALY) gained, or the cost-utility, can be ascertained and compared across all specialties, no matter how diverse. Cost-utility analysis is the instrument that allows a value-based medicine database to be created.

To date, the cost-utility of multiple ophthalmologic interventions has been studied. Because of the increasing importance of cost-utility analysis due to the awareness of the modality by policymakers,6 the authors undertook a study to ascertain the status of cost-utility analysis in the ophthalmic literature.

METHODS

A literature search was performed using the National
Library of Medicine (PubMed) database and the EMBASE database. The key words used in the search were ophthalmology, ocular, cost-effectiveness analysis, and cost-utility analysis. The years included in the search were 1992 through 2003. The search was confined solely to papers dealing with reimbursement in the United States because of the considerable differences in reimbursement schema, and thus the incomparability of healthcare economic analyses, among different countries. The personal experiences and familiarity of the authors with the value-based literature were also utilized to make the search as complete as possible.

For inclusion in the analysis, each paper was required to measure the outcome of value conferred by an intervention in terms of improvement in length of life and/or quality of life, both of which are incorporated in the $/QALY (dollars expended per quality-adjusted life-year). Thus, only papers reporting the results in $/QALY were utilized. It should be noted that some authors refer to healthcare economic analyses measured in $/QALY as cost-effectiveness analyses, whereas those in countries other than the United States refer to them as cost-utility analyses. The authors of the present study believe that a healthcare economic analysis reporting an outcome in $/QALY should be termed a cost-utility analysis. Papers reporting outcomes in the form of life-years gained or years of vision gained were excluded from the study.

Each paper found in the search was analyzed for the following variables: (1) the health-related quality of life analysis methodology utilized, (2) the source of the preferences used in the health-related quality-of-life analysis, (3) the treatment of comorbidities, (4) the general perspective of the analysis (eg, societal, third-party insurer, patient), (5) the individual perspective of analysis (reference case or age-specific), (6) the cost basis for facility, provider, and pharmaceutical expenditures, and (7) the annual discount rate(s) employed for costs and outcomes. Depending upon the year in which the study was undertaken, the authors of the present paper adjusted the dollars for general inflation, thus converting the results to real dollar form using year 2003 US dollars.

RESULTS

A total of 19 articles were found meeting the criteria outlined. Each of the articles reported results in the form of $/QALY, or dollars spent gained per quality-adjusted life-year gained. The total number of interventions evaluated in the 19 articles found was 25.

The cost-utility of the interventions ranged from $761/QALY to $65.5 million/QALY, with a median cost of $6,470/QALY. The most cost-effective intervention was treatment of threshold retinopathy of prematurity using laser photoagulation, with a $/QALY of $746. The least cost-effective treatment, at $6.5 million/QALY, was that for acute central retinal artery occlusion using anterior chamber paracentesis and in-hospital treatment with intermittent inhalation of 95% oxygen and 5% carbon dioxide.

Among the 25 interventions, 21 (84%) had a cost-utility under $100,000/QALY. An upper limit of $100,000/QALY has been suggested as the cutoff for interventions that are cost-effective.

A list of the ophthalmologic interventions studied with cost-utility analysis is shown in Table 1. The year the study was published is shown, as is the cost-utility of each intervention in year 2003 US dollars adjusted for general inflation according to the consumer price index. Twenty-two of the 25 interventions (88%) were in articles published within the 5-year period from 1999 through 2003, and three of 25 (12%) were published in the preceding 7 years from 1992 through 1998.

Other parameter variables of the analyses are shown in Table 2. Included among these parameters are the following:

• Utility value (patient preference) methodology. In 22 (88%) of 25 interventions, time tradeoff utility analysis was utilized. The quality of life measure used was not specified in two instances, and a multi-attribute utility value was used in one instance.

• Source of utility values. In 24 (96%) of 25 interventions, utility values obtained from patients with a health state under study were used in the analysis and in one of 25 (4%), the utility values were obtained from community members.

• Treatment of comorbidities. In 21 (84%) of 25 of studies, a holistic approach (utilizing a utility value that is disease-specific, rather than a multi-attribute utility value obtained from an aggregation of symptoms and signs such as pain, anxiety, or mobility) was undertaken, and comorbidities were not accounted for in the utility values employed. In three cases, it was unspecified whether comorbidities were accounted for, and in one case the utility values were multi-attribute in nature.

• Cost perspective. The third-party insurer perspective was utilized in 24 (96%) of 25 of analyses. The costs included in this perspective were the direct healthcare costs for providers, facilities, and drugs. The benefits included in the third-party insurer perspective included gains in quality of life and/or length of life. One analysis was performed from the societal point of view, and one paper included the third-party insurer perspective, the societal perspective, and the governmental perspective.

• Value perspective. With the exception of one study, 24 of the 25 studies were performed from the viewpoint...
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of the reference case. The reference case is the average case, using the average age and the average clinical course, of a person with a disease.

- **Costs.** The Medicare fee schedule for physicians, as well as the Medicare reimbursement for facilities, was utilized in 23 (92%) of 25 of interventions. Estimated average costs in the United States were used for two interventions. The average wholesale price for drugs was used to evaluate pharmaceutical costs.

- **Discount rate.** In each study, a 3% annual discount rate was used for costs and health outcomes. This is the rate recommended by the Panel for Cost-Effectiveness in Health and Medicine. In most studies, other discount rates were also analyzed with sensitivity analysis.

**DISCUSSION**

From 1992 through 2003, the cost-utility for 25

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**TABLE 1. COST-UTILITY ANALYSES FOR OPHTHALMOLOGIC INTERVENTIONS (YEAR 2003 US DOLLARS)**

<table>
<thead>
<tr>
<th>INTERVENTION</th>
<th>YEAR OF STUDY</th>
<th>PUBLICATION</th>
<th>$/QALY GAINED*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laser therapy for threshold ROP</td>
<td>1999</td>
<td>761</td>
<td></td>
</tr>
<tr>
<td>Cryotherapy for threshold ROP</td>
<td>1999</td>
<td>2,028</td>
<td></td>
</tr>
<tr>
<td>Vitrectomy for vitreous hemorrhage in type 1 diabetes</td>
<td>2001</td>
<td>2,038</td>
<td></td>
</tr>
<tr>
<td>Cataract surgery, initial</td>
<td>2002</td>
<td>2,093</td>
<td></td>
</tr>
<tr>
<td>Amblyopia detection and therapy</td>
<td>2002</td>
<td>2,395</td>
<td></td>
</tr>
<tr>
<td>Cataract surgery, second eye</td>
<td>2003</td>
<td>2,963</td>
<td></td>
</tr>
<tr>
<td>Repair of senile ectropion</td>
<td>2003</td>
<td>3,180</td>
<td></td>
</tr>
<tr>
<td>Laser therapy for DME</td>
<td>2000</td>
<td>3,309</td>
<td></td>
</tr>
<tr>
<td>Biweekly screening of, and cryotherapy for, threshold ROP</td>
<td>1993</td>
<td>3,623</td>
<td></td>
</tr>
<tr>
<td>Laser therapy for extrafoveal CNVM with histplasmosis</td>
<td>2000</td>
<td>4,528</td>
<td></td>
</tr>
<tr>
<td>Laser therapy for subfoveal CNVM with ARMD</td>
<td>2000</td>
<td>6,118</td>
<td></td>
</tr>
<tr>
<td>Laser therapy for macular edema associated with BRVO</td>
<td>2002</td>
<td>6,821</td>
<td></td>
</tr>
<tr>
<td>Normohyperglycemic DM management</td>
<td>1997</td>
<td>16,002</td>
<td></td>
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<tr>
<td>Laser therapy to prevent neovascular glaucoma with very ischemic CRVO</td>
<td>2000</td>
<td>16,657</td>
<td></td>
</tr>
<tr>
<td>Laser therapy for extrafoveal CNVM with ARMD</td>
<td>2003</td>
<td>23,640</td>
<td></td>
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<tr>
<td>Annual screening for retinopathy (vs every 2 yr) in high-risk type 2 diabetics</td>
<td>2000</td>
<td>43,254</td>
<td></td>
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<tr>
<td>Surgery for PVR, C3F8 (no previous vitrectomy)</td>
<td>2002</td>
<td>49,742</td>
<td></td>
</tr>
<tr>
<td>Surgery for PVR, C3F8 (previous vitrectomy)</td>
<td>2002</td>
<td>48,932</td>
<td></td>
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<tr>
<td>Surgery for PVR, silicone oil (no previous vitrectomy)</td>
<td>2002</td>
<td>42,667</td>
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<tr>
<td>Surgery for PVR, silicone oil (previous vitrectomy)</td>
<td>2002</td>
<td>66,126</td>
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<tr>
<td>Prophylactic oral ganciclovir treatment for CMV retinitis</td>
<td>1997</td>
<td>90,957</td>
<td></td>
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<tr>
<td>PDT for subfoveal CNVM with ARMD</td>
<td>2000</td>
<td>94,526</td>
<td></td>
</tr>
<tr>
<td>• 20/40 initial vision</td>
<td>2001</td>
<td>189,643</td>
<td></td>
</tr>
<tr>
<td>• 20/200 initial vision</td>
<td>2001</td>
<td>189,643</td>
<td></td>
</tr>
<tr>
<td>Treatment for acute CRAO</td>
<td>2000</td>
<td>366,104</td>
<td></td>
</tr>
<tr>
<td>• AC paracentesis</td>
<td>2000</td>
<td>6.5 million</td>
<td></td>
</tr>
<tr>
<td>• AC paracentesis and CO2/O2 therapy</td>
<td>2000</td>
<td>6.5 million</td>
<td></td>
</tr>
</tbody>
</table>

AC, anterior chamber; ARMD, age-related macular degeneration; BRVO, branch retinal vein occlusion; C3F8, perfluoropropane gas; CMV, cytomegalovirus; CNVM, choroidal neovascularization; CO2/O2, 5% carbon dioxide, 95% oxygen gas mixture; CRAO, central retinal artery occlusion; CNVM, choroidal neovascular membrane; CRVO, central retinal vein occlusion; DM, diabetes mellitus; DME, diabetic macular edema; PPT, photodynamic therapy; PVR, proliferative vitreoretinopathy; ROP, retinopathy of prematurity.

*$/QALY gained = dollars expended per quality-adjusted life-year gained.
ophthalmologic interventions was reported. The number of cost-utility value analyses performed on ophthalmologic interventions has increased considerably over the past decade. Three (16%) of the 19 papers were published during the first 6 years of the 12-year period, while 16 of 19 (84%) were published during the second 6-year period. This trend of an increasing prevalence of healthcare economic analyses has also been noted for healthcare interventions overall.36 The interest of top federal policymakers in incorporating cost-utility analysis and value-based medicine into policy correlates with the increased number of publications in the literature.9

The data presented herein demonstrate that most ophthalmologic interventions in the United States studied with cost-utility analysis are cost-effective using the conventionally accepted upper limit of $100,000/QALY.31,32 It should be noted, however, that the $100,000/QALY number is arbitrary and varies from country to country, depending upon the resources available to spend on healthcare services.8 As more

<table>
<thead>
<tr>
<th>TABLE 2. PARAMETER VARIABLES UTILIZED IN COST-UTILITY ANALYSIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTERVENTION</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Laser, ROP12</td>
</tr>
<tr>
<td>Cryo, ROP12</td>
</tr>
<tr>
<td>PPV, vit heme diabetes14</td>
</tr>
<tr>
<td>Cataract, first eye14</td>
</tr>
<tr>
<td>Amblyopia treatment15</td>
</tr>
<tr>
<td>Cataract, second eye15</td>
</tr>
<tr>
<td>Entropion repair16</td>
</tr>
<tr>
<td>Laser treatment, DME16</td>
</tr>
<tr>
<td>ROP, screening and cryotherapy16</td>
</tr>
<tr>
<td>Laser extrafoveal CNVM, histoplasmosis20</td>
</tr>
<tr>
<td>Laser, subfoveal CNVM, ARMD21</td>
</tr>
<tr>
<td>Laser, macular edema, BRVO22</td>
</tr>
<tr>
<td>Normoglycemic DM management23</td>
</tr>
<tr>
<td>Laser, ischemic CRVO24</td>
</tr>
<tr>
<td>Laser, extrafoveal CNVM, ARMD25</td>
</tr>
<tr>
<td>DR screening26</td>
</tr>
<tr>
<td>PVR, C3F8, no previous PPV27</td>
</tr>
<tr>
<td>PVR, silicone oil, no previous PPV27</td>
</tr>
<tr>
<td>PVR, C3F8, previous PPV27</td>
</tr>
<tr>
<td>PVR, silicone oil, previous PPV27</td>
</tr>
<tr>
<td>Prophylaxis, CMV retinitis28</td>
</tr>
<tr>
<td>PDT, subfoveal classic CNVM</td>
</tr>
<tr>
<td>•20/200 vision29</td>
</tr>
<tr>
<td>CRAO treatment30</td>
</tr>
<tr>
<td>•AC paracentesis + O2, CO230</td>
</tr>
</tbody>
</table>

AC, anterior chamber; ARMD, age-related macular degeneration; BRVO, branch retinal vein occlusion; C3FS, perfluoropropane gas; CMV, cytomegalovirus; CNVM, choroidal neovascular membrane; CO2/O2, 5% carbon dioxide, 95% oxygen gas mixture; CRAO, central retinal artery occlusion; CRVO, central retinal vein occlusion; DM, diabetes mellitus; DME, diabetic macular edema; DR, diabetic retinopathy; PDT, photodynamic therapy; PPV, pars plana vitrectomy; PVR, proliferative vitreoretinopathy; ROP, retinopathy of prematurity; TTO, time tradeoff; vit heme, vitreous hemorrhage.
healthcare interventions are studied with cost-utility analysis, it is very likely that this standard will change. The most common intraocular surgical intervention, cataract surgery, is particularly cost-effective, whether performed in the first eye ($2,093/QALY) or the second eye of a person who has already had cataract surgery in the first eye ($2,863/QALY). The majority of interventions in the posterior segment appear to be cost-effective as well. The exceptions are treatment of acute central retinal artery obstruction and photodynamic therapy for classic subfoveal choroidal neovascularization when the visual acuity at the time of initial treatment is poor (20/200). Nonetheless, when the visual acuity at the time of initial treatment is 20/40, photodynamic therapy for classic subfoveal choroidal neovascularization falls within the upper cost-effective limit of $100,000/QALY at $94,563/QALY.  

The comparability of many of the cost-utility analyses in ophthalmology results from the fact that the majority have been performed by a core group of researchers. This is very different for the rest of healthcare, in which most cost-utility analyses are not comparable to other cost-utility analyses.

Value-Based Medicine

Value-based medicine, as defined in previous reports, incorporates three essential components: (1) the highest level of evidence-based data, (2) the conversion of evidence-based data to value form using patient-based quality of life preferences (utility values), and (3) the integration of the associated costs with the value conferred by an intervention to yield a final value-based medicine result measured in $/QALY (cost per quality-adjusted life-year) (Figure 1). The majority of ophthalmologic interventions studied to date are cost-effective due to the great value patients place upon their vision.

Utility value analysis measures the quality of life associated with a health state. By convention, utility values range from 1.0 (perfect health) to 0.0 (death). The better the health state, the closer the utility value is to 1.0, while the poorer the health state, the closer the utility value is to 0.0. For example, the utility value associated with treated systemic arterial hypertension is 0.98, while that associated with a severe stroke is 0.34. Utility values associated with ocular diseases most closely correlate with visual acuity in the better seeing eye, rather than vision in the poorer seeing eye or the cause of the visual loss. As the vision in the better seeing eye decreases, the corresponding utility value decreases as well. Utility values are often referred to as preferences, since patients are given a theoretical choice of whether they prefer to (1) remain in their current health state or (2) risk or lose something of value (eg, their life, a proportion of remaining life, money) to return to a normal health state.

When an intervention is undertaken, utility analysis can quantify the improvement in quality of life conferred by that intervention. Quality-adjusted life-years measure the total value gained from an intervention. The number of quality-adjusted life-years gained is calculated by multiplying the improvement in utility value conferred by the intervention by the duration of the improvement in years. For example, if an intervention raises a utility value from 0.50 to 1.00 for 12 years, the total number of QALYs gained is (1.00 – 0.50) × 12 = 6.0. When the associated costs of an intervention are added, the cost-utility ($/QALY), or dollars spent for the value conferred by the intervention, can be ascertained.

Because value-based medicine incorporates patient-perceived quality of life parameters typically not factored into the primary outcomes of evidence-based trials, the accuracy of value-based medicine in quantifying the real benefit of an intervention to a patient can supersede that of evidence-based medicine. As an example, evidence-based data from a clinical trial for cancer chemotherapy might show a primary evidence-based result such as the improvement of the average life expectancy from 12 months to 13 months (an 8.3% gain in QALYs over no treatment). In this instance, value-based data also show an improvement of the average life expectancy from 12 months to 13 months, but additionally take into account the severe vomiting during this time that decreases the overall value of remaining life (in QALYs) by 30%. Thus, value-based medicine—incorporating patient-perceived quality of life parameters—shows that during the 13 months...
months there is an actual loss of 24.2% of value over no
treatment, even with the extra month of life taken into
account. Since value-based medicine provides a more
accurate measure of the patient-perceived worth of a
healthcare intervention than most primary, evidence-
based medicine outcomes, value-based medicine allows
for the practice of higher-quality medical care than value-
based medicine. Succinctly, value-based medicine
incorporates quality of life parameters and, most
important, from the patient point of view.

It should be noted that there is confusion in the
literature regarding cost-utility analysis and cost-
effectiveness analysis. Some authors in the United States
use the terms interchangeably, but authors in other
countries differentiate between the two. We agree with
the latter authors and believe that cost-utility analysis
should be reserved for those interventions that measure
outcome results in $/QALY. Cost-effectiveness analysis
measures outcomes in terms of dollars spent per life-year
lost, per year of good vision gained, or years of
disability obviated, but not in terms of $/QALY. When the
outcome of $/QALY is used, the study should be termed a
cost-utility analysis. Despite the fact that an analysis is a
cost-utility analysis, interventions measured using it are
referred to as more or less cost-effective, rather than more
or less cost-utilitarian.

Standardization
To date, the cost-utility literature has been largely
composed of publications involving vastly different input
parameters and methodologies. In regard to the quality of
life measures employed, multiple utility value instruments
and multiple respondents have been employed. Tengs and
Wallace, in a comprehensive literature review, found 30
different variants of quality of life instruments used to
obtain values for cost-utility analyses. These variants
include time tradeoff utility analysis, standard gamble
utility analysis, rating scales, and expert judgment. The
respondents from whom the quality of life values were
obtained included authors, experts, the general
community, patients, and others.

When one includes the possible general perspectives
of cost-utility analysis (third-party insurer, societal,
governmental), the most common discount rates that have
been utilized (0%, 3%, 5%), and the cost basis (eg,
Medicare fee schedule, average US costs, regional costs)
used, the number of possible variants conservatively rises
to 810. When the year of the analysis and the national
currency used in the analysis are factored in, there are
tens of different, possible cost-utility variants. Thus
it is no small surprise that the great majority of cost-utility
analyses are not comparable with other cost-utility
analyses. This lack of comparability of cost-utility analyses
likely accounts, in part, for the failure of value-based
medicine to be incorporated into public policy in the
United States at the current time. Nonetheless, it is very
likely that value-based medical standards will play a role in
clinical healthcare practice within the decade. The
following parameters should be standardized for one cost-
utility analysis to be comparable to another.

Evidence-Based Medical Data
The importance of standardization for cost-utility analyses
cannot be overemphasized. The highest level of evidence-
based data (α ≤ 0.05 and β ≤ 0.20) should be utilized,
preferably from randomized clinical trials with level 1
evidence.

Utility Analysis
The utility values should come from a standardized
database for comparability. Time tradeoff utility analysis,
the quality of life measure used in the majority of the cost-
utility analyses studied herein, has demonstrated the
highest reproducibility and construct validity among
preference-based quality of life instruments.

While some have suggested that quality of life values
from the community be used for cost-utility analyses
performed for healthcare resource distribution, the
authors agree with others who believe that utility values
derived from patients who have lived in a given
health state should be the criterion (“gold standard”).
Previous studies have shown that utility values obtained
from patients often differ considerably from those
obtained from physicians and the general community
when the latter two groups are asked to assume they had
the same health state as patients.

Comorbidities
Comorbidities have been incorporated into utility values
by some investigators, typically with the result of
decreasing the value of an intervention. For example, the
quality of life assessment methodologies of many
investigators would quantify cataract surgery as less
valuable in a patient with diabetes and cardiac disease
than in someone who is in otherwise excellent systemic
health. This runs counter to the Americans With
Disabilities Act of 1990, which forbids discrimination
against those who are disabled. Thus, utilization of any
quality of life instrument or methodology that biases
against those with comorbidities (disabilities) cannot be
incorporated into public policy. The majority of the
ophthalmologic analyses did not incorporate
comorbidities into the analysis, although one used a multi-
attribute technique that inherently alters utility values to
account for comorbidities.
Costs
The source of costs of a cost-utility analysis must also be standardized for a valid comparison of studies. The most standardized costs for providers and facilities in the United States are those used by Medicare for reimbursement. In regard to pharmaceuticals, the average wholesale price is considered the most standardized cost of a drug.

The cost perspective must also be standardized. Direct healthcare costs include provider costs, facility costs, and drug costs. Direct nonhealthcare costs include caregiver and travel costs, and indirect costs include disability payments and failure to contribute to the gross domestic product (GDP). The societal perspective includes all of the preceding costs, while the governmental perspective includes the preceding costs but ignores caregiver costs and travel costs. The third-party insurer perspective, which includes only the direct healthcare costs to an insurer (or some other payer), helps simplify an already complex methodology. The third-party insurer perspective has been used in the majority of ophthalmologic cost-utility analyses performed to date. While the societal viewpoint is more all-encompassing than the third-party insurer perspective, there is not uniform agreement on which costs to include in the societal perspective. The lack of conformity in this area is another reason cost-utility analysis has not been incorporated into public policy.

Discounting
It is generally agreed that both the costs and outcomes (QALYs) in cost-utility analyses be discounted at an annual rate of 3%. Each of the 25 studies analyzed herein used a 3% yearly rate, as suggested by the Panel on Cost-Effectiveness in Health and Medicine. Costs are discounted to account for the money they could have earned above inflation had they not been invested in healthcare services. There is some controversy in regard to the discounting of outcomes (QALYs gained), but the authors of the current paper believe the same concept must be applied—that good health now is worth more than good health in the future because it can be used to produce resources that can be invested to yield additional resources with time.

Table 3 shows the input parameter variables the authors believe are most appropriate for the performance of cost-utility analyses. While some may argue for other parameters, until a standardized format is undertaken, it is unlikely that cost-utility analysis will play a large role in healthcare delivery.

In summary, value-based medicine, the natural extension of evidence-based medicine, shows that the majority of the ophthalmologic interventions reported to date deliver excellent value for the resources expended. Value-based medicine incorporates the patient-perceived quality of life parameters associated with healthcare interventions that evidence-based medicine often ignores. In addition to providing a more accurate assessment of the overall worth of healthcare interventions than evidence-based medicine owing to the integration of patient-perceived quality of life improvement, value-based medicine provides a measure of cost-effectiveness. Interest by federal healthcare policymakers strongly suggests that value-based medicine will become an integral part of the healthcare system in the near future.

REFERENCES


<table>
<thead>
<tr>
<th>VARIABLES</th>
<th>RECOMMENDED PARAMETERS</th>
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<tbody>
<tr>
<td>Evidence-based data</td>
<td>From level 1 clinical trials</td>
</tr>
<tr>
<td>Utility analysis methodology</td>
<td>Time tradeoff</td>
</tr>
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<td>Utility respondents</td>
<td>Patients with a health state</td>
</tr>
<tr>
<td>Perspective</td>
<td>Third-party payer</td>
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<tr>
<td>Viewpoint</td>
<td>Reference case (average person)</td>
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<tr>
<td>Costs</td>
<td>Average CMS reimbursement</td>
</tr>
<tr>
<td>• Physicians</td>
<td>Average CMS reimbursement</td>
</tr>
<tr>
<td>• Hospitals</td>
<td>Average CMS reimbursement</td>
</tr>
<tr>
<td>• Ambulatory surgical centers</td>
<td>Average CMS reimbursement</td>
</tr>
<tr>
<td>• Pharmaceuticals</td>
<td>Average wholesale price</td>
</tr>
<tr>
<td>Discount rate</td>
<td>3% per year</td>
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</table>

CMS, Centers for Medicare and Medicaid Services.
47. Landy J, Stein JD, Brown GC, et al. Patient, community


DISCUSSION

Dr Charles P. Wilkinson. It should be obvious to all of us that we face a genuine health care crisis in the United States. The issues of increasing demand and costs combined with limited resources mandate change in the status quo and a closer look at the genuine value of the practices and procedures that are performed. The ability to reliably measure and compare costs and value gained will become increasingly important.

The authors are genuine experts in the field of health care economic analyses. As of March 15, these authors in different combinations have published at least 40 papers devoted to this topic, and of the 19 papers cited in this current study, 15 were written by one or more of these authors. For those of you interested in learning more about this arena, I would recommend beginning with an article in the 2003 Survey of Ophthalmology, and I have used this in preparation of this brief discussion.

When attempting to analyze papers regarding quality and costs of health care, a neophyte such as I becomes immediately impressed and, candidly, overwhelmed with the large number of terms that are employed by various authors. These include, but are not limited to, “utility analysis,” “decision analysis,” “cost-benefit analysis,” “cost-effectiveness analysis,” “cost-minimization analysis,” and “cost utility analysis.” Looking at so-called quality of life instruments, one will discover the terms “sickness-impact profile,” “short form or SF-36,” “quality of well-being scale,” “health utility index,” and “Euro-QOL.” Ophthalmic quality of life measures include the “VF-14” and “VFQ-25.” Things become even more confusing when one is confronted with terms such as “quality-adjusted life year,” “standard gamble,” “time-tradeoff,” “Markov modeling,” and “Monte Carlo simulation”!

Value-based medicine to me represents an attempt to link a given procedure’s effectiveness, cost, and value to the patient in a standardized manner, so that one procedure can be compared to another across many lines of health care. Effectiveness and cost appear to be at least logically measurable, and the major difficulty (at least for me) lies in calculating value, a term that in this context implies a favorable change in quality of life. It is very important to note that the authors believe that patients’ opinions regarding value are more legitimate than the values assigned by payers or healthy individuals.

Values are a function of the change in so-called “utilities” and are measured, not in dollars, but in terms of so called “quality-adjusted life years” that have been described by Dr Brown. The known costs in dollars of a given procedure are then applied to the change in quality-adjusted life years to provide a figure in dollars per quality-adjusted life year. This figure can then be compared to any other procedure.

I believe I may now understand the basics of value-based medicine at an elementary level. However, I have some concerns regarding the concepts associated with assigning utilities to various states of health and therefore to some of the values associated with differing disease states. My major difficulty may be a function of my inexperience, but I wonder about the genuine “reasonableness” of some of these utility values. We all know that patients highly value vision as a very critical quality-of-life variable. Nevertheless, I am astounded by the fact that patients allegedly assign the same utility values to breast cancer requiring lumpectomy or mastectomy and subsequent radiation therapy (0.89) and to 20/25 vision in the better eye and less than or equal to 20/40 in the other eye (0.87). In addition, I had difficulty in determining precisely how costs per QALY that were below $50,000 or $100,000 per QALY came to be defined as “reasonable.” As far as I can tell, these are very arbitrary figures that have been perpetuated.

I have four questions for Dr Brown:

1. Can you defend these relatively low utility values associated with mild losses of vision to payers and the public?
2. In these days of an aging “me generation,” is it realistic and acceptable to compare one patient’s opinions of how bad his or her specific problem to those of another patient with another problem? After all, things seem worse when they happen to us than to unknown individuals.
3. There are substantial differences in $20,000/QALY versus $50,000/QALY versus $100,000/QALY, and yet these seem to have been very arbitrarily determined. Are the literal numbers of practical importance?
4. Although I agree with the importance of patients’ views of their quality of life, it remains difficult for me to believe that the assignments of utilities are similar across all socioeconomic, educational, gender, and ethnic levels. Is this really true?
REFERENCE


Brown et al

Dr W. Banks Anderson, Jr. We had a practical application of this at our local VA hospital that has a prosthetic budget. One hip prosthesis costs about the same as 20 intraocular lenses. How would approach the administrator with that problem?

Dr George L. Spaeth. You are suggesting that your new measure is in some way more valid than old quality-of-life indicators. One of the problems that some of the studies that used old quality-of-life indicators, such as the VFQ, is that the old indicators are variable in terms of different groups. They might be reproducible, but whether or not they were valid was not determined. There's been no validation of any quality-of-life indicator because there has been no standard against which to validate it. You have not mentioned the work that's been done by Guralnik, and some of others, who have discussed performance-based measures in which the standard is what the person can actually do. Can the person read under different levels of light, find boxes in a room, and so forth? Why is it that you have not discussed the performance-based measures? Is not your “yardstick” at least partially arbitrary and is it not at least partially culturally dependent?

Dr David L. Guyton. There probably must be some regulation (rationing) of who gets what in this era of expensive techniques and surgeries. You suggest that value-based medicine might perform as an anti-rationing tool. How are you defining rationing?

Dr Rick Ferris. I’m very interested in outcome variables and it is the totality of the evidence that is important. I am sure you are not saying that we should throw away those outcome variables that are used as the basis of evidence-based medicine, but rather it is the combination of, here is what we have found and here is how it relates to the qualities. I wondered if you would comment about that. We have all seen data that suggest people would trade 10 percent of their life to throw away their glasses. How does this relate to your study? Many of the economic analyses that we have had to do in the past tend to be about income and age. If you’re over 65, it was virtually that your life after that point got zero value.

Dr Carol L. Shields. How does ophthalmology compare or rank with other subspecialties, like ENT or orthopedics, in value-based medicine?

Dr Gary C. Brown. The authors thank Dr Wilkinson for his scholarly review and will address his questions first.

Why do ocular utility values seem relatively low when associated with good vision? How can a relatively good visual acuity of 20/25 in the better-seeing eye have a utility as low as 0.89? The utility values we obtained were those from people with ocular diseases. Since utilities are all encompassing, they take fear of the future into account. For example, the average person with an ocular disease and 20/20 vision in each eye does not have a utility value of 1.0, but rather one of 0.97 because of the worry about losing vision in the future. Once people start to lose vision, they worry even more. Thus, a person with 20/20 vision in one eye and 20/40 or worse vision in the fellow eye has a utility value of 0.92, while if the vision is 20/25 in the better eye and 20/40 or worse in the fellow eye, the utility value is 0.89. Most ocular diseases are bilateral, and the concern that the second eye will be involved markedly diminishes quality of life and, therefore, the utility values.

Perspective. Dr Wilkinson brings up the point that adverse events always seem more serious to us than when they happen to other people. This is specifically why we recommend using utility values derived from patients who have lived with a disease in our cost-utility analyses. It has been shown that surrogate respondents typically rate the quality of life associated with a disease better than people who have that disease.

Cost-utility standards. Regarding cost-utility (cost-effectiveness) standards, Laupacis and colleagues suggested the number of $100,000 per quality-adjusted life-year (QALY) gained as the upper cutoff for cost-effectiveness in 1992 (Can. Med. Assoc. J., Feb 1992; 146: 473-481). This number continues to be used as the upper cutoff but is somewhat irrelevant at this point. A society will ultimately have to decide how much it will pay and what the upper cutoff will be. In large part, this is dependent upon the resources a society is willing to devote to healthcare services.

Utility values and demographic variables. Utility values appear to be innate to human nature. Therefore, the person with an 8th-grade education is willing to trade the same number of years in return for perfect health as the person with an 18th-grade education. Utility values have been demonstrated repeatedly to transcend gender, age, ethnicity, education level, income bracket and nationality.

Value comparison of disparate interventions. Dr Anderson’s question regarding whether total hip
into the hip surgery is not the best use of resources. Arthroplasty or 20 cataract surgeries, putting the money in the US healthcare system to pay for all healthcare interventions that work for everyone. However, if the only option is to perform one total hip replacement surgery is very close to that conferred by arthroplasty or 20 cataract surgeries, putting the money into the hip surgery is not the best use of resources.

Validity. In answer to Dr Spaeth, there are two types of validity: 1) criterion validity and 2) construct validity. Criterion validity assesses how well a health-related quality of life instrument measures up to the “criterion” or “gold standard” in the field. Since there is no gold standard for health-related quality of life instruments, the criterion validity of utility values cannot be measured. Construct validity assesses how well a health-related quality of life instrument measures what it is supposed to measure—health-related quality of life.

Typically, predicting events or behaviors shows construct validity. We have demonstrated the construct of systemic utility values by showing that they correlate with worsening symptoms and clinical signs associated with diseases. Ocular utility values correlate most closely with the visual acuity in the better-seeing eye rather than the underlying cause of visual loss. With decreasing vision, the construct validity of utility values is corroborated by the fact that there are especially large utility value drops associated with visual loss that correlates with: 1) loss of driving privileges, 2) the inability to read with low-vision aids and 3) the loss of navigating vision.

We believe that utility analysis evaluates function (motor, psychological, social, etc.) very well; but function isn’t enough. As a patient with a serious health condition, there is also concern about caregiver status, the well being of family, fear of the future, the economic consequences of disease, and many additional issues. There are about 30 or 40 different parameters we’ve come up with for quality of life and we believe that utility analysis is all encompassing. It takes every single one into account, and I know of none of the other quality-of-life instruments that can do that. It’s interesting that most people who developed the quality-of-life instruments, from my point of view, were never deathly sick themselves because they certainly missed a good number of quality-of-life parameters important to people who are seriously ill.

Anti-rationing tool. In response to Dr Guyton’s question, some people label cost-utility analysis as being a rationing tool, thinking about the ill-fated Oregon plan in the early ’90s. It is just the opposite. Two points merit discussion here.

We do not perform cost-utility analyses based on an individual patient’s age, but rather on the basis of the age of the reference case, or the case of the average patient with a disease. Age based standards would never be politically palatable since they discriminate against seniors. Reference case analyses prevent discrimination or any rationing based upon age.

Addressing the population of the entire country, we have 44 million uninsured people in the United States. For the people in that group, there is severe rationing of healthcare services. We believe that value-based medicine standards created using cost-utility analysis will identify interventions that confer negligible value, no value, or some that are even harmful. The resources saved from these value-less interventions can then be shifted to interventions that work for everybody. We therefore refer to value-based medicine as the “anti-rationing system.”

Evidence-based medicine and value-based medicine. Concerning Dr Ferris’ comment, it is impossible to have good value-based medicine standards without utilizing the highest level of evidence-based data from clinical trials. The evidence-based data are then converted to value-based (utility value) form. For example, what is the diminution in quality of life when the vision in the better-seeing eye decreases three lines from 20/20 to 20/40 on the Early Treatment Diabetic Retinopathy Study chart? And is the quality of life change the same as losing three lines of vision from 20/125 to 20/250? Furthermore, are the degrees of visual loss comparable? It turns out that the utility value decrease from 20/20 to 20/40 is ~0.12, while that for 20/125 to 20/250 is ~0.06. Thus the vision change from 20/20 to 20/40 causes twice the decrement in quality of life than the vision change from 20/125 to 20/250 does.

Evidence-based medicine and value-based medicine are inextricably linked. But value-based standards allow clinicians to deliver higher quality care than evidence-based standards because value-based medicine incorporates the quality of life variables often ignored in primary, evidence-based, clinical trial outcomes.

Value-based medicine and other specialties. Lastly, in regard to Dr Shields’ comments, ophthalmic interventions deliver extraordinary value to patients, often much more than we appreciate. To illustrate, we found the mean time tradeoff utility value of ophthalmologists at Wills Eye Hospital who were asked to assume they had severe macular degeneration to be 0.69. This means the average respondent was willing to trade approximately three of every 10 theoretical remaining years of life in return for
permanent normal vision. In contrast, patients with macular degeneration and counting fingers or worse vision in each eye had a mean time tradeoff utility value of 0.40, meaning that the average patient would have traded six of every 10 remaining years, or twice the proportion of the ophthalmologists! Because of the great value that ophthalmic interventions deliver, we believe they will be viewed very favorably in the era of value-based medicine.
COMPARISON OF INTRAOCULAR LENS POWER CALCULATION METHODS IN EYES THAT HAVE UNDERGONE LASER-ASSISTED IN-SITU KERATOMILEUSIS

By Li Wang MD PhD, Marc A. Booth MD, and Douglas D. Koch MD*

ABSTRACT

Purpose: To compare methods of calculating intraocular lens (IOL) power for cataract surgery in eyes that have undergone myopic laser-assisted in-situ keratomileusis (LASIK).

Methods: Eleven eyes of eight patients who had previously undergone myopic LASIK (amount of LASIK correction, \(-5.50 \pm 2.61\) D (SD); range, \(-8.78\) to \(-2.38\) D) and subsequently phacoemulsification with implantation of the SA60AT IOLs were included (refractive error after cataract surgery, \(-0.61 \pm 0.79\) D; range, \(-2.0\) to 1.0 D). We evaluated the accuracy of various combinations of (1) single-K versus double-K (in which pre-LASIK keratometry is used to estimate effective lens position) versions of the IOL formulas; the Feiz-Mannis method was also evaluated; (2) four methods for calculating corneal refractive power (clinical history, contact lens overrefraction, adjusted EffRP (EffRP_{adj}), and Maloney methods); and (3) four IOL formulas (SRK/T, Hoffer Q, Holladay 1, and Holladay 2). The IOL prediction error was obtained by subtracting the IOL power calculated using various methods from the power of the implanted IOL, and the F test for variances was performed to assess the consistency of the prediction performance by different methods.

Results: Compared to double-K formulas, single-K formulas predicted lower IOL powers than the power implanted and would have left patients hyperopic in the majority of the cases; the Feiz-Mannis method had the largest variance. For the Hoffer Q and Holladay 1 formulas, the variances for EffRP_{adj} were significantly smaller than those for the clinical history method (0.43 D² vs 1.74 D², \(P = .018\) for Hoffer Q; 0.75 D² vs 2.35 D², \(P = .043\) for Holladay 1). The Maloney method consistently underestimated the IOL power but had significantly smaller variances (0.19 to 0.55 D²) than those for the clinical history method (1.09 to 2.35 D²) \((P < .015)\). There were no significant differences among the variances for the four formulas when using each corneal power calculation method.

Conclusions: The most accurate method was the combination of a double-K formula and corneal values derived from EffRP_{adj}. The variances in IOL prediction error were smaller with the Maloney and EffRP_{adj} methods, and we propose a modified Maloney method and second method using Humphrey data for further evaluation.


INTRODUCTION

An unfortunate consequence of corneal refractive surgery is difficulty in accurately calculating intraocular lens (IOL) power in eyes undergoing cataract surgery.¹ These IOL power errors can be attributed primarily to three factors: (1) inaccurate measurement of anterior corneal curvature by standard keratometry or computerized videokeratography; (2) inaccurate calculation of corneal power from the anterior corneal measurement by using the standardized value for refractive index of the cornea (1.3375); this occurs because procedures that remove corneal tissue (eg, excimer laser photorefractive keratectomy [PRK] or laser-assisted in-situ keratomileusis [LASIK]) change the relationship between the front and back surfaces of the cornea; and (3) incorrect estimation of effective lens position (ELP) by the third- or fourth-generation formulas when the postoperative corneal power values are used;¹ the Haigis formula is an exception because it does not use the K-reading for ELP prediction.⁶

Several methods have been proposed to improve the accuracy of estimating corneal power in eyes that have undergone LASIK. These approaches can be categorized according to whether or not they require knowledge of data acquired before LASIK was performed. Those that depend upon pre-LASIK data and the specific values that are needed include the clinical history method¹ (manifest refraction and corneal power values), Feiz-Mannis...
method⁸ (manifest refraction and corneal power values), and a topographical method based on adjusting the measured EffRP \( \text{EffRP}_{\text{adj}} \)⁹ (manifest refraction) method. Methods that do not require knowledge of any of the pre-LASIK data include contact lens overrefraction, adjusting corneal power using a correcting factor,¹⁰ direct measurement using Orbscan topography,¹¹ and a method proposed by Maloney (Robert K. Maloney, MD, personal communication, October 2002).

Briefly, the calculations in methods evaluated in this study are as follows:

1. Clinical history: Postoperative corneal power is calculated by subtracting the change in manifest refraction at the corneal plane induced by the refractive surgical procedure from the corneal power values obtained prior to refractive surgery.⁷

2. Feiz-Mannis method: To first determine the IOL power as if the patient had not undergone corneal refractive surgery, IOL power is calculated using pre-LASIK corneal power values and the axial length measured just prior to cataract surgery. To this value is added the LASIK-induced change in refractive error divided by 0.7.

3. EffRP \( \text{EffRP}_{\text{adj}} \): The EffRP \( \text{EffRP}_{\text{adj}} \) is calculated by multiplying the LASIK-induced refractive change by 0.15 D and subtracting this value from the measured EffRP, which is displayed in the Holladay Diagnostic Summary of the EyeSys Corneal Analysis System (effective refractive index: 1.3375) (EyeSys Technologies, Inc, Houston, Texas).⁹

4. Contact lens overrefraction: Corneal power is calculated as the sum of the contact lens base curve, power, and overrefraction minus the spherical equivalent of the manifest refraction without a contact lens.

5. Maloney method: The corneal power at the center of the axial topographic map is modified according to this formula:

\[
\text{Central power} = \left[ \text{central topographic power} \times \left( \frac{376}{337.5} \right) \right] - 4.9
\]

In the third- or fourth-generation IOL calculation formulas, corneal power values are used in the calculation of the ELP. In eyes following myopic corneal refractive surgery, the calculated ELP will be erroneously anterior if the lower postoperative corneal power values are used; this results in implantation of a lower-power IOL, predisposing to a postoperative hyperopic refractive error. Aramberri⁴ proposed a modified IOL formula, in which the K-reading before refractive surgery is used to estimate the ELP and the K-reading after refractive surgery is used to calculate the IOL power (the so-called single-K formula), in contrast to the traditional method, in which one K-reading (the so-called single-K formula) is used for both calculations. Based on Aramberri’s work, we theoretically compared the IOL power calculated using single-K and double-K methods and found that single-K formulas underestimate the IOL power in myopic LASIK eyes and that the ELP-related prediction errors varied with the formulas, the amount of LASIK correction, and the axial length of the eye.¹² ¹³

The purpose of this study was to evaluate the accuracy of various methods of IOL power prediction using combinations of both single-K and double-K versions of four IOL formulas (SRK/T, Hoffer Q, Holladay 1, and Holladay 2), with four methods for calculating corneal power (clinical history, contact lens overrefraction, EffRP \( \text{EffRP}_{\text{adj}} \) and Maloney methods); the Feiz-Mannis method was also evaluated.

METHODS

Subjects
Upon obtaining institutional review board approval, we analyzed IOL power results in 11 consecutive eyes of eight patients who had previously undergone LASIK for myopia and underwent cataract surgery from July 2002 through July 2003. All cataract surgeries were performed in the same manner by the same surgeon (D.D.K.) using a temporal clear corneal incision, phacoemulsification, and implantation of the SA60AT IOL (Alcon Surgical, Inc, Fort Worth, Texas). Preoperatively, the clinical history and EffRP \( \text{EffRP}_{\text{adj}} \) methods were used for corneal power estimation, and the double-K Holladay 2 formula was used for IOL power calculation for all but eyes 7 and 8, for which the single-K Holladay 1 formula was used. Targeting at postoperative myopia of around 0.75 D, we selected either the average or the lower of the two IOL powers to minimize the risk of postoperative hyperopia.

IOL Power Calculation Methods
Retrospectively, we compared the IOL power implanted with the IOL power calculated by using the following combinations:

1. The single-K and double-K versions of each IOL calculation formula. For the SRK/T, Hoffer Q, and Holladay 1 formulas,¹⁴ ¹⁶ the single-K and double-K values were calculated by using the post-LASIK and pre-LASIK K-readings for the ELP prediction, respectively. In both of the single- and double-K versions, the post-LASIK K-reading was used in the vergence portion of the formulas. These two versions of formulas were implemented in the Excel spreadsheet. For the Holladay 2 formula, the single-K formula was used by entering only the post-LASIK corneal power value, whereas the
Comparison of Intraocular Lens Power Calculation Methods in Eyes That Have Undergone Laser-Assisted In-Situ Keratomileusis

double-K calculation was obtained by checking the “Previous RK, PRK…” box and then entering the pre-LASIK K-reading.

2. The Feiz-Mannis method and four methods for calculating corneal power: clinical history, contact lens overrefraction, EffRPadj, and Maloney method (central values from Humphrey Atlas, effective refractive index: 1.3375), and

3. Four intraocular lens calculation formulas: SRK/T, Hoffer Q, Holladay 1, and Holladay 2

IOL Prediction Error

The IOL prediction error was obtained by the following steps:

1. For a given combination of formula and corneal power value, determine by interpolation the IOL power that would give the actual postoperative manifest refraction after cataract surgery (predicted IOL power). The refractive error after cataract surgery was obtained at the most recent examination (range, 3 weeks to 1 year).

2. Subtract the predicted IOL power from the power of IOL implanted to get the IOL prediction error. Thus, a positive value indicates that formula predicts an IOL of lower power than the power of the implanted IOL; this would leave the patient hyperopic.

For example (see case 1), implantation of a 19 D IOL gave the postoperative refractive error of –0.75 D. For the double-K Holladay 2 formula and corneal power determined from clinical history method, the IOL power predicted to give this refractive error was 18.31 D; the IOL prediction error was +0.69 D.

The results were evaluated by four criteria:

1. Mean arithmetic IOL prediction error. Positive values indicate that the method underestimated the IOL power.

2. Mean absolute IOL prediction error.

3. Variance of the mean arithmetic IOL prediction error. A smaller variance indicates better consistency of the IOL prediction with that method; by adjusting to correct for the mean IOL prediction error, a better refractive outcome might be expected.

4. The number of eyes with certain refractive prediction error. With assumption that 1 D of IOL prediction error produces 0.7 D of refractive error at spectacle plane, the number of eyes with refractive prediction error of less than –1 D (IOL prediction error, < –1.43 D), within –1 to +0.5 D (refractive errors within this range are considered to be acceptable), and greater than +0.5 D (IOL prediction error, > +0.71 D) were computed for each method.

The mean IOL prediction errors produced by different methods were compared using the paired t test. The variance of the mean arithmetic IOL prediction error by various methods was tested using the F test for variances to assess the consistency of the prediction performance by different methods. A probability of less than 5% (P < .05) was considered statistically significant.

RESULTS

The mean age of the eight patients was 50 years (range, 37 to 60 years). The amount of LASIK-induced correction was –5.50 ± 2.61 D (SD) (range, –8.78 to –2.38 D), and the mean manifest refraction after cataract surgery was –0.61 ± 0.79 D (range, –2.0 to 1.0 D) (Table 1). The eye with the greatest amount of hyperopia was one of the two eyes for which we used the single-K Holladay 1 formula (case 7). To illustrate the spectrum of outcomes for each eye, the IOL prediction errors with Holladay 2 formula for all cases using various methods are shown in Table 2, and the mean arithmetic and absolute IOL prediction errors with the single-K and double-K versions of the four formulas are shown in Table 3. The numbers of eyes within certain refractive prediction errors at the spectacle plane are shown in Table 4.

Single-K Versus Double-K Versus Feiz-Mannis Approach

Comparing the single-K to the double-K versions of each of the formulas, the single-K versions tended to underestimate IOL power in the majority of the patients (Table 3); this would have left most patients hyperopic. The one exception was the single-K Hoffer Q formula, which had a mean prediction error of –0.12 D for clinical history and –0.09 D for EffRPadj. However, several of the eyes calculated with this approach also would have been hyperopic. The Feiz-Mannis method had a mean prediction error of –0.25 D to –0.78 D, but had high variances of 1.90 to 2.53 D2 and correspondingly high ranges of prediction errors (Table 3).

Because of the better performance of the double-K formulas, we compare below the results with the various methods for calculating corneal power only for double-K versions of these formulas. The contact lens overrefraction method was performed in six of 11 eyes and was found to be the least accurate method (Tables 2 and 3); therefore, this method was not evaluated with the double-K versions of the IOL formulas.

Methods for Calculating Corneal Power

With the double-K version of the four formulas, the mean
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arithmetic prediction errors produced by the clinical history method (range, –1.02 to –0.55 D) and EffRP adj (range, –0.98 to –0.54 D) were comparable (all P > .05), but significantly different from the mean arithmetic prediction errors predicted by the Maloney method (range, +0.45 to +0.90 D) (all P < .002) (Table 3). The highest myopic and hyperopic errors were –3.50 D and 2.38 D using the clinical history method, –2.09 D and 0.74 D using EffRP adj and –0.45 D and 2.03 D with the Maloney method (compared to –3.54 D and 2.88 D for the Feiz-Mannis method), respectively. The EffRP adj and Maloney methods tended to produce smaller mean absolute errors with the four formulas (range, 0.69 to 0.98 D, and 0.53 to 0.90 D, respectively) than did the clinical history method (range, 0.90 to 1.24 D), although these differences were not statistically significant (all P > .05).

For the double-K Hoffer Q and Holladay 1 formulas, the variances for EffRP adj method were significantly smaller than those for the clinical history method (0.43 D² vs 1.74 D², P = .018 for Hoffer Q; 0.75 D² vs 2.35 D², P = .043 for Holladay 1). The variances for the Maloney method with all four double-K formulas (range, 0.19 to 0.55 D²) were significantly smaller than those for the clinical history method (range, 1.09 to 2.35 D²) (all P < .015), but not for EffRP adj method (range, 0.43 to 0.75 D²) (all P > .05).

Of the 11 eyes, the numbers of eyes with refractive prediction error of –1.0 to +0.5 D were seven to eight eyes with the double-K clinical history method, seven to 10 eyes with the double-K EffRP adj method, and three to eight with the double-K Maloney method (Table 4). The highest number with refractive prediction error of –1.0 to

<table>
<thead>
<tr>
<th>Case</th>
<th>Prior LASIK Correction (D)</th>
<th>Axial Eye Length (mm)</th>
<th>Refraction Before Cataract Surgery (D)</th>
<th>IOL Power Implanted (D)</th>
<th>Refraction After Cataract Surgery (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>44</td>
<td>–4.25</td>
<td>25.97</td>
<td>–2.375</td>
<td>19</td>
</tr>
<tr>
<td>2</td>
<td>37</td>
<td>–2.38</td>
<td>24.36</td>
<td>–0.25</td>
<td>21.5</td>
</tr>
<tr>
<td>3</td>
<td>37</td>
<td>–2.75</td>
<td>24.40</td>
<td>0.125</td>
<td>21.5</td>
</tr>
<tr>
<td>4</td>
<td>49</td>
<td>–7.27</td>
<td>25.08</td>
<td>–0.50</td>
<td>25.5</td>
</tr>
<tr>
<td>5</td>
<td>49</td>
<td>–7.18</td>
<td>25.24</td>
<td>–0.75</td>
<td>23.5</td>
</tr>
<tr>
<td>6</td>
<td>60</td>
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<td>28.72</td>
<td>0</td>
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</tr>
<tr>
<td>7</td>
<td>57</td>
<td>–7.89</td>
<td>27.97</td>
<td>–0.25</td>
<td>18.5</td>
</tr>
<tr>
<td>8</td>
<td>44</td>
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<td>30.36</td>
<td>–1.50</td>
<td>15.5</td>
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<td>9</td>
<td>59</td>
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<td>23.90</td>
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<tr>
<td>10</td>
<td>53</td>
<td>–6.50</td>
<td>25.59</td>
<td>–4.50</td>
<td>23.5</td>
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<td>11</td>
<td>59</td>
<td>–2.50</td>
<td>24.30</td>
<td>–2.00</td>
<td>21.5</td>
</tr>
</tbody>
</table>

IOL, intraocular lens; LASIK, laser-assisted in-situ keratomileusis.

<table>
<thead>
<tr>
<th>Case</th>
<th>Single-K Holladay 2</th>
<th>Double-K Holladay 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Clinical History</td>
<td>Contact Lens</td>
</tr>
<tr>
<td>1</td>
<td>1.31</td>
<td>–0.43</td>
</tr>
<tr>
<td>2</td>
<td>0.35</td>
<td>N/A ‡</td>
</tr>
<tr>
<td>3</td>
<td>0.14</td>
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</tr>
<tr>
<td>4</td>
<td>0.93</td>
<td>1.65</td>
</tr>
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<td>6</td>
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<td>7</td>
<td>0.67</td>
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<td>8</td>
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</tr>
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<td>9</td>
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</tr>
<tr>
<td>10</td>
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</tr>
<tr>
<td>11</td>
<td>–0.41</td>
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</tr>
</tbody>
</table>

* A positive value indicates a lower power predicted than the power implanted and would leave patient hyperopic.
† Adjusted effective refractive power obtained from EyeSys corneal topography.
‡ Contact lens overrefraction was not performed.
Comparison of Intraocular Lens Power Calculation Methods in Eyes That Have Undergone Laser-Assisted In-Situ Keratomileusis

**Table 3. Mean Arithmetic and Absolute Intraocular Lens Prediction Error (D) Using Different Methods**

<table>
<thead>
<tr>
<th>METHODS</th>
<th>SINGLE-K FORMULA</th>
<th>DOUBLE-K FORMULA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CLINICAL HISTORY</td>
<td>CONTACT LENS OVERREFRACTION</td>
</tr>
<tr>
<td>SRK/T</td>
<td>Arithmetic error</td>
<td>Mean: 0.87 ± 1.27</td>
</tr>
<tr>
<td></td>
<td>Range: -1.12 to 3.07</td>
<td>0.09 to 8.54</td>
</tr>
<tr>
<td></td>
<td>Absolute error</td>
<td>Mean: 1.29 ± 0.78</td>
</tr>
<tr>
<td></td>
<td>Range: 0.34 to 3.07</td>
<td>0.09 to 8.54</td>
</tr>
<tr>
<td>Hoffer Q</td>
<td>Arithmetic error</td>
<td>Mean: -0.12 ± 1.27</td>
</tr>
<tr>
<td></td>
<td>Range: -2.64 to 2.10</td>
<td>-0.77 to 8.73</td>
</tr>
<tr>
<td></td>
<td>Absolute error</td>
<td>Mean: 0.83 ± 0.93</td>
</tr>
<tr>
<td></td>
<td>Range: 0.05 to 2.64</td>
<td>0.77 to 8.73</td>
</tr>
<tr>
<td>Holladay 1</td>
<td>Arithmetic error</td>
<td>Mean: 0.60 ± 1.46</td>
</tr>
<tr>
<td></td>
<td>Range: -1.61 to 3.41</td>
<td>-0.21 to 9.09</td>
</tr>
<tr>
<td></td>
<td>Absolute error</td>
<td>Mean: 1.25 ± 0.90</td>
</tr>
<tr>
<td></td>
<td>Range: 0.22 to 3.41</td>
<td>0.21 to 9.09</td>
</tr>
<tr>
<td>Holladay 2</td>
<td>Arithmetic error</td>
<td>Mean: 0.43 ± 0.95</td>
</tr>
<tr>
<td></td>
<td>Range: -1.14 to 2.01</td>
<td>-0.43 to 8.08</td>
</tr>
<tr>
<td></td>
<td>Absolute error</td>
<td>Mean: 0.85 ± 0.55</td>
</tr>
<tr>
<td></td>
<td>Range: 0.14 to 2.01</td>
<td>0.43 to 8.08</td>
</tr>
</tbody>
</table>

*Adjusted effective refractive power obtained from EyeSys corneal topography.*

+0.5 D was 10 eyes with EFFRP<sub>adj</sub> combined with double-K Hoffer Q formula.

**Comparison of the Double-K Formulas**

When comparing the mean arithmetic IOL prediction errors of the four IOL formulas, the SRK/T formula yielded significantly higher IOL powers with the clinical history, EFFRP<sub>adj</sub>, and Maloney methods than the corresponding IOL powers produced by Hoffer Q and Holladay 2 formulas (all \( P < .05 \)). However, there were no significant differences in the variances of prediction errors produced by different IOL formulas.

**DISCUSSION**

Reduced accuracy of IOL calculations following corneal refractive surgery is a clinical problem of growing importance. Although published studies suggest that the clinical history method is a helpful approach for calculating corneal power, the numbers of eyes were small and unacceptably large refractive surprises still occurred. Using the Holladay 2 formula, Randleman and associates evaluated the accuracy of several techniques for calculating IOL power in 10 LASIK eyes. They found that large refractive errors occurred with each of the methods investigated and that the clinical history method, contact lens overrefraction, or the average of these two methods provided the most accurate results. Argento and colleagues compared the predictability of various methods of IOL power calculation in seven cases (six post-LASIK eyes and one post-RK eye) using the Holladay 2, Hoffer Q, and SRK/T formulas and found that the clinical history method with the Hoffer Q formula provided the best results.

As described by Aramberri, the single-K version of IOL formulas predicts IOL powers that are too low,
predisposing to postoperative hyperopia. Our data confirm the greater accuracy of the double-K versions of three third-generation and the Holladay 2 fourth-generation IOL calculation formulas. Tables for performing double-K adjustments on third-generation formulas have been published; the Holladay 2 permits direct entry of two corneal power values for the double-K calculation. Another option is to use the Haigis formula, in which the corneal power is not used to estimate the ELP.

With double-K version of the formulas, the mean arithmetic IOL prediction errors were comparable for the clinical history and EffRPadj methods, whereas the variance tended to be smaller for EffRPadj, demonstrating better consistency of its performance. Reliable pre-LASIK keratometry and the amount of LASIK correction are key parameters when using the clinical history method. The larger variability of the clinical history method demonstrated in this study might be attributed to the fact that one more historical datum (pre-LASIK corneal power) is required than that in the EffRPadj. Also, the clinical history method relies more heavily on preoperative values, whereas the EffRPadj method is primarily based on the corneal power measured at the time of the cataract surgery and is altered by only 0.15 D for every diopter of LASIK-induced refractive change.

The Maloney method converts the corneal central power obtained from corneal topography back to the anterior corneal power [central topographic power \(\times (376/337.5)\)] and then subtracts the posterior corneal power (4.9 D), which is based on his own experience (Robert K. Maloney, MD, personal communication, October 2002). A major advantage is that historical data are not required. In our study, even with the double-K formulas, the Maloney method still consistently underestimated the IOL power and would have resulted in postoperative hyperopia. However, the variances of the IOL prediction error with all four formulas were significantly smaller than those by the clinical history method, indicating that with appropriate modification, this method might provide more consistent results. Based on the results of our 11 eyes, we suggest a modified Maloney method in which 6.1 D instead of 4.9 D is subtracted. In our series, this would have resulted in a mean deviation of \(-0.59 \pm 0.33\) D (range, \(-1.04\) to \(-0.02\) D) from the back calculated corneal power with the double-K Holladay 2 formula. Surprisingly, the posterior corneal power of 6.1 D found in our series is in good agreement with the average value of 6.2 D (range, 2.1 to 8.5 D) reported in a study by Seitz and colleagues, in which the posterior corneal surface in vivo was assessed in 263 normal participants by the scanning slit topography technique. Nevertheless, our proposed offset value of 6.1 D was based on this small sample, and further studies are needed to validate this modified Maloney method.

We also compared the central Humphrey values to EffRP values from the EyeSys unit. Most EffRP values were lower than the central Humphrey values (mean difference, \(-0.49 \pm 0.46\) D; range, \(-1.30\) to \(+0.27\) D). If we
recalculate the Maloney method using EffRP values and the double-K Holladay 2 formula, again aiming to have all eyes plano or myopic, remarkably, the new formula is unchanged: central power = [EffRP × (376/337.5)] –6.1. However, the variance for this calculation is slightly higher for EffRP than the central power of Humphrey (0.36 D² vs 0.11 D², respectively); also, the range was slightly greater (plano to –1.96 D for EffRP, vs plano to –1.04 D for Humphrey).

In contrast, for the 11 eyes, we calculated the effect of the LASIK-induced refractive change on the optimal Humphrey values and found a multiplier of 0.19 (vs the 0.15 value that we had found for EffRP). Thus, the central power of Humphrey device can be adjusted by decreasing it by 0.19 D for every diopter of LASIK-induced refractive change. This would give mean deviation of –0.07 ± 0.20 D (–0.39 to 0.28 D) from the back calculated corneal power with the double-K Holladay 2 formula; note the low standard deviation.

Consistent with the finding reported by Argento and associates, our results also revealed that the contact lens overrefraction was not reliable. This method was originally suggested to be used and found to be acceptable in eyes following refractive keratotomy; in contrast, in eyes following ablative corneal refractive surgery (ie, PRK or LASIK), it is not accurate as theoretically demonstrated in a recent study by Haigis. The Feiz-Mannis method yielded a mean IOL prediction error that was comparable with the double-K clinical history and EffRPₐₜₚ methods, but the corresponding variances tended to be large, indicating poorer consistency. Similar findings were reported by Randleman and associates.

As for the performance of the four double-K IOL formulas in eyes following myopic LASIK, the SRK/T formula yielded higher IOL powers than the corresponding IOL powers produced by Holladay 2 and Hoffer Q formulas, indicating that a lower amount of myopia should be targeted when the SRK/T formula is used; however, there were no significant differences in the variances of the IOL prediction error produced by these formulas. These findings indicate that in our series, the IOL formula used was less important than the method of calculating the appropriate corneal power, a finding that was also reported by Odenthal and colleagues.

In conclusion, our results demonstrated that EffRPₐₜₚ with double-K formulas predicted the most accurate IOL power and reduced the chances of hyperopic surprises. However, both methods that used Humphrey values (the modified Maloney method and the approach of reducing the central power by 0.19 D per D of LASIK-induced refractive change) are most promising. Although a larger study is indicated to validate the performance of these various approaches, our results suggest that an acceptable refractive outcome can be achieved in the majority of these challenging patients.

REFERENCES


IOL calculation methods have evolved greatly over the years. There are multiple additional variables to be considered including effective lens position, index of refraction, and different adjustments for myopic and hyperopic refractive surgery. The formulas are rather complex. There is even a small cottage industry of computer programs dealing with IOL calculations.

While there are many factors affecting the accuracy of IOL calculations after keratorefractive surgery, the primary problem is that current methods to measure the central corneal curvature (keratometry and topography) after keratorefractive surgery are inaccurate. They tend to overestimate corneal power (in previous myopes) causing the calculated IOL power to be too low, resulting in hyperopia.

There are a variety of potential solutions. Solution 1: Mathematically calculate the correct curvature. Taking the pre-operative K reading and subtracting the treatment effect perform this. This clinical history method has been touted as the best, but, not infrequently, results in mediocre refractive outcomes. It has the additional downside of requiring pre-operative, operative and stable post-operative data. Solution 2: Design a better instrument or method to directly measure corneal curvature. The hard contact lens (HCL) overrefraction method has many proponents, but it is time-consuming, does not work well for eyes with poor vision due to advanced cataract and also has mediocre refractive outcomes. New machines such as 3D topography or very high frequency ultrasonography may be able to directly measure corneal power in the future, but not as of yet. Solution 3: Use a fudge factor with an existing instrument, which is what Dr. Koch and colleagues did.

The recent literature demonstrates no clear consensus as to which method of IOL calculation is best after refractive surgery. Ladas et al found corneal topography a poor method to measure central corneal power and concluded that the clinical history method was the best. Randleman et al found corneal topography and K readings were poor methods; clinical history and HCL overrefraction were better methods, but an average of these last two was best. Kim et al determined that the clinical history was the best method with the HCL overrefraction the second best method. Argento et al found both HCL overrefraction and K readings poor methods to evaluate corneal curvature; they found the clinical history method the best, while adjusted K readings and corneal topography were second best. Stakheev and Balashevich found no methods very good and suggested using multiple methods and selecting the lowest corneal power as determined by these methods in order to decrease the chance of post-operative hyperopia.

Dr. Koch and colleagues’ study involved 11 eyes of
nine patients after LASIK for myopia (mean –5.50 D). They found the clinical history and HCL overrefraction methods not reliable. Double “K” formulas were better than single “K” formulas, as they more accurately predicted the post-operative effective lens position. They found Humphrey corneal topography values, when adjusted, were the most promising method to measure central corneal power. The modified Maloney method (adjusting Humphrey corneal topography results) was also quite good and had the great advantage of not requiring any pre-operative data.

Do these results hold true for hyperopes? Will newer instrumentation make direct calculations of central corneal power more accurate, obviating the need for “fudge factors”? Will adjustable intraocular lenses, such as the light adjustable lens discussed by Dr. Daniel Schwartz earlier today, make accurate calculations less important?

REFERENCES


Dr Douglas Koch. I entirely agree with the comments made by Dr Rapuano. We need larger trials to better evaluate current approaches, but, more importantly, we need a methodology to measure true corneal refractive power or an approach that permits safe, accurate, and clinically feasible postoperative modification of intraocular lens power. Until then, our data suggest that the most accurate approaches are those that partially or totally rely upon corneal topographic measurements obtained just prior to cataract surgery.
EFFICACY AND EFFICIENCY OF A NEW INVOLUTIONAL PTOSIS CORRECTION PROCEDURE COMPARED TO A TRADITIONAL APONEUROTIC APPROACH

BY Bartley R. Frueh MD,* David C. Musch PhD, AND Hector McDonald MB BCh FRCSC

ABSTRACT

Purpose: This was a retrospective study to compare the efficacy and efficiency of a new small anterior incision, minimal dissection ptosis procedure with that of a traditional anterior aponeurotic approach for the correction of aponeurotic ptosis.

Methods: The results of a chart and photograph review of 36 patients with 49 ptotic eyelids who had ptosis correction by a small-incision, minimal dissection procedure were compared with those of 36 patients with 49 ptotic eyelids who had ptosis correction by a traditional aponeurotic approach.

Results: The successful correction of the eyelid height and the rate of recommendation for reoperation were not significantly different for the 49 lids corrected in each arm of the study. The incidence of attaining good eyelid contour was significantly better in the small-incision group, where 41 (97.6%) of 42 lids evaluated by photographs had good contour compared with 29 (78.4%) of 37 lids in the traditional group. Operating time per lid was significantly less for the small-incision, minimal dissection group, 25.3 ± 13.0 minutes (range, 13 to 68 minutes), compared with 55.4 ± 16.6 minutes (range, 35 to 119) for the traditional group.

Conclusions: Compared with the traditional aponeurotic approach, the new small-incision, minimal dissection technique for ptosis correction is equally efficacious in correcting eyelid height, superior in producing desirable eyelid contour, and much quicker to perform.


INTRODUCTION

Shortly after the concepts of aponeurotic ptosis and aponeurotic surgery to correct it were introduced by Jones and associates1 in 1975, the concepts were accepted and surgery for acquired ptosis changed.1-3 The traditional dissection for aponeurotic ptosis correction involves a lid crease incision approximately 20 to 22 mm long. Through this, dissection is carried superiorly under the orbicularis oculi muscle across the width of the incision. When the orbital septum is identified, it is opened widely to expose the orbital fat. With the fat lifted back or excised, the levator aponeurosis is exposed. Inferiorly, the anterior surface of the upper half of the tarsal plate is cleared across the same width, by either excising overlying orbicularis oculi muscle or dissecting underneath it. The aponeurosis can then be shortened or tucked, suturing it to the exposed tarsal plate, commonly with three sutures.

We present here a small-incision, minimal dissection procedure for aponeurotic ptosis correction as originally conceived by one of the authors (H.M.). The results using the small-incision, minimal dissection approach are presented and compared with those obtained by the same surgeon (B.R.F.) using the traditional dissection procedure.

METHODS

The criteria for inclusion in this study included (1) a diagnosis of aponeurotic ptosis, (2) surgical correction either with the small-incision, minimal dissection method or by the traditional method, (3) surgery performed by one surgeon (B.R.F.) with a resident or fellow participating in the procedure, and (4) follow-up conducted by the primary surgeon. Exclusion criteria included previous surgery on the ptotic eyelid, concomitant surgery done at the time of ptosis repair, and/or a follow-up of less than 2 months.

Fifty-one charts of consecutive patients who had the small-incision, minimal dissection procedure between July 2001 and July 2003 were reviewed. Fifteen patients

*Presenter.

Bold type indicates AOS member.
were excluded: six had insufficient follow-up, five had concomitant surgery, two procedures were reoperations, and two patients were determined to have had an underlying etiology other than aponeurotic ptosis. This left 36 patients for the study, 13 of whom had bilateral surgery. Sixty-three charts of nonconsecutive hapazardly chosen patients who had the traditional procedure between March 1990 and December 1996 were reviewed to come up with an equal number of patients with the same bilaterality as the small-incision, minimal dissection group. Twenty-seven were excluded: 10 had inadequate follow-up, 10 had concomitant surgery, four procedures were reoperations, and three patients were determined not to have aponeurotic ptosis. After reviewing the first 61 charts, 25 unilateral cases had been selected and 12 bilateral cases. Only bilateral cases were then reviewed to obtain the 13th bilateral case. No charts for the interval from January 1997 to June 2001 were reviewed because the surgical methodology used in that interval did not meet the selection criteria.

Information recorded for each patient in the study included age, male or female sex, ocular diseases, previous surgery on the operated side, other medical conditions, family history of ptosis, side(s) involved with ptosis, preoperative and postoperative lid excursion for each side, levator force, preoperative and postoperative distance of each lid above the center of the pupil for each side, number of sutures used, surgical time, presence of exposure keratitis postoperatively, whether the patient was clinically considered to be overcorrected or undercorrected, the lid contour judged from postoperative photographs, and whether reoperation was recommended. The study protocol was approved by the University of Michigan’s institutional review board.

Prior to data gathering, a successful outcome was defined as each lid being within 0.5 mm of the other and the operated lid being within 2 to 4 mm above the center of the pupil with the patient looking in the primary position.

Small- Incision, Minimal Dissection Procedure
The patient, in the supine position on the operating table, is asked to look straight ahead, up at the ceiling. A vertical line is drawn on the upper lid, in line with the center of the pupil. Another line is drawn in the lid crease, centered on the vertical line and about 8 to 10 mm long. Local anesthetic, an equal mixture of 1.0% Xylocaine with epinephrine 1:100,000 and 0.75% bupivacaine, is infiltrated beneath the skin of the lid crease line. The anesthetic needle is then passed vertically through the vertical line at the center of the tarsus, through the skin and orbicularis until the tarsal plate is felt but not penetrated, and additional local anesthetic is injected. A total of less than 0.6 mL of local anesthetic is used per eyelid.

The marked lid crease is incised through the skin. A sharp scissors, aimed toward the center of the tarsal plate, is used to bluntly spread the orbicularis fibers until the tarsal plate comes into view through the semitransparent levator aponeurosis (Figure 1A). The aponeurosis is incised with sharp scissors horizontally over the tarsal plate using numerous small snips to obtain a defect that is approximately 8 mm wide (Figure 1B). The lower edge of the skin incision is pulled down, and sharp scissors are used to bluntly dissect superiorly under the cut aponeurosis until it is free from the underlying tarsal plate and Müller's muscle, a distance of about 12 to 15 mm (Figure 1C). An 8-mm spatula needle on a permanent 6-0 suture is then passed through this space, in line with the vertical lid marking, as high as it will reach, and then curved forward and brought out the upper edge of the incision just posterior to the orbicularis oculi muscle (Figure 1D). The two ends of the suture are then grasped and held inferiorly, leaving a little slack in the suture. The lights are dimmed, and the patient is requested to open his or her eyes and look up. A firm tug should be felt on the suture if it is through the aponeurosis at the upper extent of its passage. For the infrequent times that a firm tug is not felt, the suture is repassed and the pull on upgaze rechecked. After it is ascertained that the aponeurosis has been engaged by the suture, the needle is passed horizontally through the tarsal plate in mid tarsus, centered on the vertical lid mark. The suture is tied with a surgeon's knot to an estimate of the correct tension, and a slipknot is placed over the surgeon's knot.

The patient is then asked to sit up and open his or her eyes. The eyelid height and contour are inspected. The suture tension is adjusted until the height seems optimal. If the eyelid cannot be elevated sufficiently with the suture tightly tied, it is removed and replaced higher in the aponeurosis. If the lid appears low medially or laterally, the dissection is extended in that direction by incising skin, extending the aponeurosis incision, and then spreading superiorly. An additional suture is similarly placed and tied. When the lid position seems optimal with the patient seated, the patient again assumes the supine position and each suture is tied permanently. The skin is closed.

Description of Patients
The average age of the patients undergoing small-incision, minimal dissection ptosis correction was 63 years, with a range of 14 to 82 years. There were nine male and 27 female patients. The average age of the patients undergoing traditional ptosis surgery was 62 years, with a range of 17 to 92 years. There were 10 male and 26 female patients.

In the small-incision, minimal dissection group, 13
Small-incision, minimal dissection procedure. The steps from dissection through the orbicularis to passing the needle through the aponeurosis, shown in cross-section. A. After incising the skin, the orbicularis is bluntly spread to expose the aponeurosis over the midtarsal plate. B. The aponeurosis is incised. C. Dissection is bluntly carried superiorly posterior to the aponeurosis. D. The suture is passed through the dissected space through the posterior surface of the aponeurosis and out the anterior surface, posterior to the orbicularis oculi muscle.
patients had bilateral correction; of the 23 unilateral surgeries, 15 were on the right side and 8 were on the left side. In the traditional group, 13 patients had bilateral correction; of the 23 unilateral surgeries, 12 were on the right side and 11 were on the left side.

Nineteen of the patients in the small-incision, minimal dissection group had previous surgery in or around the eye on the ptotic side, and five had had more than one procedure. Previous surgeries included 11 cataract surgeries, three glaucoma surgeries, two corneal transplants, and two retinal surgeries. There were one each of dacryocystorhinostomy, LASIK, strabismus surgery, and removal of an orbital cavernous hemangioma. Twenty-one of the patients in the traditional group had previous surgery in or around the eye on the ptotic side, and six had more than one procedure. Previous surgeries included eight cataract surgeries, one glaucoma surgery, two corneal transplants, six retinal surgeries, three enucleations (with each of these patients having a secondary orbital implant), two punctual occlusions, one dacryocystorhinostomy, and one strabismus surgery.

In the small-incision, minimal dissection group, 24 patients had a history of other medical conditions. The most common were hypertension (14 patients), diabetes mellitus (five patients), stable thyroid abnormality (five patients), and rheumatoid arthritis (one patient). In the traditional group, 22 patients had a history of other medical conditions. The most common were hypertension (eight patients), diabetes mellitus (three patients), stable thyroid abnormality (six patients), and rheumatoid arthritis (three patients).

There was a family history of ptosis in three patients in the small-incision, minimal dissection group and in seven patients in the traditional group.

**Statistical Methods**

Comparison between groups of patient-level data (eg, age, sex) made use of two-sample independent statistical tests (eg, Student’s t test, chi-square test). Because lid height contrasts involved data from 26 eyelids of 13 patients who had bilateral procedures and 23 eyelids of 23 patients who had unilateral procedures in each group, comparisons of means treated subjects as a random effect and thereby adjusted for intereye dependency, using SAS Proc Mixed software. For comparisons of frequencies between groups, the generalized estimating equation was used to adjust for intereye dependency using SAS Proc Genmod software.

**RESULTS**

A summary of the results for each group and significance of differences is in Table 1. In the small-incision, minimal dissection group, the criteria of the lids being within 0.5 mm of each other and the operated lid being between 2 and 4 mm above the center of the pupil were met for 24 (66.7%) of the 36 patients, with both lids being low on one bilateral patient. In the traditional group, using the same criteria, criteria were met in 22 (61.1%) of 36 patients, with both lids being low on one bilateral patient.

The lid height difference postoperatively was ≤0.5 mm in 27 patients (75%) in the small-incision, minimal dissection group, with six of the nine failures being in the bilateral group. Of these six, one was a bilateral undercorrection, three were unilateral undercorrections (<2 mm), one was asymmetry of 1.5 mm with each lid in the acceptable range of 2 to 4 mm, and one was a unilateral overcorrection (5.0 mm above the center of the pupil). Of the three unilateral cases, two were <2 mm above the pupil, and one was 2.5 mm but 1.5 mm lower than the other side. The difference between the two sides when the difference was greater than 0.5 mm was 3.0 mm in one patient, 2.0 mm in one patient, 1.5 mm in four patients, and 1.0 mm in two patients. The lid height difference postoperatively was ≤0.5 mm in 23 patients (63.9%) in the traditional group, with four of the failures being in the bilateral group. Of these four, two were unilateral undercorrections and two had asymmetry of 1.0 mm with each lid in the acceptable range of 2 to 4 mm. For the nine unilateral cases, two were undercorrections, one was an overcorrection (5.0 mm above the center of the pupil), and six were in the range of 2.0 to 4.0 mm above the center of the pupil, but each had the operated side higher. The difference between the two sides when the difference was greater than 0.5 mm was 3.0 mm in three patients, 2.0 mm in six patients, and 1.0 mm in four patients.

The clinical decision to recommend reoperation in the small-incision, minimal dissection group was made for only the nine patients (25%) who showed >0.5 mm difference in eyelid height. There were six patients with one lid 1.5 mm above the center of the pupil and the other lid 2 mm. In two of these patients, the lowest lid was the unoperated lid, each having fallen 0.5 mm compared to the preoperative position. All six were satisfied with their lid heights, and reoperation was not recommended. In the traditional group, clinical decision for reoperation was made in 11 patients (31%), nine of whom showed >0.5 mm difference in eyelid height, one bilateral case where each lid was similarly low, and one case where the central lid height was fine but the lid was low medially. For this last patient, the medial suture was replaced in the office 1 week postoperatively. Recommendation for reoperation among patients with a lid difference of >0.5 mm was not made in four patients, three with a difference of 1.0 mm and one with a difference of 2.0 mm. Of the three with 1.0 mm difference, one patient had bilateral surgery, and in
two patients the operated side was the highest and both lids were within normal range. One 70-year-old patient with the operated lid at 3.5 mm and the unoperated lid at 1.5 mm felt she could see fine and was pleased, so additional surgery was not recommended.

The frequency of a patient not meeting the criteria of the lids being within 0.5 mm of each other and the operated lid being between 2 and 4 mm above the center of the pupil and within 0.5 mm of each other was not significantly different in the two groups. The frequency of a lid being <2 mm or >4 mm above the center of the pupil when evaluated using SAS Genmod with generalized estimating equation to adjust for intereye dependency is not significantly different between the small-incision, minimal dissection group and the traditional group ($P = .40$). The incidence of the lids not being within 0.5 mm of each other was not significantly different in the two groups.

The frequency of the clinical decision to recommend reoperation, analyzed using the chi-square test, is not significantly different in the two groups ($P = .80$).

Of the 49 lids operated on with the small-incision procedure, 34 received one suture, nine received two sutures, all placed laterally, and six received three sutures. Of the 49 lids operated on with the traditional procedure, two received one suture, three received two sutures, all placed medially, and 44 received three sutures.

The eyelid contour, as judged from postoperative photographs, was normal in all but one lid of the 32 patients (10 bilateral) with available postoperative photos in the small-incision, minimal dissection group. In that patient, the lid was lower laterally and had received three sutures. In the traditional group, the lid contour was normal in 29 lids of 27 patients (10 bilateral) with available postoperative photographs, with one flat centrally, three low medially, and four low laterally. The frequency of a good lid contour, 41 (97.6%) of 42 for the small-incision, minimal dissection group, is significantly greater than found for the traditional group, 29 (78.4%) of 37 by the Fisher exact test ($P = .01$).

The time for each procedure was obtained from the operating room records. The mean time from the beginning of the procedure to closure of the wound for the small-incision, minimal dissection group was 26.3 minutes (SE = 0.1 minute), with a range of 13 to 68 minutes. The mean operating time for the traditional group was 56.6 minutes (SE = 2.5 minutes), with a range of 35 to 119 minutes. The mean time required for the small-incision, minimal dissection group is significantly less than for the traditional group ($P < .0001$).

**DISCUSSION**

The small-incision, minimal dissection ptosis correction procedure is easy to do and to teach. The minimal dissection required means that the anatomy is less disrupted and probably explains the significantly higher rate of good eyelid contour outcome. It is a procedure that is usable usually only in eyelids that have not had previous lid surgery or trauma, because the anatomy should be in its original state to allow the blunt dissection. The first pass of the needle through the aponeurosis is nearly always effective in securing it but should always be checked by having the patient look up. One suture was sufficient in 69% of the cases, and all had a good contour outcome. The dissection to add a second suture is quick: of the patients requiring two sutures, the time of operation was less than the mean time in five, and only one of the others was an outlier, at 50 minutes. Of the six patients requiring three sutures, four were outliers, requiring 50 minutes, 68 minutes, and a bilateral case averaging 61 minutes per side. The extra time spent on these outliers suggests there was difficulty getting the contour satisfactory, although
the only nonsatisfactory contour was in one lid of a bilateral patient with three sutures per side but averaging 32 minutes per side.

Use of a single suture was introduced by Liu in 1993. Meltzer and coworkers in 2001 presented excellent results with an adjustable single suture. Lucarelli and Lemke published the first small-incision ptosis procedure and used primarily a single suture, adding additional sutures as needed, in a similar fashion as in this report. However, their dissection was similar to the traditional dissection—finding and opening the orbital septum, retracting the fat to identify the levator aponeurosis, cutting the aponeurosis from Miller's muscle, and then attaching it to tarsus. They state that the procedure requires less operative time than a full-incision external levator repair, but no data were provided.

Although there was no attempt to match the patients in the two groups beyond meeting the inclusion and exclusion criteria, and ensuring that the same number of bilateral cases were included in the traditional group as in the small-incision, minimal dissection group, they are remarkably similar in age, female-male mix, prior surgery on the affected side, and concomitant medical conditions. There is a preponderance of women in each group, 75% in the small-incision, minimal dissection group and 72% in the traditional group. In the failed cases of McCulley and coworkers, 52 (72.2%) of 72 subjects were female, and in the 125 successful cases they analyzed, 72 (57.6%) were women. In the patients of Bartley and coworkers, 9 (52.9%) of 17 were women. In the series of Lucarelli and Lemke, 11 (64.7%) of 17 patients were female.

Although this is a retrospective study, there are complete data for every aspect studied except for postoperative photographs to judge contour. Photos were available for 89% of the small-incision, minimal dissection group and 75% of the traditional group. Looking at those for whom reoperation was recommended, seven (78%) of nine patients in the small-incision, minimal dissection group and 6 (55%) of 11 of the traditional group had photographs. Circumstance (photography department was closed) or error of omission, rather than bias, accounts for the less than 100% availability of photographs, because it is our policy to get photographs of every postoperative ptosis-correction patient at the 2-month postoperative visit. The photograph evaluator (B.R.F.) was not masked to the surgical method used; evaluation by a different, masked observer would be preferable. To comply with IRB/HIPAA regulations, and not have to contact each subject for permission, each chart and accompanying photographs were examined and the data recorded, and the records were returned, with no link remaining to connect the data collected to the patient. We believe the drawbacks of less than complete availability of photographs and nonmasked evaluation do not change the conclusion that good contour was achieved in a higher percentage of the small-incision, minimal dissection group than of the traditional group.

The ideal position of a corrected ptotic lid will depend on at least three factors: whether the patient has a dry-eye problem or even reduced tear production on Schirmer's test; the height of the other eyelid, if the ptosis is unilateral; and the height of the patient's lid prior to the onset of the ptosis. These are factored in and a clinical judgment is arrived at. For this reason, we did not calculate the mean elevation or the mean final position of the operated lids. Means would not convey the desired outcomes of surgery.

Whereas follow-up time was not contrasted between groups, all patients had a minimum of 2 months of follow-up. For the past 15 years, based on prior experience, when patients have adequate lid position at 2 months, they are discharged from care. This approach is supported by the data of Doxanas, who followed 150 patients for a minimum of 3 years and up to 5 years and reported no case of late recurrence. If patients were overcorrected or undercorrected, this was evident 1 week postoperatively in his patients. Berlin and Vestal did report 15 failures in 62 patients occurring after the 6-week visit when using 6-0 polyglactin suture. Use of absorbable suture in aponeurotic ptosis correction can lead to late failure, and this knowledge had led the surgeon (B.R.F.) to sole use of permanent suture for aponeurotic ptosis correction.

Table 2 shows the outcomes of 11 investigators in relation to meeting their criteria for successful lid position when operating for acquired ptosis. Only two of the 11 reports defining success specify not only a maximum difference in lid height but a range above the pupil that is acceptable. These two reports presented the most detailed and useful information on surgical outcome. Although Table 2 shows specific success rates for the report by McCulley and coworkers, these are estimates, with a range of success within a 95% confidence limit of 71% to 83% for unilateral patients and 67% to 82% for bilateral patients. The success rates in McCulley and coworkers and Berlin and Vestal are similar to ours, given our tighter criteria for lid difference and maximum acceptable eyelid height. It seems important that both difference in lid height and an acceptable range of lid position should be specified to truly define success. Clearly, two lids that postoperatively bisect the center of the pupil represent failed operations, although there is no difference between the height of the two lids.

The acceptable difference in lid height above the center of the pupil was 1.0 mm in most of the reports. One report found a difference of 1.5 mm acceptable for a successful operation. Using that criterion, 20 (28%) of our
72 patients did not need surgery in the first place. The same report had data imbedded which showed that if a 1.0-mm difference was used, the success rate dropped from 90.1% to 83.1%, and if a 0.5 mm difference was used, it dropped to 64.3%. Only one report mentioned success as being within 0.5 mm. Tight criteria for success in this report were chosen prior to obtaining the data: we sometimes operate for 1.0 mm of ptosis, and a normal range for lid position above the center of the pupil seemed to be 2 to 4 mm. Three (6.5%) of the 46 unilateral cases had a 1 mm difference in lid height preoperatively, confirming that judgment. Berlin and Vestal made an important philosophic comment: “The success rate is determined by what one is willing to accept as a good result. Expectations were less with earlier techniques and therefore, more postoperative ptosis was tolerated than would be considered cosmetically acceptable today.” This is every bit as true 15 years later.

Reoperation rate, which was not significantly different between the two groups, has a significant subjective component that does not necessarily align with the results of eyelid height. In one instance, a patient in the traditional group with 2 mm difference in ptosis, and a normal range for lid position above the center of the pupil seemed to be 2 to 4 mm. Three (6.5%) of the 46 unilateral cases had a 1 mm difference in lid height preoperatively, confirming that judgment. Berlin and Vestal made an important philosophic comment: “The success rate is determined by what one is willing to accept as a good result. Expectations were less with earlier techniques and therefore, more postoperative ptosis was tolerated than would be considered cosmetically acceptable today.” This is every bit as true 15 years later.

Table 2. Other Reports of the Results of Surgery for Correcting Aponeurotic Ptosis

<table>
<thead>
<tr>
<th>Author et al.</th>
<th>Year</th>
<th>Difference Used and Range</th>
<th>Timing of Determination</th>
<th>No. Meeting Criteria</th>
<th>No. Done</th>
<th>% Meeting Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anderson et al.</td>
<td>1979</td>
<td>1.0 mm</td>
<td></td>
<td>10</td>
<td>12</td>
<td>83.3</td>
</tr>
<tr>
<td>Older</td>
<td>1983</td>
<td>1.0 mm</td>
<td></td>
<td>108</td>
<td>113</td>
<td>95.6</td>
</tr>
<tr>
<td>Putterman et al.</td>
<td>1986</td>
<td>1.5 mm (1.0 mm) (0.5 mm)</td>
<td></td>
<td>192</td>
<td>213</td>
<td>90.1</td>
</tr>
<tr>
<td>Berlin et al.</td>
<td>1989</td>
<td>1.0 mm and 2-4.5 mm</td>
<td>Long term, up to 6.7 mo</td>
<td>62</td>
<td>87</td>
<td>71.3</td>
</tr>
<tr>
<td>Shore et al.</td>
<td>1990</td>
<td>1.0 mm and ≥3 mm for bilat</td>
<td></td>
<td>178</td>
<td>207</td>
<td>89.5</td>
</tr>
<tr>
<td>Doxanas</td>
<td>1992</td>
<td>1.0 mm</td>
<td></td>
<td>96</td>
<td>92</td>
<td>93.5</td>
</tr>
<tr>
<td>Liu</td>
<td>1993</td>
<td>1.0 mm</td>
<td></td>
<td>162</td>
<td>169</td>
<td>95.9</td>
</tr>
<tr>
<td>Bartley et al.</td>
<td>1996</td>
<td>1.0 mm</td>
<td></td>
<td>11</td>
<td>17</td>
<td>64.7</td>
</tr>
<tr>
<td>Lucarelli et al.</td>
<td>1999</td>
<td>1.0 mm</td>
<td></td>
<td>25</td>
<td>28</td>
<td>89.3</td>
</tr>
<tr>
<td>Meltzer et al.</td>
<td>2001</td>
<td>0.5 mm</td>
<td></td>
<td>46</td>
<td>51</td>
<td>89.3</td>
</tr>
<tr>
<td>McCulley et al.</td>
<td>2003</td>
<td>1.0 mm and 2-4.5 mm</td>
<td>Unilateral</td>
<td>404</td>
<td>77.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Bilateral</td>
<td>399</td>
<td>76.0</td>
<td></td>
</tr>
</tbody>
</table>

*The success rates for McCulley and coworkers are estimates with a range, so no numbers meeting criteria are given.

While McCulley and coworkers found a similar success rate for unilateral and bilateral ptosis correction, 77% and 76% respectively, the reoperation rate was very different, at 5.2% for those having had unilateral surgery and 13% for those having had bilateral surgery. In our small-incision, minimal dissection group, 13% of the unilateral patients were recommended to have reoperation compared to 46% of the bilateral group. This divergence was not seen in the traditional group, where 30% of the unilateral and 31% of the bilateral patients were recommended to have reoperation. The numbers in our groups are too small to show these differences to be significant. However, on the basis of these data, we have increased our awareness of differences in bilateral cases.

Overcorrections occurred in only one patient in each of the two groups in this report, or two (7%) of the 28 that did not meet our strictest criteria. Bartley and coworkers reported overcorrections in four of six patients (67%) who had a difference of >1 mm between the two lids. Considering that they felt the four higher lids were each properly positioned, they had no functional overcorrections among the 17 eyelids and the likelihood of functional overcorrection was similar to ours. McCulley and coworkers reported overcorrections in 39 (54%) of 72 failures. These differences probably reflect the conservative bias of the surgeon (B.R.F.): an undercorrection is less onerous than a symptomatic dry eye from an upper eyelid that is too high.

In the United States, we are working at a time of decreasing surgical reimbursements. The Michigan Medicare screen for code 67904, correction of ptosis, external approach, has decreased 40% since 1995, from $942 to $554. Overhead has increased in that interval. That the small-incision, minimal dissection procedure can
be done in less than half the time, on average, thus has economic significance. That the standard error is less with this procedure means there is greater predictability of the operating time, facilitating scheduling. We found no other reports of operative time to use for comparison.

In conclusion, the outcome of small-incision, minimal dissection ptosis correction in terms of eyelid height is similar to that of the traditional dissection, and success in achieving a good eyelid contour is significantly greater with the small-incision, minimal dissection procedure. The small-incision, minimal dissection procedure is significantly quicker to perform. These findings should make the small-incision, minimal dissection ptosis correction procedure the surgical procedure of choice in previously unoperated cases of aponeurotic ptosis to be corrected with aponeurotic surgery.

ACKNOWLEDGMENT

The authors thank Kenneth E. Guire, MS, Department of Biostatistics, School of Public Health, University of Michigan, for his sophisticated data analysis that enabled bilaterality of some procedures to be appropriately dealt with in outcome evaluation.

REFERENCES


DISCUSSION

Dr GEORGE B. BARTLEY. When entering the operating room, it is useful to remember the aphorism “the longer you stay, the longer you stay”, as it reminds us to get the job done with as little invasion as possible, and then get out.

A corollary lesson from this fine study by Dr Frueh and coworkers might be: “the more you do, the more you do.” Specifically, the dissection used in the traditional approach to levator aponeurosis repair required more sutures—3 of them in 90% of cases—but yielded less favorable results in terms of eyelid contour. This has an interesting implication: given that the primary difference between the traditional “open sky” technique and the small incision/minimal dissection is maintenance of orbital septum integrity, perhaps the role of this structure in eyelid support and function is more important than is generally assumed. Sounds like a potential AOS thesis to me.

While we’re thinking about the orbital septum, two other caveats deserve mention. First, it’s critical for occasional ptosis surgeons who may wish to adopt the small incision technique to avoid snagging the septum when the levator aponeurosis is advanced, because doing so carries the risk of eyelid lag and lagophthalmos. Second, the small incision technique may not be practical in Asian eyelids that lack an eyelid crease. In such eyelids the orbital septum fuses with the aponeurosis much more inferiorly than in occidental eyelids, allowing the preaponeurotic fat to extend down over the tarsal plate. Transgression of the orbital septum is inevitable unless the incision is made just superior to the eyelash follicles.

Moving from anatomy to outcomes, Dr Frueh states “Objective measurements of eyelid position are a better benchmark of surgical success than re-operation rate.” This unquestionably is true when doing a comparative study, but the re-operation rate is a useful measure of patient satisfaction given that ptosis surgery has a significant subjective component. This is particularly true when surgery is performed to correct 1 mm of ptosis, cases that are done primarily for aesthetic improvement rather than to enlarge the superior visual field.

An editorial comment about reimbursement for CPT code 67904. Since moving to Florida, I have been
surprised and dismayed by how often third-party payers deny payment for the repair of ptosis, even when the eyelid rests inferior to the pupil or is completely closed, as may occur with an oculomotor nerve palsy. Because the reimbursement for ptosis repair has dropped considerably during the past decade and may well be below cost for many practices, perhaps oculoplastic surgeons should be arguing that the operation is indeed cosmetic, as this would allow charges to be set at market rates. That might raise a few eyebrows, if not eyelids.

**Dr Bartley Frueh.** I thank Dr Bartley for his cogent discussion.
VASCULAR PERFUSION OF CHOROIDAL MELANOMA BY 3.0 TESLA MAGNETIC RESONANCE IMAGING

BY Bruce M. Buerk MD, Jose S. Pulido MD MS,* Ignacio Chiong, Robert Folberg MD, Deepak P. Edward MD, Mark T. Duffy MD PhD, AND Keith R. Thulborn MD PhD

ABSTRACT

Purpose: Because signal-to-noise performance improves with increased magnetic field strength, the quality of magnetic resonance images is greater at 3.0 tesla (T) than at 1.5 T. Because of the longer T1 values at higher field strength, intravenously administered magnetic resonance contrast agents provide improved T1 enhancement at 3.0 T. We have used these factors to obtain high-quality contrast-enhanced imaging of small intraocular lesions using a standard head radiofrequency volume coil. Specifically, we have examined lesion size and magnitude of maximum contrast enhancement in a series of intraocular melanomas before and during therapy.

Methods: Eighteen patients with intraocular masses were examined by 3.0 T magnetic resonance imaging (MRI) including intravenous contrast enhancement. Precontrast images were acquired through the orbits followed by sequential post-contrast images at 1-minute intervals for 5 minutes. The magnitude of contrast enhancement of the lesion, extraocular muscles, and brain parenchyma was measured as a percentage increase in magnetic resonance signal over the pre-enhancement signal intensity.

Results: Lesions demonstrated different levels of enhancement ranging up to 130%. Three patterns of enhancement—0% to 20%, 20% to 50%, and >50%—were identified. Brain parenchyma, benign lesions, and responsive tumors following brachytherapy with 125I demonstrated enhancement of less than 20%. Four choroidal melanomas showed intermediate (20% to 50%) levels of enhancement. Four malignant lesions (three melanomas, one metastatic tumor), as well as the extraocular muscles, showed strong, rapid enhancement (>50%). Four patients who had MRI studies before and following plaque brachytherapy ultimately demonstrated a decline in the contrast enhancement following treatment.

Conclusions: Contrast enhancement of intraocular lesions measured by 3.0 T MRI demonstrates different patterns of enhancement that may be useful for indicating the degree of malignancy and in monitoring response to therapy.


INTRODUCTION

The evaluation of choroidal melanomas is based on the clinical findings by ophthalmoscopy and ultrasonography. Greater than 99% of choroidal melanomas can be correctly diagnosed by experienced observers using these methods.* Ancillary testing, such as fluorescein angiography, computed tomography, and magnetic resonance imaging (MRI), is used primarily to differentiate types of mass lesions and to define their extent.

Although most clinical scanners are still 1.5 tesla (T), recently introduced 3.0 T scanners are rapidly proving that the higher signal-to-noise performance of higher magnetic field improves image spatial and temporal resolution. Dedicated MRI studies of the orbits are now possible with the standard head coil rather than having to resort to specialized surface coils. The longer longitudinal relaxation times (T1) at higher field make the same dose of intravenous contrast agent more effective, thereby enhancing T1-weighted images that can be acquired rapidly through the orbits. Despite the small size of intraocular lesions, sequential images can be obtained following contrast administration to allow the maximum enhancement to be determined accurately. This study was undertaken to evaluate the contrast enhancement characteristics of 18 intraocular mass lesions (14 choroidal melanomas, one metastatic lesion, two subretinal hemorrhages, and a choroidal nevus).

From the Department of Ophthalmology and Visual Sciences (Drs Buerk, Pulido, Folberg, Edward, and Duffy), the Center for Magnetic Resonance Research (Mr Chiong, Dr Thulborn), and the Department of Pathology and Laboratory Sciences (Dr Folberg), University of Illinois at Chicago, Chicago, Illinois. Supported in part by grant EY10457 and Core grant EY01792 from the National Eye Institute, National Institutes of Health, as well as an unrestricted grant from Research to Prevent Blindness, Inc, New York, New York.

*Presenter.

Bold type indicates AOS member.
METHODS

We studied 18 patients from June 2000 to September 2002 who presented to the Retina Service at the University of Illinois at Chicago with intraocular masses. All patients received a complete ophthalmic examination including ultrasonography. Fluorescein angiography was performed on six of 18 patients. All patients underwent a complete metastatic evaluation by the internal medicine service.

This retrospective study of ocular MRI examinations was performed under the approval of the institutional review board. Magnetic resonance imaging with intravenous contrast enhancement was performed on a 3.0 T whole-body scanner (Signa V Hi 3T; General Electric Medical Systems, Milwaukee, Wisconsin). Sagittal T1-weighted FLAIR (fluid attenuated inversion recovery) images (TR = 2,613 ms, TE = 16 ms, FOV = 24 cm², acquisition matrix = 512 × 192, image thickness = 5 mm) through the head were used for graphic prescription of axial T2-weighted fast-spin echo images (TR = 5,000 ms, TE = 102 ms, FOV = 15 cm², acquisition matrix = 416 × 416, image thickness = 1.5 mm, gap = 0.5 mm) through the orbits. Precontrast axial T1-weighted spin echo images (TR = 400 ms, TE = 19 ms, FOV = 14 cm², acquisition matrix = 416 × 416, image thickness = 3 mm, gap = 0.5 mm) were then obtained through the ocular lesion. For lesions placed in the superior or inferior regions of the globe, images were obtained in the coronal plane for improved visualization.

Following administration of gadolinium-diethylenetriamine pentaacetic acid (DTPA) contrast (Omniscan, 0.1 mmol/kg) via an antecubital vein, 5 volumes of same T1-weighted spin echo images were acquired through the globes at 1-minute intervals for 5 minutes. The time-dependent enhancement patterns were obtained using the average signal intensity from five separate regions of interest placed over each of three locations: the ocular lesion, the brain gray matter, and the extraocular muscles, as a function of time (Functools; General Electric Medical Systems, Milwaukee, Wisconsin). The average and standard deviation of the maximum enhancement for each region were calculated as the percentage difference between the preenhancement and maximum postenhancement signal intensities divided by the preenhancement signal intensity for each tissue on the T1-weighted images (Microsoft Excel; Microsoft Corporation, Everett, Washington). The gray matter of the brain served as a measure of signal intensity stability over time, because no enhancement should occur away from blood vessels when the blood-brain barrier is intact. The muscle served as a measure of enhancement expected for normally perfused tissue.

Lesion size was documented from the contrast-enhanced T1-weighted images as the longest baseline dimension in the axial or coronal plane and the greatest thickness of the lesion from the sclera into the vitreous.

Four eyes (patients 10, 15, 17, and 18) with large uveal melanomas were treated by enucleation. Each eye was fixed in 10% neutral buffered formalin and stained with hematoxylin-eosin and the periodic acid–Schiff stain without hematoxylin counterstaining to demonstrate vasculogenic mimicry looping patterns.

RESULTS

Table 1 categorizes the patients’ clinical information (including age, race, sex), magnetic resonance characteristics of the lesion, magnetic resonance contrast enhancement characteristics, histopathologic findings, and presence or absence of microcirculatory networks. The shape and location of these lesions on MRI correlated with the clinical examination. All of the uveal lesions were well visualized on both T1-weighted images (T1WI) and T2-weighted images (T2WI). Fourteen of the lesions suspected of being choroidal melanomas were found to be hyperintense relative to vitreous on T1WI and hypointense relative to vitreous on T2WI (Figure 1). In contrast, the lesions from age-related macular degeneration and the nevus were dark on T1-weighted images. The size as measured by ultrasonography correlated well with the size measured on MRI.

The contrast enhancement of these lesions demonstrated three patterns of enhancement: <25%, 25% to 50%, and >50% (Figure 2). Benign lesions or some lesions following plaque brachytherapy with ¹²⁵I demonstrated enhancement of less than 20%. One melanoma lesion (patient 1) did not enhance, but this patient had recently undergone chemotherapy for a primary lung adenocarcinoma, presumably also treating the coincidental ocular lesion. There were four choroidal melanomas with an intermediate (25% to 50%) degree of enhancement. Eight lesions (seven melanomas, one metastatic tumor) showed strong, rapid enhancement (>50%). The four patients (patients 5, 6, 8, and 17) who had contrast enhancement studies following plaque brachytherapy (Figure 3) showed declines in contrast enhancement following treatment (range, 3 to 17 months following treatment). Two of the patients (patients 5 and 8) initially demonstrated a strong increase in perfusion following brachytherapy, followed by a decline in contrast enhancement.

Four globes (from patients 10, 15, 17, and 18) were enucleated and examined by histopathology (Table 1). Three of the four tumors demonstrated spindle B melanoma cells. One tumor was composed of mixed epithelioid and spindle B tumor cells. No microcirculatory loops or networks were identified in any of the specimens.
DISCUSSION

Although ophthalmoscopy and ultrasonography remain the primary modalities for evaluating intraocular masses, MRI can provide additional information on difficult cases regarding diagnosis and evaluation of extraocular extension. Some investigators have noted decreased effectiveness of MRI in imaging amelanotic lesions. Choroidal melanomas are hyperintense on T1WI and hypointense on T2WI relative to the vitreous humor. These findings are due to the shortened T1 and T2 relaxation times of choroidal melanomas compared with surrounding structures. The shortened relaxation times are due to the paramagnetic properties of melanin. Melanin has been shown to produce stable free radicals, which can cause proton relaxation enhancement, thereby shortening both T1 and T2 time values. In contrast, most other ocular tumors and tumors elsewhere in the body generally have prolonged T1 and T2 times. The addition of gadolinium-DTPA has been found to enhance tumor delineation by shortening the T1 relaxation time to produce increased signal intensity on T1WI. This effect is further enhanced at 3.0 T compared with 1.5 T.

This study was undertaken to evaluate whether the degree of intravenous contrast enhancement of intraocular masses, as demonstrated on a 3.0 T scanner using the standard head coil, could be used to distinguish benign from malignant lesions and to follow response of malignancies to brachytherapy. The enhancement findings of the lesions were compared with muscle, which demonstrates strong, rapid perfusion, and brain, which does not normally enhance (Figure 2). There were three patterns of perfusion noted: 0% to 25%, 25% to 50%, and >50%. Among the 0% to 25% group, the lesions were benign or previously treated choroidal melanomas. Although one patient (patient 1) had an active choroidal melanoma, she had received chemotherapy for a primary lung adenocarcinoma, which may have altered the enhancement of the choroidal melanoma. Four tumors demonstrated intermediate (25% to 50%) patterns of perfusion, and eight lesions demonstrated strong, rapid uptake of contrast (>50%), consistent with metastatic or malignant lesions.

The effect of plaque brachytherapy on degree of enhancement was evaluated. All tumors treated with...
FIGURE 1A
T1-weighted image of orbit demonstrating hyperintense mass relative to vitreous of right eye. This choroidal melanoma has an associated retinal detachment that appears slightly less hyperintense.

FIGURE 1B
T2-weighted image of orbit of same case shown in 1A, demonstrating hypointense mass relative to vitreous of right eye. Note that in the T2-weighted image, the choroidal melanoma is seen as more hypointense than the overlying retinal detachment and the vitreous cavity, which has the most hyperintense signal in the eye.

FIGURE 1C
Histopathology of the same case shown in 1A, demonstrating an array of spindle B cells and epithelioid cells. No vascular looping patterns were seen by periodic acid-Schiff staining.

FIGURE 1D
Plot of the change in vascular enhancement of the same choroidal melanoma shown in 1A over time. Note that there is a maximum enhancement of 40% over the initial level.

FIGURE 1E
T1-weighted image of the orbit of a large ciliary body melanoma. Note the shrunken right eye with the enhancing mass extending from the wall of the globe into the anterior chamber.

FIGURE 1F
T2-weighted image demonstrating the hypointensity of the large ciliary body melanoma shown in 1E, extending into the anterior chamber. The vitreous is hyperintense compared with the melanoma.
plaque brachytherapy demonstrated a decline in the percentage of enhancement. Interestingly, two of the tumors initially had an increase in enhancement after treatment followed by a subsequent decline in enhancement. We hypothesize that radiation damage to the tumor vasculature initially enhanced leakage of contrast material from the tumor circulation but later decreased as vessels underwent thrombosis. Prior studies demonstrated thickening, thrombosis, and blood vessel damage following radiation treatment to choroidal melanomas.

Four tumors were enucleated and evaluated by histopathology. Three of the tumors were composed of spindle B cells, and one tumor was composed of mixed spindle B and epithelioid tumor cells. We examined the tumor for the presence of microcirculatory loops and networks. The presence of these networks has been correlated with an increased risk of metastatic disease. No tumors had microcirculatory loops or networks. With a larger cohort of tumors, future areas of investigation may include associations between contrast enhancement of choroidal melanomas and microcirculatory patterns.

Other imaging modalities for imaging the vascular pattern of choroidal melanomas are currently being investigated. Indocyanine green (ICG) angiography is a technique utilizing the ability of long near-infrared wavelength to penetrate the retinal pigment epithelium. The ICG is highly protein-bound and therefore remains predominantly within the choroidal vessels. Confocal ICG scanning laser ophthalmoscopy has improved resolution to view vessels to 20 mm. Investigators have used the confocal ICG scanning laser to image choroidal melanoma microcirculation patterns that have been correlated with increased risk of metastatic death. These vessels have been shown to regress after plaque brachytherapy, and their presence in small melanocytic tumors is predictive for future growth.

Doppler ultrasound technology has also been used to demonstrate the vascular perfusion of choroidal tumors.
Guthoff and associates demonstrated blood flow within choroidal melanomas and a decrease in Doppler shift following plaque brachytherapy. They felt that persistent circulation in areas of tumor following plaque brachytherapy likely represented areas of tumor viability. Lieb and colleagues demonstrated no flow on Doppler ultrasound in nonmalignant lesions. They also showed lowered blood flow in choroidal melanomas after treatment with plaque brachytherapy. Silverman and associates were able to differentiate choroidal melanomas into high- and low-risk groups by correlating ultrasound spectrum analysis with histopathologic presence or absence of extravascular matrix patterns on the basis of power spectrum analysis of raw radiofrequency ultrasound data. This technique detects patterns within tumors without detecting blood flow.

None of our patients who underwent enucleation and whose tumors were available for histopathologic study (cases 10, 15, 17, and 18) have experienced metastatic disease, but the follow-up interval is short. None of these patients showed evidence of extravascular matrix patterning in their tumors, but three of these tumors (in patients 10, 17, and 18) were recorded as having “high % enhancement” by 3.0 T MRI imaging. These early data suggest that 3.0 T MRI may be measuring effects related to larger vessels and not from vasculogenic mimicry patterns.

In summary, contrast dynamic imaging has been shown to be of value at 1.5 T to evaluate the vascularity of lesions, and with even better spatial and temporal resolution at 3 T, the ability to evaluate internal tumor characteristics has improved further. This small retrospective study demonstrates that 3.0 T MRI detects different enhancement patterns for different intraocular masses. There seem to be three patterns of enhancement: 0% to 25%, 25% to 50%, and >50%. Benign or treated lesions demonstrate perfusion under 25%. Malignant lesions seem to demonstrate enhancement greater than 25%, with most choroidal melanomas demonstrating strong enhancement (>50%), suggesting a malignant process. Following plaque brachytherapy, the enhancement may initially increase, but then declines without any increase and often with a decrease in lesion size. Further studies are needed to ascertain if this contrast enhancement pattern can be used to monitor therapy for ocular malignancies.

REFERENCES

Vascular Perfusion of Choroidal Melanoma by 3.0 Tesla Magnetic Resonance Imaging


**DISCUSSION**

**DR JAMES J. AUGSBURGER.** The authors of this report ostensibly determined the vascular perfusion of choroidal melanomas and a number of other lesions using 3 Tesla magnetic resonance imaging. Regrettably, they really didn’t do what they said they did. All they did was determine the contrast enhancement of evaluated lesions. Too few lesions were evaluated to allow any meaningful conclusion about the relative enhancement patterns of choroidal melanomas and alternative tumors. The authors reported post-imaging survival data on only a few of their patients, so one cannot make any scientifically valid statement about the prognostic value of the obtained information.

Rather than dwell on specific shortcomings of this paper, I’d like to ask an important ethical question raised by this study. My question is “Who should pay for investigational clinical studies?” In any exploratory clinical research study such as this one, I believe that the cost of the studies being evaluated should be borne by the researchers or their grants and not by the patients or their insurers. The authors of this report will no doubt counter that MRI with contrast is not in and of itself an experimental technique. They will undoubtedly also assert that MRI’s of the orbits and brain are performed frequently in many centers for other indications and even sometimes for evaluation of intraocular mass lesions. I agree completely with both of these assertions. However, MRI’s of the orbits and brain or any other clinical studies (including fluorescein angiograms, ICG angiograms, OCT’s, corneal topographic maps, etc.) obtained primarily for the purpose of producing descriptive clinical data about the spectrum of findings in lesions of different types but which provide no information that changes subsequent patient management are clearly research studies. As such, they should not be billed to the patients or their insurers.

**DR GERHARD W. CIBIS.** I would like to address the issue of billing a patient for a test that may not in any way
influence the treatment or outcome of that individual. Having run an electrophysiology lab where, frequently, the answers from my electroretinograms did nothing for the treatment of the patient, I think the information gathered in that way and paid for by the insurance companies was nonetheless extremely valuable.

Dr Jose S. Pulido. I would like to thank Dr Augsburger for having sent to me a critique of an earlier manuscript. Some of his criticisms have been addressed in the submitted manuscript and it has helped to improve this groundbreaking study. In regards to the specific MR imaging methods, these have been added in this manuscript. As for the relationship between enhancement and perfusion, they are strongly associated and the MR literature oscillates between the two. For instance, Molls et al state, “The gadolinium-induced enhancement of signal intensity versus noise in the early phase after bolus injection is a reliable parameter for the perfusion in the regions of interest….Thus the pre-therapeutic investigation of blood flow (dynamic CT, MRI, Positron emission tomography) can contribute to a more individual planning of treatment (decision for or against hyperthermia in addition to radiotherapy or chemotherapy).”

Furman-Haran and her colleagues state, “The kinetics and extent of signal enhancement are related to changes in the concentration of the contrast agent….Physiological models used to analyze contrast-enhanced images and obtain perfusion parameters of tracer kinetics were based on the work of… Problem is good high temporal and comcomitant spatial resolution while at the same time maintaining a sufficient signal-to-noise ratio.”

Let me address the issue of the use of MR imaging. First, we had IRB approval. Secondly, one of the great strengths of ophthalmology is our specialization to our small area of the body. On the other hand, this can also turn into a weakness because we have to resist the urge to be insular and provincial when it comes to advances elsewhere in the body. I believe that Dr Augsburger’s criticisms come from not knowing the extensive literature regarding the clinical use of MRI for evaluation of vascularity for many other forms of tumors throughout the body and the brain.

Last year, Dr Paul Lauterbur from the University of Illinois won a Nobel Prize for his pioneering work in magnetic resonance imaging. The University of Illinois has always been at the forefront in MR imaging and the work from Dr Mahmood Mafee has set the bar for the use of MR in orbital diseases. Dr Augsburger’s misperceptions about the value of MR probably arose from the early studies using the 0.5 Tesla machines and the early 1.5 Tesla machines where the spatial resolution was poor. By the late 90s the resolution was good enough that MR imaging at least rivaled ultrasound for detection of extracocular extension.

During the same time period, the temporal resolution was being exploited by others to look at the vascularity of tumors in the breast, the brain, and other areas in the body. Indeed, Kuhl and colleagues wrote, “The diagnostic value of the early-phase enhancement rate criterion has been established by the findings of several recent studies…In practice, evaluation of lesion time course kinetics has already had considerable effect on the management of lesions in breast MR imaging.”

Furman-Haran and colleagues state, “In contrast to histological assessment, contrast-enhanced magnetic resonance imaging, which is increasingly employed in the clinic, is a highly sensitive, noninvasive technique for detecting and diagnosing the nature of breast lesions. Moreover, it has great value as a quantitative tool for mapping the unique properties of tumor vasculature.”

Another quotation showing the value of perfusion imaging was from Degani and colleagues who wrote, “It can monitor alterations in vascular parameters during tumor development, in response to treatment and during metastatic spread. The use of this methodology in the clinic has proven to be a highly efficient technique of the diagnosis and prognosis of malignant lesions.”

With the advent of 3Tesla imaging, which parenthetically, was championed by one of my co-authors, high spatial as well as temporal resolution is possible and this is becoming the unit of choice throughout the United States. We are presently working on a 9.4 Tesla machine.

Finally, considering that vascularity is a separate prognostic indicator and therapeutic indicator elsewhere in body and that work from another of my co-authors, Dr. Folberg, as referenced in the manuscript, shows that it is also important for uveal melanomas and we recommend the use of the special ultrasound machine championed by Dr Coleman or the 3Tesla imaging that we are using to monitor patients.

REFERENCES
4. Mafee MF, Peyman GA. Retinal choroidal detachments:


DOES MEDICAL TREATMENT INFLUENCE THE SUCCESS OF TRABECULECTOMY?

BY Allan Joseph Flach PharmD MD*

ABSTRACT

Purpose: Many ophthalmologists believe that long-term use of topically applied glaucoma medications can adversely affect results of fistulizing surgery. This presentation critically analyzes the published studies most often cited in support of this view to determine whether this conclusion is justified.

Methods: Morphologic effects of long-term treatment with antiglaucoma drugs on the conjunctiva and Tenon’s capsule in glaucomatous patients have been studied. The results of these studies encouraged investigators to examine the influence of prior therapy on the success of trabeculectomy performed in patients with open-angle glaucoma. From this work, many have concluded that long-term use of topically applied glaucoma medications can adversely affect the results of fistulizing surgery. These results and conclusions are summarized and critically analyzed to determine whether this conclusion is justified.

Results: Morphologic studies describe increased numbers of macrophages, fibroblasts, lymphocytes, and mast cells in conjunctival and Tenon’s capsule specimens taken from patients receiving long-term antiglaucoma drugs. These findings suggest a potential for more inflammation and subsequent scarring following trabeculectomies in these patients. Efforts to confirm the clinical relevance of these histologic findings in open-angle glaucoma patients with a history of long-term antiglaucoma medication prior to surgery have been published. These retrospective, nonrandomized, unmasked studies of open-angle glaucoma patients include treatment groups and surgeries that are not comparable. In addition, the medical treatments within these studies do not reflect our current approaches to the medical management of open-angle glaucoma.

Conclusions: At present, there is no convincing clinical evidence that long-term medical treatments influence the success of contemporary trabeculectomy surgery performed on open-angle glaucoma patients.


INTRODUCTION

Many ophthalmologists believe that long-term administration of topically applied glaucoma medications can adversely affect the results of subsequent fistulizing surgery. This belief is based on the results of histologic examinations and clinical studies of patients with open-angle glaucoma with and without prior long-term medical treatments.1-5 These studies provide histologic evidence that long-term medical treatment of open-angle glaucoma induces a subclinical conjunctival inflammation that may predispose to scarring.1-5 Furthermore, subsequent clinical studies provide results that have encouraged investigators to conclude that long-term medical treatment of open-angle glaucoma patients is detrimental to the outcome of trabeculectomy surgery.1-4 The purpose of this presentation is to summarize and critically review the results and conclusions of these studies in an effort to determine whether this conclusion is justified.

METHODS

The morphologic effects of long-term glaucoma drug treatment on the conjunctiva and Tenon’s capsule in open-angle glaucoma patients have been studied.1-2 The results of these studies indicate that long-term medical therapy, administered before fistulizing surgery is performed, increases the number of tissue inflammatory cells. These histologic observations suggest that extensive medical treatment induces a subclinical inflammation that may enhance the risk of external bleb scarring and filtration surgery failure. This clinical speculation encouraged investigators to study the influence of prior medical therapy on the success of trabeculectomy performed in...
open-angle glaucoma patients in an attempt to link the histologic laboratory observations with an undesirable surgical outcome.3,5

Two groups of patients with open-angle glaucoma, one group with a history of long-term medical treatment (1 year to 17 years) and the other with less than 8 weeks of treatment, were carefully examined after glaucoma filtering surgery. The intraocular pressures (IOPs) of all of these open-angle glaucoma patients were measured and compared 18 months after surgery. The results of this retrospective, unmasked clinical study encouraged investigators to conclude that the use of topically applied glaucoma medications for 1 or more years can adversely affect the results of fistulizing surgery.7

Furthermore, a second clinical study reports confirmation that long-term topical therapy has adverse effects on the conjunctiva and that these detrimental changes correlate with failure of trabeculectomies.3 These results and the associated conclusions are summarized and critically analyzed in an effort to determine whether it is justified to conclude that the success rate of contemporary trabeculectomy performed in open-angle glaucoma patients is jeopardized by long-term medical treatment.

RESULTS

 Conjunctival and Tenon’s capsule biopsies from patients with and without long-term medical treatment were analyzed by light microscopy.1 Group A consisted of 20 patients with a mean age of 58, all with open-angle glaucoma and a history of no more than 3 weeks of treatment with pilocarpine. These patients represent the primary surgical treatment group. Patients within group B had a mean age of 65 and were treated with at least two glaucoma medications for at least 1 year (mean, 7.7 years) before surgery. Both groups of patients submitted biopsy specimens for histologic examination by masked observers who were unaware of the patients’ prior treatments. Specimens taken from the patients who received long-term glaucoma medication (group B) demonstrated increased numbers of macrophages, fibroblasts, lymphocytes, and mast cells within conjunctiva and Tenon’s capsule as compared to specimens from patients receiving minimal treatment. In addition, there appeared to be a decrease in goblet cells in group B. Subsequently, the results of a more extensive histologic study of 126 patients comparing various medical treatment regimens and their influence inducing subclinical inflammation within the conjunctiva prior to filtration surgery supported this observation.5

These histologic observations demonstrate that more subclinical inflammation exists within the conjunctiva of patients who have received extensive medical therapy as compared to minimal medical treatment. These histologic results have encouraged speculation that the subclinical inflammation induced by long-term medical therapy may increase the chance of bleb failure after filtration surgery. This potential conclusion, in turn, suggests important questions regarding the optimum time for filtration surgery and the use of medications in the management of patients with open-angle glaucoma.

Therefore, these clinically relevant speculations prompted investigators to design clinical studies to investigate the effect of previous medical therapy on the outcome of subsequent glaucoma filtration surgery in an effort to correlate the observed histologic observations of subclinical inflammation with clinically significant scarring and fibrosis resulting in failed trabeculectomies.5 A pivotal clinical study compares two groups of open-angle glaucoma patients 18 months after filtration surgery. One group consists of 47 eyes of 47 patients with less than 8 weeks of medical treatment. This is the primary trabeculectomy (PT) group. The subjects within the PT group are taken, retrospectively, from a prior clinical study that compared the results of medical, laser, and surgical treatments of open-angle glaucoma patients.3 The second group is composed of 43 eyes of 34 patients with 1 to 17 years of medical treatment. This group is identified as the multiple treatment (MT) group. An IOP of less than 21 mm Hg without treatment is considered a surgical success, whereas an IOP greater than 21 mm Hg without treatment is considered a failure. The results of this study show a significant difference in surgical success between the MT group (9/43 failed) and the PT group (1/47 failed) with P < .005. In addition, at 3 months following surgery, the PT group had IOPs of 13.5 ± 5.7 mm Hg as compared to the MT group, which had IOPs of 18.1 ± 9.9 mm Hg (P = .008). The investigators conclude that long-term use (>1 year) of topically applied glaucoma medications can adversely affect the results of fistulizing surgery. These conclusions have influenced the clinical use of glaucoma medications during the medical treatment of open-angle glaucoma patients. Furthermore, this speculation suggests that early trabeculectomy may be a more conservative and less risky alternative to long-term medical treatment of open-angle glaucoma patients.

DISCUSSION

Medical treatment of open-angle glaucoma can induce undesirable changes within the conjunctiva and Tenon’s capsule. In addition to the transient conjunctival changes related to allergy and toxicity that most topically applied eye drops can induce, the parasympathomimetics and sympathomimetics can be associated with cicatricial pemphigoid.6,8 Early developed beta blockers such as
practolol commonly caused scarring of the conjunctiva, and even commercially available timolol has been associated with drug-induced ocular pemphigoid in at least two cases. Furthermore, carbonic anhydrase inhibitors are known to occasionally induce Stevens-Johnson syndrome with severe conjunctival scarring. Finally, investigators have reported that exposure to topical antiglaucoma medications for more than 3 years is associated with a significant foreshortening of the inferior fornix secondary to conjunctival fibrosis. These clinical observations suggest that a possibility for enhanced postoperative scarring of surgically created filtering blebs might be more likely in patients receiving long-term antiglaucoma medications. In fact, many believe that the continuing use of miotics prior to filtering surgery increases the likelihood of surgical failure. In spite of these reports and studies, there has been general agreement that surgical treatment of open-angle glaucoma is indicated only after maximally tolerated medical treatment has failed.

However, in recent years studies of conjunctiva and Tenon’s capsule have demonstrated increased inflammatory cells in patients receiving long-term open-angle glaucoma therapy as described above. These results suggest that extensive medical therapy before surgery may enhance the risk of external bleb scarring and filtration surgery failure. Therefore, investigators and clinicians have awaited a well-designed clinical study to support this speculation. Such an investigation might link the laboratory finding of subclinical inflammation within the conjunctiva to an enhanced scarring of the surgical bleb with failure of filtering surgery. This would change the approach to the medical treatment of open-angle glaucoma.

During the past 10 years, the results of clinical studies have been referenced with authority as good evidence that long-term medical therapy of open-angle glaucoma (>1 year) can adversely affect the results of fistulizing surgery. The suggestion that medical therapy for open-angle glaucoma patients may be counterproductive and encourage the failure of filtering procedures represents a major change in thinking that essentially redefines conservative treatment of open-angle glaucoma patients. This dramatic change in therapeutic attitude requires a rigorous, properly controlled, well-designed clinical study as support. However, this study does not exist.

The most commonly quoted clinical study that concludes that long-term treatment can adversely affect the results of fistulizing surgery is not randomized, prospective, or masked. Therefore, the potential for bias has not been properly controlled. In fact, there is clear evidence for bias within this study because the subjects within the PT group, patients with minimal medical treatment, were selected from a group of subjects reported within a prior study. More specifically, the 47 subjects used in this pivotal study as the minimally treated control are selected from a group of 57 subjects that were reported in a prior study. It remains unclear how and why the 10 subjects present within the initial study but absent from the subsequent study were eliminated.

In addition to potential subject-selection bias, this pivotal study is weakened because there is evidence that the two groups being compared in terms of postoperative IOP after 18 months are not comparable at baseline in several important ways. As summarized in Table 1, the MT group demonstrates more advanced visual field defects and a greater duration of disease and has more subjects with a history of laser surgery than does the PT group. Furthermore, all of the subjects in the MT group are by definition medical treatment failures. The PT group, which consists of newly diagnosed open-angle glaucoma, may have, at best, only 20% of its subjects that will ultimately become treatment failures. These differences suggest that open-angle glaucoma is present in a more advanced form in the MT group compared with the PT group. In addition, the presence of subjects with a history of laser treatment within the MT group may influence the results of trabeculectomy surgery in these patients, because eyes treated with laser trabeculoplasty before filtration surgery have been reported to have a decreased probability of successful surgery.

Although these important differences between the two groups significantly weaken the integrity of the study, an even more critical consideration is whether all of the subjects within the PT group really have open-angle glaucoma. The likelihood that this disease is not present is greater in subjects having had the diagnosis for 8 or fewer weeks than in subjects having had the diagnosis for 1 to 17 years. This possibility seems particularly reasonable in light of recent studies showing that visual field defects observed in open-angle glaucoma patients require confirmation to ensure their presence. This diagnostic requirement is much more likely to have been fulfilled within the MT group than the PT group, because the subjects within the PT group, by definition, had their diagnosis of open-angle glaucoma for only 8 or fewer weeks prior to their surgery. This limited time period is hardly sufficient to confirm visual field defects in potential open-angle glaucoma patients on three separate occasions.

It is recognized that preoperative use of miotics can influence the success of glaucoma filtering procedures. Although the anticholinesterase agents are most clearly implicated, all of the parasympathomimetics are capable of disrupting the blood-aqueous barrier and changing the chemical composition of the aqueous humor. It is possible that more of the filtering procedures within the medically treated group would have been successful if the miotics
Flach

had been discontinued at least 3 days prior to surgery, as some have recommended.\textsuperscript{12}

In addition to concerns about the comparability of the two groups prior to surgery, it appears that the surgeries provided the two groups might not be comparable. The presenting IOPs are higher in the PT group. Furthermore, consultants performed more than twice as many of the surgeries in the MT group. Finally, there were almost four times as many shallow chambers following surgery in the PT group, compared with the MT group (Table 2). These differences may have influenced the long-term results of surgery, including the number of failures observed 18 months following surgery.

A final consideration is whether the results of this study and its subsequent conclusions can be extrapolated to the current treatment of open-angle glaucoma patients. Table 3 summarizes the medications used in the MT group and, in particular, the medical treatment that the surgical failures received prior to trabeculectomy. Pilocarpine and epinephrine were used in 100% and 89%, respectively, of the surgical failures in this study. These parasympathomimetic and sympathomimetic drugs are used much less extensively as part of the current medical treatment of open-angle glaucoma. Furthermore, the guanethidine-epinephrine combination, which was used in 56% of the surgical failures, has never been used in the United States. Therefore, it is clear that the current medical therapy for open-angle glaucoma is different from the medical treatments used within this study.

A subsequent study of 124 patients was published in support of the findings of the pivotal study discussed above.\textsuperscript{4} This study concludes that its results show that topical therapy has an adverse effect on the conjunctiva and that these changes correlate with the failure of trabeculectomies. However, this investigation has the most worrisome of the shortcomings within the previously published study.\textsuperscript{3} In addition, the more recent study has fewer subjects in the minimal treatment group, has a shorter

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**Table 1. Preoperative Comparability of Primary Trabeculectomy (PT) and Multiple Treatment (MT) Groups*\textsuperscript{a}**

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>PT GROUP</th>
<th>MT GROUP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presenting visual field defects</td>
<td>Early</td>
<td>Advanced</td>
</tr>
<tr>
<td>Duration of disease</td>
<td>Days/weeks</td>
<td>1-17 years</td>
</tr>
<tr>
<td>History of failed medical</td>
<td>Early 20% of group</td>
<td>100% of group</td>
</tr>
<tr>
<td>treatment</td>
<td>destined to fail treatment</td>
<td></td>
</tr>
<tr>
<td>History of prior laser surgery</td>
<td>No</td>
<td>Yes (25% of group)</td>
</tr>
</tbody>
</table>

* PT group has less than 8 weeks of medical treatment. MT group has 1 to 17 years of medical treatment.

**Table 2. Preoperative, Operative, and Postoperative Comparability of Surgical Procedures in Primary Trabeculectomy (PT) and Multiple Treatment (MT) Groups*\textsuperscript{a}**

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>PT GROUP</th>
<th>MT GROUP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preoperative intraocular</td>
<td>Higher</td>
<td>Lower</td>
</tr>
<tr>
<td>Consultant performed surgery</td>
<td>8%</td>
<td>20%</td>
</tr>
<tr>
<td>Shallow postoperative anterior</td>
<td>23%</td>
<td>6%</td>
</tr>
</tbody>
</table>

* PT group has less than 8 weeks of medical treatment. MT group has 1 to 17 years of medical treatment.

**Table 3. Glaucoma Medications Used by Subjects in Multiple Treatment (MT) Group*\textsuperscript{a}**

<table>
<thead>
<tr>
<th>MEDICATION</th>
<th>DRUGS USED BY ALL SUBJECTS IN MT GROUP</th>
<th>DRUGS USED BY ALL SURGICAL FAILURES IN MT GROUP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pilocarpine</td>
<td>91%</td>
<td>100%</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>60%</td>
<td>89%</td>
</tr>
<tr>
<td>Timolol</td>
<td>54%</td>
<td>67%</td>
</tr>
<tr>
<td>Guanethidine/epinephrine</td>
<td>27%</td>
<td>56%</td>
</tr>
</tbody>
</table>

* MT group has 1 to 17 years of medical treatment with multiple medications.
follow-up period, and includes a group with minimal treatment that is even more highly selected than the previous study. Therefore, it is difficult to accept the publication's conclusion that good evidence exists showing that early surgery is more likely to be successful and more likely to reduce disease progression in open-angle glaucoma patients.\(^4\)

In summary, there is no convincing evidence that long-term medical treatment as used today influences the success of contemporary trabeculectomy surgery. Existing evidence makes it appropriate to agree with investigators who believe that preoperative use of topical medication does not influence the outcome of surgery.\(^11\) The studies that attempt to justify early surgery are all potentially biased, based on postoperative comparisons of groups that are not comparable at baseline, and include medical treatments that do not reflect current treatment regimens. Therefore, it seems only prudent to employ long-term medical treatment of open-angle glaucoma in our open-angle glaucoma patients, delaying filtering surgery until maximal tolerated medical treatment fails.

REFERENCES


DISCUSSION

Dr Max Forbes. Although the inflammatory changes in the conjunctiva reported by the Moorfields group are quite real, the comparative surgical outcomes are unreliable and probably overblown as Dr Flach indicated. There is no reason to change the conventional therapeutic strategy in open-angle glaucoma from medication-first to trabeculectomy-first on the basis of unproven results.

The case seems closed, but not quite. Just because an adverse effect of chronic topical antiglaucoma medical therapy on filtration surgery has not been proven does not necessarily mean that such an effect is not true. In fact, there is a current ongoing prospective, randomized, controlled clinical investigation, the Collaborative Initial Glaucoma Treatment Study, that is comparing the long-term outcomes of trabeculectomy-first versus medication-first, laser trabeculoplasty-next, and then trabeculectomy, if needed. Dr Paul R. Lichter, the Study Chairman, is the co-author of an interim five-year report.\(^1\) When a sufficient number of the medication-first eyes have undergone filtration surgery to permit comparison, it will be very interesting to learn whether or not they responded as well as the trabeculectomy-first eyes bearing in mind that interim deployment of laser trabeculoplasty will be a confounding variable.

An additional issue to consider is the subsequent finding by the same Moorfields group that both the conjunctival inflammatory changes and the impaired success of trabeculectomy could be reversed by preoperative treatment with topical fluorometholone combined with cessation of sympathomimetic agonists for a period of one month.\(^2\) That reversal was also accomplished without even resorting to anti-fibrotic agents that reduce the rate of failure of filtration surgery in eyes that are prone to postoperative scarring. Therefore, even if it should turn out that medical therapy does adversely influence the success of trabeculectomy, the means to overcome that effect are available, and there would appear to be no reason to alter therapeutic strategy on that account. On the other hand, this does not preclude the possibility of switching to surgery as the initial step on the basis of some other develop-

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opment, such as a major advance in operative methodology and efficacy.

REFERENCES


Dr Allan J Flach. I want to thank Dr Forbes for his kind comments. I certainly agree with him that the results of the Collaborative Initial Glaucoma Treatment Study Trial will be of great interest to all of us.
THE PARAMETERS OF INFORMED CONSENT

BY Edward L. Raab MD JD*

ABSTRACT

Purpose: To describe the components of a proper informed consent; which risks must be disclosed and which need not; additional safeguards for incapacitated persons, minors, and research subjects; and where the law will imply consent that is not otherwise obtained.

Methods: Summarization of current law obtained from legal treatises, reports of recent cases, and personal experience as a reviewer and expert.

Results: Lack of informed consent can reinforce a claim of medical malpractice or serve as an alternative point of attack when the case is otherwise weak. Special requirements must be met when patients are the subjects of clinical research.

Conclusion: Demonstration of a well-conducted process, not merely of a paper, not only protects the physician from exposure to liability, but increases the patient's autonomy in decisions concerning health and encourages compliance with treatment.


INTRODUCTION

This presentation reviews informed consent as it applies not only to the practice of ophthalmology but to the practice of all areas of medicine. In most instances the informed consent process flows naturally from the “partnership” between physician and patient; however, when this does not occur, serious legal and ethical consequences may result.

METHODS

This discussion is based on review of legal decisions and commentary and on my personal experience as a reviewer and expert in medical malpractice cases. I have utilized case reports and several informative writings that have appeared in the New York Law Journal and The Journal of Legal Medicine, as well as selections from a vast amount of material available on the LexisNexis database. This report is not intended to be specific advice on any private legal matter.

RESULTS

Lack of informed consent is a claim that can win for the plaintiff even when the malpractice claim is weak. Better understanding of the informed consent process is likely to protect and advance the interests of both patient and doctor.

DISCUSSION

Battery

Informed consent begins with consideration of the tort of battery, one of the oldest forms of legally disfavored conduct. It consists of unpermitted, privileged, intentional contact with another’s person. The contact need not result in bodily harm; the intended contact itself is the harm.

Society’s original interest in consent was as a peacekeeping device. Battery provoked revenge, which threatened the public peace. The offensive conduct became acceptable if its recipient consented and thereby waived revenge.

In the medical context, it is legally well established that everyone of sufficient age and soundness of mind has the right to decide what is to be done to his or her body, even when survival is implicated. Treatment with no consent at all, actual or implied, treatment substantially different from that to which the patient consented, or unauthorized substitution of one treater for another come within the definition of battery, especially when

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*Presenter.

Bold type indicates AOS member.
involving invasive procedures. That an unpermitted medical treatment may be lifesaving or curative, except in situations where consent would be implied (discussed below), does not excuse battery. The following actual case is illustrative:

A physician determined that a patient required surgery on the right ear. The patient gave informed consent to the procedure. With the patient under general anesthesia, the surgeon operated on the left ear, which at the subsequent trial he said required the same procedure. There were complications and resulting damage from the surgery. The patient took an easier route than proving negligence by obtaining a verdict of battery, despite the surgeon's best intentions.

In this case, consent to treatment of the right ear did not imply consent for the other ear despite the similarity in indications. Had life or hearing been immediately threatened, the result might have been otherwise. A point of practical significance is that as an intentional tort, battery is not within the scope of the physician's malpractice liability coverage, and because it is considered a wrong against society at large requiring a strong deterrent, there is the possibility of punitive damages.

**Informed Consent**

The patient's consent distinguishes permitted from unpermitted treatment. Whereas this effectively precludes a claim of battery under present-day conventions, it otherwise is insufficient to eliminate all legal risk. The right of choice under the concepts of autonomy and personal dignity is now legally recognized to require more. Some authors have advocated deeming lack of informed consent as an invasion of these rights, actionable as battery. A claim of lack of informed consent usually accompanies an allegation of medical malpractice for wrongful diagnosis or treatment. It differs importantly from malpractice in not requiring that the treatment be a departure from the standard of care. The elements of the claim are (1) the physician did not present the risks and benefits of the proposed treatment and of alternative treatments; (2) with full information, the patient would have declined the treatment; and (3) the treatment, even though appropriate and carried out skillfully, was a substantial factor causing the patient's injuries.

That the physician acted entirely in good faith is not a defense. Expert testimony usually is required to establish what the patient should have been told, but jurisdictions are divided on whether expert opinion is required as to what the patient would have decided if properly informed.

Although the legal analyses for malpractice and informed consent are similar, these are distinctly separate causes of action. Malpractice addresses the patient's interest in competent care, whereas lack of informed consent implicates self-determination. Success on the latter issue will enhance a favorable verdict for the plaintiff and is a convenient fallback position when the malpractice case is weak. Some attorneys feel that juries are more comfortable with lack of informed consent as a basis for an award, because it avoids a malpractice branding of the defendant physician while addressing a sentiment that the patient should be compensated.

In contrast to the Canadian position that consent requires only broad discussion, at least some US courts have held that consent is a nullity unless it is obtained completely consistent with the informed consent process, that is, there is either informed consent or no consent at all. That this is not the prevailing sentiment is largely due to public policy considerations, so as not to be harsh enough on physicians to possibly affect delivery of medical care.

**Disclosure**

Almost all courts scrutinize the adequacy of informed consent under principles of negligence. Courts apply either of two standards for adequate disclosure, depending on the jurisdiction. Whether either one is breached is a question for the jury. One refers to what a reasonable physician would consider important to the patient's decision (the "reasonable practitioner" standard). The other is whether the physician has disclosed what a reasonable person in the patient's position would want to know in order to make a considered decision (the "prudent patient" standard). The latter issue will enhance a favorable verdict for the plaintiff.

Ideally, these standards should coincide, but in practice they are differing legal concepts, applied on a state-by-state basis. The reasonable practitioner standard, or "doctor knows best," tends to ignore the concept of patient autonomy to decide what is acceptable regardless of the type and likelihood of risks involved. Some English courts have held that this does not include the right to disclosure of any alternative treatment considered by the patient's own doctor to be contraindicated, a throwback to Hippocrates and Plato, who believed that comprehension of medical matters was irredeemably beyond people's capacity. The law has progressed from this type of paternalism, although it survived well into the 20th century, but today this remains as the standard in about half of the US states, in part as a response to the malpractice insurance
The Parameters Of Informed Consent

A young woman agreed to a recommendation of surgery for her injured knee. In preparation for the procedure, the surgeon referred her for an arthrogram (arthroscopy was not yet in vogue) to a radiologist who obtained informed consent and included among the disclosed risks the possibility of an adverse inflammatory reaction to the injected contrast medium. This occurred and was incorrectly diagnosed as a joint infection by a different orthopedic surgeon. Continuous infusion of antibiotics into the patient’s knee for the supposed joint infection led to that very outcome.

At trial, the radiologist could not produce the patient’s signed consent for the arthrogram, but his sworn testimony as to his invariable custom and practice of holding a thorough informed consent conversation persuaded the jury that he had indeed carried out this process. The first orthopedic surgeon, who was not responsible for performance of the arthrogram nor for holding the informed consent dialogue for this procedure with the patient, and whose own diagnosis and proposed treatment were entirely within the standard of care, was found by the jury on the basis of the patient’s testimony to have omitted informing her that intensive physical therapy without surgery or the need for an arthrogram was a treatment option.

There are important lessons in this case. First, the radiologist could have been defended much more easily had his consent form been available to support his testimony. He won because he could convince the jury of a thorough conversation outlining the risks, benefits, and in this instance the limited alternatives to achieve visualization of the interior of the joint. Second, each participant in a treatment must describe his or her own aspect of it, but not that of any other participant. Discussing the pros and cons of the proposed surgery was not part of the radiologist’s obligation. Likewise, the orthopedic surgeon was not required to make disclosure about the arthrogram. The jury found him liable for not presenting an alternative to the operation, which the patient testified she would have elected.

Must all risks be disclosed? The law requires disclosure only of material, not trivial, risks that are reasonably foreseeable. “One in a million” occurrences, risks obvious even to a layperson, and those that clearly would not result in refusal of treatment had they been disclosed constitute other exceptions. As in malpractice, these situations turn on their particular fact contexts.

There also is an allowance for risks whose disclosure, in the physician’s best judgment, would be emotionally harmful. Since assertion of this defense is clearly self-serving and not accepted by all courts, it is best avoided.

Some courts and commentators have considered the degree of the physician’s experience with a treatment to be a material risk factor requiring disclosure with or without a query. Other courts have not adopted this approach, stating that information regarding the skill and experience of a doctor is not relevant to understanding the risks of the treatment itself. Other potential concerns that have led to similar debate involve personal medical factors that could affect the treating physician’s skill and judgment.

Another familiar dilemma is what a doctor should tell a patient about the risks of general anesthesia. Although undeniably a profound consequence, the risk of anesthetic death to a patient whose general health is reasonably good takes the inquiry into the range of unforseeability, yet clearly a reasonable patient would want to be aware of it. But what if disclosure would result in refusal of what, under the particular circumstances, clearly would be the best treatment alternative? There is no definite answer to this difficult problem.

A proper informed consent dialogue requires that the patient receive the information in ordinary terms and in his or her customary language, translated if necessary. There must be the opportunity to decide free of duress, although this does not prevent the physician from offering a recommendation based on expertise and judgment. Disclosure should always include the possibility of no treatment at all and the anticipated consequences of that course. Any undisclosed treatment alternatives, or withholding the option to do nothing, can be construed as an imposition of the physician’s choices upon the patient’s power to decide.

Documentation

The informed consent process has been criticized as concentrating more on avoidance of physician liability than on truly educating patients so that they might make self-determined medical decisions. This observation cites the consent form for support of this proposition, as it shapes what is intended as a process of dialogue and discussion into a discrete paper-signing event.

Instead, these critics advocate an autonomy-enhancing model in which the patient, by way of continuous
acquisition of information from the physician, remains master of the consent process. This approach emphasizes “informed” over mere “consent” and is facilitated by providing the patient a full opportunity to raise questions. As a beneficial side product, studies have shown that this model of informed consent, by giving the patient a greater sense of control, encourages compliance with treatment.31 To date, this view has not prevailed either in customary medical encounters or in the courts.

Minors and Incapacitated Persons
For both adults and minors requiring emergency life- or function-preserving treatment, the law implies full consent except where it is clear from prior information that this has affirmatively been refused. This exception applies also to the extension of a planned surgical procedure on an anesthetized patient because of circumstances becoming evident only during the course of the operation.

In our practices, sometimes we are confronted with an unaccompanied minor presenting for routine examination. Even without invasive treatment, the examination represents “contact” for which the physician has no permission, especially for the use of cycloplegic drugs.

Parents or a legal guardian are responsible for health care decisions for an underage child. Many states allow adolescents to consent for purposes such as obtaining contraceptive devices, prenatal care, or screening and treatment for sexually transmitted diseases. Minors who are married, pregnant, parents, self-supporting, or a member of the military (so-called emancipated minors) generally are fully able to give consent in all health matters.

Unless the child fits into one of these categories, the best course in this situation would be to require written authority or consent by telephone. The latter presupposes a good faith belief that the appropriate person at the remote location has been contacted. In urgent but not emergency situations, a relative or adult sibling is deemed to have this authority without formal delegation.

Persons whose ability to understand and choose appropriately is questionable, unless already under judicially decreed guardianship, may require psychiatric consultation, as a New York court recently has held.28

Informed Consent for Research Subjects
Courts have evolved from the strong presumption that all nontraditional practices are outside the standard of care13,34 to a recognition that investigation requires a different analysis.25 Originally, the intent was to control quack remedies. Beginning in about 1935, the necessity for medical experimentation became more evident.

Initially, clinical research was largely unregulated and ethical matters such as consent and safety monitoring remained in the hands of the investigators. Special concerns arose in the aftermath of Nazi medical experiments during World War II, which resulted in the Nuremberg Code and the Declaration of Helsinki.36 In the 1970s, certain research abuses led to Congressional action to protect human subjects,27,28 leading to comprehensive regulations, known as the Common Rule, under which most therapeutic trials are now conducted,39 including those involving drugs or devices regulated by the Food and Drug Administration.40 The regulations meticulously spell out the informed consent requirements and provide that they be monitored by local institutional review boards.35

In addition, there is an in-between category of “innovative therapy,” a single or limited number of unproven interventions, such as an off-label use of a drug, intended to solve an immediate clinical problem in an individual patient when the usual treatment options have not been effective or appropriate.11,42 Penalization for the treatment of amblyopia, now well accepted, was at one time such an innovation. Without prior formal study of safety and efficacy, these nonvalidated practices also expose patients to greater risk, and it has been urged but not required that their use should be within the framework of a research protocol.

The difference between the conventional treatment and research settings is not only the comparative uncertainty of risks and benefits, but also the difference in objectives. To be approved by institutional review boards, the informed consent process must make the patient recognize that such studies are done to develop new knowledge that later may be valuable to a broader population of patients.11,42 The purpose of the trial is not to make the individual subject well, although this may occur fortuitously.11

The investigator’s first loyalty is to the protocol, not to the patient. Subjects may have to forego all treatment or an adjustment in dosage, or may experience unexpected and unpleasant side effects.11 As some authors42,43 have pointed out, this itself is a conflict of interest requiring meticulous disclosure. It is entirely possible that the subject will not be told of treatment alternatives outside the experimental protocol that could be elected by declining to participate. If a subject enrolls solely because of hoped-for benefits, any informed consent process has failed.

Recently, there has been a trend toward lawsuits for harm to research subjects,44 including an action for enrolling premature infants into a high-oxygen study without parental knowledge and consent.45 As the widely known DES litigation3 illustrated, courts formerly analyzed informed consent mostly under the standard for conventional treatment, although now claiming to recognize that there is a special duty of care.10,44,46,47 but the judi-
cial response has not always been consistent with the sentiment that loss of the right to personal dignity is a separate and unique wrong to which traditional malpractice and informed consent principles do not fully apply,31-35.

The main informed consent safeguards are that the patient know that he or she is an experimental subject and possibly a control36; that the effects of the treatment are not entirely known and that withdrawal from the study is permissible36; that there is a Data and Safety Monitoring Board and that the study will be discontinued if, as occurred in the high-oxygen study,45 the treatment and that the study will be discontinued if, as occurred in the high-oxygen study,45 the treatment convincingly shows an adverse effect; that there is a state-ment of the subject's rights and that they may not be waived36; and that there is a study privacy policy. Some authors36 have stated that for informed consent to be complete, discussion of risks should include remotely suspected as well as known risks, because in this special context it is the patient who should determine which risks are material to the decision to participate.

Informed consent authority for minors and adolescents follows similar rules to those applicable to the usual clinical situation. Institutional review boards generally have specified that obtaining assent of the child in addition to that of the responsible guardian is an integral part of the process, but in the absence of specific regulatory guidelines, the threshold age for this requirement has varied.31-33.

It has been suggested that the federal regulations, rather than the views of experts, should constitute the standard for disclosure. There is precedent for this for ophthalmology in cases involving intraocular lens implantation when this technique still was in the experimental stage.34,35 Expert opinion on compliance with the standard remains appropriate, and compliance should provide a partial if not a complete defense.34,36

To summarize, informed consent should be thought of as a process, not as a paper. The document is helpful to memorialize what should have occurred before the patient's signature is obtained. The process should be appropriate whether the setting is one of conventional clinical practice or of an investigative study, and requires disclosure of information and its implications to a person with capacity who understands what is disclosed and voluntarily makes a decision. This is sound both legally and ethically.

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DISCUSSION

Dr Richard L. Abbott. In his paper, Dr Raab discusses important parameters of the informed consent process that are often neglected or overlooked by the busy clinician and can be used against the physician in a medical malpractice case. Some of these components include disclosure of risks and possible alternatives, the experience of the physician, risks from anesthesia, and off-label uses of a device or medication.

He emphasizes that malpractice and informed consent issues are distinct causes of action and that a claim of inadequate or improper informed consent is a convenient fallback position when the malpractice component of the claim is weak. In a recently published study looking at malpractice predictors and risk factors for ophthalmologists performing PRK and LASIK surgery, improper informed consent was found as the main cause for negligence in 30% of the claims and suits studied.1

The social goals of medical malpractice litigation are to deter unsafe practices, compensate patients injured through negligence, and exact corrective justice.2 Many consumer groups view litigation as an indispensable form of protection against medical carelessness. Recently, it was estimated that one of six practicing physicians are faced with a malpractice claim each year.

For Ophthalmologists, the probability of incurring a claim or suit during a 35 year career is 95%. (Ghezzi T, unpublished data, 1998) Several factors have been linked to patients’ decisions to file malpractice claims against their physician. Most commonly these have been dissatisfaction with treatment outcome and inadequate physician communication and interpersonal skills.3 Unfortunately, busy schedules and time consuming administrative duties often make it difficult for ophthalmologists to develop the dialogue necessary to minimize unrealistic patient expectations. The ACGME has recognized the importance of interpersonal and communication skills by including these qualities as one of the six general competencies for assessment in the Maintenance of Certification process. Since informed consent is based on a shared decision between the physician and the patient, the ability of the physician to communicate effectively with the patient is crucial to the process.

Dr Raab raises many key points in his discussion of informed consent, emphasizing the importance of disclosure and in what detail a patient must be told regarding the nature of the risks, benefits, alternatives, and complications involved in the recommended treatment. In my experience, this issue often is debated regarding whether or not the patient needed to know all possible complications and what specifically needed to be documented in the medical record.

A second issue that is raised by Dr Raab in his paper discusses the disclosure of the physician’s experience with a particular treatment and whether that should be offered to the patient as part of the informed consent process. This discussion with the patient has both ethical and legal implications and no clear-cut answer to this difficult dilemma emerges in the paper.

Finally, a review of the issues unique to the informed consent process for research subjects enrolled under IRB protocols and the use of off-label treatments leads the reader to consider several difficult and complex issues. Among these are: conflict of being an investigator and one’s loyalty to the protocol (rather than the patient); inconsistent judicial response regarding special duty to care; failure of the informed consent process if a subject has enrolled in the study solely because of hoped for benefits.

REFERENCES


**Dr Robert C. Drews.** Informed consent, by definition, consists of education of the patient. I would suggest that the education process in any other setting always includes an examination or quiz to see if the patient understood the material that was being presented. It is disconcerting to find out how poorly patients understand all of the material you so diligently presented to them. A written copy of the quiz can go a long way toward satisfying the needs of an informed consent.

**Dr Dennis M. Robertson.** We published a report on informed consent about 25 years ago in which patients were given a questionnaire after informed consent about retinal surgery. Patients remembered about half of what they were told even though we asked them questions relative to information in the informed consent discussion within 48 hours of the surgery. They also denied ever hearing about issues that we had clearly covered, and they confabulated. You may be interested to know that Mayo Clinic in Rochester has no written informed consent. The patients do not sign informed consent for surgery. It’s different for IRB protocols involving research since the patients must sign an informed consent. But, for other surgical procedures and interventions, we merely write in our notes that the patient was informed about the process, about the disease, about the interventions, the risks and uncertainties, and the natural course. This is different at Mayo Clinic in Jacksonville, Florida, where, by state law, patients must sign an informed consent for surgical procedures.

**REFERENCE**


**Dr Arthur Jampolsky.** If one tells a patient that at worst you may die or may you lose the eye or go blind, and then fills in with some of the major things, does that add anything to coverage of things that might be omitted?

**Dr David L. Guyton.** Some of the angriest patients I see in my strabismus practice are those who see double after cataract surgery and occasionally after posterior segment retinal scleral buckle surgery as well. We now know that the incidence of anesthetic myotoxicity causing double vision after cataract surgery from retrobulbar or peribulbar anesthesia is about one in 200, and after retinal scleral buckle surgery, maybe as high as five to 10 percent, although that’s hidden in the other causes of diplopia and tropias after scleral buckle surgery. Invariably, these patients state that they were not told about possible double vision after cataract surgery. Is this level of incidence something that really needs to be brought to the profession’s attention? If it is not in such informed consent or informed consent documents, should it be, at that level of one in 200 cases?

**Dr David R. Stager, Sr.** A refractive surgeon from Canada spoke at our group bimonthly meeting recently and he had a list of eight questions that he asks all his potential refractive surgery patients. He said that this gives him a profile of the patient who is destined to be unhappy. Is there a way of predicting patients for elective surgery who may be at a much higher risk of being unhappy, no matter what is done or what consent form is provided?

**Dr Edward L. Raab.** I enjoyed Dr Abbott’s thoughtful discussion, as he is very knowledgeable in this subject. The evident interest in this topic, shown by the number and content of the other discussants’ remarks, also is extremely satisfying.

To respond to Dr Drews, I do not think that the patient has to be given a set quiz, but certainly the informed consent process includes asking the patient to raise any questions he or she may have once the doctor’s part of the dialogue is over.

Several discussants said that we give the patient informed consent. In fact, you take the patient’s informed consent. What you give is the information; the consent is what comes back to you. Informed consent is a process that should be documented in your charts. In the research setting, it is mandated by your IRBs, which are responding to regulations that have been set forth by the federal government.

Dr Jampolsky asks whether, if you tell a patient the worst-case scenario, that embraces everything in between? My answer to that is “no,” and I do not tell patients they can die under anesthesia unless in response to a direct question. The worst-case scenario is almost in the realm of the unforeseeable, and all the risks that are short of that are more foreseeable. Those are the ones that the law requires you to particularly inform about.

Dr Guyton wonders if a one-in-200 risk is something that should be revealed to the patient. There’s no number, no bright line on something like that. For me, a one-in-200 risk is really not a high-level risk. But you will never find me, nor will I find you, in a jury box. Jurors are lay-
people and lawyers spin arguments. Although I don’t think that’s a very large risk, numerically, I probably would include it.

Dr Stager asks whether there is a profile of the liable-to-be-unsatisfied patient? I think we all have our gut feelings about this, but probably it is a question for a psychologist.

Thank you for the opportunity to contribute to our program.
FINAL RESULTS OF THE EARLY TREATMENT FOR RETINOPATHY OF PREMATURITY (ETROP) RANDOMIZED TRIAL

BY William V. Good MD,* on behalf of the Early Treatment for Retinopathy of Prematurity Cooperative Group

ABSTRACT

Purpose: To present the final results of the Early Treatment for Retinopathy of Prematurity Study.

Methods: Infants with bilateral high-risk prethreshold retinopathy of prematurity (ROP) (n = 317) had one eye randomized to early retinal ablative treatment and the fellow eye managed conventionally (control eye). In asymmetric cases (n = 84), the eye with high-risk prethreshold ROP was randomized to early or to conventional management. High risk was determined using a model based on the Cryotherapy for Retinopathy of Prematurity natural history cohort. The primary outcome was visual acuity assessed by masked testers using the Teller acuity card procedure. Structural examinations were performed at 6 and 9 months.

Results: Grating acuity results showed a reduction in unfavorable visual acuity outcomes with earlier treatment, from 19.8% to 14.3% (P < .005). Unfavorable structural outcomes were reduced from 15.6% to 9.0% (P < .001) at 9 months. Further analysis supported retinal ablative therapy for eyes with type I ROP, defined as zone I, any stage ROP with plus disease; zone I, stage 3 ROP without plus disease; or zone II, stage 2 or 3 with plus disease. The analysis supported a “wait and watch” approach to type II ROP, defined as zone I, stage 1 and 2 without plus disease, or zone II, stage 3 without plus disease. These eyes should be considered for treatment only if they progress to type I ROP or threshold.

Conclusion: Early treatment of high-risk prethreshold ROP significantly reduced unfavorable outcomes in both primary and secondary (structural) measures.


INTRODUCTION

Despite the success of peripheral retinal ablation in reducing the risk of retinal detachment, vision impairment still remains common in infants with severe retinopathy of prematurity (ROP).1 In the Cryotherapy for Retinopathy of Prematurity (CRYO-ROP) study, peripheral retinal ablation was administered when ocular findings indicated a risk of approximately 50% for retinal detachment.2 This degree of severity was termed the threshold for treatment of ROP, and was defined as at least five contiguous or eight cumulative sectors (clock hours) of stage 3 retinopathy of prematurity (ROP) in zone I or II in the presence of plus disease (a degree of dilation and tortuosity of the posterior retinal blood vessels meeting or exceeding that of a standard photograph).2 Treatment at threshold results in approximately a 50% reduction in the rate of retinal detachment.

With the hope of improving this rate of unfavorable outcome, the timing indications for treatment of ROP have been questioned, with some investigators advocating earlier treatment and others advocating conventionally timed treatment.3,4 One concern with earlier treatment is the expected increase in surgical intervention in eyes with ROP that would otherwise regress spontaneously. This concern has led to efforts to identify treatment selection criteria that would result in earlier treatment only in those eyes at highest risk for developing threshold ROP and/or an unfavorable visual or structural outcome in the absence of treatment.

In 1999, the National Eye Institute funded a cooperative agreement to study early treatment for ROP (ETROP).5,6 In the study, eyes of infants were randomized...
to early peripheral retinal ablation or standard treatment (conventional management) if they developed prethreshold ROP and if RM-ROP2, a risk analysis program based on natural history data from the CRYO-ROP study, indicated a high risk for an unfavorable outcome. Prethreshold ROP was defined as any ROP in zone I that was less than threshold; or in zone II stage 2 with plus disease (dilation and tortuosity of posterior pole retinal vessels in at least two quadrants, meeting or exceeding that of a standard photograph); or zone II, stage 3 disease without plus disease; or zone II, stage 3 with plus disease but fewer than five contiguous or eight cumulative clock hours. This paper presents the final results of this study, including implications for the timing of retinal ablative treatment for ROP. Preliminary results were published previously.

**METHODS**

Study protocols were approved by the review boards of all participating institutions, and parents provided written informed consent prior to infants’ enrollment into the study and again at randomization. Details of the study design and laser technique are described elsewhere. Infants with birth weights less than 1,251 g and birth dates between October 1, 2000, and September 30, 2002, were screened at 26 participating centers. If an infant developed ROP, parents were asked to consent to data collection and possibly increased frequency of examinations. Study-certified ophthalmologists conducted serial examinations to detect rate of progression of ROP, development of prethreshold ROP, and development of threshold ROP. If at least one eye reached prethreshold ROP, the infant’s demographic and ROP information was entered into the RM-ROP2 risk model to determine the likelihood of progression to an unfavorable outcome in the absence of treatment.

The risk determination was made at the coordinating center, using the RM-ROP2 model to evaluate data provided by the clinical center. If the risk of progression to an unfavorable outcome in the absence of treatment was calculated to be ≥15%, consent for the randomized trial was obtained, and randomization occurred. These eyes that had a risk of ≥15% were termed “high-risk” prethreshold. Eyes with <15% risk were termed “low-risk” and were followed every 2 to 4 days for at least 2 weeks until the ROP regressed, or the risk progressed to ≥15%. If both eyes were eligible for randomization, one eye was assigned at random to earlier treatment with ablative therapy within 48 hours of the first diagnosis of high-risk prethreshold ROP. Treatment was generally laser therapy, but cryotherapy was allowed. The fellow eye served as the control and was managed conventionally, which meant that it was observed either until it reached threshold and was treated or until the ROP regressed without progressing to threshold. In cases where only one eye had reached high-risk prethreshold ROP, that eye was randomized to treatment within 48 hours or to serve as a conventionally managed control, receiving treatment only if the ROP progressed to threshold severity. Infants in whom either eye had developed threshold ROP prior to randomization were excluded from the study.

For the analyses, eyes with prethreshold ROP that remained at low risk were categorized by the lowest zone and highest stage of ROP that ever developed. Eyes in the randomized group were classified according to the zone and stage of ROP that were present at the time of randomization, as determined by the confirming examiner’s observations.

**Functional Outcome**

The functional outcome of each randomized eye at 9 months corrected age was evaluated by assessment of monocular grating acuity, conducted by one of two testers masked to the eye’s treatment assignment, who traveled to the study centers for testing.

The technique employed to evaluate grating acuity was the Teller acuity card procedure as used previously in the CRYO-ROP study. Acuity was scored as the spatial frequency of the finest grating to which the infant showed a consistent fixation response. Eyes in which visual acuity was too poor to be quantified in this way were categorized as having no light perception (NLP), light perception only (LP), or detection of the grating on the low vision (LV) card only. The LV card has 2.2-cm-wide black-and-white stripes covering one half of the card. It was not used to quantify vision, but only to determine whether the infant had pattern vision. The tester was permitted to move the LV card and/or to present it at any distance and at any location in the infant’s visual field.

Visual acuity data were included in analyses only if the following criteria were met: (1) an acuity result (measurable acuity, detection of the grating on the LV card, LP, or NLP) was obtained for each eye of bilateral high-risk prethreshold cases or the randomized eye of asymmetric cases; (2) treatment for amblyopia, if present, had been prescribed for at least 4 weeks prior to the acuity test; and (3) refractive error, if present in either eye of bilateral high-risk prethreshold cases or in the randomized eye in asymmetric cases, had been corrected for at least 2 weeks prior to the acuity test. The criteria for correction of refractive errors were myopia greater than –4.00 diopters (D), hyperopia greater than +5.00 D, and/or astigmatism greater than 2.50 D in one or both eyes. Correction of anisometropia greater than 1.50 D spherical equivalent or 1.50 D cylinder was required only if the examining physi-
cian found evidence of amblyopia.

The visual acuity outcome was divided into four categories of functional response: normal, defined as greater than or equal to 3.70 cycles per degree\(^{13,14}\); below normal, defined as 1.85 to less than 3.70 cycles per degree (from approximately 4 to ≥2 standard deviations below the mean grating acuity for a 9-month-old child\(^{14}\)); poor, if less than 1.85 cycles per degree but measurable with one of the standard acuity cards (not the LV card); and blind/low vision (NLP, LP only, or LV only). These functional outcome categories of grating acuity results were further grouped into “favorable” and “unfavorable” designations. The favorable grouping included eyes in the normal and below normal categories. The unfavorable grouping included eyes in the poor and blind/low vision categories, which would be expected to have a poor long-term prognosis for visual function.\(^{15}\)

### Structural Outcome

Structural outcome was documented with a dilated fundus examination at 6 months and 9 months corrected age by study-certified examiners. Complete ophthalmologic examinations were performed at both of these ages; at the 9-month examination, a developmental questionnaire (DDST\(^{16}\); results not reported in this article) was conducted. Refractive errors were determined by cycloplegic retinoscopy after instilling 1% cyclopentolate hydrochloride. When there was a medical contraindication to this drop, either 0.5% cyclopentolate or 1% tropicamide was used. At 6 months, an unfavorable outcome was defined as (1) a posterior retinal fold involving the macula, (2) a retinal detachment involving the macula, or (3) retrolental tissue or “mass” obscuring the view of the posterior pole. If an infant required a vitrectomy or scleral buckle, the 6-month examination was conducted prior to the surgery. At the 9-month examination, eyes that had received a vitrectomy or scleral buckle were classified for study purposes as having an unfavorable structural outcome.

### Statistical Analyses

The ETROP study was designed to detect a 35% reduction in the percentage of eyes having an unfavorable structural outcome with a type 1 error rate of 0.05 and a power of 80%.\(^*\) Using data from the CRYO-ROP study, the percentage of unfavorable eyes managed conventionally was predicted to be 20%.\(^*\) If earlier treatment produced a 35% reduction, 13% of the earlier treated eyes would have an unfavorable outcome. Taking into account that approximately 80% of infants were expected to have both eyes eligible for the study, the number of infants needed for the study was 370.\(^*\) The primary outcome for this study was visual function, for which there are limited data on which to conduct sample size calculations. Therefore, we based sample size on structural outcome. This was a conservative approach, since in the CRYO-ROP study, unfavorable functional outcome rates were approximately 50% higher than unfavorable structural outcome rates at ages at which functional outcome was tested.\(^{17,18}\)

The statistical technique used to compare the eyes treated at high-risk prethreshold with the conventionally managed high-risk prethreshold eyes was developed and used in the CRYO-ROP study.\(^{19}\) It combines the data from infants with bilateral disease (both eyes eligible) and asymmetric disease (one eye eligible) into one overall chi-square analysis of outcome differences between the two treatment groups. Although not part of the original study design, functional and structural results are also presented by the International Classification of ROP (ICROP) and by RM-ROP2 categories to allow a more detailed examination of the data.

A Data and Safety Monitoring Committee of researchers, clinicians, and an ethicist not directly involved in the ETROP study met in person every 6 months to review adverse event and outcome data and to monitor study progress. The committee approved the protocol and monitored the performance of participating centers.

### RESULTS

At the 26 clinical sites, 828 infants whose parents had given consent for systematic follow-up of ROP were identified as having prethreshold disease in one or both eyes. Among the 828 infants with prethreshold ROP, there were 499 (60%) whose eye or eyes were classified as high-risk and who were thereby eligible for the randomized trial (Figure 1). Among these 499 infants, consent for randomization was not obtained for 40 infants, and high-risk prethreshold ROP was not confirmed by the required second study-certified examiner or for other reasons in 58 infants.
infants. Thus, 401 infants were enrolled into the randomized trial. The remaining 329 infants, who had prethreshold ROP that was judged to be low-risk, were followed as clinically indicated and then underwent follow-up study examinations at 6 months corrected age to determine retinal outcomes.

Table 1 shows the distribution of prethreshold eyes by RM-ROP2 risk classification and by severity of prethreshold ROP according to the ICROP20 characteristics. One eye per infant is represented in Table 1, and that is the eye with the higher risk, according to the RM-ROP2 model.7

Table 1 shows that the ICROP can serve as a good indicator of most of the high-risk prethreshold eyes. For prethreshold zone I eyes with plus disease, 100% were high-risk; when stage 3 was present without plus disease, 95.7% were high-risk; and with stage 1 or 2, without plus disease, 92.3% were high-risk. In zone II, 95.2% of eyes that were classified as stage 3 with plus disease and 83.3% of eyes with stage 2 with plus disease were high-risk, whereas only 2.6% of the 303 eyes that were zone II, stage 3, without plus disease were high-risk. The parallels between the RM-ROP2 model and ICROP are particularly striking even though the former takes into account a number of demographic and disease-related factors that are not part of ICROP.

Table 2 provides baseline characteristics for the 401 infants who entered the randomized trial. The mean birth weight was 703 g and the mean gestational age was 25.3 weeks. At the time of randomization, 79.1% of the infants had RM-ROP2 high-risk prethreshold disease bilaterally. The remaining 20.9% of infants had asymmetric disease, with high-risk prethreshold ROP in only one eye; the fellow eye had less severe ROP.

Table 3 shows the distribution of eyes treated at high-risk prethreshold and conventionally managed (control) eyes by ICROP categories at randomization, along with the percentage of conventionally managed eyes that reached threshold ROP. Zone I disease accounted for approximately 40% of randomized eyes. The largest categories of high-risk prethreshold eyes were those with zone II, stage 3, with plus disease (42.1% of prethreshold treated eyes and 43.7% of conventionally managed eyes), and those with zone I, stage 1 or 2 with no plus disease (27.4% of prethreshold treated eyes and 26.1% and conventionally managed eyes). Table 3 also indicates that 66.4% of eyes in the conventionally managed group progressed to threshold and underwent peripheral retinal ablation at that time.

The average age at high-risk prethreshold treatment was 35.2 weeks postmenstrual age (SD, 2.3; range, 30.6 to 42.1 weeks) and 10.0 weeks chronological age (SD, 2.0). The average age for treatment of eyes in the conventionally managed group that went on to threshold was 37.0 weeks postmenstrual age (SD, 2.5; range, 31.9 to 46.6 weeks) and 11.9 weeks chronological age (SD, 2.2). Only

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**Table 1. Risk status by ICROP category for the eye with higher risk of poor structural outcome for all 828 infants with prethreshold retinopathy of prematurity in one or both eyes***

<table>
<thead>
<tr>
<th>ICROP Category</th>
<th>Prethreshold</th>
<th>Risk in Prethreshold Eye</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low Risk</td>
<td>High Risk</td>
</tr>
<tr>
<td>Zone</td>
<td>No. of Patients</td>
<td>No. of Patients</td>
</tr>
<tr>
<td>I 3 Yes</td>
<td>29</td>
<td>0</td>
</tr>
<tr>
<td>I 3 No</td>
<td>23</td>
<td>1</td>
</tr>
<tr>
<td>I 1 or 2 Yes</td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td>I 1 or 2 No</td>
<td>117</td>
<td>9</td>
</tr>
<tr>
<td>II 3 Yes</td>
<td>271</td>
<td>13</td>
</tr>
<tr>
<td>II 3 No</td>
<td>303</td>
<td>295</td>
</tr>
<tr>
<td>II 2 Yes</td>
<td>66</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>828</td>
<td>329</td>
</tr>
</tbody>
</table>

ICROP, International Classification of Retinopathy of Prematurity.20

*Risk of poor structural outcome was based on the RM-ROP2 risk model analysis for each eye (a risk analysis program based on natural history data from the Multicenter Trial of Cryotherapy for Retinopathy of Prematurity Study).7 Low risk was less than 0.15; high risk was 0.15 or greater. Plus disease was defined as a degree of dilation and tortuosity of the posterior retinal blood vessels meeting or exceeding that of a standard photograph.2

**Table 2. Baseline characteristics of 401 randomized patients**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with bilateral high-risk prethreshold ROP</td>
<td>79.1</td>
</tr>
<tr>
<td>Birth weight, mean ± SD, g</td>
<td>703 ± 148</td>
</tr>
<tr>
<td>Gestational age, mean ± SD, wk</td>
<td>25.3 ± 1.4</td>
</tr>
<tr>
<td>Male</td>
<td>54.4</td>
</tr>
<tr>
<td>Singleton births</td>
<td>71.1</td>
</tr>
<tr>
<td>Born in the study hospital</td>
<td>80.3</td>
</tr>
<tr>
<td>Race</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>63.8</td>
</tr>
<tr>
<td>African American</td>
<td>18.0</td>
</tr>
<tr>
<td>Hispanic</td>
<td>14.7</td>
</tr>
<tr>
<td>Other</td>
<td>3.5</td>
</tr>
</tbody>
</table>

ROP, retinopathy of prematurity.

*Data are presented as percentage unless otherwise indicated.
one eye received cryotherapy at threshold ROP as the primary treatment for ROP, with the others receiving laser retinal ablation. Some eyes received supplementary cryotherapy at the time of the initial treatment. Retreatment was conducted in 13.9% of eyes treated at high-risk prethreshold ROP and in 11.0% of conventionally managed eyes treated at threshold ROP.

**Primary Outcome**

In this final report, grating visual acuity data have been obtained from 372 infants (98.2% of patients that survived). Data were not obtained from 22 infants who died prior to the 9-month examination and seven infants whose parents did not bring them for the examination. Average corrected age (age from expected date of delivery) at the time of grating acuity assessment was 10.3 months (SD, 1.8).

Table 4 presents the proportion of randomized eyes with unfavorable grating acuity outcomes at 9 months. Overall, there was a significant benefit of treatment of eyes at high-risk prethreshold ROP, with a reduction in unfavorable visual acuity outcome from 19.8% to 14.3% ($P < .005$). Within-subject comparison afforded a powerful opportunity to examine treatment effects while controlling for individual characteristics. Results from the 33 infants with bilateral disease in whom there were discordant outcomes in the two eyes provide even stronger evidence of a beneficial effect of treatment at high-risk prethreshold ROP ($P < .005$). Thirty-seven infants with bilateral high-risk prethreshold disease had an unfavorable outcome in both eyes.

Table 5 provides a more detailed presentation of the grating acuity results. Although differences were not statistically significant in these smaller categories, there were more high-risk prethreshold treated eyes than conventionally managed eyes that had grating acuity in the normal range for age ($P = .38$). In addition, fewer eyes randomized to high-risk prethreshold treatment than conventionally managed eyes were designated as blind or low vision ($P = .07$).

**Secondary Outcomes**

Structural outcome data have been obtained from 366 infants (94.8% of patients that survived) at 6 months...
corrected age and 372 infants (98.2% of patients that survived) at 9 months corrected age. Six-month data were not obtained from 15 infants who died prior to the examination and 20 infants whose parents did not bring them in for the examination. Average corrected age at the 6-month examination was 5.5 months (SD, 2.2). At 9 months, structural outcome data were not obtained from 22 infants who died prior to the examination and seven infants whose parents did not bring them in for the examination. Average corrected age at the 9-month examination was 9.8 months (SD, 1.4). This is younger than the average age for acuity testing because the final acuity data were sometimes collected during retesting after the 9-month structural examination.

The results for the 9-month structural outcome are presented in Table 6. Data indicate a statistically significant benefit of treatment of eyes with high-risk prethreshold ROP, with unfavorable structural findings reduced from 15.6% in conventionally managed eyes to 9.0% in high-risk prethreshold treated eyes ($P < .001$). As is the case for grating acuity outcome, results from infants with bilateral disease in whom there were discordant outcomes in the two eyes provide strong evidence of a beneficial effect of treatment at high-risk prethreshold.

Among the 30 high-risk prethreshold treated eyes that had an unfavorable structural outcome at 9 months, two had a partial retinal detachment involving the macula, 23 had undergone a vitrectomy or a scleral buckle, and five had total retinal detachment. Among the 51 conventionally managed eyes with an unfavorable outcome, four had a partial retinal detachment involving the macula, 43 had undergone a vitrectomy or a scleral buckle, and four had total retinal detachment.

Structural outcome results at the 6-month examination for eyes randomized at high-risk prethreshold ROP were similar to those at 9 months, as shown in Table 7. Six-month structural outcome data were also collected for low-risk prethreshold eyes (determined by the RM-ROP2 program to have a <15% risk for an unfavorable outcome). Among this group of 329 infants, 51 (15.5%) had at least

### Table 5. Distribution of Nine-Month Grating Acuity Outcomes Among Randomized Eyes by Treatment Assignment*

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Eyes Treated at High-Risk Prethreshold (n = 336)</th>
<th>Conventionally Managed Eyes (n = 328)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAVORABLE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal ($\geq$3.70 cycles per degree)</td>
<td>219 (65.2)</td>
<td>203 (61.9)</td>
</tr>
<tr>
<td>Below normal (1.85 to &lt;3.70 cycles per degree)</td>
<td>69 (20.5)</td>
<td>60 (18.3)</td>
</tr>
<tr>
<td>UNFAVORABLE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poor (measurable but &lt;1.85 cycles per degree)</td>
<td>15 (4.5)</td>
<td>18 (5.5)</td>
</tr>
<tr>
<td>Blind/LV (NLP, LP only, LV card only)</td>
<td>33 (9.8)</td>
<td>47 (14.3)</td>
</tr>
<tr>
<td>Total</td>
<td>336 (100)</td>
<td>328 (100)</td>
</tr>
</tbody>
</table>

LP, light perception; LV, low vision; NLP, no light perception.
*Data are presented as number (percentage).

### Table 6. Nine-Month Structural Outcome for Randomized Patients*

<table>
<thead>
<tr>
<th>Eyes Treated</th>
<th>Eyes Treated at High-Risk Prethreshold (n = 336)</th>
<th>Conventionally Managed Eyes (n = 328)</th>
<th>$\chi^2$</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilateral</td>
<td>290† (10.3)</td>
<td>291† (17.2)</td>
<td>11.7‡</td>
<td>$&lt;.001$</td>
</tr>
<tr>
<td>Asymmetric</td>
<td>42§ (0)</td>
<td>36 (2.8)</td>
<td>1.2</td>
<td>.28</td>
</tr>
<tr>
<td>Total</td>
<td>332 (9.0)</td>
<td>327 (15.6)</td>
<td>12.6</td>
<td>$&lt;.001$‡</td>
</tr>
</tbody>
</table>

*Data are presented as number (percentage unfavorable) unless otherwise indicated.
†Less than 293 because of inability to determine the structural outcome.
‡Based on discordant pairs (25 infants with favorable outcomes in earlier treated eyes and unfavorable outcomes in conventionally managed eyes; 6 infants with unfavorable outcomes in earlier treated eyes and favorable outcomes in conventionally managed eyes).
§Less than 43 because of inability to determine the structural outcome.
¶Twenty-four eyes with partial retinal detachment not including the macula (stage 4A) had vitrectomy or a scleral buckling procedure prior to the 9-month examination and are included in this Table as having an unfavorable outcome. When the analysis was based on the structural outcome of these examinations, $P = .002$. Stage 4B or 5 eyes were a priori considered unfavorable in this study.
one eye that progressed to the conventional threshold for treatment and was treated accordingly. An unfavorable outcome occurred in only 1.3% of the 302 low-risk prethreshold eyes for which 6-month structural outcome data were available.

**Relationship to ICROP Classification**

Tables 8 and 9 present the visual acuity and structural outcomes for randomized eyes, stratified by ICROP category and by RM-ROP2 risk category. The greatest benefit of treatment at high-risk prethreshold ROP versus conventional management occurred in eyes that had zone I, stage 3 ROP, with and without plus (30.8% unfavorable versus 53.8% unfavorable). A relative benefit from intervention at high-risk prethreshold ROP for both visual acuity and structural outcomes was also seen among eyes that had zone I, stage 1 or 2 ROP without plus disease, and among eyes that had zone II, stage 3 ROP with plus disease.

As shown at the bottom of Tables 8 and 9, examination of outcome by RM-ROP2 risk category showed greater benefit in both grating acuity and structural outcomes for earlier treatment in high-risk prethreshold eyes with ≥30% risk than in high-risk prethreshold eyes with 15% to <30% risk.

**Other Ocular and Clinical Findings**

The distribution of refractive errors at the 9-month examination was similar between the high-risk prethreshold treated eyes that received early treatment and those that were conventionally managed.

Cataract/aphakia that was not associated with total retinal detachment or vitrectomy was found in four eyes (1.2%) in the group treated at high-risk prethreshold and in four eyes (1.2%) in the conventionally managed group. Nystagmus occurred in 22% of randomized infants with...
bilateral high-risk ROP.

Table 10 compares other ocular and systemic complications of treatment among eyes and infants treated at high-risk prethreshold versus conventionally managed high-risk prethreshold eyes that progressed and later underwent treatment at threshold ROP. Ocular complication rates were similar in the two groups. Systemic complications were higher following treatment at high-risk prethreshold. High-risk prethreshold eyes randomized to early treatment received peripheral retinal ablation at an average postmenstrual age of 35.2 weeks, compared with an average postmenstrual age of 37.0 weeks in conventionally managed eyes that underwent peripheral retinal ablation at threshold ROP.

**DISCUSSION**

This study of treatment for high-risk prethreshold ROP showed a benefit of earlier treatment compared to conventional management in the primary outcome measure of grating visual acuity at 9 months corrected age, and a much greater benefit for structural outcome at 6 and 9
months corrected age. Whereas the rates of ophthalmologic complications were similar among the two treatment arms, infants in the high-risk prethreshold treatment group were more likely to experience systemic complications of apnea, bradycardia, or reintubation following earlier treatment than with treatment at conventional threshold, perhaps due to the earlier average postmenstrual age at which treatment was conducted. There was no mortality or known permanent morbidity attributed to treatment in either group.

This report includes all the 9-month visual acuity and structural outcome examinations (98.2% of infants followed to age 9 months corrected). The beneficial effect of treatment at high-risk prethreshold ROP on structural outcome and on visual acuity outcome is even stronger than originally reported and provides further support for treatment of certain prethreshold eyes (type 1, see below) and careful observation of other prethreshold eyes (type 2).8

In the ETROP study, a novel risk model that was developed based on natural history data from the CRYO-ROP study was used to identify infants at high risk for adverse outcomes from ROP, and only those infants were randomized.3-7 The model is available at http://www.sph.uth.tmc.edu/rmrop/riskcalc/disclaimer.aspx. The model used demographic characteristics of the infant and clinical features of the ROP to classify eyes with prethreshold ROP as high-risk or low-risk. The validity of the model is demonstrated in the finding that high-risk prethreshold eyes that received conventional management showed a much higher percentage of progression to threshold disease than those at low risk (66.4% versus 15.5%, respectively) and a much higher percentage of unfavorable structural outcome (10.0% versus 1.3%, respectively, at 6 months). Eyes with low-risk prethreshold ROP were managed conventionally, with treatment administered if conventional threshold was reached. Overall, study data support treatment of only selected eyes that develop prethreshold ROP.

In the CRYO-ROP study, only about 9.6% of eyes in the natural history cohort with prethreshold ROP had zone I disease, and of these eyes, 33.3% had an unfavorable structural outcome.21 This produced strong weighting on the risk factor, presence of disease in zone I, in the RM-ROP2 model. In the ETROP study, 22.7% of eyes with prethreshold ROP had zone I disease, and because of the strong weighting of zone I disease in the risk model, 94.7% of these eyes were classified as high-risk. These eyes represented about 40% of eyes in the ETROP randomized trial.

The difference between the CRYO-ROP study and the ETROP study in frequency of zone I disease and in the more benign course of zone I disease in the ETROP study is noteworthy. It is tempting to attribute the large number of zone I cases to advances in neonatal care and improved survival rates of the smallest premature infants. However, a thorough analysis (not presented here) of the data from the two studies (CRYO-ROP and ETROP) showed that even when the effects of birth weight and gestational age are controlled, the number of zone I eyes in the ETROP cohort is still significantly higher than in the CRYO-ROP study. Perhaps other changes in the care of premature infants, as yet unrecognized, have given rise to an increase in zone I disease and a decrease in its severity.

An alternative explanation is that examiners may now be more attentive to diagnosing zone I ROP than they were previously. The CRYO-ROP study showed a clear benefit of retinal ablative therapy, but the results in zone I eyes were not impressive, since most of these eyes developed unfavorable visual and structural outcomes even after receiving treatment at threshold. After publication of the CRYO-ROP results, it is possible that eyes were more carefully monitored and observed by ophthalmologists, and that some eyes diagnosed as zone I today might have been categorized as zone II in the era before treatment was proven effective. Additionally, some prior investigators have considered posterior zone II and zone I eyes to be in the same category.4 An assignment of posterior zone II eyes to the zone I category could have the effect of increasing the number of zone I eyes in this study. These subtle factors could explain both the increased frequency and the improved outcome of zone I eyes in ETROP subjects compared to CRYO-ROP subjects.

For all groups of eyes in the ETROP study, the effect of treatment at high-risk prethreshold is more pronounced for structural outcomes than for visual acuity outcomes. A similar discrepancy between the magnitude of the difference between treatment groups for visual acuity versus structural outcomes was also observed in the CRYO-ROP study.11,17 The ETROP study chose visual acuity as its primary outcome because vision is the most important measure of a treatment designed to prevent visual loss from severe ROP. Also, there was a safety concern that treatment at high-risk prethreshold with laser could have some previously unrecognized deleterious effect on visual acuity. The Teller acuity card procedure was selected as the assessment tool for measurement of visual acuity at the 9-month examination because it allows quantification of visual acuity in infants and because it had been used successfully to test infants of a similar age in the CRYO-ROP study.11,17

As in the CRYO-ROP study,11,17,18 the finding of a discrepancy between the magnitude of the treatment group differences in visual acuity versus structural outcomes in the ETROP study is likely to be due, in part, to non-ROP-related ophthalmologic and neurologic problems that can occur in very premature infants.
with severe ROP may develop visual impairment secondary to neural insult or other cerebral factors. In addition, nystagmus, which reduces visual acuity, was found in over 20% of randomized infants with bilateral disease. These non-ROP-related factors may have resulted in reduced acuity in conventionally managed eyes, as well as in eyes treated at high-risk prethreshold ROP, thereby decreasing the difference in visual acuity outcome between the two groups of eyes.

Another likely contributor to the difference between functional and structural outcomes in this study is the immaturity of the visual system at 9 months post-term. Because the visual acuity of a 9-month-old infant is well below that of a normal adult, it is possible that some visual deficits that result from structural abnormalities will not be apparent until older ages, when acuity in normal eyes has improved to near-adult levels. Follow-up testing using recognition (letter) visual acuity charts at older ages would be expected to reveal these deficits in visual acuity, as it did in the CRYO-ROP study.

In evaluating the benefit of treatment at high-risk prethreshold, it is important to take into account possible adverse effects and trade-offs related to earlier treatment. These include an increased rate of systemic complications, potential long-term risks of earlier treatment, an increase in the number of eye examinations needed to detect prethreshold ROP, and an increased frequency of treatment of eyes that would otherwise have undergone spontaneous regression of ROP. In the following paragraphs, we discuss these issues.

In the ETROP study, systemic complications, including apnea, bradycardia, and reintubation, occurred more frequently when peripheral retinal ablative therapy was performed at high-risk prethreshold than at conventional threshold, probably because of the younger average postmenstrual age at which the treatment of high-risk prethreshold eyes occurred. Ophthalmic complications following retinal ablative therapy were comparable in eyes treated at high-risk prethreshold and conventionally managed eyes, as were ophthalmic complications (other than retinal detachment) when the entire group of conventionally managed eyes was compared to the group of eyes treated at high-risk prethreshold. One potential deleterious effect of earlier treatment that was not evaluated in this study is the effect of peripheral retinal ablation on peripheral vision. It is possible that ablation in zone I will result in a greater loss in visual field extent than peripheral ablation in zone II.

Another issue related to earlier treatment of ROP concerns the treatment of eyes that would have undergone spontaneous involution without treatment. The question arises: “How many eyes must receive treatment unnecessarily in order to achieve the benefit of earlier treatment for those eyes that need it?” Based on the structural outcome data at 6 months for a cohort of eyes with prethreshold ROP of all degrees of severity that were conventionally managed, it is possible to determine the number of eyes with high-risk prethreshold ROP that had a favorable outcome without peripheral retinal ablation, as illustrated in Appendix I. Table A in Appendix 1 summarizes the results of an analysis of data from the natural history cohort of prethreshold eyes in the ETROP study. The table shows that 136 (36.6%) of the 372 high-risk prethreshold eyes in the conventionally managed group that were examined at 6 months had favorable structural outcomes and never developed threshold ROP. That is, these eyes met the criteria for early treatment, yet, without treatment, went on to a favorable outcome at 6 months.

To reduce the number of eyes treated that would have had a favorable outcome without intervention, additional strategies for selecting eyes for earlier treatment were explored using this same cohort of prethreshold eyes. Table B in Appendix 1 indicates that eyes with zone I, stage 1 or 2 without plus disease, as well as eyes with zone II, stage 3 without plus disease, had lower rates of progressing to threshold or unfavorable outcome than eyes in the other ICROP categories. And, when treated at the conventional threshold, those two groups of eyes had less than 5% unfavorable structure outcomes.

The results of this analysis, along with the results in Tables 8 and 9, led to a clinical algorithm in which treatment should be considered for eyes with zone I, any stage ROP with plus disease; eyes with zone I, stage 3 ROP without plus disease; and eyes with zone II, stage 2 or 3 with plus disease. As shown in Table C of Appendix 1, use of this ICROP-based, limited selection algorithm would have resulted in treatment of 91 eyes that showed favorable outcomes and never reached threshold disease. This is a reduction of 33% from the 136 such eyes that would have been treated using the RM-ROP2 risk model as applied in the ETROP study (Table A in Appendix 1).

If it is assumed that conventional threshold ROP continues to occur in 6% of infants weighing less than 1,251 g at birth, as in the CRYO-ROP study, the early treatment algorithm based on RM-ROP2 would result in treatment of 9% of infants. The ICROP-based, limited selection criteria described in the preceding paragraph would result in treatment of 8% of infants while retaining the advantage of early treatment for eyes at highest risk of adverse outcomes.

An alternative approach to using the ICROP-based, limited selection algorithm is to base treatment on the RM-ROP2 risk model using a risk of ≥30%, instead of ≥15% as the criterion for early treatment. Since the absolute risk of an unfavorable outcome is low in the risk...
range from 0.15 to <0.30 (Tables 8 and 9) and therefore the relative benefit is not as great as in the higher-risk categories, use of the higher-risk criterion for treatment would reduce treatment of eyes that would not progress to threshold, while maintaining the benefit of earlier treatment in higher-risk eyes. However, using such a model may be more difficult in a clinical setting than using a revised treatment algorithm based on the eye findings (ICROP) alone.

In the ETROP protocol, timely identification of high-risk prethreshold ROP was important to the successful application of an early treatment program; hence, infants were followed on a weekly basis after developing zone II, stage 2 ROP or if they had retinal vessel immaturity with vessels ending in zone I, but no ROP in zone I. Infants with low-risk prethreshold disease were followed twice weekly and managed conventionally unless a change in status caused by development of more severe ROP resulted in advancement into the high-risk category. Thus, a screening program aimed at identifying eyes for treatment prior to conventional threshold may require an increase in the number of screening examinations conducted in the neonatal nursery.

The long-term effects of earlier treatment for ROP are as yet unknown. Because there is considerable development of visual function that occurs between infancy and childhood, and because it is possible to measure aspects of visual function in childhood that are not assessed easily in infancy, the National Eye Institute has funded continued follow-up of randomized children in the ETROP study to the age of 6 years. At that age, recognition visual acuity will be measured with Early Treatment Diabetic Retinopathy Study charts. Visual field extent, contrast sensitivity, and ocular status will also be evaluated, and each child’s developmental status will be assessed. This longer follow-up will give a more detailed evaluation of the impact of earlier treatment on the visual, ophthalmologic, and general developmental status of study participants.

Clinical Implications
The results of this study show that it is possible to identify characteristics of ROP that predict which eyes are most likely to benefit from early peripheral retinal ablation. Based on study data, a clinical algorithm was developed to identify for early treatment eyes with prethreshold ROP that are at highest risk for retinal detachment and blindness, while minimizing treatment of prethreshold eyes likely to show spontaneous regression of ROP. The use of this algorithm circumvents the need for computer-based calculation of low risk or high risk, as was used in this study.

The clinical algorithm shows that, in most circumstances, peripheral retinal ablation should be considered for any eye with:

Type I ROP
• Zone I, any stage ROP with plus disease or
• Zone I, stage 3, with or without plus disease or
• Zone II, stage 2 or 3 ROP, with plus disease

Plus disease, in this instance, requires at least two quadrants (usually six or more clock hours) of dilation and tortuosity of the posterior retinal blood vessels and, hence, the presence of significant disease. The algorithm does not take into account all of the other known risk factors (eg, extent of stage 3, birth weight), and therefore some clinical judgment is required in applying this initial step to the management of ROP.

The clinical algorithm also indicates that continued serial examinations, as opposed to peripheral retinal ablation, should be considered for any eye with:

Type II ROP
• Zone I, stage 1 or 2 with no plus disease or
• Zone II, stage 3 with no plus disease

Treatment should be considered for an eye with type II ROP when progression to type I status or threshold ROP occurs.

It is important to note that even with the addition of early treatment of selected eyes with prethreshold ROP, some eyes will still progress to an unfavorable visual and/or structural outcome. Thus, additional research is needed to identify better methods for the prevention and treatment of severe ROP.

APPENDIX 1

The results presented in this Appendix are based on data from 664 infants who had one eye identified at prethreshold ROP that was not treated unless the ROP progressed to threshold. This natural history cohort was examined to consider alternative treatment strategies for managing prethreshold eyes. Included in this natural history cohort were all control eyes in the asymmetric randomized group, the conventionally managed eyes of infants with bilateral high-risk prethreshold disease, and one eye selected at random from the infants with low-risk prethreshold disease. Table A categorizes these eyes by RM-ROP2 high-risk and low-risk classification, and by whether or not they progressed to threshold ROP for treatment and/or had an unfavorable outcome.

Table B shows this cohort of eyes classified by ICROP categorization and indicates that nearly 100% of zone I eyes were classified as high-risk. However, among eyes...
with zone I, stage 1 or 2 ROP with no plus disease, a much lower percentage progressed to threshold and/or an unfavorable outcome than occurred in all other categories of zone I disease. Among zone II eyes, those with zone II, stage 3 with no plus disease did much better than eyes in other categories. These data led to a proposed grouping of the eyes by the ICROP classification into type I and type II ROP. The outcome results achieved by dividing the cohort into type I and type II ROP based on the ICROP classification are shown in Table C.

**APPENDIX 2**

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REFERENCES


Good's paper detailing the outcome of the Early Treatment for ROP (ETROP) Randomized Clinical Trial, the latest in a series of successful trials to ask and answer important questions about the disease ROP. Surely this must place such trials, CRYO-ROP, LIGHT ROP, STOP ROP, and ETROP among the most successful intervention trials supported by taxpayer monies through the medium of the National Eye Institute.

When discussing such a massive trial in a few short minutes it seems to me best to try to focus on a few questions that get to the heart of the effort involving 26 centers, 240 investigators and 823 infants studied at a cost to the taxpayer of 13 millions of dollars.

Is the disease studied important? The answer is yes. ROP once thought banished as an ophthalmic and pediatric curiosity is back with a vengeance. It is among the three top causes of blindness in infancy and is almost world-wide (first and second world) in its distribution. It is very much a paradox of medical progress: As modern neonatology has advanced in its sophistication, the result has been the salvage of many infants well under a kilogram in birth weight and 30 weeks gestational age. This has provided an almost inexhaustible pool of infants vulnerable to the most severe forms of ROP in numbers never seen before.

Is the question asked an important one? Once again the answer is yes, emphatically yes. Should the threshold for treatment be lowered to include infants previously classed as not yet at threshold but at pre-threshold? Should they be treated at this new level of severity of disease? The downside risk here would be subjecting some (an unknown number) of these infants to a treatment that might not indeed be necessary.

Is the study design adequate to provide an unequivocal answer? The Randomized Clinical Trial is the gold standard in assessing the benefit of intervention or treatment outcomes in medicine today. This is a lesson learned early and well by the cadre of investigators who have become addicted to the disease (ROP) and its treatment over the course of the last two decades.

Are the results significant? Both clinically and statistically they are. Most importantly what gives them that significance clinically? This study includes in its sample 188 eyes (23%) with Zone 1 disease in contrast to CRYO-ROP that had 33 eyes (12%) eyes with Zone 1 disease. The results of treatment of Zone 1 eyes in the CRYO study were dismal; 87% had an unfavorable response to treatment, little better than no therapy at all, where 93% were unfavorable. In the current trial, Zone 1 high risk eyes had seven more discordant pairs where the early treatment produces a favorable response in that eye compared to an unfavorable outcome in its fellow eye treated at threshold versus only one pair where the

DISCUSSION

Dr John T. Flynn. It is a privilege to discuss Dr Bill
threshold treated eye had a favorable response compared to the unfavorable response in its fellow early treated eye. An added benefit is the clinical algorithm for Zone 1 disease that has arisen from the data with regard to treatment. This algorithm is one that will be useful to the hundreds of clinicians throughout the world who are and will continue to be faced with making very tough decisions about whether and when to treat these infants.

Are there any downsides we should be aware of in this otherwise well done study? Lest we come away thinking this study has no downside, it does. It is something Bill Good, as PI, and all of the investigators are well aware of. Treating these pre-threshold infants on an average of 2 weeks earlier than those treated at threshold carries levels of risk of side effects, both ocular and systemic, not encountered with infants treated at threshold. The results (of any treatment trial) can be summarized in this regard from two standpoints: the number needed to treat to see the benefit of the new treatment. It is simply the reciprocal of the absolute difference in benefit between the two treatments. In this case it is the difference between 19.8% and 14.3% or 5%. So one must treat 20 infants before a difference in outcome is seen in one infant. The same logic holds for side effects or harm as well. And here the two weeks earlier treatment plays a critical role—just 2 weeks in age between the two groups it makes a tremendous difference. How many infants must be treated until one observes a difference in the systemic side effects of therapy? The answer is, if one treats two infants, one will see a potentially serious systemic side effect in one. The systemic complication will occur twice as often in the early treated infants. This should give us a measure of circumspection in our decision-making regarding applying the results of this study in the clinic.

Dr Good and his co-investigators have brought home another in a series of successful treatment trials in the prevention of this potentially devastating disease. The effect though small is significant. As important, if not more so, they have been able to derive rules for dealing with Zone 1 disease that may prove to be the most important outcome of the study.

Dr Allan J. Flach. Could you put the quality of life of these tiny infants into a clearer perspective? How do these kids do in later life?

Dr Gerhard W. Cibis. I have a study interest in children that were treated with lasers and then developed maculopathies in situations that the laser treatment did not directly involve the posterior pole. I am concerned with reflective phototoxicity of the laser treatment itself. As we narrow the criteria to where we have to treat more and more children in order to achieve a 5 percent improvement, I have a concern that when we’re only comparing the laser-treated against the laser-untreated, we may be creating some phototoxicity maculopathies with long-range vision problems that are lost in the study because that’s not looking at that.

Dr Edward L. Raab. What you do in the study for following Zone 1 immature vessels with no ROP at one-week intervals? That might have been something to enhance recruitment, or it might actually have made a difference whether you followed those individuals at one week or at two weeks, particularly if you’re examining them closer to 28 days or closer to 42 days. Did you conclude that it is necessary to follow nearly incomplete vascularization in Zone II at intervals of one week?

Dr George R. Beauchamp. This might be an ideal disease to apply the tools of value-based medicine to get some context of how much this intervention is going to improve the lives of these children. This disease is perhaps the highest-value intervention in all of medicine, let alone ophthalmology. I suggest that some of us consider with you extending the evidence that you have to the value-based tools to get these answers.

Dr William V. Good. No doubt it makes an enormous difference to a child, regardless of neurocognitive or neurologic outcome, to have sight. In the CRYO-ROP Study, where many infants who had favorable retinal outcomes still have visual acuity less than 20/40, there’s no argument that it is far better to have, say, 20/100 visual acuity than to be blind.

If one looks at the overall outcome for premature infants in the birth weight category <1000 grams, about 40 percent of these children have neurocognitive problems, and about 20 percent have cerebral palsy. The mortality rates have gone down in this birthweight group, but the morbidity rates have stayed the same or increased. ROP rates appear to be approximately the same if you just look at the data that we present in this paper.

Thank you to Dr Beauchamp. I would be interested in pursuing value-based medicine further with him and others.

Any treatment intervention carries a side-effect profile that needs evaluation. We can debate the number needed to treat in the ETROP Study. The control infants in this Study were often treated, as part of their conventional management. About 66 percent of control infants went to threshold and had to have the control eye treated with laser or cryotherapy. Therefore, we’re not comparing treatment to no treatment; we’re comparing earlier treatment to conventional management and treatment. So, this number needed to treat doesn’t hold. To avoid over-treat-
ment, we adopted the Type I/Type II algorithm, to reduce the rate of unnecessary earlier treatment. Guidelines from the ETROP Study indicate that certain eyes can avoid earlier treatment, and be observed for signs of progression of disease.

We are concerned about reflective phototoxicity and other potential side effects of treatment, particularly for Zone I eyes. From personal experience looking at some of the Zone I eyes that had treatment with resulting favorable structural and favorable visual acuity outcome, there is a lot of ablated retina. We will have the opportunity to follow these children at least to the age of 6 to learn more about what the long-term potential effects are from such extensive ablation.

Concerning the question about Zone I immature vessels and the frequency with which these children were followed, we did follow these children on a weekly basis. This was a study design issue. It is not necessarily a recommendation for clinical practice. We wanted to identify pre-threshold disease at the earliest possible time point. We are evaluating our data now to determine if we can come up with evidence-based screening guidelines. We hope to determine whether the eyes that were followed more frequently were at risk for getting pre-threshold and at what time sequence they were at risk so that we might be able to offer additional guidelines useful to clinicians.
EFFECTS OF LATRUNCULIN B ON OUTFLOW FACILITY, INTRAOCULAR PRESSURE, CORNEAL THICKNESS, AND MIOTIC AND ACCOMMODATIVE RESPONSES TO PILOCARPINE IN MONKEYS

BY Mehmet Okka MD, Baohe Tian MD, AND Paul L. Kaufman MD*

ABSTRACT

Purpose: To determine if low doses of topical latrunculin B (LAT-B) will increase outflow facility and decrease intraocular pressure (IOP) without adversely affecting the cornea, and inhibit miotic and accommodative responses to pilocarpine, in ocular normotensive monkeys.

Methods: Intraocular pressure was measured by Goldmann tonometry before and after one and nine dose(s) of 0.005% and 0.01% topical LAT-B/vehicle given twice daily on successive weeks. Outflow facility was then measured by perfusion following 15 doses. Central corneal thickness was measured by ultrasonic pachymetry before and after one and nine dose(s) of 0.01% LAT-B/vehicle. Pupillary diameter (calipers) and accommodation (refractometry) before and after one dose of 0.005% and 0.02% LAT-B were determined.

Results: LAT-B dose-dependently decreased IOP, multiple doses more than a single dose. Maximal hypotension after one dose was 2.5 ± 0.3 mm Hg (0.005% LAT-B; n = 8; P < .001) or 2.7 ± 0.6 mm Hg (0.01% LAT-B; n = 8; P < .005); maximal hypotension after nine doses was 3.2 ± 0.5 mm Hg (0.005% LAT-B; n = 8; P < .001) or 4.4 ± 0.6 mm Hg (0.01% LAT-B; n = 8; P < .001). Outflow facility was increased by 75 ± 13% (n = 7; P < .005). Central corneal thickness was not changed after one or nine dose(s) of 0.01% LAT-B. The miotic and accommodative responses to intramuscular pilocarpine were dose-dependently inhibited. At 0.02% LAT-B, the inhibition of miosis was essentially complete when compared with the pre-LAT-B value, whereas the inhibition of accommodation was only about 25%. At 0.005% LAT-B, the effects were trivial.

Conclusions: In ocular normotensive monkeys, 0.005/0.01% LAT-B administered topically increases outflow facility and/or decreases IOP, but does not affect the cornea. Multiple doses reduce IOP more than a single dose. LAT-B dose-dependently relaxes the iris sphincter and ciliary muscle, with some separation of the miotic and accommodative effects.

INTRODUCTION

Latrunculins, macrolides isolated from the marine sponge Latrunculia magnifica, are specific and potent actin-disrupting agents that sequester monomeric G-actin, leading to the disassembly of actin filaments.1-3 Latrunculins A and B (LAT-A and B) are two common latrunculins, which cause reversible dose- and time-dependent destruction of actin bundles and associated proteins in varieties of cultured cells, including human trabecular meshwork cells.4 In living monkeys, both LAT-A and LAT-B increase outflow facility and decrease intraocular pressure (IOP).5,6,9 LAT-B also increases outflow facility in organ-cultured anterior segment of porcine eyes,3 suggesting a direct effect on outflow resistance in the conventional drainage pathway. The latter has been confirmed by a recent morphologic study of the trabecular meshwork in the live monkey eye (Tian B, et al, ARVO, 2004; abstract). Since LAT-B, compared with LAT-A, is more potent in increasing outflow facility6,8 and produces smaller transient increases in aqueous humor formation, corneal endothelial permeability, and protein concentration in the anterior chamber,9 LAT-B may be a better candidate than LAT-A as a potential antiglaucoma medication. However, a single dose of 20 µL of 500 µM
(-0.02%) LAT-B administered topically, which decreases IOP in living monkeys, still produces a transient increase in corneal thickness when applied to the central cornea as four drops of 5 µL volume. Presumably, multiple treatments with the high concentration of LAT-B might induce more apparent side effects in the cornea.

We hypothesized that repetitive lower concentrations and total doses in higher-solution volumes, spread out over the entire corneal or conjunctival surface in the larger human eye, might minimize or avoid corneal toxicity induced by high concentrations of cytoskeletal drugs without attenuating their effects on outflow resistance. To test this hypothesis, we determined the effects of a single dose or multiple doses of 0.005/0.01% topical LAT-B on outflow facility, IOP, and/or central corneal thickness in normotensive monkey eyes. To learn more about the drug-induced changes in the anterior segment physiology, the pupil diameter and accommodation following 0.005/0.02% topical LAT-B were also determined.

METHODS

Animals and Anesthesia

Twenty-seven adult normal cynomolgus monkeys (Macaca fascicularis) of both sexes, weighing 3 to 8 kg, were studied. All experiments were conducted in accordance with University of Wisconsin and National Institutes of Health guidelines, and with the ARVO Statement for the Use of Animals in Ophthalmic and Visual Research. All monkeys were free of anterior chamber cells and flare by slit-lamp biomicroscopy when studied. Anesthesia for tonometry or pachymetry was induced with intramuscular ketamine (10 mg/kg) and maintained with supplemental intramuscular injections as required (usually 5 mg/kg every 30 to 45 minutes). Anesthesia for anterior chamber perfusion or refractometry was induced with intramuscular ketamine (10 mg/kg), followed by intravenous pentobarbital sodium (15 mg/kg).

Drug Preparation and Administration

LAT-B was obtained from Sigma Chemical Co (St Louis, Missouri) and stored as a 2 mM stock solution in dimethyl sulfoxide (DMSO; Sigma Chemical Co) at -20°C. LAT-B solutions for topical administration were freshly prepared in Bárány’s solution with 25% DMSO. Twenty microliters of 0.005% (1 µg/20 µL), 0.01% (2 µg/20 µL), or 0.02% (4 µg/20 µL) LAT-B were composed of 1.26, 2.53, or 5.00 µL of 2 mM LAT-B stock solution and 3.74, 2.47, or 0.00 µL of DMSO plus 15 µL of Bárány’s solution. The 25% DMSO served as a vehicle control. In IOP protocols, the drug or vehicle solution was administered to the central cornea of opposite eyes of either ketamine-anesthetized (day 1 and day 5; 4×5 µL drops) or fully conscious and manually restrained monkeys (day 2 through day 4; 2×10 µL drops) twice daily for 4.5 days at 8 AM and 4 PM. Eye drops were administered at 30- to 60-second intervals with blinking prevented between drops. The same eyes of the same animals were treated with the drug in two IOP protocols, with the 0.01% LAT-B experiment conducted the week immediately following the 0.005% LAT-B experiment. Following the 0.01% LAT-B IOP experiment, the monkeys were treated with 0.01% drug/vehicle solution at 4 PM on day 5, and then once (days 6 and 7) or twice (day 8) daily (2×10 µL) for three additional days while fully conscious and manually restrained. On day 9, these monkeys were treated again with the same dose of the drug (4×5 µL) under ketamine anesthesia 2 hours before the anterior chamber perfusion. For pachymetry, different monkeys were treated with 0.01% LAT-B twice daily for 4.5 days under ketamine anesthesia. For refractometry and pupil-diameter measurement, monkeys were treated with 0.005/0.02% LAT-B (4×5 µL) one time under ketamine plus pentobarbital anesthesia. Administering the drug/vehicle solution to fully conscious and manually restrained monkeys in the IOP/outflow facility protocol was designed to reduce any potential cumulative effect of repeated ketamine administration on IOP or outflow facility during the multiple treatments (Bunch TJ, et al, ARVO, 2003; abstract).

IOP Measurement

Intraocular pressure was determined on day 1 (before and after the first dose) and day 5 (before and after the ninth dose) with a minified Goldmann applanation tonometer, using “Half and Half” creamer solution (Borden Inc, Columbus, Ohio) as the tear film indicator, with the monkey lying prone in a head holder. For each eye, three IOP readings were averaged as a baseline or pretreatment IOP before administration of the first or ninth dose of 0.005/0.01% LAT-B or vehicle, and single IOP readings were taken after the drug/vehicle administration hourly for 6 hours.

Outflow Facility Measurement

Total outflow facility was determined by two-level constant pressure perfusion of the anterior chamber with Bárány’s mock aqueous humor, using a one-needle technique and correcting for internal apparatus resistance. Outflow facility was measured for 90 minutes 2 hours after the 15th dose of 0.01% LAT-B or vehicle on day 9.

Central Corneal Thickness Measurement

Central corneal thickness was determined by ultrasonic pachymetry (DGH-1000 ultrasonic pachymeter, DGH Technology, Inc, Solana Beach, California) on day 1 (before and after the first dose) and day 5 (before and...
after the ninth dose). For each eye, three readings were averaged as a baseline or pretreatment value before administration of the first or ninth dose of 0.01% LAT-B or vehicle, and single readings were taken after the drug/vehicle administration every 30 minutes for 4 hours and then hourly for 2 hours.

**Pupil and Accommodation Measurement**

Accommodation (difference between baseline and post-drug refraction) was determined with a Hartinger coincidence refractometer. Pupil diameter was measured with vernier calipers under normal room light (350 lux). Baseline refraction, pupillary diameter, or both were measured, followed by topical application of 2.5% phenylephrine (stimulates the iris dilator muscle without influencing the iris sphincter and ciliary muscle,\textsuperscript{14,15} facilitating measurement of miosis and accommodation\textsuperscript{8}). Refraction and/or pupillary diameter were measured again approximately 30 minutes later, after which 20 µL (4×5 µL) of 0.005/0.02% LAT-B was administered topically to one eye and vehicle to the other. Refraction and pupillary diameter were determined 85 minutes after LAT-B. Five minutes later, approximately 3 mL of pilocarpine solution was infused intramuscularly in the thigh (1.5 mg/kg) over 10 minutes. Refraction was determined every 5 minutes after pilocarpine infusion until stable, and final pupillary diameter was then measured.

**Slit-lamp Examination**

Slit-lamp biomicroscopy was performed before drug administration, during IOP measurement (1, 3, and 6 hours after drug administration), and before pachymetry and anterior chamber perfusion. The integrity of the corneal epithelium and endothelium, the presence of flare or cells in the anterior chamber, and the clarity of lens were noted. All animals were free of preexisting ocular abnormalities when studied.

**Data Analysis**

Data are given as mean ± SEM for n eyes or animals. Predrug or postdrug treated versus contralateral control; postdrug or postvehicle versus ipsilateral baseline; and baseline corrected postdrug treated versus control comparisons were made using the two-tailed paired \(t\) test for differences versus 0.0 or ratios versus 1.0.

**RESULTS**

**Intraocular Pressure**

A single dose of 0.005% LAT-B lowered IOP from 19.3 ± 0.8 to 16.4 ± 0.7 mm Hg within 6 hours. After adjustment for baseline and contralateral IOP, the maximal hypotension of 2.5 ± 0.3 mm Hg (\(n = 8, P < .001\)) occurred at hour 6. Multiple doses (nine doses) of 0.005% LAT-B reduced IOP similar to a single dose, but the significant IOP reduction occurred earlier (hour 1 versus hour 3) and the maximal ocular hypotension was slightly greater (3.2 ± 0.5 mm Hg; \(P < .001\)). Intraocular pressure at 16 hours after the eighth treatment (IOP at 0 hours on day 5) in the LAT-B–treated eye was significantly lower than that in the contralateral control eye (–1.4 ± 0.3 mm Hg; \(P < .005\)) (Figure 1A). A single dose of 0.01% LAT-B lowered IOP from 18.8 ± 0.7 to 15.7 ± 0.8 mm Hg within 6 hours. After adjustment for baseline and contralateral IOP, the maximal hypotension of 2.7 ± 0.6 mm Hg (\(n = 8, P < .005\)) occurred at hour 3. Multiple doses (nine doses) of 0.01% LAT-B induced a greater IOP reduction than a single dose, with the maximal hypotension of 4.4 ± 0.6 mm Hg (\(P < .001\)) at hour 4. The pre-ninth-treatment IOP (IOP at 0 hours on day 5) in the LAT-B–treated eye tended to be lower than that in the contralateral control eye (–1.7 ± 0.7 mm Hg; \(P = .056\)). Although the monkeys had not received any treatment for 3 days after the ninth treatment with 0.005% LAT-B, the baseline IOP (IOP at 0 hours on day 1) in the LAT-B–treated eye in the 0.01% LAT-B protocol (Figure 1B) did not return to the level before the first treatment with 0.005% LAT-B (Figure 1A).

**Outflow Facility**

LAT-B significantly increased outflow facility by 75 ± 13% (\(n = 7, P < .005\)) during the overall 90-minute post-drug perfusion beginning 2 hours after the 15th treatment of 0.01% LAT-B. The reason \(n = 7\) rather than 8 is that one monkey died on day 6 due to an unrelated disease. In analysis per three 30-minute perfusion periods, the drug increased outflow facility by 35 ± 14%, 69 ± 14%, and 100 ± 14% in the first, second, and third 30-minute durations, respectively (Table 1; Figure 2).

**Corneal Thickness**

On day 1, baseline central corneal thickness was 456.3 ± 17.0 µm in the LAT-B–treated eye and 457.7 ± 18.2 µm in the contralateral control eye. Central corneal thickness after the first treatment varied between 454.6 ± 17.2 and 462.4 ± 17.0 µm in the LAT-B–treated eye, and between 453.4 ± 15.4 and 458.6 ± 18.7 µm in the contralateral control eye during 6 hours pachymetry. On day 5, pre-ninth-treatment central corneal thickness was 448.4 ± 17.9 µm in the LAT-B–treated eye and 455.9 ± 18.6 µm in the contralateral control eye. The central corneal thickness after the ninth treatment varied between 454.4 ± 16.4 and 462.2 ± 18.2 µm in the LAT-B–treated eye, and between 452.2 ± 18.8 and 457.2 ± 19.6 µm in the contralateral control eye during 6 hours pachymetry. Collectively, the central corneal thickness in the LAT-
The B–treated eye was only 0.9 to 8.1 µm (P = .08–.82) or 3.1 to 7.1 µm (P = .07–.37) thicker than that in the vehicle-treated eye after one dose or nine doses of 0.01% LAT-B, after adjustment for ipsilateral baseline (Figure 3).

**Pupil and Accommodation Measurement**

**Pupillary Diameter**

Baseline pupil diameters of both eyes in all monkeys were similar (Figure 4A and C). Twenty-five minutes after phenylephrine administration, both pupils dilated equally (in the 0.02% LAT-B protocol: 7.2 ± 0.3 mm versus 7.2 ± 0.3 mm, n = 8; P = NS, Figure 4A; in the 0.005% LAT-B protocol: 7.0 ± 0.3 mm versus 7.0 ± 0.3 mm, n = 6, P = NS, Figure 4C). Eighty-five minutes after topical administration of 20 µL of 0.02% LAT-B, the pupils in the LAT-B–treated eyes dilated further relative to the contralateral controls (to 8.0 ± 0.3 mm versus 7.0 ± 0.4 mm, P < .005, Figure 4A). However, 85 minutes after 20 µL of 0.005% LAT-B, the pupil in the LAT-B–treated eye was only slightly larger than that in the vehicle-treated eye. When pilocarpine was infused intramuscularly in the thigh, the control pupils constricted but the pupils after 0.02% LAT-B did not (5.6 ± 0.3 mm in controls versus 7.0 ± 0.4 mm in LAT-B–treated eyes, P < .001, Figure 4A). The inhibition of miosis was essentially complete when compared with the pre-LAT-B value (7.0 ± 0.4 versus 7.2 ± 0.3). This miosis was only slightly inhibited by 0.005% LAT-B (5.0 ± 0.4 mm in the LAT-B eye versus 4.3 ± 0.3 mm in the control eye).

**Accommodation**

No significant differences between pilocarpine-induced accommodation in LAT-B–treated versus control eyes were observed initially after 20 µL of 0.02% LAT-B (Figure 4B). However, the accommodation plateau in the LAT-B–treated eye occurred earlier than that in the control eye (30 versus 40 minutes after the intramuscular pilocarpine). A statistically significant difference between eyes was observed during the period of 30 to 40 minutes after intramuscular pilocarpine, with the LAT-B–treated eyes accommodating approximately 2.5 ± 0.5 diopter (± 25 ± 8%) less than the controls eventually (8.9 versus 11.4 D; n = 5; P < .01; Figure 4B). The accommodation was only

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**TABLE 1. EFFECT OF LAT-B ON OUTFLOW FACILITY IN MONKEYS**

<table>
<thead>
<tr>
<th>PERFUSION PERIOD</th>
<th>LAT-B</th>
<th>VEHICLE</th>
<th>LAT-B / VEHICLE</th>
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<tr>
<td>90 min</td>
<td>0.93 ± 0.19</td>
<td>0.51 ± 0.08</td>
<td>1.75 ± 0.13</td>
</tr>
<tr>
<td>First 30 min</td>
<td>0.58 ± 0.10</td>
<td>0.43 ± 0.05</td>
<td>1.35 ± 0.14†</td>
</tr>
<tr>
<td>Second 30 min</td>
<td>0.59 ± 0.19</td>
<td>0.51 ± 0.06</td>
<td>1.69 ± 0.14‡</td>
</tr>
<tr>
<td>Third 30 min</td>
<td>1.19 ± 0.28</td>
<td>0.57 ± 0.11</td>
<td>2.00 ± 0.14§</td>
</tr>
</tbody>
</table>

LAT-B, latrunculin B.

*Following 15 doses of 0.01% LAT-B/vehicle (Figure 2), outflow facility was measured by two-level constant pressure perfusion for 90 minutes. No baseline outflow facility was determined, but all monkeys were selected from those that had similar baseline facilities in both eyes per previous studies. Data are mean ± SEM (µL/min/mm Hg for outflow facility) for seven animals. Ratios are unitless. Difference between eyes was tested for ratios ≠1.0 by the two-tailed paired t test.

†P < .005.
‡P < .05.
§P < .001.
Slit-lamp Examination
During IOP measurement, most monkeys had mild punctate corneal epithelial defects at 3 to 6 hours after the drug administration, but the defects in LAT-B–treated eyes were similar to that in control eyes. Additionally, the punctate corneal epithelial defects seen during tonometry after the first treatment on day 1 had disappeared in both eyes of almost all monkeys at approximately 16 hours after the eighth dose (before tonometry on day 5). No other abnormality was observed in any monkey in any protocol during slit-lamp examination.

DISCUSSION
This study has shown that LAT-B administered topically decreases IOP in normotensive monkeys in a dose-dependent manner, with multiple doses producing greater IOP reduction than a single dose. This is consistent with many current clinical and experimental antiglaucoma drugs that have greater effects following multiple treatments in both normotensive17,18 and glaucomatous19,20 monkeys. Some ocular hypotensive effect of multiple administrations of LAT-B appears to last more than 16 hours, evidenced by the lower IOP in the LAT-B–treated eye than in the vehicle-treated eye at 16 hours after the eighth treatment in both the 0.005% and 0.01% LAT-B protocols (Figure 1A and B), and by the tendency toward slightly lower baseline in the drug-treated eye than in the control eye 3 days after the ninth treatment of 0.005% LAT-B (Figure 1B). In a previous study,9 a single dose of 20 µL of 500 µM (~0.02%) LAT-B maximally decreased IOP by 3.1 mm Hg, slightly greater than the maximal IOP reduction (~2.7 mm Hg) induced by a single dose of 0.01% LAT-B, and apparently smaller than the IOP reduction (~4.4 mm Hg) induced by multiple doses of 0.01% LAT-B, in the current experiments. This further indicates that LAT-B dose-dependently decreases IOP and that multiple doses of LAT-B are more effective than a single dose. In the present study, 15 treatments with 0.01% LAT-B significantly increase outflow facility in the monkey eye, which, in conjunction with our previous findings,5,8 suggests that LAT-B decreases IOP by reducing outflow resistance in the trabecular meshwork.
In the previous study, a single dose of 0.02% topical LAT-B also transiently increased the central corneal thickness of the monkey eye by up to 47 µm within 3 hours. Unlike the higher dose studied previously, a single and multiple dose(s) of 0.01% LAT-B administered topically in the present study do not change the central corneal thickness. This indicates that the 0.01% concentration of the drug does not significantly affect the corneal endothelium. By slit-lamp biomicroscopy, 0.01% LAT-B is also less toxic to the corneal epithelium than the higher dose studied before. The LAT-B doses used in this study do not produce any additional punctate corneal epithelial defects in the LAT-B–treated eye compared with the vehicle-treated eye. The mild punctate corneal epithelial defects in both eyes, occurring 3 to 6 hours after the drug administration, is a common phenomenon during tonometry in ketamine-anesthetized animals, presumably due to reduced blinking under ketamine anesthesia and frequent IOP measurements. All these seem to support our hypothesis from previous studies that repetitive lower concentrations and total doses in higher-solution volumes, spread out over the entire corneal or conjunctival surface, may minimize or avoid corneal toxicity.

A recent morphologic study (Tian B, et al, ARVO, 2004; abstract) has revealed that LAT-B induces formation of numerous cytoplasmic projections of the subcanalicular cells and massive “ballooning” of the JXT region, leading to a substantial expansion of the space between the subcanalicular cell layer and the trabecular collagen beams. Additionally, LAT-B also significantly increases the junction-to-junction distance of the inner wall cells of Schlemm’s canal (Tian B, et al, ARVO, 2004; abstract), although the increase is not as great as that after the serine-threonine kinase inhibitor H-7. All these structural changes in the trabecular meshwork may be consequent to the drug-induced cellular relaxation and account for the drug-induced decrease of outflow resistance in the trabecular meshwork. The current physiology data indicate that LAT-B dose-dependently relaxes intraocular smooth muscles. This further supports that cellular relaxation could be an important mechanism by which LAT-B decreases outflow resistance in the trabecular meshwork, since H-7, which decreases outflow resistance primarily by relaxing the trabecular meshwork, also relaxes the iris sphincter in vivo and ciliary muscle strips in vitro. More interestingly, although 0.02% LAT-B almost completely inhibits the miotic response of the monkey eye to pilocarpine, it inhibits the accommodative response to pilocarpine by only up to 25%. The reason for the separation is not clear, but a pharmacokinetic explanation seems plausible. Pilocarpine is a classic antiglaucoma medication, which indirectly increases outflow facility by contracting the ciliary muscle. However, the induced miosis, which reduces vision especially in elderly patients with incipient cataract, restricts its usage. The relative dissociation of miotic and accommodative responses to pilocarpine after LAT-B provides a possibility that the combination of a low but still facility-effective dose of pilocarpine with a facility-effective and cornea-safe dose of LAT-B may induce a facility increase greater than that induced by either drug alone, without damaging the cornea and constricting the pupil.

Collectively, the fact that 0.005% and 0.01% topical LAT-B increases outflow facility and/or decreases IOP without adversely affecting the cornea suggests that a low dose of topical LAT-B may have potential as a safe and trabecular meshwork–selective antiglaucoma medication.

REFERENCES


DISCUSSION

Dr Robert Ritch. Sponges are extremely interesting creatures. The phylum Porifera consists of about 15,000 species of one of the earliest forms of metazoan development. They consist of about 10 different cell types and can be disaggregated into a single cell suspension by removing calcium from the medium. When calcium is re-added, the cells reaggregate into clumps, each of which can develop again into a mature sponge. Sponges are sessile and produce numerous toxic substances, perhaps to discourage predators, but more likely to protect the space comprising their environment from settlement by competitive species. Some of the many compounds isolated from sponges have potential beneficial effects for humans, including compounds with respiratory, cardiovascular, gastrointestinal, anti-inflammatory, antitumor, and antibiotic activities. To these, we can hopefully add the latrunculins.

Transient disruption of the cellular structure of the trabecular meshwork offers a potential new approach to increasing aqueous outflow. Inhibition of aqueous production, the basis of most of our presently used drugs, may be detrimental to trabecular function in the long term, a concept well reviewed a decade ago by Becker.1 Pilocarpine, the first drug used to treat glaucoma, is usually prescribed 4 times daily for lowering IOP, although nasolacrimal occlusion can effectively make this a twice daily drug.2 Nevertheless, its use has been largely abandoned in favor of newer agents.

A generation ago, Anders Bill et al3 perfused eyes with EDTA. This produced distention of the juxtacanalicuicular meshwork, wash-out of extracellular material, and disintegration of the denuded trabecular cores. Its toxicity prevented clinical use. In 1982, Kaufman and Erickson showed similar effects with cytochalasins.4 However, a great idea is a great idea, and Dr. Kaufman never gave up.

The authors have shown that low concentrations of topically administered latrunculin-B in single or multiple doses decrease intraocular pressure (IOP) and increase the coefficient of aqueous outflow in normotensive monkeys. Importantly, the cornea was not adversely affected, suggesting that this drug may have potential as an antiglaucoma drug which can be targeted specifically at the trabecular meshwork.

I would ask a few questions. In these experiments, the latrunculin was dissolved in a medium containing 25% DMSO? Is this necessary for solubilization and, if so, how would a formulation be prepared for human use? Have the authors performed the same studies yet in glaucomatous monkeys? In 1986, Epstein et al described acute reduction of outflow in cynomolgus monkey eyes when perfused with pigment particles isolated from the iris and ciliary body.5 Have the authors considered pretreating a group of monkeys with latrunculin-B to see if this reduction can be prevented when compared to perfusion into a control group and would they think such a study worthwhile?

From my perspective, the most important potential application of latrunculin would be in eyes with exfoliation syndrome (XFS), which is, overall, the most common identifiable cause of open-angle glaucoma worldwide, accounting for the majority of the open-angle glaucoma in
some countries. We have estimated that there are about 60 million people with XFS, of whom 15 million have elevated IOP and 5 million have glaucoma. Elevated IOP with or without glaucomatous damage occurs in approximately 25% of persons with XFS, or about 6 to 10 times the rate in eyes without XFS. Glaucoma in XFS has a more serious clinical course and worse prognosis than primary open-angle glaucoma. Exfoliative glaucoma is associated with an increase in aqueous outflow resistance and elevated IOP. The most likely mechanism responsible is blockage of the meshwork by a combination of exfoliation material and liberated iris pigment.

Increased trabecular pigmentation is prominent and is apparent in virtually all patients with clinically evident disease. In virtually all studies of patients with clinically unilateral XFS, the trabecular pigment is almost always denser in the involved eye. Eyes with exfoliative glaucoma tend to have greater pigmentation than both eyes with XFS but without glaucoma and eyes with POAG. There appears to be a highly significant correlation between elevated IOP and the degree of pigmentation of the meshwork.

I have hypothesized that, if these eyes were perfused with latrunculin, transient disruption of the trabecular meshwork might allow release of trapped exfoliation material and pigment and, when the meshwork is reconstituted, markedly improved function and lowered IOP. Indirect evidence suggesting this possibility are reports that suctioning of the meshwork in eyes with XFS and trabeculotomy have greater success in eyes with XFS than those with primary open-angle glaucoma. It is possible that a single treatment might have a prolonged effect, needing to be repeated perhaps only at widely spaced intervals. Certainly such a breakthrough would be a welcome therapeutic advance benefiting millions of people.

I would like to commend the authors for an elegant investigation and urge them to attempt to bring this drug to fruition for clinical use.

REFERENCES

it becomes a long-term drug, and considering the potential action of the drug, it will be necessary to look at other things about the cornea with regard to toxicity and, particularly, corneal stem cells, which have the potential to create a variety of favorable effects. It may take time to see the adverse outcome in terms of poor epithelial stasis, static state, and epithelial repair.

Dr Paul L. Kaufman. Dr Ritch alluded to a concept that Dr Bernard Becker also had a number of decades ago about reduction of aqueous flow possibly affecting outflow facility adversely. We just published a paper in Experimental Eye Research in monkeys showing that chronic secretory suppression reduces aqueous outflow facility in the live, nonhuman primates. It is even more so if you use prostaglandin treatment as well because that redirects fluid away from the trabecular meshwork and out the ciliary muscle. In fact, there may be long-term downsides to these approaches.

Is DMSO necessary? These were proof-of-principle studies and also LAT-B is a relatively insoluble molecule. This is a problem for the pharmaceutical industry to solve. Whether you change the molecule or whether you change the vehicle, you can actually get away with a little bit less of a concentration of DMSO than I showed in these studies. DMSO itself, at this concentration and for this period of time, does not seem to have an adverse effect. We would certainly like to have a vehicle or perhaps an altered molecule that doesn't require an extraordinary solubilizing agent. We have not done this in glaucomatous monkeys yet but we have done it in glaucomatous monkeys with some other compounds on the contractility side. It does work. I would just remind all of you that the glucoma monkey model that we use now, the laser glaucoma model, is a scar model so the physiology may be completely different. It would be great if we had a reliable steroid glaucoma model in the monkey, but unfortunately, we do not.

In terms of the pigment and exfoliation obstruction type of pathology that Dr Ritch alluded to, we haven't used pigment and obviously we don't have monkeys with exfoliation. We have created an excess of extracellular matrix in the monkey trabecular meshwork by chronic therapy with phospholine iodide. That goes back to another series of research projects that we've done and published. These compounds do work under those circumstances so that at least, in principle, this might work. We have not done that in this experiment. I think we would like to wash out all excess extracellular material at one time. If that's what the pathophysiology of primary open-angle glaucoma or exfoliation and pigmentary glaucoma really is, this might be an approach. The monkeys that we've used so far are normotensive monkeys except for this little study with echothiophate. You do get a return of normal resistance after a period of time. If we were using glaucoma eyes, whether you'd get a return of pathological resistance, we just don't know. We're not going to know that until we get into clinical trials.

The more physiologic way of doing larger drop volume does not seem to give us the corneal changes. We had a clue in that, if you look at the peripheral corneal thickness, you did not see the thickening. We measured corneal thickness centrally and peripherally, and the peripheral cornea was not thickened. Therefore we knew it was a very high concentration of drug that was sitting in the center of the cornea. Gravity boots and neckties have not been done in a monkey. We could do so, but it seems inadvisable.

In terms of ultrastructure, neither the iris, the ciliary muscle, nor the ciliary epithelium as well, have shown changes in these acute experiments. These were single-dose intracameral infusions to look for acute toxicity; we didn't see any. What happens with weeks or months or years will not be known with any drug until you actually get out into clinical trials. The FDA often mandates, as it did with the prostaglandins, Phase 4 post-marketing studies, not for purposes of advertising the drug, but for purposes of following long-term toxicity. Unfortunately, at this stage I do not have answers to a lot of these questions. We will just have to see what happens, as you do with any new class of drugs.

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POSTERS
TWO PROMINENT OPHTHALMOLOGISTS FROM CINCINNATI

BY Taylor Asbury MD and James J. Augsburger MD

Purpose: To discuss two of the most important and influential ophthalmologists of their eras, both of whom achieved prominence in Cincinnati and were distinguished members of the AOS.

Methods: Historical review.

Results: Dr. Elkanah Williams (1822-1888) was born in Indiana and attended Asbury College (Indiana) and the University of Louisville School of Medicine. He was one of the first physicians in the United States to limit his practice to ophthalmology and among the first to use an ophthalmoscope (1855). He became Chairman of Ophthalmology at the Miami Medical College in Cincinnati in 1865 and held this position until shortly before his death. He was an original member of the AOS (1864). Dr. Derrick T. Vail, Jr. (1878-1973) grew up in Cincinnati and attended Yale University and Harvard Medical School. After ophthalmology residency at the Massachusetts Eye and Ear Infirmary, he traveled to India to perform cataract surgery with Colonel Henry Smith. He became Chairman of Ophthalmology at the University of Cincinnati in 1937. During World War II, he served as Senior Eye Consultant for all American Armed Forces in Europe. After the war, Dr. Vail accepted the position of Chairman of Ophthalmology at Northwestern University. He achieved great national recognition as long-term editor of the American Journal of Ophthalmology (1940-1965). He was a leading member of the AOS (1948-1977) and served as its president in 1959.

Conclusion: Drs. Williams and Vail were giants of ophthalmology of different eras who had lasting impacts on the specialty and the AOS.

LOCALIZED CHOROIDAL MELANOCYTOSIS: A DISTINCT CLINICAL ENTITY?

BY James J. Augsburger MD, Vrinda Hershberger MD, and JoAnn Chang MD

Purpose: To describe what appears to be a distinct clinical entity, localized choroidal melanocytosis (a limited form of congenital ocular melanocytosis), in a series of patients encountered in a single referral practice.

Methods: Retrospective descriptive analysis of seven patients with a localized choroidal patch of confluent but flat melanotic hyperpigmentation measuring at least 5 mm in maximal basal diameter.

Results: The seven patients ranged in age at initial diagnosis from 2 months to 83 years (median 17 years). Two of the patients were male and five were female. All patients were asymptomatic, and all lesions were detected on dilated fundus examination prompted by other issues. None of the lesions had measurable thickness by ultrasonography. The melanotic choroidal patch ranged from 7.5 mm to 23 mm in maximal arc length basal diameter (median maximal basal diameter 14.5 mm). It was located entirely posterior to the equator in five patients and straddled the equator in two. It extended beneath the fovea in four patients and to the optic disc margin in two. None of these lesions that have been followed for more than six months have enlarged appreciably.

Conclusions: Localized choroidal melanocytosis appears to be a distinct clinical entity that probably represents a limited form of congenital ocular melanocytosis. This fundus lesion must be distinguished from choroidal nevus, choroidal melanoma, and other darkly pigmented fundus tumors. Patients with this form of ocular melanocytosis may have an increased risk of developing uveal melanoma from the hypermelanotic choroidal patch.
NEW TREATMENT OPTIONS FOR MANAGING ENDOGENOUS FUNGAL ENDOPHTHALMITIS USING VORICONAZOLE AND CASPOFUNGIN

BY M. Gilbert Grand MD, Seenu M. Hariprasad MD, William F. Mieler MD, Gaurav K. Shah MD, Sean Breit MD, AND Russell Van Gelder MD

Purpose: Voriconazole, a new generation triazole, has been shown to achieve therapeutic intraocular levels after oral administration. Caspofungin is the first approved agent from a new class of antifungals, the echinocandins. This series describes the experience at two centers in using these novel antifungals to treat endogenous fungal endophthalmitis.

Methods: A retrospective review of four patients with Candida endophthalmitis from the Barnes Retina Institute and the Cullen Eye Institute. Post-mortem intraocular voriconazole concentrations on a fifth patient will be presented as well.

Results: All patients had systemic cultures positive for Candida species. Our first two patients had prompt resolution of intraocular mycosis with systemic voriconazole and caspofungin. The third patient received 100mcg of intravitreal voriconazole (final vitreous concentration 25 mcg/ml) followed by oral voriconazole and responded favorably. Our fourth patient with bilateral disease responded well to intravenous voriconazole and caspofungin but had a recurrence after being discharged on oral voriconazole and IV caspofungin. This patient had a bowel resection with an ileostomy; therefore, absorption of oral voriconazole may have been inadequate. Bilateral amphotericin-B intravitreal injection ultimately resolved this infection. Our fifth patient had multi-system failure and passed away one week after initiating intravenous voriconazole for candidemia. Post-mortem HPLC analysis of the aqueous and vitreous revealed voriconazole concentrations of 1.52 mcg/ml and 1.12 mcg/ml, respectively (MIC90 of C. albicans is 0.06 mcg/ml).

Conclusions: Voriconazole and caspofungin appear to be powerful weapons to add to our existing armamentarium against fungal endophthalmitis. Further studies are warranted to precisely define the role of these new agents.

THE DRAGGED-FOVEA DIPLOPIA SYNDROME

BY David L. Guyton MD, M. Elaine De Pool MD, AND Sheena O. Broome OC(C) COMT

Purpose: To identify the clinical characteristics of the “dragged-fovea diplopia (DFD) syndrome,” to introduce a simple diagnostic test to identify this syndrome, and to provide a simple treatment option to provide relief from the diplopia in some of these cases.

Methods: We reviewed the records of our patients with a diagnosis of maculopathy, internal limiting membrane contracture, or “dragged fovea,” and complaint of diplopia.

Results: Fifty-two affected eyes in 47 patients met the criteria for inclusion in the study. In 32 patients, the small-field central fusion test (viewing a single white letter on the black background of a distance visual acuity display) showed central fusion with the room lights off, but showed peripheral fusion with central diplopia with the room lights on. Diplopia recurred despite prism trial, as the patient adapted to the prism power in seconds to minutes. In 15 eyes, a pars plana vitrectomy with membrane peeling had been performed. In 6 of these patients, the diplopia was noticed only after the pars plana vitrectomy with membrane peeling. In 17 of the 32 patients, diplopia was effectively relieved using a piece of Scotch™ “Satin” tape.

Conclusions: The dragged-fovea diplopia syndrome consists of central diplopia in the presence of peripheral fusion that does not respond to prism therapy or eye muscle surgery. The small-field central fusion test (“lights on/off test”) can be used to confirm the diagnosis in patients with clinical characteristics compatible with the DFD syndrome. A strip of Scotch “Satin” tape, applied vertically on the rear surface of the spectacle lens of the non-preferred eye, provides an aesthetic form of monocular occlusion. Epiretinal membrane peeling surgery can unmask or precipitate the development of the DFD syndrome.
CORRELATION OF THE SUBJECTIVE AND OBJECTIVE RETINAL ARTERY PULSE: POSSIBLE HOME MONITORING OF OCULAR PERFUSION

BY James S. Kelley MD

Purpose: To compare subjective patient response to ophthalmodynamometry (ODM) with objective observer’s endpoint.

Methods: Patients were positioned at the slit lamp. Using a standard ODM device on the upper lid, pressure was raised until patients noted the “blinking” of the arterial pulse. Entopically, this was compared with observed onset of pulsation seen with a 60-diopter lens. Testing included 120 eyes of 75 patients.

Results: The subjective pulsation correlated exactly with the objective pulsation, when observed. In 10 percent of cases, there was no clear subjective pulse. Pulses in the choroids were visible to the observer but not to the patient.

Conclusions: Many patients could monitor their own diastolic ophthalmic artery pressures at home using a device similar to the Proview Pressure Monitor. Alternatively, a technician or non-ophthalmologist could estimate the perfusion pressure in the eye noting the patient’s entopic pulsating endpoint.

DOES A 2.6 MM INCISION CAUSE LESS SURGICALLY-INDUCED ASTIGMATISM (SIA) THAN 3.0 TO 4.0 MM INCISIONS?

BY John C. Merriam MD, Joanna E. Merriam, AND Lei Zheng

Purpose: To compare surgically induced astigmatism (SIA) following a temporal 2.6 mm corneal incision to SIA following 3.0 to 4.0 mm incisions.

Methods: This retrospective study compares the effect on corneal curvature of inserting three-piece IOLs [Acrysof MA60AC (n = 81), Acrysof MA30BA (n = 172), AMO SI40 (n = 103)] with a lens forceps and 3 to 4 mm incisions with the injection of a one-piece acrylic IOL (Acrysof SA60AT, n = 125) via a 2.6 mm incision. Unoperated eyes (n=134) from these surgical groups served as controls. Corneal curvature was measured with a keratometer. We compared groups by calculating SIA with vector analysis, and by comparing absolute change on the horizontal and vertical meridians of each group. All groups were followed for at least 18 months.

Results: A linear equation (y = a + bx) describes change in SIA and the corneal meridians. Best-fit parameters and the 95 percent confidence intervals were calculated for each IOL type and control eyes. Surgical groups did not differ significantly from each other or control eyes.

Conclusions: As measured by keratometry, all these incisions appear to be astigmatically neutral. The clinician may prefer the smallest possible incision to maintain a stable chamber and to hasten recovery, but there is no detectable advantage of a sub-3.0 mm incision on SIA.

Bold type indicates AOS member.
SUPRANORMAL MULTIFOCAL ELECTRORETINOGRAMS (MFERGS) FOLLOWING ACUTELY ELEVATED INTRAOCULAR PRESSURE (IOP) IN MONKEYS AND IN VARIOUS HUMAN RETINAL DISORDERS

BY T. Michael Nork MD, Charlene B. Y. Kim, Paul L. Kaufman MD, AND James N. Ver Hoeve

Purpose: To describe a phenomenon of increased mfERG waveforms that is associated with a variety of pathologic conditions.

Methods: Three rhesus monkeys underwent cannulation of the anterior chamber followed by elevation of the intraocular pressures to 50 mmHg for up to five hours. One of the animals had a unilateral optic nerve transection (ONT). The stimulus consisted of an array of 241 equal-sized hexagonal elements. We also retrospectively reviewed our database of human patients and found four with markedly supranormal traces. Their diagnoses (ages) were as follows: multiple evanescent white dot syndrome (29), acute idiopathic blind spot enlargement (40), acute zonal occult outer retinopathy (30), and chloroquine retinopathy (42). A stretched, 103-element stimulus array was used for all of the patients. Their responses were compared quantitatively with 20 adult control subjects.

Results: K1 response amplitudes and oscillatory potentials increased rapidly and markedly in all three of the monkeys after IOP elevation. The central retina appeared to be more affected than the mid-periphery. Response amplitudes returned towards baseline levels within four weeks after the IOP returned to normal. The human patients all had supranormal early K1 waveforms that were ≥3 standard deviations above normal. In most cases, the waveforms decreased either to normal or below normal within a few months.

Conclusions: Retinal disease most often results in depression of the mfERG. However, given the right circumstances, the retina is capable of generating supranormal responses. Even so, these supranormal traces are associated with significant retinal pathology and may be an indicator of impending, irreversible damage.

PATHOLOGY IN EYES WITH ANTERIOR STROMAL DYSTROPHIES UNDERGOING EXCIMER LASER PHOTOTHERAPEUTIC KERATECTOMY

BY Christopher J. Rapuano MD

Purpose: To evaluate the use of high frequency ultrasound biomicroscopy (UBM) in determining the depth of corneal pathology in eyes undergoing excimer laser phototherapeutic keratectomy (PTK) for primary or recurrent anterior stromal corneal dystrophies.

Methods: Twenty eyes of 14 patients with anterior stromal corneal dystrophies were treated with PTK. Eyes were evaluated pre- and six to eight weeks post-operatively with slit lamp biomicroscopy, manifest refraction, keratometry, computerized corneal topography, ultrasound pachymetry, and UBM.

Results: Nineteen of 20 corneas (95 percent) had greatly improved corneal clarity after PTK. Mean uncorrected Snellen vision improved from 20/102 to 20/69, and best-corrected vision improved from 20/62 to 20/38. Nine eyes (45 percent) improved two or more lines of uncorrected vision, and 13 eyes (65 percent) improved two or more lines of best-corrected vision. Mean change in spherical equivalent was just -0.92 diopters (SD: 4.3 diopters), although the range was large (-13 to +3.88 diopters). UBM measurement of central corneal pathology did not correlate significantly with the actual PTK ablation depth (P=0.07).

Conclusions: PTK resulted in improvements in corneal clarity and visual acuity in most patients with superficial corneal stromal dystrophies. UBM was not an effective tool to accurately measure the depth of corneal pathology pre-operatively. PTK is a very good minimally invasive technique to improve vision in eyes with anterior stromal corneal dystrophies.
RECURRENT NANOLITER PUSH-PULL PERFUSION SAMPLING OF THE RAT VITREORETINAL INTERFACE

BY Scott A. Shippy PhD, Jose S. Pulido MS MD, AND Sumith Kottegoda

Purpose: We describe the use of a nanoliter push-pull perfusion system for the recurrent sampling of amino acids at the vitreoretinal interface.

Methods: Amino acid levels from 10 animals were quantified in samples collected by low-flow push-pull perfusion. The low-flow push-pull perfusion probe is constructed of concentric fused silica capillaries (outer diameter 170-micron) and fits through a 29-gauge needle for insertion into the vitreous. After visual verification of placement at the vitreoretinal interface with indirect ophthalmoscopy, saline is perfused through the capillary probe construction at 20-50 nL/min flow rates. Samples are collected every 10-15 minutes for three hours and the amino acid content of 500-nL samples is found via a capillary electrophoresis separation.

Results: The basal levels at the vitreoretinal interface are determined for 11 amino acids. The amino acids quantified include major neurotransmitters including glutamate, glycine, GABA, and D-Ser. The basal levels have similar proportions as found in plasma but are lower than levels reported following more invasive vitreous collection methods. The probe construction is significantly small so that no damage is seen to the eye. Further, the small perfusion region allows spatially targeted and recurrent sampling of the vitreoretinal interface.

Conclusions: These results demonstrate a new method for determining chemical composition of the rat vitreoretinal interface. The basal levels of major neurotransmitter amino acids are determined. Profiling the chemical composition of the vitreoretinal interface in normal and disease states may elucidate potential treatment targets and advance an understanding of disease progression.

Bold type indicates AOS member.
THESSES
COMPUTERIZED EXPERT SYSTEM FOR EVALUATION OF AUTOMATED VISUAL FIELDS FROM THE ISCHEMIC OPTIC NEUROPATHY DECOMPRESSION TRIAL: METHODS, BASELINE FIELDS, AND SIX-MONTH LONGITUDINAL FOLLOW-UP

BY Steven E. Feldon MD MBA

ABSTRACT

Purpose: To validate a computerized expert system evaluating visual fields in a prospective clinical trial, the Ischemic Optic Neuropathy Decompression Trial (IONDT). To identify the pattern and within-pattern severity of field defects for study eyes at baseline and 6-month follow-up.

Design: Humphrey visual field (HVF) change was used as the outcome measure for a prospective, randomized, multi-center trial to test the null hypothesis that optic nerve sheath decompression was ineffective in treating nonarteritic anterior ischemic optic neuropathy and to ascertain the natural history of the disease.

Methods: An expert panel established criteria for the type and severity of visual field defects. Using these criteria, a rule-based computerized expert system interpreted HVF from baseline and 6-month visits for patients randomized to surgery or careful follow-up and for patients who were not randomized.

Results: A computerized expert system was devised and validated. The system was then used to analyze HVFs. The pattern of defects found at baseline for patients randomized to surgery did not differ from that of patients randomized to careful follow-up. The most common pattern of defect was a superior and inferior arcuate with central scotoma for randomized eyes (19.2%) and a superior and inferior arcuate for nonrandomized eyes (30.6%). Field patterns at 6 months and baseline were not different. For randomized study eyes, the superior altitudinal defects improved \( (P = .03) \), as did the inferior altitudinal defects \( (P = .01) \). For nonrandomized study eyes, only the inferior altitudinal defects improved \( (P = .02) \). No treatment effect was noted.

Conclusions: A novel rule-based expert system successfully interpreted visual field defects at baseline of eyes enrolled in the IONDT.


INTRODUCTION

The Ischemic Optic Neuropathy Decompression Trial (IONDT) was a randomized prospective study designed to establish the safety and efficacy of optic nerve sheath decompression as a treatment for nonarteritic anterior ischemic optic neuropathy (NAION), as well as to document the natural history of NAION.\(^1\) Based upon visual acuity as the primary outcome measure, the IONDT demonstrated that optic nerve sheath decompression is not effective and may be harmful.\(^1\)

For NAION, characterized clinically as causing visual field loss, basing conclusions about efficacy of treatment and natural history solely on visual acuity may be inadequate. For this reason, the Humphrey visual field (HVF) was included in the study as a secondary outcome measure. Quantitative assessment of visual field function has been aided considerably by the advent of computerized automated static perimeters such as the HVF analyzer (Humphrey Instruments, Dublin, California). These instruments provide a standardized testing environment, quantitative assessment of threshold sensitivity to spots of light at fixed points throughout the visual field, and some data regarding reliability of patients’ responses.

Initial visual field evaluation based only on a readily computed global measure, mean deviation, failed to
distinguish any difference between surgical and observational management. However, mean deviation alone may not be an adequate measure for assessment of eyes with NAION. The classic patterns of defect encountered in this disease may shift without changing average loss. Furthermore, there may be important changes in sensitivity within small areas of the visual field corresponding to nerve fiber bundle defects. These may not be detected when averaged into the mean deviation calculation. Therefore, a more detailed analysis of the quantitative visual field testing is important, even though such an analysis was not originally part of the IONDT methodology.

Whereas visual acuity based upon the logMAR charts developed for the Early Treatment Diabetic Retinopathy Study is a simple, well-tested measure of visual function, visual field assessment is complex and well beyond the scope of the original analysis. Prospective glaucoma trials have utilized a number of approaches for evaluating progression, but the algorithms seldom include classifications based upon the type of defect. The Optic Neuritis Treatment Trial categorized visual field defects, but the methodology did not involve strict definitions for classification. Furthermore, patterns of field loss were qualitatively rather than quantitatively determined. In the context of a prospective clinical trial, an automated, objective classification may be preferable.

For the current study, a Visual Field Analysis Committee was formed in 1998, consisting of neuroophthalmologists from six of the 26 participating Clinical Centers. As an initial step, each member of this group classified the visual fields of patients with NAION but not enrolled in the study. Using an iterative process to achieve consensus, a set of rules was devised to categorize the visual defects encountered in the disease. These rules were then incorporated into a computerized expert system to analyze the study fields in a consistent, reproducible manner.

The validated computerized expert system, described in detail herein, was used to determine the pattern of defect present as well as the density of defect within each pattern for both enrolled and fellow eyes. Based upon the categorization from the computerized expert system, a detailed evaluation of baseline visual field defects noted by HVF for patients enrolled in the IONDT was performed. The 6-month visual field for each patient was then compared quantitatively to the visual field at baseline, allowing for short-term fluctuations in each data set. The following questions were addressed:

1. Are the pattern and severity of visual field defects found in eyes randomized to treatment similar to those randomized to careful follow-up?
2. Is the pattern or severity of visual field defects

found in eyes with better than 20/64 visual acuity substantially different from those with worse acuity who were eligible for randomization?
3. Are there preexisting conditions (eg, diabetes, hypertension) that affect the pattern and severity of visual field defects?
4. What is the relationship between global indices of HVF performance and the pattern or severity of visual field defects?
5. What is the relationship between visual acuity and pattern or severity of visual field defect?
6. Are visual field defects present in fellow eyes without known optic neuropathy that may indicate the presence of subclinical optic disk ischemia?
7. Are there changes in the visual fields between baseline and 6-month visits for randomized eyes, nonrandomized study eyes, and fellow eyes?
8. Are there changes in the visual fields for eyes randomized to surgery as compared to careful follow-up?

**METHODS**

**Study Protocol**

The eligibility criteria, randomization procedure, and visit protocols are extensively described in prior publications. Briefly, patients aged 50 or above were eligible for randomization into surgical and careful observation groups if they had symptoms and signs characteristic of NAION and their visual acuity was 20/64 or less. A Late Entry subset of the randomized patients included eyes for which acuity in the study eye was better than 20/64 at baseline but lost acuity to below this level within 30 days. Patients with acuity better than 20/64 were followed but not randomized. Fellow eyes were tested, and the results were recorded. At the time of examination, the clinician determined whether the fellow eye had optic neuropathy (of any type) or not. Visual fields were obtained at baseline, 6 months, 12 months, and at closeout of the study. Although multiple replications of the fields at some or all evaluation visits might have been helpful in minimizing training effects, fatigue effects may have increased because only time-consuming standard threshold strategies were available at the outset of the study. All centers utilized protocols approved by investigational review boards at their respective institutions. Patients were enrolled between 1992 and 1994. At that time the Data Safety and Monitoring Committee halted further recruitment, because surgery was found not to be effective.

**Organizational Structure**

The Data Coordinating Center maintained the IONDT database that included visual field results from HVFs.
Study (CIGTS) intra-test reliability rating. If fixation criteria of the Collaborative Initial Glaucoma Treatment analysis if they were deemed reliable, using the four basic excluded from analysis. Visual fields were included for patients might have contained useful information, the recorded. Although unreliable fields in severely affected false-negative responses, and fixation losses were only those points also represented on the 24-2 HVF test. The number and percentages of false-positive responses, corresponding with the protocol were analyzed by utilizing

centered expert system, based on an agreed-upon set of rules for identification of the various types of field defects. The VFAC maintained the digital file inventory of all analyzed fields that were then forwarded to the Data Coordinating Center.

Determination of Quality

Visual fields were evaluated for compliance with the protocol, that is, that the field was a 24-2 performed on a HVF using test stimulus size III with the foveal sensitivity switch “on.” The IONDT did not utilize 30-2 visual fields because the additional peripheral test points were considered too variable. A few 30-2 visual fields that otherwise corresponded with the protocol were analyzed by utilizing only those points also represented on the 24-2 HVF test. The number and percentages of false-positive responses, false-negative responses, and fixation losses were recorded. Although unreliable fields in severely affected patients might have contained useful information, the interpretation would not be meaningful; thus, they were excluded from analysis. Visual fields were included for analysis if they were deemed reliable, using the four basic criteria of the Collaborative Initial Glaucoma Treatment Study (CIGTS) intra-test reliability rating. If fixation losses were greater than 20% for more than 20 trials, one point was added. If false-positives for eight or more trials were 33% or greater, one point was added. A similar criterion was applied for false-negatives. Short-term fluctuation (dB) was rated at zero for less than or equal to 4.0, one if greater than 4.0 but less than or equal to 6.0, two if greater than 6.0 but less than or equal to 7.0, and three if greater than 7.0. A rating of less than four was considered reliable.

Classification of Visual Fields

Validation of a system for classifying visual fields is complex. Given that there is no “gold standard,” experts will likely disagree on interpretation. This problem is well known in medicine. For instance, studies validating the use of computer-assisted diagnosis tools suggest that the differences between computer diagnosis and human expert diagnosis vary to about the same extent as human experts disagree among themselves. Given that computerized diagnosis may be no better than that of an expert panel, the principal reason for utilizing a computerized expert system in the context of a clinical trial is to reduce inconsistency by eliminating intraobserver and interobserver variability.

In the absence of a “gold standard,” validation of a classification system for visual fields requires several steps. First, an expert panel needs to achieve consensus on a set of rules. Second, the experts should be able to apply these rules in such a manner that the rate of disagreement is not different from that reported for similar classifications in other medical contexts. Third, the consistent application of the rules by a computerized expert system should produce classifications that do not disagree with the panel more than the expert panel disagrees with itself. Finally, the computerized expert system should reach reasonable clinical interpretations, such that major disagreements with the expert panel are rare.

The number of experts required on the panel was determined after a statistical computation determined that the chance of all six experts agreeing on ten patterns by guessing would be 0.00001. A majority of the experts needed to agree to categorize a field defect as a specific pattern. The chance of this degree of concordance occurring by guessing alone was .01215. For any field in which the agreement among panelists was not significantly better than guessing, the field was called “nonclassifiable.”

A sequence similar to that used by Molino and associates was developed to facilitate derivation of the rules, as shown in Figure 1. The information associated with this sequence was formulated into “sets” and included an evaluation set, a training set, and a validation set.

Evaluation Set

The Visual Field Steering Committee developed an evaluation set for each of the six expert panelists. It consisted of instructions to the panelists, a set of proposed definitions for 13 types of defects and for severity accompanied by a
A series of 19 examples thought to correspond to the proposed definitions, a grading form, and a set of sample visual fields from nonstudy patients with AION for analysis.

Using preliminary definitions as modified by the expert panel, an initial classification was made for the 19 visual fields provided in the evaluation set. Many of these fields contained more than one type of pattern defect. The panelists then independently reported the degree to which they agreed with the classification. In addition, the panelists were instructed to categorize the density of the defect as mild, moderate, severe, or absolute. Results are shown in Table 1. Based upon this exercise, additional revisions of the definitions were deemed necessary. Of importance, three separate categories of scotoma were identified: peripheral, paracentral, and central. Also, an admonition was added to the instructions that the category of “other” be utilized only for visual fields that were impossible to fit into a specific category.

**Training Set**

Using the final instructions and forms derived from the evaluation set, the expert panel analyzed 120 masked, representative nonstudy NAION fields. IONDT fields were not utilized for training in order to preserve the integrity of the classification tool. To assess the ability of the panelists to accurately apply the rules, 10 of these fields were duplicates and 10 were example fields from the evaluation set. For 55 of the analyzed fields, at least 83% agreed on the categorization without any collaboration among experts. Agreement on classification of the remaining 65 fields was achieved through a series of four interactive reconciliation meetings of the expert panel, held either by teleconference or face-to-face. These resulted in refinement of the pattern definitions and consensus on interpretation of the fields in the training set. These rules were then programmed into the computerized expert system.

The consensus derived from the training set included identification of 14 different field types, shown in Table 2. Severity was restricted to mild, moderate, or severe. General rules for classification included the following:

1. If a field is noted as normal or as an absolute defect, no other notations can be made.
2. A depressed point is defined as equal to or greater than 4-dB loss.
3. An attempt should be made to classify fields even though they may appear unreliable from the indices.
4. Severity is based upon subjective judgment. Only the arcuate/altitudinal category can have more than one severity with a separate severity assignable to the arcuate and the altitudinal components.

**Validation Set**

Having determined the rules for classification, another set of 95 non-IONDT visual fields was sent to the expert panel as a validation set. The classification results from each panelist were determined (Table 3). The agreement obtained from the panel was then compared with the classifications obtained from the computer program. The

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**Figure 1**

Sequence developed to facilitate derivation of the rules used for classification system for visual fields.
level of agreement is shown in Table 4. There was a large percentage of internal disagreement among the panelists as to classification of fields within the validation set, despite a common set of rules derived by consensus. In turn, there was disagreement between the panel and the computer.

The inability of the experts to independently agree with each other or the computer is consistent and of the same order of magnitude as reported in the literature for instances for which no gold standard exists. This result reinforces the need, within the context of a large clinical trial, to have a computerized system for consistent application of clinically meaningful rules.

Due to the lack of consensus, a second method of validation was performed. In this method, the computer program evaluated all the fields, and the panelists were asked to agree or disagree with the computer results. This method changed the question asked of the experts from “How would you try to apply rules to classify a defect?” to “Does the consistent application of consensus-derived rules applied by the computer result in a classification that is clinically acceptable?”

With the new method of validation, in only four instances was there initial disagreement with the computer by half or more of the panelists, as shown in Table 5. These were determined to be secondary to data entry errors in two instances and due to computer criteria differentiating altitudinal from arcuate defects in two instances. In the latter instances, investigation revealed that further manipulation of the computer algorithm to allow concordance with the panel would result in other classification errors; therefore, these discrepancies were allowed to stand. There was majority agreement between the computer and the expert panel in 91 (94%) of 95 fields, indicating that incorporation of the rules into a computer program unanimously agreed upon by the expert panel was a valid method for analysis of the patterns and severities of the visual field data collected by the IONDT.

The Computer-Based Expert System

Data Entry

Humphrey visual field data consisted of five components: (1) visual field identification (patient number, visit number, and eye examined), (2) reliability indices (fixation losses, false-positives, and false-negatives), (3) foveal sensitivity (dB) and highest point of sensitivity for the whole field (dB), (4) dB loss at each of the 52 data points on the total deviation plot, and (5) short-term fluctuation. For each visual field, the location and dB loss (if any) at

<table>
<thead>
<tr>
<th>PATTERN</th>
<th>EXCELLENT</th>
<th>GOOD</th>
<th>UNCERTAIN</th>
<th>POOR</th>
<th>BAD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>83%</td>
<td>17%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute defect</td>
<td>100%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild diffuse depression</td>
<td>50%</td>
<td>17%</td>
<td>33%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe diffuse depression</td>
<td>17%</td>
<td>66%</td>
<td>17%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild superior altitudinal</td>
<td>66%</td>
<td>50%</td>
<td>17%</td>
<td>17%</td>
<td></td>
</tr>
<tr>
<td>Moderate superior and inferior altitudinal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe superior altitudinal</td>
<td>100%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild inferior altitudinal</td>
<td>33%</td>
<td>67%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate inferior altitudinal</td>
<td>17%</td>
<td>50%</td>
<td>33%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe inferior altitudinal and moderate superior arcuate</td>
<td>83%</td>
<td>17%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate superior arcuate</td>
<td>67%</td>
<td>17%</td>
<td>17%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe superior and inferior arcuates</td>
<td>33%</td>
<td>33%</td>
<td>17%</td>
<td>17%</td>
<td></td>
</tr>
<tr>
<td>Mild inferior arcuate</td>
<td>100%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate inferior arcuate</td>
<td>67%</td>
<td>17%</td>
<td>17%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe inferior arcuate</td>
<td>67%</td>
<td>33%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate inferior nasal step</td>
<td>83%</td>
<td></td>
<td>17%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild paracentral scotoma</td>
<td>33%</td>
<td>17%</td>
<td>50%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate central scotoma</td>
<td>67%</td>
<td>33%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe central scotoma</td>
<td>50%</td>
<td>50%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### TABLE 2. TYPES OF FIELD DEFECTS BASED ON CONSENSUS OF INTERPRETATION

<table>
<thead>
<tr>
<th>Type</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>No quadrants depressed or only a few points in no specific pattern. One depressed point in a location surrounding the blind spot is normal unless it is part of another defined field defect.</td>
</tr>
<tr>
<td>Absolute defect</td>
<td>No response (sensitivity = zero) was recorded for all points in all quadrants or if only one point is less than or equal to 9 dB sensitivity and all other points are zero. If the retest is zero, then the point sensitivity is zero. Foveal sensitivity must be equal to zero.</td>
</tr>
<tr>
<td>Diffuse depression</td>
<td>Entire visual field equally depressed, including fixation as defined as presence of both a superior and an inferior altitudinal defect that are equally depressed and a central scotoma.</td>
</tr>
<tr>
<td>Superior altitudinal</td>
<td>Upper half of field equally depressed as defined as all points in the superior two quadrants approximately equally depressed, excluding those nasal to the blind spot (ie, points 11 and 19 on the visual field map). Depression should extend down to horizontal meridian including approximate equal involvement of the superior paracentral points (points 21 and 22 on the visual field map).</td>
</tr>
<tr>
<td>Inferior altitudinal</td>
<td>Lower half of field equally depressed as defined as all points in the inferior two quadrants approximately equally depressed, excluding those nasal to the blind spot (ie, points 27 and 35 on the visual field map). Depression should extend up to horizontal meridian, including approximate equal involvement of the superior paracentral points (points 29 and 30 on the visual field map).</td>
</tr>
<tr>
<td>Superior arcuate</td>
<td>Peripheral defect (at least four peripheral points must be depressed within one quadrant) that appears in either or both superior quadrants with relative sparing of either one or both of the superior paracentral points, or either one of the superior paracentral points is less depressed in comparison to the superior periphery in either quadrant and it is not a nasal step. Superior periphery is defined as all points in the superior two quadrants except points 21 and 22.</td>
</tr>
<tr>
<td>Inferior arcuate</td>
<td>Peripheral defect (at least four peripheral points must be depressed within one quadrant) that appears in either or both inferior quadrants with relative sparing of either one or both of the inferior paracentral points, or either one of the inferior paracentral points is less depressed in comparison to the inferior periphery in either quadrant and it is not a nasal step. Inferior periphery is defined as all points in the inferior two quadrants except points 29 and 30.</td>
</tr>
<tr>
<td>Superior nasal step</td>
<td>An isolated superior nasal quadrant defect which preferentially involves the peripheral points (points 18, 25, and 26) adjacent to the horizontal meridian. Cannot be part of a superior arcuate defect and there cannot be an arcuate defect in the superior temporal quadrant. Superior nasal points adjacent to the vertical meridian (points 3, 8, 15, and 22) are relatively spared.</td>
</tr>
<tr>
<td>Inferior nasal step</td>
<td>An isolated inferior nasal quadrant defect which preferentially involves the peripheral points (points 33, 34, and 42) adjacent to the horizontal meridian. Cannot be part of an inferior arcuate defect and there cannot be an arcuate defect in the inferior temporal quadrant. Inferior nasal points adjacent to the vertical meridian (points 30, 39, 46, and 51) are relatively spared.</td>
</tr>
<tr>
<td>Central scotoma</td>
<td>Decreased sensitivity of the fovea by 5 dB relative to the least depressed point in the rest of the field or the foveal sensitivity is less than 10 dB.</td>
</tr>
<tr>
<td>Paracentral scotoma</td>
<td>Focal depression of the visual field not corresponding to any other pattern and located within the paracentral region (points 20, 21, 22, 28, 29, 30) adjacent to the blind spot, but sparing fixation (ie, no central scotoma). One isolated, depressed paracentral point next to the blind spot (point 20 or 25) is not a paracentral scotoma. If there is a central scotoma and, as defined, a paracentral scotoma, then the defect is categorized as a central scotoma.</td>
</tr>
<tr>
<td>Superior arcuate/altitudinal</td>
<td>Both superior paracentral points (points 21 and 22) are equally depressed, but the superior periphery is more depressed than the paracentral. Superior paracentral points must differ substantially from the inferior paracentral points (points 29 and 30), ie, no central or paracentral scotoma involving these points.</td>
</tr>
<tr>
<td>Inferior arcuate/altitudinal</td>
<td>Both inferior paracentral points (points 21 and 22) are equally depressed, but the inferior periphery is more depressed than the paracentral. Inferior paracentral points must differ substantially from the superior paracentral points (points 29 and 30), ie, no central or paracentral scotoma involving these points.</td>
</tr>
<tr>
<td>Other</td>
<td>Pattern defect that does not fit any of the above definitions, eg, shifted field. Use this category only if you are certain that you cannot categorize the defect using the other 13 categories.</td>
</tr>
</tbody>
</table>
each of the 52-point locations is entered into an Excel database on a personal computer (Figure 2). In addition, the foveal sensitivity was recorded, and in instances of diffuse depression, the absolute sensitivity of each point was entered. Although digitized data in the form of floppy disks and flat files were available, changes in computer hardware and software precluded their use in constructing the database. Therefore, the printed visual fields were transcribed into a Microsoft 97/00 Excel compatible database, using double-entry verification.

Software Structure

The computer-based expert system was constructed as a rule-based system on an Excel platform, running under Windows 98, evaluating each field quadrant-by-quadrant. Each rule consisted of a logical statement that could be found true or false, taking the form “if…then.” A truth table was utilized to define specific types of field defects, based upon definitions of the expert panel. Two forms of logical statements were used to identify pattern defects. The first form was based upon average dB loss within a quadrant corresponding to a particular pattern. If no average depression was present, then the number of disturbed points within a quadrant was used to determine the presence of pattern defects. Thus, the numbers of disturbed points were used primarily to find mild defects that were missed by averaging. A listing of the algorithms utilized by the expert system is included in the Appendix.

For instance, if the average dB loss is greater in the periphery than in the central field by 5 dB, then an arcuate defect is present in that quadrant. If the central dB loss is greater by 5 dB than the periphery, then a central or paracentral defect is present. If no pattern defect can be found by averaging, then disturbed point algorithms are used to find mild or smaller pattern defects within a quadrant. A disturbed point is defined as >3-dB loss. If there are a predetermined number of disturbed points within the boundary of a pattern, then the defect is detected. Some pattern defects are determined by the presence or absence of other defects. For example, if there is a superior and an inferior altitudinal defect and a central scotoma, then the pattern is a diffuse depression. If there is a paracentral scotoma and a central scotoma, then there is just a central scotoma. Average dB loss within a pattern defect is used to determine severity, as shown in Table 6. In the instance of determining absolute defect, the expert computer system operator reviews all fields noted to have diffuse depression; then, actual sensitivity rather than relative sensitivity loss is used to determine whether or not the field has an absolute defect.

Calculation of Pattern Changes Between 6-month and Baseline Visits

Longitudinal evaluation of changes in the visual fields for individual patients between baseline and the 6-month follow-up visit was determined by modification of the computerized expert system to correct for short-term fluctuation. The joint short-term fluctuation (SF) was calculated as follows: SF = \sqrt{(SF_{baseline}^2 + SF_{6-month}^2)}.

The baseline visit HVF sensitivities were not altered. The HVFs from the 6-month visit were altered ±1.96 SF at test loci that differentiate arcuate from altitudinal (points 21 and 22 or points 29 and 30 in Figure 2), central from paracentral defects (foveal sensitivity), and absence of defect from arcuate, altitudinal, central, and paracentral defects. For instance, let us assume an eye has a superior

| TABLE 3. AGREEMENT IN CLASSIFICATION OF 95 VISUAL FIELDS AMONG READERS |
|----------------|---------|-------------|
| AMOUNT OF AGREEMENT | NO. OF FIELDS | % OF TOTAL FIELDS |
| 6 of 6 readers agree | 7 | 7 |
| 5 of 6 readers agree | 14 | 15 |
| 4 of 6 readers agree | 23 | 24 |
| 3 of 6 readers agree | 22 | 23 |
| 2 of 6 readers agree | 25 | 26 |
| None agree | 4 | 4 |
| Total | 95 | 100 |

| TABLE 4. AGREEMENT BY COMPUTER IN CLASSIFICATION OF 66 FIELDS WHERE THERE WAS AGREEMENT OF AT LEAST 3 OF 6 READERS IN DEFECT CLASSIFICATION |
|----------------|---------|-------------|
| NO. OF FIELDS FOR STATED LEVEL OF AGREEMENT BETWEEN EXPERTS | NO. OF FIELDS FOR WHICH COMPUTER AND PANEL AGREED | % OF FIELDS FOR WHICH COMPUTER AND PANEL AGREED |
| In 7 fields where 6 of 6 readers agreed | 7 | 100 |
| In 14 fields where 5 of 6 readers agreed | 10 | 71 |
| In 23 fields where 4 of 6 readers agreed | 16 | 70 |
| In 22 fields where 3 of 6 readers agreed | 16 | 73 |
| Total (in 66 visual fields with agreement) | 50 | 66 |
arcuate scotoma at baseline but an altitudinal defect at the 6-month visit. Since relative sparing of paracentral points 21 and 22 for the upper visual field distinguishes an arcuate from an altitudinal defect, the two paracentral points from the 6-month visit were increased in sensitivity by 1.96 times the SF for that field. If the difference in field pattern persisted despite this manipulation, then the follow-up field was determined as “changed.” If the difference in field pattern disappeared due to this manipulation, then the follow-up visual field was determined as “not changed.”

Statistical Methods
Data analysis was carried out using Stata statistical software for Windows (StataCorp 2001, Stata Statistical Software: Release 7.0; Stata Corporation, College Station, Texas). SPSS software for Windows was used to produce some of the tables (SPSS Inc 1998, SPSS: Release 8.0.1; SPSS, Chicago, Illinois). All statistical tests were two-tailed. Techniques included paired and unpaired Student t tests, the Fisher exact test, Kendall tau-b test for association of ordinal variables, Mantel-Haenszel extension test, and Stuart-Maxwell test of marginal homogeneity producing a chi-square test with 2 degrees of freedom, as appropriate.

RESULTS

Baseline Visual Fields
Visual fields were available on 247 randomized eyes, 158 nonrandomized study eyes, 79 fellow eyes with optic neuropathy at baseline, and 326 fellow eyes with no optic neuropathy at baseline (total 810). A total of 13 fields could not be scored for reliability, three fields were determined to be unreliable, and four fields were 30-2 programs for which data were unavailable for analysis. The frequency of unreliable visual fields was similar to the 1% found by Katz and associates,4 using the same CIGTS criteria. Of the remaining reliable fields, 38 had no foveal sensitivity. Baseline visual fields were available for analysis on 229 study eyes randomized to either surgery or careful follow-up. Baseline visual fields were also available on an additional 147 eyes with vision better than 20/64 followed with careful observation. Fellow eyes were also evaluated at baseline. Of the 376 fellow eyes, 75 were identified at baseline as having optic neuropathy and 301 were identified at baseline as having no optic neuropathy. These data included four 30-2 visual fields for which points not covered by the 24-2 field were not analyzed and 16 Fast-Pac program fields.

Distribution of Field Defect Patterns by Category
The distribution of defect patterns for the various categories of eyes is summarized in Tables 7A and 7B. There was no detectable difference between the frequency of various patterns of field defect comparing eyes randomized to surgery and careful follow-up. Therefore, all randomized eyes are shown as a single group. Differences

<table>
<thead>
<tr>
<th>Severity</th>
<th>n</th>
<th>Average dB Loss</th>
<th>95% CI</th>
<th>Average No. Points</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>1</td>
<td>6.2</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>5</td>
<td>18.2</td>
<td>12.9-23.5</td>
<td>25</td>
<td>24.3-25.7</td>
</tr>
<tr>
<td>Severe</td>
<td>17</td>
<td>27.43</td>
<td>26.3-28.5</td>
<td>26</td>
<td>25.8-26.2</td>
</tr>
</tbody>
</table>

CI, confidence interval; IONDT, Ischemic Optic Neuropathy Decompression Trial.
were observed between the randomized and the observation groups. Central scotomas were much more frequent in the randomized study eyes than in the observation study eyes. The most commonly observed defect in the randomized group of 229 eyes was a superior and inferior arcuate defect with central scotoma in 44 eyes (19.2%), followed by superior arcuate defect and inferior altitudinal defect with central scotoma in 39 eyes (17.0%).

Central scotoma is part of the definition for both diffuse depression in 30 randomized eyes (13.1%) and absolute defect in 30 randomized eyes (13.1%). Excluding diffuse defects, central or paracentral scotoma was present, either isolated or in combination, in 138 eyes (60.3%) (Table 7B). By comparison, the most commonly observed visual field in the 147 nonrandomized study eyes was a combined superior and inferior arcuate defect in 45 eyes (30.6%) followed by superior arcuate and inferior altitudinal defect in 17 eyes (11.6%) and isolated inferior arcuate defect in 16 eyes (10.9%), none of which included central scotoma. Central or paracentral scotoma, isolated or in combination, occurred in only 48 of the nonrandomized study eyes (32.7%). The difference in distribution is not surprising, because central scotoma includes foveal sensitivity as part of its definition. Another notable difference was that there was only a single instance of diffuse defect for the study observation group.

Fellow eyes with optic neuropathy were more widely distributed regarding the patterns of field loss encountered, possibly because these 75 eyes were not segregated according to visual acuity (Table 7A). Using the established criteria, the computerized expert system identified only 47 (15.6%) of 301 fellow eyes without optic neuropathy as having normal visual fields. The most commonly encountered abnormalities were isolated superior arcuate defects in 35 eyes (11.6%), isolated inferior arcuate defects in 20 eyes (6.6%), and, especially, combined superior and inferior arcuate defects in 169 eyes (56.1%). Severity of 35 superior arcuate defects in nonoptic neuropathy fellow eyes was mild in 33 (94.3%), and severity of 169 combined superior and inferior arcuate defects was mild in 100 eyes (59.2%) (Table 8). A severe defect was included in only 9 (5.3%) of 169 fellow eyes that had superior and inferior arcuate defects with no optic neuropathy. Scotomas were noted in only 18 eyes in this group (6%). Diffuse defects were even more rare, present in only 5 eyes (1.7%) (Table 7B).

Characteristics of Visual Fields for Late-Entry Randomized Eyes

The patterns of visual field defects for eyes randomized at regular entry (n = 175) were compared to those randomized at late entry due to progression of acuity loss (n = 54). Results are summarized in Table 9. Diffuse depression was present in 27 (15.4%) of the eyes randomized to regular entry, and an absolute defect was present in 24 (13.7%) of these eyes. By comparison, diffuse depression was present in only 3 (5.6%) of the eyes randomized to late entry, and an absolute defect was present in 6 (11.1%) of these eyes. The combination of superior altitudinal and inferior arcuate defects with central scotoma was noted in 11 eyes (6.3%) randomized to regular entry compared to 8 eyes (14.8%) of those randomized in the late-entry group. Although there was a trend for the patterns of defects seen in the regular-entry and late-entry groups to be different, this difference did not reach statistical significance (Fisher’s exact test, P = .078).

Relationship Between Severity of Defect and Global Indices of Visual Function

The severity of field defects for the study eye was compared to global indices of visual field abnormality provided by the HVF. Both the mean deviation and the corrected pattern standard deviation (CPSD) were included in the evaluation (Table 10). Fields may have had more than one defect with differing severity (eg, mild superior arcuate and severe inferior altitudinal). Therefore, visual fields were divided into normal, mild only, mild and moderate, moderate only, mild and severe, mild-moderate and severe, moderate and severe, and severe only. The average mean deviation for randomized study eyes was –21.47, compared to –14.51 for nonrandomized study eyes, and –4.70 for fellow eyes without optic neuropathy. Corrected pattern standard deviation was largest for the observation group, averaging 10.18, compared to 7.03 for the randomized group and 3.50 for the fellow eye group without optic neuropathy. Across all categories, the mean deviations tended to decline with increasing severity of field defect. However, the decline was not monotonic for any of the categories. Corrected pattern standard deviation tended to be small for mild-only and severe-only disease but did not differ systematically for the other categories of severity.

Relationship Between Visual Acuity and Category of Visual Field Defect

Analysis was performed to evaluate the visual acuity at baseline for the various patterns of field defects. For the 374 study eyes (Tables 11A and B), visual acuity was almost equally distributed for visual acuity 20/10 to <20/64, 133 eyes; 20/64 to <20/200, 108 eyes; and 20/200 or worse, 133 eyes. Visual acuity better than 20/64 was associated with isolated superior arcuate in three eyes (100% of all superior arcuate defects), inferior arcuate defects in 15 eyes (88.2%), combined superior and inferior arcuate defects in 41 eyes (80.4%), and combined superior arcuate with inferior altitudinal defects in 13 eyes (65%). Visual acuity 20/200 or worse was found most

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Computerized Expert System for Evaluation of Automated Visual Fields From the Ischemic Optic Neuropathy Decompression Trial

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frequently for 24 eyes with diffuse depression (77.4%) or 29 eyes with absolute defects (96.7%). Other patterns of field defects, including those with central scotomas, were divided between the 20/64 to 20/200 and the 20/200 and worse categories. Intermediate visual loss of 20/64 to 20/200 was most characteristic of the following visual field patterns: superior and inferior arcuate defects with paracentral scotoma in 10 eyes (50.0%) and central scotoma in 29 eyes (53.7%). For the fellow eye without optic neuropathy (Table 11C), 284 eyes (95.6%) had visual acuity of 20/64 or better. Acuity of 20/64 to 20/200 was noted in eight eyes (2.7%), and acuity worse than 20/200 in four eyes (1.4%).

As shown in Table 12A, for the study eye mild defects were associated with good acuity in 15 of 18 eyes (83.3%), whereas severe defects were associated with good acuity in 284 eyes (95.6%).
Relationship of Age to Pattern and Severity of Visual Field Defect

Relationship of Age to Pattern and Severity of Visual Field Defect

The relationship of age to the pattern and severity of visual field defect was evaluated for patients over 65 years and compared to those 65 years or under. Results are summarized in Table 13.

Based on a total of 220 patients aged 65 or over and 156 patients under age 65, combined superior and inferior arcuate defects were the most frequently encountered (n = 28) in the under 65 age group for the study eye (17.9%). They were less common (n = 23) in the 65 or over age group (10.5%). Second most common in the younger age group were the 21 eyes with superior and inferior arcuate defects with central scotoma (13.5%), but this did not differ in frequency much from the 33 eyes in the older age group (18.9%). The most frequently encountered pattern of defect in the older age group was a superior arcuate and inferior altitudinal defect with central scotoma in 35 eyes (15.9%). Only 12 eyes (7.7%) of the under age 65 group demonstrated a similar defect. As shown in Table 13B, evaluating study eyes based upon the worst severity of field defects encountered within a single visual field demonstrated that only four eyes in patients 65 or over (1.8%) had mild defects compared to 14 eyes in the under 65 group (9%). Conversely, there were 108 eyes (69.2%) in the under age 65 group manifesting severe field defect compared to 150 eyes (81.8%) in the 65 or over age group. This pattern was statistically significant (Kendall’s tau-b = 3.041, P = .002).

In the fellow eye without optic neuropathy (Table 13B), a few differences in the frequency of pattern defect were noted related to age. Normal fields were found in 29 eyes (21.6%) in the younger age group compared to 18 eyes in the 65 or over age group (10.8%). The frequency of other types of field defects did not vary based upon the age range in which they fell. Greatest severity at the moderate level (Table 13D) was present in 49 eyes in the older age group (29.7%) but in only 25 eyes in the younger age group (18.9%). The differences in severity of field defects based upon age were statistically significant (Kendall’s tau-b = 3.313, P = .001).

Relationship of Vascular Conditions to Pattern and Severity of Visual Field Defect

Several risk factors were evaluated, including history of hypertension, stroke, myocardial infarction, angina, and transient ischemic attack. No differences in pattern were noted among the various vascular risk factors, and the numbers for some categories were small; therefore, these risks were combined. Results are summarized in Table 13.

The effect of vascular conditions at baseline on pattern of visual field in the study eye was assessed (Table 13A). No important differences were found. Patients with vascular conditions at baseline numbered 222, and those without numbered 154. The most commonly encountered defects for eyes with or without vascular conditions were superior and inferior arcuate defects (n = 28 with, 23 without), superior and inferior arcuate defects with central scotoma (n = 31 with, 23 without), and superior arcuate and inferior altitudinal defects with central scotoma (n = 30 with, 17 without). The percentage of total field patterns for any one pattern ranged from 11.0% to 14.9%. In evaluating greatest magnitude of visual field defect (Table 13B), the distribution did not appear to differ between the group with or without vascular conditions at baseline (Kendall’s tau-b = –.367, P = .714). In the vascular category, 169 of 222 eyes (76.1%) had a greatest magnitude of visual field defect that was severe, similar to the same severity group in the nonvascular category of 119 of 154 eyes (77.3%).

The effect of vascular conditions at baseline on pattern of visual field in the nonstudy eye without optic neuropathy was assessed as well (Table 13C). In the vasculopathy group of 180 patients, normal fields were found in 22 (12.2%). In the nonvasculopathy group of 121 patients, normal fields were found in 25 (20.7%). The
frequency of other types of field defects did not vary based upon the presence or absence of vasculopathic risk factors. Of the 177 eyes with vascular conditions at baseline, 53 (29.9%) had a greatest magnitude of visual field defect that was moderate. In contrast, of the 120 eyes with no vascular conditions at baseline, 21 (17.5%) had a greatest magnitude of visual field defect that was moderate (Table 13D). In the category of greatest magnitude of field defect of severe, only five eyes (2.8%) were in the vascular condition group compared to nine eyes (7.5%) in the no vascular condition group. However, there was no statistically significant difference in overall distribution of field defect magnitude between the two groups (Kendall’s tau-b = .095, \( P = .087 \)).

### Longitudinal Follow-up: Comparing 6-Month to Baseline Visual Fields

#### Overall Description

There were 605 eyes with visual fields performed at both the baseline and the 6-month visit. Of these, 571 had data at both time points and had foveal sensitivity. After unreliable fields were excluded, 559 eyes were available for
### TABLE 10. GLOBAL INDICES AT BASELINE BY SEVERITY OF DEFECT AND PATIENT GROUP AND EYE

<table>
<thead>
<tr>
<th>CATEGORIZATION OF EYES FOR BASELINE PAPER</th>
<th>STUDY EYE: RANDOMIZED</th>
<th>STUDY EYE: OBSERVATION</th>
<th>FELLOW EYE: NO OPTIC NEUROPATHY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MEAN</td>
<td>CORRECTED</td>
<td>MEAN</td>
</tr>
<tr>
<td></td>
<td>DEVIATION SD</td>
<td>PATTERN SD</td>
<td>DEVIATION SD</td>
</tr>
<tr>
<td>Normal field</td>
<td>n</td>
<td>MEAN</td>
<td>SD</td>
</tr>
<tr>
<td>1</td>
<td>-0.56</td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td>Mild only</td>
<td>3</td>
<td>-7.93</td>
<td>5.74</td>
</tr>
<tr>
<td>Mild and moderate</td>
<td>22</td>
<td>-9.64</td>
<td>4.31</td>
</tr>
<tr>
<td>Mild and severe</td>
<td>62</td>
<td>-22.29</td>
<td>3.85</td>
</tr>
<tr>
<td>Mild, moderate, and severe</td>
<td>53</td>
<td>-23.69</td>
<td>8.51</td>
</tr>
<tr>
<td>Moderate and severe</td>
<td>18</td>
<td>-14.40</td>
<td>6.23</td>
</tr>
<tr>
<td>Severe only</td>
<td>61</td>
<td>-27.06</td>
<td>5.68</td>
</tr>
<tr>
<td>Total</td>
<td>229</td>
<td>-21.47</td>
<td>8.22</td>
</tr>
</tbody>
</table>

*Total is not 301 because four patients have missing severity.

### TABLE 11A. VISUAL ACUITY AT BASELINE BY CATEGORY OF FIELD DEFECT, STUDY EYE

<table>
<thead>
<tr>
<th>VISUAL ACUITY AT BASELINE</th>
<th>20/10 TO &lt;20/64</th>
<th>20/64 TO &lt;20/200</th>
<th>20/200 AND WORSE</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>PATTERN</td>
<td>n</td>
<td>20/10 ROW %</td>
<td>20/64 ROW %</td>
<td>20/200 ROW %</td>
</tr>
<tr>
<td>Normal</td>
<td>1</td>
<td>50.0</td>
<td>1</td>
<td>50.0</td>
</tr>
<tr>
<td>Superior arcuate isolated</td>
<td>3</td>
<td>100.0</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Inferior arcuate isolated</td>
<td>15</td>
<td>88.2</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Superior + inferior arcuate</td>
<td>41</td>
<td>80.4</td>
<td>8</td>
<td>15.7</td>
</tr>
<tr>
<td>Superior + inferior arcuate + paracentral scotoma</td>
<td>9</td>
<td>45.0</td>
<td>10</td>
<td>50.0</td>
</tr>
<tr>
<td>Superior + inferior arcuate + central scotoma</td>
<td>10</td>
<td>18.5</td>
<td>29</td>
<td>53.7</td>
</tr>
<tr>
<td>Superior arcuate + inferior altitudinal</td>
<td>13</td>
<td>65.0</td>
<td>4</td>
<td>20.0</td>
</tr>
<tr>
<td>Superior arcuate + inferior altitudinal + central scotoma</td>
<td>6</td>
<td>13.0</td>
<td>18</td>
<td>39.1</td>
</tr>
<tr>
<td>Superior altitudinal + inferior arcuate</td>
<td>6</td>
<td>54.5</td>
<td>5</td>
<td>45.5</td>
</tr>
<tr>
<td>Superior altitudinal + inferior arcuate + central scotoma</td>
<td>3</td>
<td>13.6</td>
<td>6</td>
<td>27.3</td>
</tr>
<tr>
<td>Superior + inferior altitudinal</td>
<td>4</td>
<td>22.2</td>
<td>5</td>
<td>27.8</td>
</tr>
<tr>
<td>Diffuse depression</td>
<td>1</td>
<td>3.2</td>
<td>6</td>
<td>19.4</td>
</tr>
<tr>
<td>Absolute defect</td>
<td>0</td>
<td>0.0</td>
<td>1</td>
<td>3.3</td>
</tr>
<tr>
<td>Other: isolated defect</td>
<td>4</td>
<td>57.1</td>
<td>1</td>
<td>14.3</td>
</tr>
<tr>
<td>Other: 2 or more defects</td>
<td>17</td>
<td>40.5</td>
<td>14</td>
<td>33.3</td>
</tr>
<tr>
<td>Total</td>
<td>133</td>
<td>35.6</td>
<td>108</td>
<td>28.9</td>
</tr>
</tbody>
</table>
longitudinal analysis. Of these, 203 (36.3%) were randomized study eyes, 75 (13.4%) were nonrandomized study eyes, 57 (10.2%) were fellow eyes with optic neuropathy, and 224 (40.1%) were fellow eyes without optic neuropathy.

The SFs at baseline and at the 6-month follow-up field were compared. For all 559 eyes, the mean SF at baseline was 2.43 (1.45 SD) compared to 2.27 (1.49 SD) at 6 months. For the randomized eyes, mean SF at baseline was 2.58 (1.80 SD), and at 6 months it was 2.57 (1.78 SD). For the nonrandomized study eyes, the mean SF was 2.56 (1.24 SD) at baseline and 2.60 (1.35 SD) at 6 months. For fellow eyes without optic neuropathy, the mean SF at baseline was 2.16 (0.89 SD) and at 6 months, 1.81 (0.78 SD).

Visual Field Changes Between 6-Month and Baseline Visits for Randomized Study Eyes
In evaluating the 203 randomized study eyes, the pattern of defect in the superior and inferior visual fields did not change significantly between baseline and 6-month follow-up (Table 14A). For superior visual fields, the Stuart-Maxwell test of marginal homogeneity had a chi-square of 3.40, \( P = .18 \); for inferior visual fields the chi-square was 0.88, \( P = .64 \). A statistically significant change in central field was noted for randomized eyes with chi-square of 11.43, \( P = .003 \). However, there was not a consistent direction of change. For instance, of 160 central field defects at baseline, 29 (18.1%) changed to neither a central nor a paracentral defect and 14 (8.8%) changed to paracentral defects. Of 28 eyes with neither central nor paracentral defects at baseline, 14 (50.0%) developed central defects.

For those randomized eyes that maintained the same pattern of defect at 6 months and at baseline, the severity of defect was compared (Table 15A). Superior arcuate defect severity did not vary (\( t = -0.098, P = .92 \)) in the
interval with a mean loss for 83 eyes of 14.71 dB (7.86 SD) at baseline and of 14.63 dB (8.01 SD) at 6 months. However, superior altitudinal defects for 85 eyes did improve significantly ($t = –2.24, P = .028$).

Inferior field changes mirrored the superior field changes. For inferior arcuate defects of 70 eyes, the mean depression at baseline was 18.42 dB (8.66 SD) compared to a mean sensitivity loss of 19.20 (8.26 SD) at 6 months. The difference was not significant ($t = 1.09, P = .28$). For inferior altitudinal defects, the baseline mean sensitivity loss was 28.84 dB (3.35 SD) compared to a 6-month sensitivity loss of 27.84 (4.28 SD). The mean difference of –.99 (3.68 SD) was statistically significant ($t = –2.63, P = .01$).

Paracentral visual field defects did not differ in severity between baseline and 6 months for randomized study eyes ($t = –1.05, P = .32$), nor did central defects ($t = –0.82, P = .41$). Of nine paracentral scotomas, the mean depression was 18.81 dB (4.62 SD) at baseline compared to 16.72 (6.51 SD) at 6 months. Of 117 central scotomas, the mean depression was 6.17 dB (8.34 SD) at baseline compared to 5.37 (8.56 SD) at 6 months.

**Visual Field Changes Between 6-Month and Baseline Visits for Nonrandomized Study Eyes**

In evaluating the 75 nonrandomized study eyes, the pattern of defect in the superior and inferior visual fields did not change significantly between baseline and 6-month follow-up (Table 14B). For superior visual fields, the Stuart-Maxwell test of marginal homogeneity had a chi-square of 0.55, $P = .76$; for inferior visual fields the chi-square was 3.67, $P = .16$. A statistically significant change in central field was noted for nonrandomized eyes with chi-square of 6.03, $P = .05$. The numbers within each cell of the 3x3 table were too small to consider the direction of change.

For those nonrandomized eyes that maintained the same pattern of defect at 6 months and at baseline, the severity of defect was compared (Table 15B). Superior arcuate defect severity worsened ($t = 2.98, P = .005$) in the interval with a mean loss for 36 eyes at baseline of 11.85 dB (6.73 SD) and at 6 months of 15.68 dB (8.05 SD). However, superior altitudinal defects did not change significantly ($t = –2.07, P = .093$), with a mean at baseline of 23.89 dB (7.75 SD) and at 6 months of 22.02 dB (7.63 SD). Inferior altitudinal defects improved ($t = –2.83, P = .017$). For inferior arcuate defects of 51 eyes, the mean depression at baseline was 18.29 dB (7.81 SD) compared to a mean sensitivity loss of 18.41 (7.97 SD). The difference was not significant ($t = 0.13, P = .90$). For inferior altitudinal defects, the baseline mean sensitivity loss for 12 eyes was 26.69 dB (5.50 SD) compared to a 6-month sensitivity loss of 25.13 (5.89 SD). The mean difference of –1.57 (1.92 SD) was statistically significant ($P = –2.83, P = .016$). Paracentral visual field defects

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**Table 12A. Visual Acuity at Baseline by Worst Defect Severity, Study Eye**

<table>
<thead>
<tr>
<th>Severity</th>
<th>20/10 to &lt;20/64</th>
<th>20/64 to &lt;20/200</th>
<th>20/200 and Worse</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Row %</td>
<td>n</td>
<td>Row %</td>
</tr>
<tr>
<td>Worst</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>1</td>
<td>50.0</td>
<td>1</td>
<td>50.0</td>
</tr>
<tr>
<td>Mild</td>
<td>15</td>
<td>83.3</td>
<td>1</td>
<td>5.6</td>
</tr>
<tr>
<td>Moderate</td>
<td>36</td>
<td>52.9</td>
<td>22</td>
<td>32.4</td>
</tr>
<tr>
<td>Severe</td>
<td>81</td>
<td>28.3</td>
<td>84</td>
<td>29.4</td>
</tr>
<tr>
<td>Total</td>
<td>133</td>
<td>35.6</td>
<td>108</td>
<td>28.9</td>
</tr>
</tbody>
</table>

**Table 12B. Visual Acuity at Baseline by Worst Defect Severity, Fellow Eye: No Optic Neuropathy**

<table>
<thead>
<tr>
<th>Severity</th>
<th>20/10 to &lt;20/64</th>
<th>20/64 to &lt;20/200</th>
<th>20/200 and Worse</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Row %</td>
<td>n</td>
<td>Row %</td>
</tr>
<tr>
<td>Worst</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>47</td>
<td>100.0</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Mild</td>
<td>157</td>
<td>97.5</td>
<td>4</td>
<td>2.5</td>
</tr>
<tr>
<td>Moderate</td>
<td>69</td>
<td>93.2</td>
<td>4</td>
<td>5.4</td>
</tr>
<tr>
<td>Severe</td>
<td>11</td>
<td>78.6</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Total</td>
<td>284</td>
<td>95.9</td>
<td>8</td>
<td>2.7</td>
</tr>
</tbody>
</table>
improved in severity between baseline and 6 months for 10 nonrandomized study eyes ($t = -2.40, P = .04$), but 4 central defects did not ($t = 1.98, P = .14$). Of 10 paracentral scotomas, the mean depression was 14.55 dB (7.08 SD) at baseline compared to 12.98 (6.95 SD) at 6 months. Of four central scotomas the mean depression was 12.25 dB (14.43 SD) at baseline compared to 21.00 (12.06 SD) at 6 months.

**Visual Field Changes Between 6-Month and Baseline Visits for Fellow Eyes Without Optic Neuropathy**

In evaluating the 224 fellow eyes without optic neuropathy, there was a significant change in pattern of defect in the superior and inferior visual fields between baseline and 6-month follow-up (Table 14C). For superior visual fields, the Stuart-Maxwell test of marginal homogeneity had a chi-square of 27.02, $P < .0001$; for inferior visual fields the chi-square was 27.60, $P < .0001$. Central fields were too few to be analyzed. The direction of change for both upper and lower fields was primarily that of an arcuate defect at baseline changing to no defect at the 6-month visit, seen for 27.4% of 164 eyes with a superior arcuate defect at baseline and for 29.9% of 154 eyes with an inferior arcuate defect at baseline.

For those fellow eyes that maintained the same pattern of defect at 6 months and at baseline, the severity

**TABLE 13A. CATEGORY OF FIELD DEFECT AT BASELINE BY RISK FACTOR, STUDY EYE**

<table>
<thead>
<tr>
<th>PATTERN</th>
<th>AGE</th>
<th>HX of 1+ VASCULAR CONDITIONS AT BASELINE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤65</td>
<td>&gt; 65</td>
</tr>
<tr>
<td>Normal</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Superior arcuate isolated</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Inferior arcuate isolated</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>Superior + inferior arcuate</td>
<td>28</td>
<td>23</td>
</tr>
<tr>
<td>Superior + inferior arcuate + paracentral scotoma</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>Superior + inferior arcuate + central scotoma</td>
<td>21</td>
<td>33</td>
</tr>
<tr>
<td>Superior arcuate + inferior altitudinal</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>Superior arcuate + inferior altitudinal + central scotoma</td>
<td>12</td>
<td>35</td>
</tr>
<tr>
<td>Superior altitudinal + inferior arcuate</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Superior + inferior arcuate + central scotoma</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>Superior arcuate + inferior altitudinal</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>Diffuse depression</td>
<td>9</td>
<td>22</td>
</tr>
<tr>
<td>Absolute defect</td>
<td>7</td>
<td>23</td>
</tr>
<tr>
<td>Other: isolated defect</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Other: 2 or more defects</td>
<td>23</td>
<td>19</td>
</tr>
<tr>
<td>Total</td>
<td>156</td>
<td>220</td>
</tr>
</tbody>
</table>

**TABLE 13B. WORST DEFECT SEVERITY AT BASELINE BY RISK FACTORS, STUDY EYE**

<table>
<thead>
<tr>
<th>SEVERITY</th>
<th>AGE</th>
<th>HX of 1+ VASCULAR CONDITIONS AT BASELINE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤65</td>
<td>&gt; 65</td>
</tr>
<tr>
<td>Normal field</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Mild</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>Moderate</td>
<td>32</td>
<td>36</td>
</tr>
<tr>
<td>Severe</td>
<td>108</td>
<td>180</td>
</tr>
<tr>
<td>Total</td>
<td>156</td>
<td>220</td>
</tr>
</tbody>
</table>

Feldon
of defect was compared (Table 15C). Superior arcuate defect severity improved ($t = -4.08, P = .0001$) in the interval with a mean loss for 113 eyes at baseline of 9.63 dB (5.98 SD) and at 6 months of 7.68 dB (5.13 SD). For inferior arcuate defects of 104 eyes, the mean depression at baseline was 9.66 dB (5.85 SD) compared to a mean sensitivity loss of 7.24 (5.36 SD) at 6 months. The difference was highly significant ($t = -4.78, P < .0001$).

Altitudinal defects, paracentral scotomas, and central scotomas were few.

**Comparison of Careful Follow-up and Surgery Treatments for Randomized Patients**

**Visual Field Patterns.** The pattern of defects in the superior visual fields for the study eyes randomized to careful follow-up showed no difference between baseline and the 6-month visit using the Stuart-Maxwell test of marginal homogeneity ($\chi^2 df = 1.43, P = .49$). Similarly, the pattern of defects in the superior fields for the study eyes randomized to surgery showed no difference between baseline and the 6-month visit ($\chi^2 df = 2.57, P = .28$). However, seven of the eight patients who received surgery in their study eye and who had no superior defect at baseline developed either an arcuate or an altitudinal defect at 6 months. Table 16 compares the proportion of eyes that declined, stayed the same, and improved, wherein improvement is defined as changing from an altitudinal defect to an arcuate defect and decline

---

**Table 13C. Category of Field Defect at Baseline by Risk Factor, Fellow Eye: Optic Neuropathy, No Optic Neuropathy**

<table>
<thead>
<tr>
<th>PATTERN</th>
<th>&lt;65</th>
<th>&gt;65</th>
<th>NO</th>
<th>YES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>29</td>
<td>18</td>
<td>25</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>21.6</td>
<td>10.8</td>
<td>20.7</td>
<td>12.2</td>
</tr>
<tr>
<td>Superior arcuate isolated</td>
<td>15</td>
<td>20</td>
<td>14</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>11.2</td>
<td>12.0</td>
<td>11.6</td>
<td>11.7</td>
</tr>
<tr>
<td>Inferior arcuate isolated</td>
<td>12</td>
<td>4.8</td>
<td>10</td>
<td>5.6</td>
</tr>
<tr>
<td></td>
<td>9.0</td>
<td>4.8</td>
<td>8.3</td>
<td>5.6</td>
</tr>
<tr>
<td>Superior + inferior arcuate</td>
<td>67</td>
<td>102</td>
<td>61</td>
<td>108</td>
</tr>
<tr>
<td></td>
<td>50.0</td>
<td>61.1</td>
<td>50.4</td>
<td>60.0</td>
</tr>
<tr>
<td>Superior + inferior arcuate + paracentral scotoma</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>0.0</td>
<td>1.8</td>
<td>0.8</td>
<td>1.1</td>
</tr>
<tr>
<td>Superior + inferior arcuate + central scotoma</td>
<td>2</td>
<td>5</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>3.0</td>
<td>1.7</td>
<td>2.8</td>
</tr>
<tr>
<td>Superior arcuate + inferior altitudinal</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>0.0</td>
<td>0.6</td>
<td>0.0</td>
<td>0.6</td>
</tr>
<tr>
<td>Superior altitudinal + inferior arcuate</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>0.6</td>
<td>1.7</td>
<td>1.1</td>
</tr>
<tr>
<td>Superior altitudinal + inferior arcuate + central scotoma</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>0.0</td>
<td>0.6</td>
<td>0.0</td>
<td>0.6</td>
</tr>
<tr>
<td>Superior + inferior altitudinal</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>0.0</td>
<td>1.2</td>
<td>0.0</td>
<td>1.1</td>
</tr>
<tr>
<td>Diffuse depression</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.0</td>
<td>0.6</td>
<td>0.8</td>
<td>0.0</td>
</tr>
<tr>
<td>Other: isolated defect</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>1.8</td>
<td>2.3</td>
<td>1.1</td>
</tr>
<tr>
<td>Other: 2 or more defects</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>3.7</td>
<td>1.2</td>
<td>1.7</td>
<td>2.8</td>
</tr>
<tr>
<td>Total</td>
<td>134</td>
<td>167</td>
<td>121</td>
<td>180</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

**Table 13D. Worst Defect Severity Baseline by Risk Factors, Fellow Eye: Optic Neuropathy, No Optic Neuropathy**

<table>
<thead>
<tr>
<th>SEVERITY</th>
<th>&lt;65</th>
<th>&gt;65</th>
<th>NO</th>
<th>YES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Worst</td>
<td>29</td>
<td>18</td>
<td>25</td>
<td>22</td>
</tr>
<tr>
<td>Normal field</td>
<td>22.0</td>
<td>10.9</td>
<td>20.8</td>
<td>12.4</td>
</tr>
<tr>
<td>Mild</td>
<td>74</td>
<td>53</td>
<td>65</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>56.1</td>
<td>33.3</td>
<td>54.2</td>
<td>54.8</td>
</tr>
<tr>
<td>Moderate</td>
<td>25</td>
<td>29.7</td>
<td>21</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>18.9</td>
<td>29.7</td>
<td>17.5</td>
<td>29.9</td>
</tr>
<tr>
<td>Severe</td>
<td>4</td>
<td>6.1</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>6.1</td>
<td>7.5</td>
<td>2.8</td>
</tr>
<tr>
<td>Total</td>
<td>132</td>
<td>165</td>
<td>120</td>
<td>177</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>
is defined as the opposite. Using the Mantel-Haenzel extension test (M-H), no treatment effect was noted when comparing the pattern changes for the superior field described for careful follow-up and for surgery to each other ($\chi^2 = 0.49, P = .48$).

The pattern of defects in the inferior visual fields for the study eyes randomized to careful follow-up showed no difference between baseline and the 6-month visit ($\chi^2 = 1.47, P = .48$), and neither did the study eyes randomized to surgery ($\chi^2 = 0.70, P = .71$). As shown in Table 16, no treatment effect was noted when comparing the pattern changes for the inferior field described for careful follow-up and for surgery to each other, (M-H test, $\chi^2 = 0.26, P = .61$).

There was no significant shift in the patterns of

<table>
<thead>
<tr>
<th>TABLE 14A. FREQUENCY OF VISUAL FIELD DEFECTS AT BASELINE AND AT 6-MONTH FOLLOW-UP: RANDOMIZED EYES</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUPERIOR VISUAL FIELD</td>
</tr>
<tr>
<td>6- MONTH FOLLOW-UP FIELD</td>
</tr>
<tr>
<td>BASELINE FIELD</td>
</tr>
<tr>
<td>NO DEFECT</td>
</tr>
<tr>
<td>No defect (n)</td>
</tr>
<tr>
<td>Row %</td>
</tr>
<tr>
<td>Column %</td>
</tr>
<tr>
<td>Arcuate</td>
</tr>
<tr>
<td>Row %</td>
</tr>
<tr>
<td>Column %</td>
</tr>
<tr>
<td>Altitudinal</td>
</tr>
<tr>
<td>Row %</td>
</tr>
<tr>
<td>Column %</td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>Row %</td>
</tr>
<tr>
<td>Column %</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TABLE 14B. FREQUENCY OF VISUAL FIELD DEFECTS AT BASELINE AND AT 6-MONTH FOLLOW-UP: NONRANDOMIZED EYES</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUPERIOR VISUAL FIELD</td>
</tr>
<tr>
<td>6- MONTH FOLLOW-UP FIELD</td>
</tr>
<tr>
<td>BASELINE FIELD</td>
</tr>
<tr>
<td>NO DEFECT</td>
</tr>
<tr>
<td>No defect (n)</td>
</tr>
<tr>
<td>Row %</td>
</tr>
<tr>
<td>Column %</td>
</tr>
<tr>
<td>Arcuate</td>
</tr>
<tr>
<td>Row %</td>
</tr>
<tr>
<td>Column %</td>
</tr>
<tr>
<td>Altitudinal</td>
</tr>
<tr>
<td>Row %</td>
</tr>
<tr>
<td>Column %</td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>Row %</td>
</tr>
<tr>
<td>Column %</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TABLE 14C. FREQUENCY OF VISUAL FIELD DEFECTS AT BASELINE AND AT 6-MONTH FOLLOW-UP: NONRANDOMIZED EYES</th>
</tr>
</thead>
<tbody>
<tr>
<td>INFERIOR VISUAL FIELD</td>
</tr>
<tr>
<td>6- MONTH FOLLOW-UP FIELD</td>
</tr>
<tr>
<td>BASELINE FIELD</td>
</tr>
<tr>
<td>NO DEFECT</td>
</tr>
<tr>
<td>No defect (n)</td>
</tr>
<tr>
<td>Row %</td>
</tr>
<tr>
<td>Column %</td>
</tr>
<tr>
<td>Arcuate</td>
</tr>
<tr>
<td>Row %</td>
</tr>
<tr>
<td>Column %</td>
</tr>
<tr>
<td>Altitudinal</td>
</tr>
<tr>
<td>Row %</td>
</tr>
<tr>
<td>Column %</td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>Row %</td>
</tr>
<tr>
<td>Column %</td>
</tr>
</tbody>
</table>

Marginal homogeneity (Stuart-Maxwell)

<table>
<thead>
<tr>
<th>TABLE 14C. FREQUENCY OF VISUAL FIELD DEFECTS AT BASELINE AND AT 6-MONTH FOLLOW-UP: NONRANDOMIZED EYES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marginal homogeneity (Stuart-Maxwell)</td>
</tr>
<tr>
<td>chi-square</td>
</tr>
<tr>
<td>3.40</td>
</tr>
<tr>
<td>0.88</td>
</tr>
</tbody>
</table>
### TABLE 14C. FREQUENCY OF VISUAL FIELD DEFECTS AT BASELINE AND AT 6-MONTH FOLLOW-UP: FELLOW EYES WITHOUT OPTIC NEUROPATHY

<table>
<thead>
<tr>
<th>Superior Visual Field</th>
<th>Inferior Visual Field</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BASELINE FIELD NO DEFECT</strong></td>
<td><strong>BASELINE FIELD NO DEFECT</strong></td>
</tr>
<tr>
<td><strong>ARCUATE ALTITUDINAL OTHER TOTAL</strong></td>
<td><strong>ARCUATE ALTITUDINAL OTHER TOTAL</strong></td>
</tr>
<tr>
<td><strong>SUPERIOR VISUAL FIELD 6-MONTH FOLLOW-UP FIELD</strong></td>
<td><strong>INFERIOR VISUAL FIELD 6-MONTH FOLLOW-UP FIELD</strong></td>
</tr>
<tr>
<td><strong>NO DEFECT ARCUATE ALTITUDINAL OTHER TOTAL</strong></td>
<td><strong>NO DEFECT ARCUATE ALTITUDINAL OTHER TOTAL</strong></td>
</tr>
<tr>
<td><strong>No defect (n)</strong></td>
<td>44</td>
</tr>
<tr>
<td><strong>Row %</strong></td>
<td>83.02</td>
</tr>
<tr>
<td><strong>Column %</strong></td>
<td>48.89</td>
</tr>
<tr>
<td><strong>Arcuate</strong></td>
<td>45</td>
</tr>
<tr>
<td><strong>Row %</strong></td>
<td>27.44</td>
</tr>
<tr>
<td><strong>Column %</strong></td>
<td>50</td>
</tr>
<tr>
<td><strong>Altitudinal</strong></td>
<td>0</td>
</tr>
<tr>
<td><strong>Row %</strong></td>
<td>0.00</td>
</tr>
<tr>
<td><strong>Column %</strong></td>
<td>0.00</td>
</tr>
<tr>
<td><strong>Other defect</strong></td>
<td>1</td>
</tr>
<tr>
<td><strong>Row %</strong></td>
<td>25</td>
</tr>
<tr>
<td><strong>Column %</strong></td>
<td>1.11</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>90</td>
</tr>
<tr>
<td><strong>Row %</strong></td>
<td>40.18</td>
</tr>
<tr>
<td><strong>Column %</strong></td>
<td>100</td>
</tr>
</tbody>
</table>

#### Marginal homogeneity

<table>
<thead>
<tr>
<th>(Stuart-Maxwell)</th>
<th>chi-square</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Superior Visual Field</strong></td>
<td>27.02</td>
<td>3</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td><strong>Inferior Visual Field</strong></td>
<td>27.6</td>
<td>3</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

### TABLE 15A. SEVERITY OF VISUAL FIELD DEFECT AT BASELINE AND 6 MONTHS, FOR EYES WITH SAME DEFECT AT BOTH VISITS: RANDOMIZED EYES

<table>
<thead>
<tr>
<th>Superior arcuate</th>
<th>Inferior arcuate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Examination</strong></td>
<td><strong>Examination</strong></td>
</tr>
<tr>
<td><strong>n</strong></td>
<td><strong>mean</strong></td>
</tr>
<tr>
<td>6 month</td>
<td>83</td>
</tr>
<tr>
<td>baseline</td>
<td>83</td>
</tr>
<tr>
<td>difference</td>
<td>83</td>
</tr>
<tr>
<td><strong>Paired t test</strong></td>
<td><strong>Paired t test</strong></td>
</tr>
<tr>
<td><strong>t</strong></td>
<td><strong>P</strong></td>
</tr>
<tr>
<td>-0.098</td>
<td>.92</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Superior altitudinal</th>
<th>Inferior altitudinal</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Examination</strong></td>
<td><strong>Examination</strong></td>
</tr>
<tr>
<td><strong>n</strong></td>
<td><strong>mean</strong></td>
</tr>
<tr>
<td>6 month</td>
<td>85</td>
</tr>
<tr>
<td>baseline</td>
<td>85</td>
</tr>
<tr>
<td>difference</td>
<td>85</td>
</tr>
<tr>
<td><strong>Paired t test</strong></td>
<td><strong>Paired t test</strong></td>
</tr>
<tr>
<td><strong>t</strong></td>
<td><strong>P</strong></td>
</tr>
<tr>
<td>-2.24</td>
<td>.028</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Paracentral scotoma</th>
<th>Central scotoma</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Examination</strong></td>
<td><strong>Examination</strong></td>
</tr>
<tr>
<td><strong>n</strong></td>
<td><strong>mean</strong></td>
</tr>
<tr>
<td>6 month</td>
<td>9</td>
</tr>
<tr>
<td>baseline</td>
<td>9</td>
</tr>
<tr>
<td>difference</td>
<td>9</td>
</tr>
<tr>
<td><strong>Paired t test</strong></td>
<td><strong>Paired t test</strong></td>
</tr>
<tr>
<td><strong>t</strong></td>
<td><strong>P</strong></td>
</tr>
<tr>
<td>-1.05</td>
<td>.32</td>
</tr>
</tbody>
</table>
central defects assigned to careful follow-up ($\chi^2 = 4.19$, $P = .12$), whereas there was a significant shift for study eyes assigned to surgery ($\chi^2 = 7.41$, $P = .025$), with 24.51% of eyes improving and only 7.84% of eyes worsening. On the other hand, comparing the patterns of change in central visual fields of patients in the careful follow-up and surgical treatment groups, no treatment difference was noted, wherein improvement in Table 16 is defined as a change from a central to a paracentral defect and decline as the opposite (M-H test, $\chi^2 = 0.57$, $P = .45$).

**Visual Field Severity.** For those visual fields that showed no change in pattern of defect over time, the severity of visual field loss at baseline and at the 6-month visit were compared using Student’s $t$ tests (Table 17). In the careful follow-up group, only inferior altitudinal defects showed a significant change in mean defect, with an improvement of $-1.13$ dB ($P = .02$). In the surgery group, only the superior altitudinal defects showed a significant change in mean defect, with an improvement of $-1.69$ dB ($P = .007$). It is worth noting, however, that all mean differences between the baseline and the 6-month visit in the careful follow-up group showed some improvement, whereas in the surgery group the superior arcuate and inferior arcuate mean severity of defect actually worsened slightly. However, in evaluating the changes in severity between the two groups (Table 18), the differences were not significant.

### DISCUSSION

#### Discussion of Methods
Automated perimetry facilitates the collection of quantitative data on the pattern and severity of visual field defects. A standard for automated interpretation of these defects requires development of decision criteria. There are three evaluations of importance in the interpretation of visual fields—detection, progression, and characterization. Difficulties in detection relate primarily to distinguishing appropriately between short-term and long-term fluctuation. This problem is further compounded in various disease states, such as glaucoma, wherein the pathologic process itself produces fluctuation in sensitivity. The Ocular Hypertension Treatment Study, wherein multiple confirmation fields were required to diagnose the presence or absence of a defect, provides an example of a method to deal with detection of visual field defects.

Progression of field defects is a common end point for glaucoma studies. The issue, once again, is determining change, but from an abnormal as opposed to a normal baseline. Katz\(^a\) has reviewed scoring methods employed
### TABLE 15C. SEVERITY OF VISUAL FIELD DEFECT AT BASELINE AND 6 MONTHS, FOR EYES WITH SAME DEFECT AT BOTH VISITS: FELLOW EYES WITHOUT OPTIC NEUROPATHY

<table>
<thead>
<tr>
<th>Examination</th>
<th>n</th>
<th>mean</th>
<th>SE</th>
<th>SD</th>
<th>Examination</th>
<th>n</th>
<th>mean</th>
<th>SE</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Superior arcuate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><strong>Inferior arcuate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 month</td>
<td>113</td>
<td>7.68</td>
<td>0.48</td>
<td>5.13</td>
<td>6 month</td>
<td>104</td>
<td>7.24</td>
<td>0.53</td>
<td>5.36</td>
</tr>
<tr>
<td>Baseline</td>
<td>113</td>
<td>9.63</td>
<td>0.56</td>
<td>5.98</td>
<td>Baseline</td>
<td>104</td>
<td>9.66</td>
<td>0.57</td>
<td>5.85</td>
</tr>
<tr>
<td>Difference</td>
<td>113</td>
<td>-1.95</td>
<td>0.48</td>
<td>5.08</td>
<td>Difference</td>
<td>104</td>
<td>-2.42</td>
<td>0.51</td>
<td>5.16</td>
</tr>
<tr>
<td>Paired t test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Paired t test</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>t</td>
<td></td>
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<td></td>
<td>t</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-4.08</td>
<td>&lt;.0001</td>
<td></td>
<td></td>
<td></td>
<td>-4.78</td>
<td>&lt;.0001</td>
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<td></td>
</tr>
</tbody>
</table>

### Superior altitudinal

<table>
<thead>
<tr>
<th>Examination</th>
<th>n</th>
<th>mean</th>
<th>SE</th>
<th>SD</th>
<th>Examination</th>
<th>n</th>
<th>mean</th>
<th>SE</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 month</td>
<td>3</td>
<td>8.31</td>
<td>3.44</td>
<td>5.96</td>
<td>6 month</td>
<td>2</td>
<td>9.48</td>
<td>3.66</td>
<td>5.18</td>
</tr>
<tr>
<td>Baseline</td>
<td>3</td>
<td>9.99</td>
<td>1.45</td>
<td>2.51</td>
<td>Baseline</td>
<td>2</td>
<td>9.87</td>
<td>1.97</td>
<td>2.79</td>
</tr>
<tr>
<td>Difference</td>
<td>3</td>
<td>-1.68</td>
<td>2.19</td>
<td>3.79</td>
<td>Difference</td>
<td>2</td>
<td>-0.39</td>
<td>1.69</td>
<td>2.39</td>
</tr>
<tr>
<td>Paracentral scotoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Central scotoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Examination</td>
<td>n</td>
<td>mean</td>
<td></td>
<td></td>
<td>Examination</td>
<td>n</td>
<td>mean</td>
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<td></td>
</tr>
<tr>
<td>6 month</td>
<td>1</td>
<td>11.17</td>
<td></td>
<td></td>
<td>6 month</td>
<td>2</td>
<td>23.5</td>
<td>0.5</td>
<td>0.71</td>
</tr>
<tr>
<td>Baseline</td>
<td>1</td>
<td>11.33</td>
<td></td>
<td></td>
<td>Baseline</td>
<td>2</td>
<td>14.5</td>
<td>1.5</td>
<td>2.12</td>
</tr>
<tr>
<td>Difference</td>
<td>1</td>
<td>-0.17</td>
<td></td>
<td></td>
<td>Difference</td>
<td>2</td>
<td>9</td>
<td>2</td>
<td>2.83</td>
</tr>
</tbody>
</table>

### Table 16. Comparison of the proportion of eyes that declined, stayed the same, and improved: Careful Follow-up versus Surgery

<table>
<thead>
<tr>
<th>Status</th>
<th>Careful Follow-up</th>
<th>Surgery</th>
<th>Odds</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Superior portion of the eye</strong>&lt;sup&gt;*&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decline</td>
<td>8</td>
<td>12</td>
<td>1.50†</td>
<td>0.61</td>
</tr>
<tr>
<td>Same</td>
<td>87</td>
<td>84</td>
<td>0.97†</td>
<td>0.72</td>
</tr>
<tr>
<td>Improve</td>
<td>6</td>
<td>6</td>
<td>1.00†</td>
<td>0.32</td>
</tr>
</tbody>
</table>

| **Inferior portion of the eye**<sup>‡</sup> |                    |         |        |          |
| Decline                 | 8                 | 7       | 0.88†  | 0.32     | 2.41     |
| Same                    | 81                | 87      | 1.07†  | 0.79     | 1.45     |
| Improve                 | 12                | 8       | 0.67†  | 0.27     | 1.63     |

| **Central portion of the eye**<sup>§</sup> |                    |         |        |          |
| Decline                 | 10                | 8       | 0.80†  | 0.32     | 2.03     |
| Same                    | 70                | 69      | 0.99†  | 0.71     | 1.37     |
| Improve                 | 21                | 25      | 1.19†  | 0.67     | 2.13     |

<sup>*</sup>Extension of M-H test: chi square, 1 df = 0.49, P = .48.

<sup>†</sup>For surgery group compared with careful follow-up.

<sup>‡</sup>Extension of M-H test: chi square, 1 df = 0.26, P = .61.

<sup>§</sup>Extension of M-H test: chi square, 1 df = 0.57, P = .45.
by two multicenter clinical trials, the Advanced Glaucoma Intervention Study and the CIGTS. These studies utilize a cumulative score (0 to 20), based on depression of adjacent points occurring within specified regions of the visual field. Depression is defined by total deviation plot on the HVF printout in the Advanced Glaucoma Intervention Study and by probability values in the CIGTS.

More advanced models of visual field perturbations have also been investigated. De la Rosa and colleagues utilized an approach for rapid assessment of glaucomatous field defects based on multiple correlations. McNaught and coworkers developed a linear model of pointwise sensitivity values against time to identify progression in normal tension glaucoma. By any of these methods, detection and progression can be determined operationally, based upon the sensitivity and reliability to be required in any particular study.

In contrast to detection and progression of visual field defects, characterization is a more complex task. It requires pattern recognition open to multiple interpretations, depending upon “lumping” versus “splitting” biases, as well as conformity to established biases formed from nonrigorous clinical observations. In one of the few clinical trials to utilize pattern recognition as an outcome for visual field testing, the Optic Neuritis Treatment Trial established 15 monocular types of field defects (14 local

---

**Table 17. Comparison of Changes in Severity of Visual Fields for Various Defects, for Eyes with Same Defect at Both Visits**

<table>
<thead>
<tr>
<th>Defect Type</th>
<th>Careful Follow-up Group</th>
<th>Surgery Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline 6 Months</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n MEAN SE MEAN SE MEAN DIFF * P VALUE</td>
<td></td>
</tr>
<tr>
<td>Superior arcuate</td>
<td>41 16.62 1.21 15.14 1.24 -1.48 0.2</td>
<td></td>
</tr>
<tr>
<td>Superior altitudinal</td>
<td>44 25.59 0.95 24.96 0.95 -0.63 0.44</td>
<td></td>
</tr>
<tr>
<td>Inferior arcuate</td>
<td>33 18.94 1.53 18.50 1.59 -0.44 0.57</td>
<td></td>
</tr>
<tr>
<td>Inferior altitudinal</td>
<td>46 28.44 0.58 27.31 0.65 -1.13 0.02</td>
<td></td>
</tr>
<tr>
<td>Paracentral</td>
<td>3 22.33 2.62 20.83 4.02 -1.50 0.76</td>
<td></td>
</tr>
<tr>
<td>Central</td>
<td>62 5.89 1.04 5.68 1.08 -0.21 0.58</td>
<td></td>
</tr>
<tr>
<td>Superior arcuate</td>
<td>42 12.84 1.18 14.13 1.26 1.29 0.26</td>
<td></td>
</tr>
<tr>
<td>Superior altitudinal</td>
<td>41 27.75 0.43 26.06 0.76 -1.69 0.007</td>
<td></td>
</tr>
<tr>
<td>Inferior arcuate</td>
<td>37 17.95 1.42 19.83 1.30 1.88 0.11</td>
<td></td>
</tr>
<tr>
<td>Inferior altitudinal</td>
<td>49 29.21 0.39 28.35 0.59 -0.86 0.15</td>
<td></td>
</tr>
<tr>
<td>Paracentral</td>
<td>6 17.66 1.57 14.67 2.35 -2.39 0.37</td>
<td></td>
</tr>
<tr>
<td>Central</td>
<td>55 6.49 1.15 5.02 1.17 -1.47 0.30</td>
<td></td>
</tr>
</tbody>
</table>

* Mean difference = mean at 6 months minus mean at baseline.

---

**Table 18. Comparison of Differences in Severity of Visual Field Defects, for Eyes with Same Defect at Both Visits: Careful Follow-up Versus Surgery**

<table>
<thead>
<tr>
<th>Defect Type</th>
<th>Careful Follow-up</th>
<th>Surgery</th>
<th>Difference *</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superior arcuate defects</td>
<td>41 -1.48 7.25 42</td>
<td>1.29 7.38 -2.78 0.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superior altitudinal defects</td>
<td>44 -0.63 5.43 41</td>
<td>1.69 3.79 1.06 0.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inferior arcuate defects</td>
<td>33 -0.44 4.46 37</td>
<td>1.88 7.00 -2.32 0.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inferior altitudinal defects</td>
<td>46 -1.13 3.24 49</td>
<td>-0.86 4.09 -0.27 0.72</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paracentral scotomas</td>
<td>3 -1.50 7.42 6</td>
<td>-2.39 5.89 0.89 0.55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central scotomas</td>
<td>62 -0.21 10.71 55</td>
<td>-1.47 10.39 1.26 0.52</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Difference is baseline to 6-month change for careful follow-up minus baseline to 6-month change for surgery.
and diffuse) of three different severities occurring in optic neuritis. After an initial review by the Director and Associate Director of the Visual Field Reading Center separately, they then reviewed them together to “reach a consensus on the final classification for each visual field.” Initial agreement was noted for 76.3% of the HVF, 81.5% on the location, and 74% on the shape. Complete agreement in every category was achieved in only 47.4% of the 309 affected eyes. In a masked retest, the shapes were in agreement in 76.2% of the 42 cases.\(^1\)\(^2\)

Neural networks have been touted as providing a means for allowing computers to “learn” how to correctly categorize visual fields, even in the absence of specified rules. In the supervised class of artificial neural networks, the systems require a training set of “correctly” categorized visual fields to allow learning to occur.\(^10\)\(^11\) Thus, there is a tautology in that, in the absence of rules, how is such a training set derived? Henson and associates\(^16\) suggest that unsupervised neural networks can be used to resolve this dilemma, as they are self-classifying. However, the patterns correspond to the number of nodes used in the neural network and do not necessarily correspond to clinically identified field defects.

As demonstrated by the experts impaneled on the VFAC for the IONDT, rule-based criteria are not easily divine. There is considerable interobserver as well as intraobserver variability, even when there is agreement on the rules for decision making. The diagnostic variability in this study is similar to performance of humans and computers in validations of other expert systems, ranging from 50% to 70%.\(^5\)\(^7\) Important in the present study, experts recognize that more than one interpretation is possible for a given distribution of disturbed points on a visual field, so that discarding a computerized determination as inconsistent with clinical interpretation is an easier end point upon which to achieve consensus than independently derived agreement. Once incorporated into an expert computer system, the criteria for categorizing the pattern and severity of visual field defects are, according to Hirshbrunner and colleagues,\(^19\) “explicit, obvious, and standardized.” Such attributes are essential within the context of prospective clinical trials.

One of the potential limitations of a highly deterministic computerized expert system is that it may not allow for changes in pattern that occur solely on the basis of short-term fluctuation. We addressed this issue of pattern changes over time by determining the joint SF for visual fields of an individual at baseline and 6-month visits. The ±2 SF was then applied to those individual test points in the visual field that determined the pattern of defect.

**Pattern of Visual Loss**

Visual field defects are noted in 63% to 100% of NAION patients.\(^20\)\(^23\) The classic presentation of NAION often involves the sudden loss of lower or, less commonly, upper visual field. Central scotomas, arcuate defects, and quadrant defects may also occur.\(^14\)\(^20\) Repka and colleagues\(^24\) tabulated the location and type of visual field defect by Goldmann perimetry and found that 46% of NAION patients had an inferior altitudinal defect and 20% had isolated central scotomas. Hayreh and Podhajsky\(^25\) reported inferior nasal or inferior altitudinal defects in 57% of NAION patients and central scotoma in 25%. Traustason and colleagues\(^26\) quantitatively classified field defects performed by Octopus perimetry and found that, although 55% of AION patients demonstrated a significant altitudinal field loss, the “spared” hemifields routinely showed some loss of sensitivity.

In the present study, a pure field defect confined to the upper or lower hemisphere was relatively unusual. This is consistent with prior observations that automated perimetry frequently demonstrates defects, even in asymptomatic hemifields.\(^26\) The clinical perception that field involvement is primarily altitudinal is likely derived from differential severity of involvement in the upper and lower hemifield and by ignoring the presence of central scotoma. In the present study, field defects were evaluated separately as to pattern and severity rather than together. The simultaneous evaluation of 12 identified types of defects with three levels of severity would result in insufficient data points for meaningful analysis.

**Comparability of Data for Eyes Randomized to Surgery or to Careful Follow-up**

The complexity of the data set, the interaction between disease state and test reliability, and the effects of learning on responses impact visual field data. Consistency and comparability at the baseline visit are important for classifying the visual fields seen with the onset of NAION, for interpreting subsequent changes, and for assessing the status of the fellow eye. Within the group randomized either to surgery or to careful follow-up, there was no difference noted in either the pattern or the severity of visual field defects. Also, there were no differences within the randomized group of eyes related to regular versus late entry (progressive NAION). Thus, any changes in subsequent visual field examinations can be reasonably attributed to treatment effect.

**Differences Between the Randomized and Careful Observation Groups**

The distribution of field defects in the nonrandomized patients, having visual acuity better than 20/64, demonstrates a pattern of defect substantially different from randomized eyes. The frequency and density of central scotomas are markedly reduced in this observational
group. Because decreased foveal sensitivity is a major criterion in the expert system definition of central scotoma, a poor central acuity is likely to be associated with proportional decrease in foveal sensitivity. The rarity of diffuse depression of the visual field in the observational group is explained by the necessity of having equal loss of sensitivity throughout the visual field. Sparred foveal sensitivity in eyes with better acuity precludes designation as diffuse depression except when the depression is mild.

**Effect of Systemic Processes**

Numerous studies have identified vasculopathic, anatomic, and pharmacologic risk factors associated with the onset of AION. In the IONDT, diabetes was significantly associated with NAION in the fellow eye. Prior evaluation of the IONDT cohort based upon visual acuity demonstrated that poorer acuity was associated with diabetes and hypertension. In a previous study of visual field loss in AION, diabetics were especially prone to severe diffuse depression. However, the results described in the present study showed that no differences were encountered in pattern or severity of pattern defect for the study eye related to systemic processes. Younger patients did tend to have milder defects and less involvement of the central visual field.

**Comparison of Pattern Defects and Severity With Global Indices**

Global measures of visual field function such as the mean deviation and the CPSD offer information regarding the average severity and localization of visual field loss, respectively. They are frequently utilized in prospective studies, because they are simple, quantitative measures that lend themselves to an analysis analogous to visual acuity. In the Ocular Hypertension Treatment Study, visual field end points included an abnormal CPSD at the \( P < .05 \) level. The mean deviation was utilized as a secondary outcome measure in reporting the results of the IONDT. CPSD was not evaluated initially but has been utilized as a measure of uneven distribution of disturbed points within the visual field.

In this study, the mean deviation was more depressed in the randomized than in the careful observation group. Thus, patients with worse visual acuity loss also tended to have more overall visual field disturbance. However, the mean deviation did not necessarily progress with progressively worse categories of field depression within specific patterns of defect. For instance, for the randomized eyes, the “moderate only” category had about the same mean deviation as the “moderate and severe category.” Therefore, mean deviation may not be a good overall indicator of severity of specific field defects.

The CPSD did not correspond to the overall severity of field defects encountered, especially for the randomized eyes. The CPSD was highest when multiple defects with differing severities were encountered. The frequency of diffuse field defects in the randomized eyes was likely responsible for the lower CPSD noted in that group compared with the observation group. Thus, CPSD was, as might be expected, more an indicator of nonhomogeneity within the visual fields than of severity per se.

The inconsistent results for both mean deviation and for CPSD related to the severity of pattern defects suggest that such global measures have limited value for comparing visual fields in a longitudinal study.

**Relationship of Visual Field Defects to Visual Acuity**

In interpreting the relationship between visual fields and visual acuity, it is important to keep in mind that depressed visual acuity and the presence of a central scotoma are likely to be highly correlated, but are not always coincident. Each is determined through different testing methods, based upon different psychophysical principles. Furthermore, with many defects splitting or otherwise only partially affecting foveal function, scanning strategies used by patients for assessment of acuity might differ from fixation strategies (eg, eccentric fixation) used for assessment of visual fields. Thus, some patients with good acuity may have only central scotoma, and other patients with poor acuity may have no central scotoma.

As expected, the presence of a central scotoma as part of the overall visual field pattern corresponded with loss of visual acuity. Poor acuity was almost routinely observed for those fields with diffuse or absolute field defects. Classic arcuate and altitudinal defects were found primarily in eyes with good acuity. However, in about one fourth of cases, severe peripheral field defects were detected in eyes with relatively preserved acuity. The results do suggest, however, that visual acuity may be a useful, though coarse, surrogate for overall severity of visual field involvement in AION.

**Late Entry**

Early studies promoting the benefit of optic nerve sheath fenestration for the treatment of NAION suggested that patients with a progressive course might be better candidates for the procedure than those who showed no progression. As defined by the IONDT, the late entry group constituted a subset of the randomized patients whose visual acuity in the study eye was better than 20/64 at baseline but lost acuity to below this level within 30 days. The late entry group did not benefit from optic nerve sheath fenestration, based upon visual acuity. That progressive AION is no different from the more common, nonprogressive form is supported by the similarity in
pattern and pattern-specific severity between these two subgroups of randomized patients.

The Fellow Eye
Two retrospective studies have compared the visual fields in patients with bilateral NAION, but with conflicting conclusions. WuDunn and associates\(^3\) found that the pattern of visual field loss in the second eye correlated poorly with that of the first eye. They also reported that mean deviation tended to be less in the second eye that in the first eye for older patients. On the other hand, Boone and associates\(^6\) found that in 75% of 16 patients with bilateral NAION, the mean deviation on HVF testing did not differ by more than 5 dB. There were 75 fellow eyes with optic neuropathy included in the cohort. However, a direct comparison of visual field involvement in the study eye and fellow eye with optic neuropathy for the same patient was not performed. This controversy in the literature will be addressed in a future analysis of IONDT data.

One surprising result of this study was the high percentage of abnormal patterns of visual fields in eyes without optic neuropathy. This finding was also reflected in the depressed mean deviation for the group. The vast majority of anomalous patterns were mild superior or inferior arcuate defects. These may have been artifactual, owing to learning effects, or secondary to non-optic nerve–related eye disease that differed from the age-matched normal population included in the HVF normative data set. Artifacts and learning effects should tend to normalize in subsequent visual field examinations, whereas field defects related to other eye diseases should be stable or progress. Analysis of the results at 6 months suggests that almost 30% of defects present at baseline normalize in follow-up; furthermore, the remaining 113 superior and 104 inferior arcuate defects become significantly less severe.

The possibility that “normal” HVFs are being classified as “abnormal” by a flawed computerized expert system should be considered. However, these criteria were set and the software algorithms validated by the expert panel. At a minimum, these criteria were consistent with the appearance of statistically significant pattern deviations being present on the HVF printout, based upon age-matched normal controls. Therefore, if the range of normal in the expert analysis is inaccurate, the HVF normal values would similarly have to be considered as inaccurate. An explanation based upon truncation of “tails” in the distribution of normal values provided by the manufacturer would not explain the frequency with which HVF sensitivities were depressed in fellow eyes.

In clinical practice, mild defects that do not correspond with clinical impressions based upon other parameters (eg, disk appearance, media opacity, retinal findings) are common and often discounted. The design of the expert system utilized in the study did not include such additional information in the analysis. Molino and associates,\(^5\) in their validation of a knowledge-based expert system, emphasize the impact of such “hidden” information.

The possibility exists, however, that some of the mild and more severe field defects are real and, therefore, suggestive of a subclinical form of NAION. This impression is supported by the preservation of good visual acuity even in those few eyes with more advanced field defects, so media-related diffuse depression is less likely. Low-tension glaucoma is known to produce visual field defects similar to those found in NAION. However, the classification of the fellow eye without optic neuropathy suggests that none of these eyes had typical disk changes associated with any alternative disease process.

Since second episodes of NAION in the same eye are rare,\(^6\) one important test of subclinical disease would be a lower frequency of clinically apparent NAION in those fellow eyes having visual field defects than in those that do not. Such an assessment would require follow-up beyond the scope of the currently available data set. If validated in future studies, the presence of subclinical NAION might support a hypothesis that the crowding associated with a disk at risk is capable of producing minimal, clinically unapparent, acute or chronic axonal loss. Such subclinical infarction might, in turn, provide more space for remaining axons and associated vascular supply, thereby precluding profound infarction of the nerve head.

Changes in Visual Fields Between 6-Month Visit and Baseline Visit
Quantitative assessment of longitudinal changes in automated visual fields is complex. For example, Katz and colleagues\(^4\) compared the proportion of glaucoma visual fields identified as progressing using the methods of the Advanced Glaucoma Intervention Study, the CIGTS, and the Early Manifest Glaucoma Treatment study on 67 eyes of 56 patients followed with Humphrey 30-2 visual fields. Rate of progression was 11% for the Advanced Glaucoma Intervention Study, 22% for the CIGTS, and 23% for the Early Manifest Glaucoma Treatment study methods. Even for the comparable rates of progression of the CIGTS and Early Manifest Glaucoma Treatment study, the patients identified were the same only 50% of the time.

In the Optic Neuritis Treatment, 383 subjects underwent Humphrey 30-2 visual field testing. Sensitivities of each of 76 test locations at 6 months were compared with those at baseline, using the normal fellow eye sensitivity at each location as a control for learning effect.\(^6\) The percentage improvement was calculated for each test point. Fields were then divided into concentric rings with radii of 3, 9, 15, 21, and 27 degrees from fixation, and the average improvement was computed for each eccentricity.
There was no attempt to compare patterns or changes within patterns.

Little is published regarding the natural history of visual field changes in NAION. Arnold and Hepler performed acute and convalescent visual fields in 27 patients with NAION, using the Octopus perimeter program 32. Mean visual field performance worsened in 22.3% of visual fields and improved in 24% of visual fields. Of 21 “stable” patients, 31.6% showed late improvement in visual field. The IONDT previously reported no change in mean deviation of visual fields at 6 months between surgical and careful follow-up groups.

The pattern of visual loss does not change very much in individuals over time. One shift in distribution for randomized eyes seems to be the appearance of arcuate and altitudinal defects in areas that had no defect at baseline. This pattern shift did not alter the overall frequency of defects sufficient to reach statistical significance. The superior visual field of the nonrandomized study eyes showed a similar tendency for appearance of new arcuate and altitudinal defects. At the same time, of the 12 superior altitudinal defects, five changed to arcuate defects and one resolved by 6 months. Onset of new visual field defects may have been the result of progressive disease associated with increasing ischemia. Improvement in pattern of field may have reflected recovery of visual functions in areas of borderline ischemia or a training effect, such as that identified for the fellow eyes without optic neuropathy.

For visual fields of randomized and nonrandomized study eyes that did not change in pattern, there was a significant improvement in the severity of inferior altitudinal defects, as well as significant improvement in superior altitudinal defect for randomized patients only. One may speculate whether the eyes showing improvement of 3 lines or more in visual acuity, noted in 42.7% of patients with AION, also showed improvement in field severity; but testing this association was beyond the scope of the current study.

There was no constant relationship between global indices of HVF performance (mean deviation and CPSD) and the pattern or severity of visual field defects. Thus, such indices were not utilized in the longitudinal analysis. On the other hand, more severe and more diffuse visual field defects were associated with poor acuity, suggesting that acuity may be a reasonable surrogate for visual field defects due to NAION.

One surprising finding was that fellow eyes without known optic neuropathy frequently demonstrated mild, arcuate field defects. Although testing and other artificial causes must be excluded, subclinical NAION may exist in some fellow eyes. If so, longitudinal studies should demonstrate persistence of the defect and, perhaps, protection from future clinical involvement with NAION. If not, longitudinal studies will demonstrate that these defects disappear or become highly variable. Further studies utilizing the full 5-year dataset of the IONDT are planned.

The IONDT developed a rule-based expert system capable of consistently defining valid patterns and severity of visual field defects encountered in NAION, essential for the purposes of a multicenter prospective clinical trial. Expansion of this rule-based model to account for short-term fluctuations facilitated meaningful interpretation of change over time in individual patients.

Baseline visual fields obtained from the IONDT demonstrated comparability between eyes randomized to surgery or to careful follow-up. This result supported the validity of testing visual field outcomes between treatment groups in the longitudinal analysis. The visual fields in nonrandomized eyes with better than 20/64 visual acuity were less likely to demonstrate diffuse depression and central scotomas, compared with randomized eyes with worse acuity. Classic arcuate and altitudinal defects were also more common in these eyes. The severity and pattern of visual field defects did not vary in relationship to vasculopathic risk factors, but eyes of younger patients had milder defects than older patients.

REFERENCES

2. Ischemic Optic Nerve Decompression Trial Research Group. Optic nerve decompression surgery for nonarteritic anterior ischemic optic neuropathy (NAION) is not effective and may be harmful. *JAMA* 1995;273:625-632.


**APPENDIX**

**EXPERT SYSTEM SOFTWARE ALGORITHMS**

The ultimate defect is coded as a 0 (Mot present) or 1 (Present). Defect severity is not classified as Severe, Moderate or Mild in this program. Instead, a series of variables, each starting with “Pt” (e.g. *PtSup_Arc*, *PtInf_Arc*, and *PtSup_Alt*) are used to store the average dB loss over the relevant area for that defect. The thresholds associated with classifications of Severe, Moderate, and Mild are noted as comments.

First define several variables that are used later to classify the defects:

```plaintext
gen Quad1_Avg_loss = (pt1+pt2+pt5+pt6+pt7+pt11+pt12+pt13+pt14+pt19+pt20+pt21)/12

gen Quad2_Avg_loss = (pt3+pt4+pt8+pt9+pt10+pt15+pt16+pt17+pt18+pt22+pt23+pt24+pt25+pt26)/14

gen Quad3_Avg_loss = (pt27+pt28+pt29+pt35+pt36+pt37+pt38+pt43+pt44+pt45+pt49+pt50)/12

gen Quad4_Avg_loss = (pt30+pt31+pt32+pt33+pt39+pt40+pt41+pt42+pt46+pt47+pt48+pt51+pt52)/14


gen AvgParaCenLoss = (pt20+pt21+pt22+pt28+pt29)/6


gen AvgSupPeriphery = (Peripheral1 + Peripheral2)/2

gen AvgInfPeriphery = (Peripheral3 + Peripheral4)/2

gen Quad1_Diff = Peripheral1 - pt21

gen Quad2_Diff = Peripheral2 - pt22

gen Quad3_Diff = Peripheral3 - pt29

gen Quad4_Diff = Peripheral4 - pt30
```
gen str9 Quad1_Sup_Arc = "" 
gen str9 Quad2_Sup_Arc = "" 
gen str9 Quad3_Inf_Arc = "" 
gen str9 Quad4_Inf_Arc = "" 
replace Quad1_Sup_Arc = "Sup.Arc." if Quad1_Diff >= 5 
replace Quad2_Sup_Arc = "Sup.Arc." if Quad2_Diff >= 5 
replace Quad3_Inf_Arc = "Inf.Arc." if Quad3_Diff >= 5 
replace Quad4_Inf_Arc = "Inf.Arc." if Quad4_Diff >= 5 

gen str9 SupBiquadArc = "" 
gen str9 InfBiquadArc = "" 
replace SupBiquadArc = "SupBiquadArc" if (Quad1_Sup_Arc=="Sup.Arc." & Quad2_Sup_Arc=="Sup.Arc.") 
replace InfBiquadArc = "InfBiquadArc" if (Quad3_Inf_Arc=="Inf.Arc." & Quad4_Inf_Arc=="Inf.Arc.") 

gen str9 DepQuad1 = "" 
gen str9 DepQuad2 = "" 
gen str9 DepQuad4 = "" 
gen str9 DepQuad3 = "" 
replace DepQuad1 = "SupBiquadArc" if (Quad1=="Depressed" & Quad2_Sup_Arc=="Sup.Arc." & Quad1_Sup_Arc=="") 
replace DepQuad2 = "SupBiquadArc" if (Quad2=="Depressed" & Quad1_Sup_Arc=="Sup.Arc." & Quad2_Sup_Arc=="") 
replace DepQuad3 = "InfBiquadArc" if (Quad3=="Depressed" & Quad4_Inf_Arc=="Inf.Arc." & Quad3_Inf_Arc=="") 
replace DepQuad4 = "InfBiquadArc" if (Quad4=="Depressed" & Quad3_Inf_Arc=="Inf.Arc." & Quad4_Inf_Arc=="") 

gen Quad1_Per_Scot = (pt1+pt2+pt5+pt6+pt7+pt11+pt12+pt13+pt14+pt19)/10 
ngen Quad3_Per_Scot = (pt27+pt35+pt36+pt37+pt38+pt43+pt44+pt45+pt49+pt50)/10 

gen Quad1_Paracent = pt21 
gen Quad3_Paracent = pt29 

gen Quad1_Diff_Paracentral = Quad1_Per_Scot - Quad1_Paracent 
gen Quad3_Diff_Paracentral = Quad3_Per_Scot - Quad3_Paracent 

gen Sup_Avg_loss = (Quad1_Avg_loss + Quad2_Avg_loss)/2 
gen Inf_Avg_loss = (Quad3_Avg_loss + Quad4_Avg_loss)/2 
gen Sup_Quad_Diff = abs(Quad1_Avg_loss - Quad2_Avg_loss) 
gen Inf_Quad_Diff = abs(Quad3_Avg_loss - Quad4_Avg_loss) 

gen Sup_Inf_Diff = abs(Sup_Avg_loss - Inf_Avg_loss) 

gen Sup_Nasal_Pts = (pt18+pt25+pt26)/3 
gen Inf_Nasal_Pts = (pt33+pt34+pt42)/3 

gen SupNasQuad = (pt4+pt9+pt10+pt16+pt17+pt18+pt23+pt24+pt25+pt26)/10 
gen InfNasQuad = (pt52+pt47+pt48+pt40+pt41+pt42+pt31+pt32+pt33+pt34)/10 

gen SupNasVert = (pt3+pt8+pt15+pt22)/4 
gen InfNasVert = (pt51+pt46+pt39+pt30)/4 

gen SupNasDiff = SupNasQuad - SupNasVert 
gen InfNasDiff = InfNasQuad - InfNasVert
gen InfNasDefect = 0
gen SupNasDefect = 0

gen PtsBelowHorz = (pt28+pt29+pt30+pt31+pt32+pt33+pt34)/7
gen SupPtsRemainder = (pt12+pt13+pt14+pt15+pt16+pt17+pt18)/7
gen InfPtsRemainder = (pt36+pt37+pt38+pt39+pt40+pt41+pt42)/7

gen SupShiftDiff = PtsAboveHorz - SupPtsRemainder
ngen InfShiftDiff = PtsBelowHorz - InfPtsRemainder

gen AboveBelowDiff = abs(PtsAboveHorz - PtsBelowHorz)
gen Sup_ParaPt_Diff = abs(pt21-pt22)
gen Inf_ParaPt_Diff = abs(pt29-pt30)
gen TempParacenDiff = abs(pt29-pt21)
gen NasalParacenDiff = abs(pt30-pt22)

/*
Test for Central Scotoma and Altitudinal defects before finalizing definitions for disturbed points and Paracentral Scotoma

gen CentScot = 0
gen Fovea_Diff = (Foveal_Sens - Point_Sens)
replace CentScot = 1 if (Fovea_Diff< -5 | Foveal_Sens < 10)

Altitudinal Defects

gen Sup_Alt=0
ngen Inf_Alt=0
replace Sup_Alt = 1 if (Quad1=="Depressed" & Quad2=="Depressed" & Quad1_Diff<5 & Quad2_Diff<5 & Sup_Quad_Diff<=-11.4 & Sup_ParaPt_Diff<=13)
replace Inf_Alt = 1 if (Quad3=="Depressed" & Quad4=="Depressed" & Quad3_Diff<5 & Quad4_Diff<5 & Inf_Quad_Diff<=-11.4 & Inf_ParaPt_Diff<=13)

Paracentral Scotoma Elements

gen str9 Quad1_ParaScot = ""
gen str9 Quad2_ParaScot = ""
gen str9 Quad3_ParaScot = ""
gen str9 Quad4_ParaScot = ""
replace Quad1_ParaScot = "ParaScot" if (Quad1_Diff_Paracentral <=-5 & CentScot==0 & Sup_Alt==0)
replace Quad2_ParaScot = "ParaScot" if (Quad2_Diff<=-5 & CentScot==0 & Sup_Alt==0)
replace Quad3_ParaScot = "ParaScot" if (Quad3_Diff_Paracentral <=-5 & CentScot==0 & Inf_Alt==0)
replace Quad4_ParaScot = "ParaScot" if (Quad4_Diff<=-5 & CentScot==0 & Inf_Alt==0)

Disturbed Points Definitions
gen Quad1_Arcuate_DistPt = 0
gen Quad2_Arcuate_DistPt = 0
gen Quad3_Arcuate_DistPt = 0
gen Quad4_Arcuate_DistPt = 0
gen ParaCentral_DistPt = 0

Order of variables in file is as follows:

Number of points in each quadrant that are disturbed, excluding the central point. Point is considered disturbed if dB >= 4

for var pt1-pt20: replace Quad1_Arcuate_DistPt = Quad1_Arcuate_DistPt+1 if X >= 4
for var pt3-pt18 pt23-pt26: replace Quad2_Arcuate_DistPt = Quad2_Arcuate_DistPt+1 if X >= 4
for var pt27-pt28 pt35-pt50: replace Quad3_Arcuate_DistPt = Quad3_Arcuate_DistPt+1 if X >= 4
for var pt31-pt52: replace Quad4_Arcuate_DistPt = Quad4_Arcuate_DistPt+1 if X >= 4
for var pt20 pt21 pt22 pt25 pt29 pt30: replace ParaCentral_DistPt = ParaCentral_DistPt+1 if X >= 4

gen str9 Quad1_DistPt_SupArc = ""
gen str9 Quad2_DistPt_SupArc = ""
gen str9 Quad3_DistPt_InfArc = ""
gen str9 Quad4_DistPt_InfArc = ""
replace  Quad1_DistPt_SupArc = "Sup.Arc." if (Quad1_Arcuate_DistPt>=4 & Sup_Alt==0 & Quad1_Sup_Arc=="" & Quad2_Sup_Arc=="")
replace  Quad2_DistPt_SupArc = "Sup.Arc." if (Quad2_Arcuate_DistPt>=4 & Sup_Alt==0 & Quad1_Sup_Arc=="" & Quad2_Sup_Arc=="")
replace  Quad3_DistPt_InfArc = "Inf.Arc." if (Quad3_Arcuate_DistPt>=4 & Inf_Alt==0 & Quad3_Inf_Arc=="" & Quad4_Inf_Arc=="")
replace  Quad4_DistPt_InfArc = "Inf.Arc." if (Quad4_Arcuate_DistPt>=4 & Inf_Alt==0 & Quad3_Inf_Arc=="" & Quad4_Inf_Arc=="")
gen str9 DistPt_SupBiquadArc = ""
gen str9 DistPt_InfBiquadArc = ""
replace  DistPt_SupBiquadArc = "SupBiquadArc" if (Quad1_DistPt_SupArc=="Sup.Arc." & Quad2_DistPt_SupArc=="Sup.Arc.")
replace  DistPt_InfBiquadArc = "InfBiquadArc" if (Quad3_DistPt_InfArc=="Inf.Arc." & Quad4_DistPt_InfArc=="Inf.Arc.")
gen str9 DistPt_ParaScot = ""
replace  DistPt_ParaScot = "ParaScot" if (ParaCentral_DistPt>=3 & Quad1_ParaScot=="" & Quad2_ParaScot=="" & Quad3_ParaScot=="" & Quad4_ParaScot=="" & CentScot==0 & Inf_Alt==0 & Sup_Alt==0 & Quad1_Sup_Arc=="" & Quad2_Sup_Arc=="" & Quad3_Inf_Arc=="" & Quad4_Inf_Arc=="" & Quad1_DistPt_SupArc=="" & Quad2_DistPt_SupArc=="" & Quad3_DistPt_InfArc=="" & Quad4_DistPt_InfArc=="")

ArcAlt Defects, including defect based on disturbed points
The separate disturbed point ArcAlt variables are needed for severity calculations

gen arcalt = 0
gen arcalt = 0
gen DistPt_SupiorArcAlt = 0
gen DistPt_InferiorArcAlt = 0
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```
replace Sup_arcalt = 1 if (Quad1_Sup_Arc=="Sup.Arc." & Quad2_Sup_Arc=="Sup.Arc." & pt21>=4 & pt22>=4 & Sup_ParaPt_Diff<=5 & NasalParacenDiff>=5 & TempParacenDiff>=5 & Quad1_ParaScot=="" & Quad2_ParaScot=="" & Quad3_ParaScot=="" & Quad4_ParaScot=="" & CentScot==0)
replace Inf_arcalt = 1 if (Quad3_Inf_Arc=="Inf.Arc." & Quad4_Inf_Arc=="Inf.Arc." & pt29>=4 & pt30>=4 & Inf_ParaPt_Diff<=5 & NasalParacenDiff>=5 & TempParacenDiff>=5 & Quad1_ParaScot=="" & Quad2_ParaScot=="" & Quad3_ParaScot=="" & Quad4_ParaScot=="" & CentScot==0)
replace DistPt_SuperiorArcAlt = 1 if (Quad1_DistPt_SupArc=="Sup.Arc." & Quad2_DistPt_SupArc=="Sup.Arc." & pt21>=4 & pt22>=4 & Sup_ParaPt_Diff<=5 & NasalParacenDiff>=5 & TempParacenDiff>=5 & Quad1_ParaScot=="" & Quad2_ParaScot=="" & Quad3_ParaScot=="" & Quad4_ParaScot=="" & CentScot==0)
replace DistPt_InferiorArcAlt = 1 if (Quad3_DistPt_InfArc=="Inf.Arc." & Quad4_DistPt_InfArc=="Inf.Arc." & pt29>=4 & pt30>=4 & Inf_ParaPt_Diff<=5 & NasalParacenDiff>=5 & TempParacenDiff>=5 & Quad1_ParaScot=="" & Quad2_ParaScot=="" & Quad3_ParaScot=="" & Quad4_ParaScot=="" & CentScot==0)
replace Sup_arcalt = 1 if DistPt_SuperiorArcAlt == 1
replace Inf_arcalt = 1 if DistPt_InferiorArcAlt == 1

Arcuate Defects. Checks for BiQuad Arcs included in VF program are not included here since they are redundant after checking for individual arcs

gen Sup_Arc = 0
gen Inf_Arc = 0

replace Sup_Arc =1 if (Quad1_Sup_Arc=="Sup.Arc." | Quad2_Sup_Arc=="Sup.Arc." | Quad1_DistPt_SupArc=="Sup.Arc." | Quad2_DistPt_SupArc=="Sup.Arc." | DepQuad1=="SupBiquadArc" | DepQuad2=="SupBiquadArc")
replace Inf_Arc =1 if (Quad3_Inf_Arc=="Inf.Arc." | Quad4_Inf_Arc=="Inf.Arc." | Quad3_DistPt_InfArc=="Inf.Arc." | Quad4_DistPt_InfArc=="Inf.Arc." | DepQuad3=="InfBiquadArc" | DepQuad4=="InfBiquadArc")

Nasal Step defect, including defect based on disturbed points
The separate disturbed point nasal step variables are needed for severity calculations

gen Sup_Nasal_Step = 0
gen Inf_Nasal_Step = 0
gen DistPt_SupNasStep = 0
gen DistPt_InfNasStep = 0

replace Sup_Nasal_Step = 1 if (SupNasDefect==1 & Quad1_Sup_Arc=="" & Quad1_DistPt_SupArc=="")
replace Inf_Nasal_Step = 1 if (InfNasDefect==1 & Quad3_Inf_Arc=="" & Quad3_DistPt_InfArc=="")
replace DistPt_SupNasStep = 1 if (SupNasDiff >=14.5 & pt18 >=4 & pt25 >=4 & pt26>=4 & Quad1_Sup_Arc=="" & Quad1_DistPt_SupArc=="")
replace DistPt_InfNasStep = 1 if (InfNasDiff >=14.5 & pt33 >=4 & pt34 >=4 & pt42>=4 & Quad3_Inf_Arc=="" & Quad3_DistPt_InfArc=="")
replace Sup_Nasal_Step = 1  if DistPt_SupNasStep ==1
replace Inf_Nasal_Step = 1  if DistPt_InfNasStep ==1

Summary Paracentral Scotoma /

gen ParaScot    = 0
for var  Quad1_ParaScot Quad2_ParaScot Quad3_ParaScot Quad4_ParaScot  DistPt_ParaScot: replace ParaScot = 1 if X=="ParaScot"

Diffuse Depression

gen DiffuseDepression= 0
gen deprflag = 0
```
replace deprflag = 1 if (Sup_Alt == 1 & Inf_Alt == 1 & CentScot == 1 & Sup_Inf_Diff <= 10)
replace DiffuseDepression = 1 if deprflag==1
replace Sup_Alt = 0 if deprflag==1
replace Inf_Alt = 0 if deprflag==1
replace CentScot = 0 if deprflag==1
drop deprflag

gen Absolute_defect = 0
gen Check_Absolute = 0
replace Check_Absolute = 1 if (DiffuseDepression==1 & Total_Avg_Quad_loss >= 21.5 & Foveal_Sens==0)

Cannot do check for Absolute without dB data from sensitivity plot, pt_1 thru pt_52
At present the sensitivity plot data is not included in the Stata file response = pt > 0
Count how many points have any response (i.e pts >0)
If no responses code as absolute
If only one response and no points are >9 code as absolute
If only one response but there is one or more point >9 keep as Diff Depression
If more than one response keep as diff Depression

gen response = 0
gen pts_above_9 = 0
replace response = . if Check_Absolute == 0
replace pts_above_9 = . if Check_Absolute == 0
for var pt_1-pt_52: replace response = response + 1 if X > 0 & Check_Absolute == 1
for var pt_1-pt_52: replace pts_above_9 = pts_above_9 + 1 if X > 9 & Check_Absolute == 1
replace Absolute_defect = 1 if Check_Absolute == 1 & response == 0
replace Absolute_defect = 1 if Check_Absolute == 1 & response == 1 & pts_above_9 <= 0
replace DiffuseDepression = 0 if Absolute_defect == 1

Normal Field

gen Normal = 1
for var Sup_Nasal_Step  Inf_Nasal_Step:  replace Normal=0 if X==1
for var DiffuseDepression  Sup_Alt Inf_Alt Sup_Arc Inf_Arc Inf_arcalt Sup_arcalt ParaScot CentScot : replace Normal=0 if X==1
replace Normal = 0 if Total_Avg_Quad_loss >1.55

gen Oth_Sup = 0
gen Oth_Inf = 0
gen Oth_Whole = 0
replace Oth_Sup =1 if (Sup_Alt== 1 & AboveBelowDiff<=4 & InfShiftDiff>=10)
replace Oth_Inf =1 if (Inf_Alt== 1 & AboveBelowDiff<=4 & SupShiftDiff>=10)
replace Oth_Whole =1 if (Total_Avg_Quad_loss>1.55 & ParaScot==0 & CentScot==0 & Sup_Alt==0 & Inf_Alt==0 & Sup_Arc==0 & Inf_Arc==0 & Sup_Nasal_Step==0 & Inf_Nasal_Step==0 & Sup_arcalt==0 & Inf_arcalt==0 & Absolute_defect==0 & DiffuseDepression==0 & Normal==0)

Code for missing foveal sensitivity

for var Absolute_defect DiffuseDepression Sup_Alt Inf_Alt Sup_Arc Inf_Arc Inf_arcalt Sup_arcalt ParaScot CentScot Sup_Nasal_Step  Inf_Nasal_Step Oth_Sup Oth_Inf Oth_Whole : replace X=9 if Fov_sens_string == “Missing”

Severity measures  Store average loss over appropriate area for paired t-test
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Do not code into Severe, Moderate and Mild at this time

/*
Arcs
Thresholds were: Severe >=22.5; Moderate <22.5 & >10 ; Mild <10

gen PtSup_Arc = .
replace PtSup_Arc = AvgSupPeriphery if DepQuad1=="SupBiquadArc" | DepQuad2=="SupBiquadArc" | SupBiquadArc=="SupBiquadArc" | DistPt_SupBiquadArc=="SupBiquadArc"
replace PtSup_Arc = Peripheral1 if PtSup_Arc == . & Quad1_Sup_Arc=="Sup.Arc."
replace PtSup_Arc = Peripheral2 if PtSup_Arc == . & Quad2_Sup_Arc=="Sup.Arc."
replace PtSup_Arc = Peripheral1 if PtSup_Arc == . & Quad1_DistPt_SupArc=="Sup.Arc."
replace PtSup_Arc = Peripheral2 if PtSup_Arc == . & Quad2_DistPt_SupArc=="Sup.Arc."

gen PtInf_Arc = .
replace PtInf_Arc = AvgInfPeriphery if DepQuad3=="InfBiquadArc" | DepQuad4=="InfBiquadArc" | InfBiquadArc=="InfBiquadArc" | DistPt_InfBiquadArc=="InfBiquadArc"
replace PtInf_Arc = Peripheral3 if PtInf_Arc == . & Quad3_Inf_Arc=="Inf.Arc."
replace PtInf_Arc = Peripheral4 if PtInf_Arc == . & Quad4_Inf_Arc=="Inf.Arc."
replace PtInf_Arc = Peripheral3 if PtInf_Arc == . & Quad3_DistPt_InfArc=="Inf.Arc."
replace PtInf_Arc = Peripheral4 if PtInf_Arc == . & Quad4_DistPt_InfArc=="Inf.Arc."

Severity measures for nasal steps
Thresholds were: Severe >=20; Mod < 20 & >10; Mild <=10

gen Pt_Sup_NasStep = .
gen Pt_Inf_NasStep = .
replace Pt_Sup_NasStep = SupNasQuad     if Sup_Nasal_Step == 1 & DistPt_SupNasStep == 0
replace Pt_Sup_NasStep = Sup_Nasal_Pts  if Pt_Sup_NasStep == . & DistPt_SupNasStep == 1
replace Pt_Inf_NasStep = InfNasQuad     if Inf_Nasal_Step == 1 & DistPt_InfNasStep == 0
replace Pt_Inf_NasStep = Inf_Nasal_Pts  if Pt_Inf_NasStep == . & DistPt_InfNasStep == 1

Severity for paracentral scotomas were previously by quadrant. (Columns CD,CE,CF,CG,CH)
Thresholds were: Severe >=20; Mod < 20 & >10; Mild <=10

For paired t-test between 2 time points use avg ParaCenloss, since the quadrant involved could be different each time

gen Pt_ParaScot = AvgParaCenLoss

Central scotoma
Thresholds were: Severe Foveal sensitivity <=10; Mod Foveal sensitivity >10 & <=25 ; Mild Foveal sensitivity >25

gen Pt_CentralScot = Foveal_Sens

Severity for Diffuse depression (column K)
Thresholds are >=21.5 ; < 21.5 & >15; <=15

gen PtDiffuseDepr = Total_Avg_Quad_loss
SupAlt and InfAlt
Thresholds are $\geq 24.5$ ; $< 24.5 \& > 15$ ; $\leq 15$

gen PtSup_Alth = Sup_Avg_loss
ngen PtInf_Alth = Inf_Avg_loss

SupArcAlt and InfArcAlt thresholds are $\geq 24.5$ ; $< 24.5 \& > 15$ ; $\leq 15$

gen PtSup_arcaAlt = Sup_Avg_loss
ngen PrInf_arcaAlt = Inf_Avg_loss

drop Quad1_Avg_loss - NasalParacenDiff Quad1_ParaScot DistPt_ParaScot

drop DistPt_SuperiorArcAlt DistPt_InferiorArcAlt DistPt_SupNasStep DistPt_InfNasStep
INTRAVITREAL HUMAN IMMUNE GLOBULIN IN A RABBIT MODEL OF STAPHYLOCOCCUS AUREUS TOXIN-MEDIATED ENDOPHTHALMITIS: A POTENTIAL ADJUNCT IN THE TREATMENT OF ENDOPHTHALMITIS

BY Dennis P. Han MD

ABSTRACT

Objectives: To test the feasibility of human immune globulin (IG, Gamimune N, 10%) as a new treatment for endophthalmitis, the ocular tolerance, distribution, and ability of intravitreal IG to attenuate the toxic effects of *Staphylococcus aureus* culture supernatant were evaluated in a rabbit model.

Methods: Effects of intravitreally injected IG were assessed histologically and with Western blot analysis performed 1 to 5 days after injection. IG reactivity to products of *S aureus* strain RN4220 was tested by Western blotting, using known toxins (beta hemolysin and toxic shock syndrome toxin-1) and a concentrated culture supernatant containing *S aureus* exotoxins (pooled toxin, PT). Endophthalmitis was induced by intravitreal PT injection. For treatment, IG and PT were mixed and injected simultaneously, or IG was injected immediately after, or 6 hours after, PT injection. PT toxicity was graded clinically and histologically over 9 days.

Results: IG persisted intravitreally at least 5 days, inducing no clinical inflammation and minimal mononuclear cell infiltration. In the endophthalmitis model, toxicity from PT was significantly reduced when IG was mixed with PT and injected simultaneously, or when IG was delivered immediately after PT. Only minimal clinically detectable reductions were observed when IG delivery was delayed 6 hours.

Conclusions: Intravitreal IG is well tolerated in the rabbit eye and attenuates the toxicity of culture supernatant containing *S aureus* exotoxins. Because toxin elaboration likely occurs gradually in true infection, reduced effects observed with delayed treatment in this toxin-injected model do not preclude clinical application. IG may represent a novel adjunct in endophthalmitis treatment.


INTRODUCTION

Infectious endophthalmitis can be a devastating complication of ocular surgery or trauma. Despite treatment parameters established by the Endophthalmitis Vitrectomy Study (EVS), moderate to severe visual loss remains a common outcome. Infections caused by *Staphylococcus aureus*, streptococcal species, and gram-negative organisms were associated with poor visual outcomes. *S aureus* constituted 10% of isolates obtained in cases of post–cataract extraction endophthalmitis. It also represents an infrequent but important cause of bleb-related and post-traumatic endophthalmitis.

Poor visual outcomes and organism virulence appear to be strongly associated. For some bacteria, exotoxins are a critical component of virulence because they enhance bacterial propagation through host tissue. Tissue destruction in *S aureus* endophthalmitis results in part from the combined effects of several exotoxins.

Attempts to mitigate inflammatory tissue destruction with steroids have been unsuccessful in treating experimental *S aureus* endophthalmitis. Improvements in the treatment of *S aureus* endophthalmitis may be achieved by targeting secreted toxins. Such an approach has been suggested for other bacteria. Experimentally, staphylococcal toxins can be neutralized with monoclonal and polyclonal antibodies. Clinically, intravenous immunoglobulins containing antibodies capable of neutralizing toxins have been used to help manage both staphylococcal and streptococcal toxin-mediated illnesses.

The intravitreal use of toxin-specific antibodies repre-
resents a novel approach to the management of endophthalmitis. Data is presented herein demonstrating that pooled human immune globulin (IG) injected into the vitreous penetrates the retina and persists in the vitreous and retina for up to 5 days without producing clinically detectable inflammation. A “proof of principle” investigation is presented determining whether pooled human IG binds proteins in \textit{S. aureus} culture supernatant and two purified \textit{S. aureus} exotoxins, and whether IG delivered into the vitreous reduces the tissue destruction and inflammatory effects produced by an intravitreal injection of \textit{S. aureus} culture supernatant. The therapeutic implications of this approach to endophthalmitis and its potential limitations are discussed.

**Background and Rationale**

**Historical Aspects**

Over the past 30 years, management of infectious endophthalmitis has changed dramatically. Previously, dismal outcomes were generally associated with this condition. However, with the introduction of intravitreal antibiotic therapy, there was a marked improvement in visual prognosis. Soon after the advent of posterior vitrectomy in the early 1980s, clinical experience with this modality suggested benefit in severe cases of endophthalmitis. However, its exact role in endophthalmitis management remained controversial until 1995, when the EVS demonstrated improved outcome from immediate vitrectomy in acute endophthalmitis after cataract surgery or secondary intraocular lens implantation in patients with presenting visual acuity of only light perception. Nevertheless, despite undergoing vitrectomy and intravitreal antibiotic injection, 20% of such patients continued to experience permanent, severe visual acuity loss to 5/200 or worse.1

**Microbiological Factors and Role of Bacterial Toxins**

It has long been recognized that certain species of microorganisms are associated with poorer prognoses, with gram-negative organisms, streptococci, enterococci, and bacillus species being the most pathogenic. Protocol measurement of outcome in the EVS allowed a rigorous comparison of relative pathogenicity among bacterial species, identifying particularly virulent groups. These organisms, primarily gram-positive organisms other than \textit{Staphylococcus epidermidis (S. aureus}, streptococci, enterococci, \textit{Bacillus cereus}), but also gram-negatives, were associated with a statistically significant worsened clinical presentation and prognosis. The poorer outcome was observed even after management with aggressive therapy for infectious endophthalmitis, underscoring the continued importance of virulence factors. The role of such factors in mediating retinal tissue destruction and loss of function has been clearly demonstrated in studies of isogenic toxin-producing and toxin-nonproducing \textit{Enterococcus faecalis}, and of other known toxin-producing organisms associated with poor prognoses, including \textit{B. cereus}, \textit{S. aureus}, and \textit{Streptococcus pneumoniae}.

**Clinical Observations**

In the EVS, visual acuity at presentation was the most potent independent risk factor for permanent visual loss, suggesting that intervention prior to severe visual loss was of utmost importance. A strong association between known toxin-producing species and poor presenting acuity was also observed, consistent with the role of toxins as a determinant in outcome. The time interval during which their imputed toxic effects might be reversed, retarded, or stabilized has not been determined. Clinically, during the first 24 to 48 hours after administration of intravitreal antibiotic therapy, visual acuity may worsen and inflammation may persist, even in cases successfully sterilized. The EVS protocol, arguably a proxy for leading authorities in the field, allowed for a substantial reduction in visual acuity after initial intravitreal antibiotic therapy prior to instituting additional measures. In some cases, visual acuity was allowed to drop to less than 5/200, in cases not already below this level, over a 36- to 60-hour interval. Thus, it can be speculated that ongoing retinal damage even after initial antibiotic therapy, from delayed bacterial killing or continued activity of toxins, might be of clinical significance. In a rabbit model of \textit{E. faecalis} endophthalmitis, toxins produced retinal damage equivalent in severity to untreated infection, even in the presence of intravitreal dexamethasone therapy and antibiotic sterilization of the vitreous cavity.

**Corticosteroid Therapy in Endophthalmitis**

The use of corticosteroids is both controversial and relevant, particularly as applied to endophthalmitis caused by toxin-producing organisms. Failure of intravitreal corticosteroids to moderate the tissue effects of toxins has been shown in rabbit models of endophthalmitis employing \textit{E. faecalis}, \textit{S. aureus}, and pseudomonas. However, a potential benefit has been observed in other studies. In an illuminating study using isogenic toxin-producing and toxin-nonproducing strains of \textit{E. faecalis}, Jett and coworkers showed that effects of intravitreal dexamethasone differed, depending on the degree to which toxins were produced. Moderation of inflammatory response and ERG b-wave amplitude loss was observed only in eyes infected with the toxin-nonproducing strain. In the human clinical studies, there is conflicting evidence on the benefit of intravitreal corticosteroids. No study has definitively shown an improved visual outcome when this treatment was applied to a large, possibly heterogeneous,
group of endophthalmitis cases.\textsuperscript{41,42} Thus, of the microbiological factors that exist, toxin-mediated tissue destruction remains a relatively unmanaged factor in endophthalmitis.

**Staphylococcus aureus endophthalmitis and toxins**

The large number of pathogenic toxins elaborated by *S. aureus* fall into at least two categories—hemolysins/leukocidins and pyrogenic toxin superantigens (PTSAgs). In addition, various lipases, nucleases, and proteases are produced. The hemolysins consist of four classes: alpha, beta, gamma, and delta hemolysins. Hemolysins and leukocidins are characterized by their ability to disrupt cell membranes, and most show powerful lethality when administered systemically in animal models. PTSAgs include toxic shock syndrome toxins and the family of staphylococcal enterotoxins, and are characterized by their pyrogenicity, enhancement of lethal endotoxin shock, and ability to stimulate T-lymphocyte proliferation (independent of the antigen specificity of these cells).\textsuperscript{15}

The known pathogenic mechanisms and systemic manifestations have been reviewed extensively.\textsuperscript{43-47}

Although the individual contributions of the various *S. aureus* toxins to clinical endophthalmitis are unknown, their role as a group in mediating virulence is undisputed.\textsuperscript{7} Studies of experimental endophthalmitis infected with isogenic mutant strains deficient in the accessory gene regulator (agr) and staphylococcal accessory regulator (sar), which control expression of the PTSAgs and some hemolysins, demonstrate a clear attenuation of virulence compared to the wild strain.\textsuperscript{11,12,14} A study of a gamma-toxin-deficient strain suggested that gamma toxin has proinflammatory effects in the rabbit eye, but must be accompanied by unidentified proinflammatory molecules other than alpha-toxin, beta-toxin, or leukocidin of Panton-Valentine, since the proinflammatory strain evaluated was also deficient in these toxins.\textsuperscript{13} Gilmore and coworkers found that the alpha, beta, and gamma toxins make slight contributions to the pathogenesis of experimental *S. aureus* endophthalmitis, but that strains with knockouts in any single gene (or even double and triple knockouts of more than a single gene) fail to reduce virulence to the level of agr/sar deficient strains (M.S. Gilmore, PhD, written communication, March 28, 2000).

Therefore, the pathogenicity of *S. aureus* toxins in endophthalmitis appears to be multifactorial.

The multifactorial character of *S. aureus* endophthalmitis is probably shared by other infectious endophthalmitides. For example, the streptococci and gram-negative organisms, capable of causing fulminant endophthalmitis, produce numerous toxins of great systemic pathogenicity, for which ocular toxicity remains largely unexplored.\textsuperscript{44,46} *Bacillus cereus* produces a variety of toxins potentially damaging to the eye, including hemolysin BL, a tripartite enterotoxin with known ocular toxicity.\textsuperscript{34,48} However, no single toxin can explain its virulence,\textsuperscript{49} which appears to be multifactorial and related to both toxin production\textsuperscript{49} and organism motility.\textsuperscript{49} In contrast, knockout of only a single toxin, cytolysin, dramatically attenuates virulence in *E. faecalis* experimental endophthalmitis.\textsuperscript{9,20}

Because of the large microbiologic spectrum of virulent organisms encountered in clinical endophthalmitis\textsuperscript{1} and the multiplicity of toxins elaborated by various species, empiric therapy for toxin-mediated virulence would require simultaneous neutralization of many toxins of various bacterial origins. Because *S. aureus* endophthalmitis occurs with relatively high frequency, and because its multifactorial pathogenicity is shared by other microbial forms of endophthalmitis, a model of *S. aureus* toxin-mediated endophthalmitis appears to be a reasonable prototype for study.

**Immunoglobulin Therapy for Toxin Neutralization**

Previous Studies. Immunoglobulins have potential for moderating the damaging effects of bacterial toxins. Inhibition of toxic effects by toxin-specific antibodies has been demonstrated by in vitro models evaluating cytotoxicity\textsuperscript{30,51} and in animal models evaluating systemic toxicity and cardiovascular collapse.\textsuperscript{21,50} Similarly, vaccine toxoids known to stimulate antibody responses against bacterial exotoxins have a protective effect against toxic shock syndromes in experimental rabbit models.\textsuperscript{52,53} The author is aware of only a single study evaluating antitoxin antibodies in endophthalmitis. In a rabbit model of *B. cereus* toxin-induced endophthalmitis, Beecher and coworkers\textsuperscript{34} observed a tissue protective effect of antiserum against the L\textsubscript{2} component of hemolysin BL injected intravitreally.

**Clinical Experience.** Until recently, most human clinical applications of immunoglobulins in infection have consisted of intravenous administration directed at either prophylaxis or treatment of established infections using polyspecific immunoglobulin G pooled from multiple donors.\textsuperscript{54} The putative mechanism of action is immunoglobulin binding of the infectious agent specifically, facilitating opsonization, phagocytosis, lysis, and stimulation of polymorphonuclear cell function, including agglutination, chemotaxis, and oxidative metabolism.\textsuperscript{54}

Clinical applications in which immunoglobulin preparations are administered specifically to neutralize microbial toxins are relatively new, representing a paradigm shift in immunoglobulin therapy for infection management. In this case, the presumed mechanism is immunoglobulin binding of toxins and not the infecting agent. Applications involve treatment of specific manifestations of toxin release (eg, toxic shock syndromes) with polyspecific intravenous IgG in conjunction with antibiotic therapy. Intravenous immunoglobulin G (IVIG) administration is
associated with reduced morbidity and mortality from streptococcal toxic shock syndrome,33,35 septic shock,36,37 and, on a more anecdotal basis, staphylococcal toxin-mediated illness.24 Thus, the concept that antibodies from polyspecific, pooled immunoglobulin can be used to neutralize the toxic effects of presumably numerous bacterial toxins acting concurrently in vivo appears clinically validated. Such an approach to endophthalmitis, which may be similarly multifactorial, appears rational.

**Intravenous Immunoglobulin G**. Preparations of IVIG are commercially available and have been extensively characterized.38-61 One preparation available for intravenous use in the United States is Gamimmune N, 10%, which has been approved by the Food and Drug Administration for replacement therapy of IgG in immune-deficiency syndromes and for the treatment of idiopathic thrombocytopenic purpura. It is isolated from pooled human plasma, is preservative-free, and consists of a 10% solution of IgG in water. It is greater than 99% IgG, 95% of which is monomeric. Anticomplement activity is removed by reduction and alkylation of disulfide bridges. Concentrated IVIG preparations are pooled from 10,000 to 15,000 donors and contain a vast assortment of idiotypes. It is estimated that one gram of IVIG contains approximately $4 \times 10^{18}$ molecules, capable of recognizing more than 10 million antigenic determinants.54 Nearly all commercial forms of IVIG have been demonstrated to have high titers of antibodies capable of neutralizing activity against PTSAgs and hemolysins (P.M. Schlievert, PhD, written communication, April 10, 2000).

**IVIG Intraocular Distribution.** The blood-retinal barrier has been held responsible for poor penetration of most drugs and high-molecular-weight substances into the posterior segment of the eye, either from the blood or from the pericellular tissues. The blood-retinal barrier exists at the level of the retinal pigment epithelium and the retinal capillary endothelial cells. However, recent reports have suggested that high-molecular-weight compounds such as dextrans (150 kd) and IgG (approximately 150 kd) can diffuse across the sclera into both the choroid and the retina.61-64 In the reverse direction, molecules as large as 38.9 kd have been shown to diffuse across the internal limiting membrane of the retina.64 However, studies on the distribution and fate of intravitreally placed IgG molecules are scarce.65 Because intraocular penetration of intravenously administered immunoglobulins is likely to be quite limited based on the above considerations, intravitreal administration of IG would likely be necessary to attenuate toxin-mediated damage in endophthalmitis.

**Present Study**

**Hypothesis**

The hypothesis of this study is that intravitreally injected human IG attenuates the inflammatory and tissue destructive effects of virulence factors elaborated by *S aureus*. The hypothesis is tested in an experimental rabbit model of noninfectious *S aureus* toxin-mediated endophthalmitis. A requisite to the testing of this hypothesis is a demonstration that intravitreally injected human IG is of itself relatively noninflammatory and that it persists in the eye of sufficient duration to have a therapeutic effect.

**Aims**

The aims of the study are twofold: (1) to assess the intraocular distribution, persistence, and histologic effects of intravitreally injected human IG, and (2) to establish proof of principle for a neutralizing effect on the clinical and histologic manifestations of toxin-induced endophthalmitis by polyspecific, pooled human IG. The evaluations that follow are described in two parts, I and II.

**1. INTRAVITREAL INJECTION OF HUMAN IMMUNE GLOBULIN: INTRAOCULAR DISTRIBUTION AND RETINAL RESPONSE BY IMMUNOHISTOCHEMICAL EVALUATION IN THE RABBIT EYE**

**General Approach**

In this study, characterization of the ocular response to intravitreally injected IG in the normal rabbit eye was performed by histologic evaluation. Immunohistochemistry was used to identify the intraocular distribution and persistence of IG. These evaluations were performed at intervals up to 5 days after injection, a time period during which visual outcome in acute endophthalmitis in humans is strongly determined.66 Protein expression by Müller cells was used as an indicator of a pathologic retinal state. Under physiological conditions, Müller cells in the human and rabbit retina contain intermediate filaments such as vimentin but not glial fibrillary acidic protein (GFAP), whereas in pathologic conditions such as retinal injury and retinal degeneration, they have been shown to contain both GFAP and vimentin.67,68 The expression of vimentin and GFAP by Müller cells was used to assess the response of nonneural retinal cells to intravitreal IG injection. Western blotting was used to demonstrate whether intact IgG molecules persisted within the vitreous.

**Methods**

**Intraocular Injections and Tissue Processing**

Institutional Animal Care and Use Committee approval from the Medical College of Wisconsin was obtained prior to initiating all animal experiments. New Zealand White rabbits, 2 to 3 kg, were housed and handled in accordance with the ARVO Statement for the Use of Animals in
Ophthalmic and Visual Research. Prior to intravitreal injection, animals were anesthetized with intramuscular ketamine (20 mg/kg) and xylazine (1 mg/kg), and topical proparacaine hydrochloride. The entire procedure was performed with full aseptic precautions and under adequate visualization. Pupils were dilated with phenylephrine 2.5% and tropicamide 1% eye drops.

A total of 24 rabbit eyes received intravitreal injections of varied doses (0.5 mg, 2.5 mg, 10 mg, 20 mg, 30 mg in volumes of 100 µL to 300 µL, depending on dose) of commercially available IG (Gamimmune N, 10%, Bayer Corporation, Elkhart, Indiana) or with diluent (balanced salt solution, BSS; Alcon, Fort Worth, Texas). The eyes were harvested on postinjection days (PIDs) 1, 2, 3, and 5. Of these, two eyes each were harvested on days 1, 2, and 3 in the 10-mg and 20-mg groups. The remaining 12 eyes were harvested on day 5, with two eyes each in the five dosage groups and two eyes with BSS injection. Prior to each intravitreal injection, 100 to 300 µL of aqueous humor was aspirated from the anterior chamber to reduce the risk of elevated intraocular pressure. Additionally, two normal rabbit eyes (no injections) were included in this study as negative controls. For all animals, only one eye was used. No intravitreal hemorrhage was observed with indirect ophthalmoscopy following injection. Animals were euthanized with intracardiac injections of pentobarbital (25 mg/kg) at the above stated intervals. Immediately after enucleation, 0.5 to 1 mL of vitreous fluid was aspirated from nine eyes with IG injection and seven control eyes and stored at –20°C. Immediately following enucleation, the eyes were fixed in 10% formalin.

Histopathology and Immunohistochemistry
Emulsinated globes were processed and the central papillary optic disk portion of the globe was embedded in paraffin. Five-micron paraffin sections were mounted on poly-l-lysine coated slides. Sections were stained with hematoxylin and eosin for light microscopy. All eyes were evaluated for histologic changes including inflammatory cell response.

After deparaffinization and hydration, endogenous peroxidase activity was blocked by a 5-minute incubation in 3% hydrogen peroxide (Sigma Chemical, St Louis, Missouri). The sections were then incubated with a serum-free protein blocking solution (DAKO, Carpinteria, California). Biotinylated rabbit antihuman IgG, monoclonal mouse antiall fibrillar acidic protein, and monoclonal mouse antivimentin (all from DAKO) were used as the primary antibodies. For the monoclonal antibodies, biotinylated antimouse IgG (Vector Laboratories, Burlingame, California) was used as the secondary antibody. The immunolabeled sites were visualized using an avidin-biotin-peroxidase kit (Vector Laboratories) with diaminobenzidine tablets (Sigma) used at the development stage. All sections were allowed to develop for 10 minutes, then immediately washed and counterstained with Mayer’s hematoxylin (Sigma). Controls included normal rabbit eyes, rabbit eyes injected with diluent only (BSS), and human tonsil tissue (as positive control). Negative controls included omission of the primary antibody.

Western Blot of Vitreous
Vitreous was removed by aspiration immediately after enucleation, collected in Eppendorf tubes, and frozen at –20°C until analysis. Vitreous samples were mixed with an equal volume of 2X Laemmli’s SDS electrophoresis buffer,19 boiled, and loaded on gels in triplicate, using three different volumes, followed by electrophoresis in 10% SDS-polyacrylamide gels (Biorad Criterion precast gel). IG samples were electrophoresed in parallel as a positive control. Western blots were prepared by standard methods and probed with a 1:50,000 dilution of peroxidase-conjugated antihuman IgG antibody (Jackson ImmunoResearch Laboratories, Inc, West Grove, Pennsylvania). Bands were visualized with the Enhanced Chemiluminescence (EML) detection system (Amersham, Piscataway, New Jersey).

Results
Histopathology
Ten of the 24 eyes showed minimal mononuclear cell infiltration. These cells were predominantly located in the prepapillary (Figure 1) or preretinal area and were first noted on the second day. This cellular response was independent of the IG dose. None of the eyes showed intraretinal inflammatory cells. One eye showed red blood cells in the vitreous.

Immunohistochemistry and Western Blot
Intense IG labeling was localized to the vitreous in all eyes and was seen at all time points studied (days 1, 2, 3, and 5), irrespective of the amount of IG injected. There was no appreciable subjective decrease in intensity of staining from day 1 to day 5. In the anterior uvea, the posterior layer of the iris epithelium and the inner layer of the ciliary epithelium (Figure 2) showed intense staining. On day 1, IG was distributed throughout the retina extending from the internal limiting membrane to the outer border of the photoreceptor layer. Staining was more intense in the inner nuclear layer (both diffuse extracellular and intracellular) (Figure 3) and in the outer extent of the outer segments layer. In some sections, the intracellular labeling in the inner nuclear layer showed a dendritic pattern; however, it did not show a distinct transretinal vertical configuration, characteristic of the distribution of...
the Müller cell processes. The staining pattern in the retina persisted into day 5. The retinal pigment epithelium (RPE) also showed positive staining for IG, but no staining could be detected in the sub-RPE space or in the choroid (Figure 4). The RPE staining was noted at all time points and was independent of the dosage used. Intense staining was also noted in the axonal bundles of the optic nerve head around the major vascular channels (Figure 5).

Vimentin immunolabeling was observed in the retina as radially oriented processes extending from the internal limiting membrane to the outer limiting membrane, representing Müller cell morphology. This staining was seen in both IG-injected, control (BSS), and normal eyes. However, the Müller cells did not express GFAP in all the eyes studied (including control eyes). GFAP staining was noted only in the optic nerve head and in the innermost retina adjacent to the optic nerve head. The immunostaining results were confirmed by Western blotting of the vitreous, which showed full-length IgG in the vitreous throughout the 5-day time course of the study (not shown).

**Discussion**

Systemically administered IG penetrates poorly into the retina in patients with an intact blood-retinal barrier. Under physiological conditions, the blood-retinal barrier includes the retinal vascular endothelium, the retinal pigment epithelium, and the tight junctions at the base of the inner layer of the ciliary epithelium. Like the blood-brain barrier, these effectively block the penetration of plasma proteins, including immunoglobulins, from the blood into the retina. Raising the serum IG concentration has not been associated with significant increase in cerebrospinal fluid concentration and is therefore unlikely to work for intraocular application. Direct intravitreal injection of IG would circumvent the blood-retinal barrier, but there are no data on the fate of IG molecules in the vitreous. The results of this study provide new information on the fate of IG molecules following intravitreal injection, including host inflammatory response, and on the response of Müller cells to the injection.

In this study the distribution of intravitreally injected IG was identified by immunohistochemical localization of IG molecules in the rabbit eye at various intervals after injection. The results indicate that IG rapidly diffuses throughout the neural retina by day 1 and can be detected for the duration of the study (5 days). The IG molecules are distributed diffusely through the extracellular space and are also internalized by cell types in the inner nuclear layer, RPE cells, and the inner layer of the ciliary epithelium. The 150-kd IgG molecules are able to cross the intact internal limiting membrane, which therefore does not constitute an efficient barrier to the IgG molecules injected into the vitreous. This is consistent with previously published data on diffusion of IgG molecules across the internal limiting membrane in the peripapillary area.

Although the immunohistochemical technique used in this study detects an IgG epitope (and not the whole molecule), it is reasonable to conclude that IgG molecules have not undergone any cleavage over the time period studied. Western blot analysis of the vitreous at PID 5 shows intact IgG molecules. Similarly, the intraretinal IgG is also likely to be intact, given that pharmacokinetic studies of IG preparations following intravenous administration in healthy subjects show a half-life of 14 to 24 days. Of note is that the in vivo half-life of Gamimmune N (used in this study) as reported by the manufacturer exceeds or equals the 3-week half-life reported for IgG in the literature. It is also known that when the blood-retinal barrier is circumvented, IgG molecules placed in the periocular space do diffuse across the sclera into both the choroid and the retina in significant amounts and into the vitreous and aqueous in negligible amounts. The reasons for the rapid and significant intra-retinal diffusion noted in this study are twofold: the IG molecules were placed directly in the vitreous, and relatively large molar quantities of IG were injected.

The host response following intravitreal IG injection was also studied by evaluating inflammatory cell infiltration and expression of GFAP by Müller cells. Müller cells in rabbits and humans express vimentin constitutively, but they express GFAP only in response to retinal injury or degeneration. GFAP was therefore used as a marker to assess the response of the nonneural retinal elements to intravitreal injection of IG. Only 10 of the 24 eyes showed minimal mononuclear cell infiltration in the prepapillary vitreous, and none in the retina itself. Also, even at PID 5, and with up to 30 mg of IG injection, Müller cells expressed only vimentin and not GFAP. Minor trauma such as that caused by a scleral puncture (similar to the technique used for the intravitreal injection used here) does not lead to GFAP upregulation by Müller cells. GFAP immunoreactivity in rabbit retina has been reported to vary with the type of fixation technique used. In this study, fixation artifact did not explain absence of GFAP expression by Müller cells, since GFAP immunoreactivity was noted in nonneural, non-Müller elements in the papillary region. Therefore, absence of retinal response by nonneuronal cells to intravitreal injection of IG most likely represented the absence of GFAP up-regulation in Müller cells.

This study shows that intravitreal injection of IG is well tolerated and intact IG persists in the vitreous and retina for at least 5 days postinjection. Also, IG molecules at the dose range employed in this study diffuse rapidly.
into all layers of the neural retina. The ocular tolerance, tissue penetration, and persistence of intravitreal IG are desirable characteristics that might allow attenuation of toxin-mediated damage in acute endophthalmitis.

II. INTRAVITREAL INJECTION OF HUMAN IMMUNE GLOBULIN: ATTENUATION OF THE EFFECTS OF STAPHYLOCOCCUS AUREUS CULTURE SUPERNATANT IN A RABBIT MODEL OF TOXIN-MEDIATED ENDOPHTHALMITIS

General Approach

Evaluations were performed to establish proof of principle for a neutralizing effect of IG on the manifestations of toxin-induced endophthalmitis. In this study, immunoreactivity of IVIG against known, toxic, components of S. aureus supernatant (pooled toxin, PT) was demonstrated by Western blotting. In a rabbit model of toxin-mediated endophthalmitis induced by intravitreal PT, clinical and histopathologic measures were used to determine whether IG neutralized the effects of PT.

Methods

Pooled Bacterial Toxin Preparation

S. aureus RN4220 is a derivative of strain 8325-4 modified for genetic manipulation which primarily produces beta hemolysin (molecular weight [MW]: approximately 35 kilodaltons [kd]). Strain RN4220 was chosen because of extensive laboratory experience with this line and the relative ease with which some aspects of toxin production can be manipulated. The particular RN4220 used in this study also produces toxic shock syndrome toxin-1 (TSST-1; MW: approximately 22 kd), alpha hemolysin (MW: approximately 32 kd), and delta hemolysin (MW: approximately 3 kd) in small amounts (P.M. Schlievert, PhD, oral communication, October 2000). The technique for collecting culture supernatant has been previously described. Bacteria were propagated overnight with aeration at 37°C in 1,200 mL of beef heart extract medium (BHM). Briefly, this medium is prepared from a tryptic digest of beef hearts that is dialyzed across 4,000 to 6,000 MW cutoff dialysis tubing. The insoluble residue is discarded, and the dialysate is sterilized, supplemented with glucose buffer, and inoculated with RN4220. After bacterial growth, extracellular proteins were precipitated from 100 mL of culture medium with four volumes of ethanol chilled overnight at 4°C. The precipitate was resuspended in 10 mL of double distilled water. The suspension was then centrifuged at 10,000g for 20 minutes to remove cellular debris. The supernatant was removed and dialyzed (12,000 to 14,000 MW cutoff) against 2 L of water overnight at 4°C. This concentrated culture supernatant (pooled toxin, PT) contained approximately 60 mg protein/mL. Bacteria-free BHM was similarly precipitated to produce a toxin-free control solution. Sterility was maintained during the entire procedure.

Purified Bacterial Toxin Preparation

Beta hemolysin was purified from RN4220 culture supernatant following the initial steps outlined above. Once the culture supernatant was collected, beta hemolysin was separated using an isoelectric focusing technique. TSST-1 was derived from an RN4220 line containing the vector pCE107. Following collection of culture fluids, TSST-1 was separated with isoelectric focusing using successive gradients of pH 3.5 to 10 and pH 6 to 8.

Western Blotting: Evaluation of Reactivity of IG Against Known S. aureus Toxins

Western blotting was performed to determine whether IG (Gamimmune N, 10%) binds to known toxins produced by S. aureus (beta hemolysin and TSST-1; source: laboratory of Patrick M. Schlievert, PhD, Minneapolis, Minnesota) and to proteins, including toxins, in PT. Known toxins, PT, and control BHM were subjected to SDS-polyacrylamide gel electrophoresis under reducing conditions (5 mM β-mercaptoethanol) using 12.5% separating gels. Protein loading was empirically determined and is reported with the “Results.” Separated proteins were transferred to nitrocellulose membranes and blotted overnight with a 1:10,000 dilution of IG. Bands were visualized with the Enhanced Chemiluminescence (ECL) detection system (Amersham Biosciences, Piscataway, New Jersey) after exposure to a donkey antihuman secondary antibody (Figure 6).

Intraocular Injections of Pooled Toxin and Clinical Examination

Preliminary studies indicated that an intravitreal injection of PT containing 330 µg protein reproducibly created intraocular inflammation with a red reflex but no ophthalmoscopically detectable fundus details. This pathologic outcome was chosen because it closely mirrors the degree of inflammation reported in a rabbit model of S. aureus endophthalmitis involving live bacteria. It was also desirable to create a model of intraocular inflammation similar to that typically seen in a clinical case of S. aureus endophthalmitis with presenting visual acuity of count fingers to hand motions.

Thirty-six rabbits were divided into three groups, each with six experimental and six control animals, in which the timing of the intravitreal injections was varied. In group 1 (simultaneous), PT and IG were mixed and delivered simultaneously; in group 2 (sequential), IG was injected immediately after PT; and in group 3 (delayed),
Light micrograph showing the optic nerve head region: mononuclear cells are seen in the prepapillary vitreous following intravitreal injection of human immune globulin (hematoxylin-eosin, ×125).

Photomicrograph showing immunolocalization of immunoglobulin molecules following intravitreal injection: intense staining is localized to the inner epithelial layer of the ciliary processes (×125).

Distinct immunoglobulin-specific intracellular staining is seen in the inner nuclear layer of the retina following intravitreal injection of human immune globulin. All the other layers of the neurosensory retina show diffuse staining (×250).

Photomicrograph of the peripapillary retina in the rabbit eye showing diffuse immunoglobulin-specific staining in all the retinal layers. Staining is more intense in the vitreous and in the outer extent of the outer segment layer. The choroid does not show any staining (×250).

Immunoglobulin-specific staining seen in the prepapillary vitreous and around major blood vessels in the optic nerve head, following intravitreal injection of human immune globulin (×125).

Western blot of Staphylococcus aureus culture supernatant (pooled toxin) and purified exotoxins. Nitrocellulose membranes probed and then visualized with ECL detection system. Primary antibody: 1:10,000 dilution of 10% Gammunone; secondary antibody: donkey antihuman. Lanes 1 and 2: toxic shock syndrome toxin-1 (TSST-1) 0.2 and 0.5 µg; lanes 3 and 4: beta hemolysin (BHL) 0.2 and 1.25 µg; lanes 5 and 6: beef heart media; lanes 7 and 8: concentrated culture supernatant 0.5 and 1.0 µg. Molecular weight markers (Bio-Rad) indicated on the right (in kilodaltons) are serum albumin (66), ovalbumin (45), carbonic anhydrase (31), trypsin inhibitor (21), and lysozyme (14). The upper arrow indicates BHL. The lower arrow indicates TSST-1.
IG was injected 6 hours after PT. Group 1 data consisted of two sets each of three experimental and three control rabbits that were sacrificed on either PID 7 or 9. All rabbits in groups 2 and 3 were sacrificed on PID 9.

The volume of IG used in this study was determined by two competing factors regarding the nonvitrectomized eye. Though it was desirable to inject the largest possible volume of IG to maximize IG and toxin interaction, injection volumes were limited to less than 250 µL by associated intraocular pressure elevation. Although removal of up to 300 µL of aqueous was possible, it was determined that 200 µL of aqueous could be safely aspirated from the anterior chamber without risk of lens or iris damage. Therefore, an IG volume of 145 µL was chosen for this portion of the study, and other parameters were adjusted accordingly.

Prior to intravitreal injection, 150 µL (group 1) or 200 µL (groups 2 and 3) of aqueous humor was aspirated with a tuberculin syringe to reduce intraocular pressure and reduce the risk of vascular occlusion. For group 1, 5 µL of the concentrated PT (containing 330 µg protein) was mixed with 145 µL IG (14.5 µg) and injected within 10 minutes through the pars plana into the midvitreous using a 30-gauge needle and a tuberculin syringe. For the other groups, 5 µL of the concentrated PT was diluted to 50 µL with BSS and injected into the midvitreous. (Increasing the 5 µL volume to 50 µL allowed for reliable toxin delivery into the midvitreous cavity using a tuberculin syringe.) IG (145 µL) was then injected at a site 90 degrees away, either immediately (group 2) or 6 hours after (group 3) PT injection. Control animals in all groups received 145 µL BSS in lieu of IG. Materials were prepared and injected under sterile conditions. For all animals, one eye only was used. Immediately following injection, all eyes were examined for intraocular complications.

For all animal groups, slit-lamp biomicroscopy and indirect ophthalmoscopy were performed four times over 9 days postinjection by one investigator and two masked vitreoretinal surgeons experienced with the management of clinical endophthalmitis. In group 1 and group 2, examinations were performed on PIDs 1, 3, 5, and 8. Group 3 examinations were performed on PIDs 2, 4, 6, and 9. At each time point, examination of six experimental and six control eyes was performed except for PID 8 in group 1 when there were only three experimental and three control eyes.

The anterior chamber reaction and fundus reflex were graded for evidence of ocular inflammation using an adaptation of a 0 to 4 grading scale reported by others (Table 1). The mean score for each parameter was determined for the six eyes in each group, and control and experimental groups were analyzed for statistically significant differences (P < .05) using the Mann-Whitney test at each time point except for the final day in group 1 when there were only three eyes in each of the experimental and control groups.

### Histopathology

Animals were killed by an intracardiac injection of pentobarbital (25 mg/kg) on PID 7 or 9, and the treated eyes were enucleated and fixed in 10% formalin for histopathologic analysis. Eyes were embedded in paraffin, sectioned, and stained with hematoxylin and eosin by standard protocols. Sections were examined and scored by an investigator masked to the identity of the treatment group. Each eye received scores for four tissues using grading scales for severity changes adapted from the work of others. The cornea, anterior chamber, and vitreous each received a single score of 0 to 3 (Table 2). For the retina, a template was used to divide each retinal section into six regions, each of which was graded separately using a 0 to 4 scale (Table 2). A single retinal score per eye was obtained by taking the mean of the six regional scores, and the Mann-Whitney test was used to determine statistically significant differences (P < .05) between experimental and control groups.

### Results

**Reactivity of IG With S aureus Products**

When IG was used as a primary antibody for Western blot analysis, we observed reactivity with numerous proteins in the PT, including a prominent unidentified high-molecular-weight protein (Figure 1). The absence of comigrating bands in the control BHM suggests that the IG immunoreactive proteins in the PT are bacterial products. To determine whether the IG can react with any known

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### TABLE 1. CLINICAL GRADING SCHEME FOR SEVERITY OF OCULAR INFLAMMATION

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S aureus toxins, which may constitute a small fraction of the protein in the total PT and therefore contribute little to the blotting signal, beta hemolysin and TSST-1 were purified from bacterial supernatants and also blotted with IG. As shown (Figure 1), IG shows reactivity with both beta hemolysin and TSST-1.

Clinical Examination

Pooled Toxin Alone. Intravitreal delivery of PT alone produced a grade 3 to 4 anterior chamber reaction on PID 1. This reaction declined until it resolved by days 5 to 7. The fundus reflex was diminished in all eyes by PID 1. Despite the obscuration of most retinal details in all eyes on PID 1, intraretinal hemorrhages could be observed in some. Over the first 5 days postinjection the fundus reflex worsened in most eyes and remained diminished at approximately the same level on PIDs 5 through 9. The initial decline in the reflex was attributed to vitreal inflammation. To a variable extent, a white membrane would form on the posterior surface of the lens by PID 2, would continue to proliferate on PIDs 2 to 4, and then would stabilize. This membrane would continue to mature even though anterior segment inflammation was resolving, and it prevented adequate evaluation of the posterior segment. The fundus reflex appearance on PID 7 is illustrated in Figure 7 (top), and scores for the fundus reflex over the 8- to 9-day time course are shown in Figure 8 (broken lines).

Pooled Toxin and Simultaneous Injection of IG. When PT was mixed with IG prior to injection, an anterior chamber reaction was seen in only three of six eyes and was limited to grade 1. The fundus reflex (Figure 7, bottom) was essentially normal throughout the time course, and retinal details were easily seen. Fundus reflex scores over the time course are graphed in Figure 8 (top). Compared to eyes receiving PT and BSS, the average fundus reflex scores from eyes receiving IG were significantly lower at all time points (Figure 8, top).

Pooled Toxin and Sequential Injection of IG. When IG was injected immediately following PT, the expected inflammatory response was attenuated compared to eyes receiving PT alone (Figure 8, middle). The difference in the fundus reflex scores between the two groups became greater over the 9-day course and could be attributed to a worsening of the fundus reflex in the eyes not receiving IG. The differences were statistically significant at all but the second time point. Compared with eyes receiving simultaneous injections of PT and IG, the fundus scores of eyes receiving sequential injections were higher (ie, more diminished), particularly at earlier time points (Figure 8, top and middle).

Pooled Toxin and Delayed Injection of IG. When injection of IG was delayed by 6 hours, only a slight attenuation of the expected inflammatory response was observed. The fundus reflex was diminished more than in eyes receiving simultaneous or sequential injections of IG (Figure 8). Despite the diminished reflex, fundus scores were significantly lower at all but the first time point when compared with eyes not receiving IG (Figure 8, bottom). Retinal details were obscured throughout the time course.

Histopathology

Pooled Toxin Alone. Essentially no inflammatory response was found in the cornea and anterior chamber of these eyes (Figure 9). The vitreous cavity was partially filled with inflammatory cells. Some eyes had abscesses and others did not. Full-thickness retinal disruption was evident with ganglion cell loss, increased vacuolization of inner nuclear layers, complete loss of photoreceptors, and choroidal thickening observed in most sections (Figure 10, top). Interspersed areas of focal retinal thinning due to loss of cellular elements were observed.
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Pooled Toxin and Simultaneous Injection of IG. In these eyes, a mild inflammatory response in the vitreous cavity was observed. Compared to eyes receiving PT alone, there was a marked preservation of retinal architecture with the presence of distinct layers composed of intact cells (Figure 10, bottom). In some sections, vacuolization of the inner nuclear layers and choroidal thickening were observed.

Pooled Toxin and Sequential Injection of IG. When IG was injected immediately following PT, an attenuation of the expected histologic response in the retina but not in the vitreous was observed (Figure 9, middle). Compared to eyes receiving PT simultaneously with IG, the retinal architecture was slightly more disrupted but still significantly less than in eyes not receiving IG. Focal areas of inner nuclear layer vacuolization, disruption of the ganglion cell layer, retinal edema, photoreceptor loss, and choroidal thickening were noted. In other sections, preserved retinal architecture was observed.

Pooled Toxin and Delayed Injection of IG. When IG was injected 6 hours after PT, the histologic appearance of all tissues was indistinguishable from eyes receiving PT alone (Figure 9, bottom). There was full-thickness retinal disorganization in most sections with ganglion cell loss, increased vacuolization of inner nuclear layers, complete loss of photoreceptors, and choroidal thickening. As in eyes receiving PT alone, some sections contained focal areas of retinal thinning due to cell loss.
The rabbit model has been extensively used in the study of endophthalmitis. The rabbit eye may differ from the human eye in its response to intravitreally injected bacteria. For instance, the rabbit eye almost always self-sterilizes after intravitreal injection of live *S. epidermidis* organisms, a phenomenon not observed to the same degree in the human eye. Nevertheless, the severity of infection in experimental endophthalmitis in the rabbit eye seems to mirror that observed clinically in the human eye across bacterial species varying in their degrees of virulence and ability to elaborate toxins. To the extent that bacterial toxins are responsible for a similarity in host response, it seems reasonable to suggest that the inflammation and retinal damage observed in the rabbit eye from injected *S. aureus* culture supernatant (or its exotoxins) would also likely occur in the human eye, although not necessarily at the same dosage or concentration. Such damage would likely be of clinical significance.

Using Western blotting techniques, it was determined that IG could bind purified toxins as well as numerous proteins from *S. aureus* RN4220 culture supernatant (or its exotoxins) would also likely occur in the human eye, although not necessarily at the same dosage or concentration. Such damage would likely be of clinical significance.

Discussion

The rabbit model has been extensively used in the study of endophthalmitis. The rabbit eye may differ from the human eye in its response to intravitreally injected bacteria. For instance, the rabbit eye almost always self-sterilizes after intravitreal injection of live *S. epidermidis* organisms, a phenomenon not observed to the same degree in the human eye. Nevertheless, the severity of infection in experimental endophthalmitis in the rabbit eye seems to mirror that observed clinically in the human eye across bacterial species varying in their degrees of virulence and ability to elaborate toxins. To the extent that bacterial toxins are responsible for a similarity in host response, it seems reasonable to suggest that the inflammation and retinal damage observed in the rabbit eye from injected *S. aureus* culture supernatant (or its exotoxins) would also likely occur in the human eye, although not necessarily at the same dosage or concentration. Such damage would likely be of clinical significance.
*Staphylococcus aureus* RN4220 is a modified laboratory strain that may prove to be more or less virulent than other *S. aureus* strains, it has been well studied and can be manipulated to produce different toxins, thus providing a framework for further study involving antitoxin therapy.

Quantitative pharmacokinetics of IG remain largely unknown, but most certainly influence the therapeutic effects of IG in this model. When IG was injected immediately following PT, the toxic effects of PT were definitely attenuated clinically and histologically, but less so when compared with eyes receiving PT simultaneously injected with IG. This observation would suggest that optimizing the mixing of IG with bacterial virulence factors elaborated in vivo might facilitate a therapeutic effect. The influence of vitreous humor on the interaction between IG and PT remains unexplored.

The rapidity with which IG is capable of inactivating the biological activity of PT would suggest that a mode of administration that promotes interaction between IG and bacterial toxin could ameliorate the destructive effects of the toxins if given at an opportune time. In *S. aureus* infection, significant toxin production typically does not occur until the postexponential growth phase, providing a potential window of opportunity for therapeutic intervention. In a rabbit model of endophthalmitis produced by the intravitreal injection of *S. aureus*, bacteria grew exponentially during the first 24 hours. Intraocular inflammation was observed at 24 to 48 hours postinjection, whereas attenuation of the electroretinography recording did not begin to occur until 48 hours postinjection. Thus, treatment that occurs within this time frame, or perhaps not much later than this, might favorably influence the course of endophthalmitis. The importance of timely intervention prior to bacterial release of large quantities of exotoxins is also suggested by our study. Little difference was noted clinically, and none histologically, when IG injection was delayed 6 hours following PT injection, most likely indicative of the rapidity of tissue destruction from abrupt exposure to a suprathreshold dose of toxin. Reduced effect with delayed treatment might relate to the marked fulminance of this noninfectious model and differ from human endophthalmitis. Because clinical endophthalmitis is most likely associated with a gradually increasing concentration of a variety of toxins, rather than a bolus dose, the concept of toxin neutralization being of potential benefit early in the course of endophthalmitis appears valid. In streptococcal and staphylococcal toxic shock syndromes, intervention with IVIG appears helpful even after onset of disease manifestations, suggesting that therapeutic opportunity might also exist in endophthalmitis cases already clinically manifest.

The commercial availability of pooled, polyspecific IG (IVIG) is a distinct advantage for its potential therapeutic application in the human eye. The risk-benefit ratio for such therapy would require that the risks of local toxicity, immune response, and systemic side effects be considered. Risks include that of transmission of bloodborne pathogens, because the product is a biological product derived from plasma pooled from human donors. Human immunodeficiency virus (HIV) and hepatitis B and C are agents of greatest concern, with cases of hepatitis B and C transmission being reported with systemic administration. However, preparations after May 1994 are considered to have a high degree of safety with respect to viral contamination due to screening of donors and adoption of procedures that inactivate viruses, with no cases of hepatitis C virus transmission having occurred since about that time. In addition, there has been no transmission of HIV by any preparation of IVIG, though reverse transcriptase of possible viral origin was found in the serum of two recipients in 1986. These issues have been discussed at length by Grob and Yap. It might be speculated that the above risks of IVIG-associated disease transmission might be reduced, though not absolutely eliminated, because of the smaller dosages likely to be used intravitreally and the relatively poor access to the systemic circulation offered by such a route.

Serious side effects ascribed to systemic administration of IVIG include headache and aseptic meningitis (1% to 11%), vasomotor reactions, thromboembolic events, and acute renal failure. Most have occurred after multiple intravenous infusions, mostly with dosages of approximately 0.35 to 1 gm/kg per day, huge amounts in comparison to a dosage that might be proposed for the eye (milligrams to decigrams). Limited experience with IVIG injected intraventricularly and intrathecally for treatment of viral CNS infections has shown no associated serious toxicity, suggesting that intraocular injection of IVIG might similarly be associated with low morbidity.

Because of the polyspecific nature of standard preparations of IVIG, relatively large intravenous doses are required to achieve systemic effect. In addition, variability in reactivity between lots of commercially available IVIG has been observed, with some containing low levels of bacterial antibodies. Correlation between titers of bacterial antibodies and those against their elaborated toxins has not been evaluated to the author's knowledge. To increase potency, hyperimmune globulins (HYPERIVIG) have been formulated, which contain high titers of specific antibodies from plasma of donors immunized with appropriate vaccines or from donors screened for naturally occurring high antibody titers. Systemic administration has been associated with promising results. Such preparations have been made for relatively few bacterial species targets, however. Significant clinical need is proposed for HYPERIVIG for treatment of systemic staphylococcal, streptococcal, and gram-negative infections. If developed, HYPER-
IVIG might have potential applications to endophthalmitis in the human eye, particularly if it contains a corresponding increase in antibody titers against elaborated toxins. The availability of recently developed rapid tests for various bacterial infections, if proven applicable to clinical endophthalmitis, would facilitate use of such targeted therapy.

SUMMARY

This is the first study of which the author is aware that evaluates the effect of polyclonal immunoglobulin therapy against bacterial exotoxins in endophthalmitis. The ocular tolerance, tissue penetration, and persistence of intravitreally injected IG observed in this study appear compatible with its proposed application as antitoxin therapy in acute endophthalmitis. Further investigation is required evaluating such factors as the interaction of ocular tissues with IG-bound toxin, efficacy of IG in infected bacterial models of endophthalmitis, the ocular immune host response, immune-complex formation, interaction of IG with antibiotics, effect of vitrectomy, and timing of intervention.

This study supports the hypothesis that intravitreally injected human immune globulin reactive to S aureus virulence factors can attenuate their inflammatory and tissue destructive effects. Pooled human IG might therefore represent a novel adjunctive therapy in the management of S aureus endophthalmitis.

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REFERENCES

Intravitreal Human Immune Globulin in a Rabbit Model of Staphylococcus Aureus Toxin-Mediated Endophthalmitis


THE ACCURACY OF DIGITAL-VIDEO RETINAL IMAGING TO SCREEN FOR DIABETIC RETINOPATHY: AN ANALYSIS OF TWO DIGITAL-VIDEO RETINAL IMAGING SYSTEMS USING STANDARD STEREOSCOPIC SEVEN-FIELD PHOTOGRAPHY AND DILATED CLINICAL EXAMINATION AS REFERENCE STANDARDS

BY Mary Gilbert Lawrence MD MPH

ABSTRACT

Purpose: To evaluate the accuracy of two digital-video retinal imaging (DVRI) systems to detect diabetic retinopathy.

Methods: A prospective, masked, technology assessment was conducted for two DVRI systems at a tertiary care Veterans Affairs Medical Center. Group A (n = 151 patients) was imaged with a 640×480 resolution system and group B (n = 103 patients) with an 800×600 resolution system. Four retinal evaluations were performed on each patient: DVRI with undilated pupils using one imaging field (U-DVRI), DVRI with dilated pupils using three imaging fields (D-DVRI), dilated clinical examination, and Early Treatment Diabetic Retinopathy Study stereoscopic seven-field photography (ETDRS-P). Two analyses of accuracy were conducted, one using ETDRS-P as a “gold standard” (ETDRS-GS) and one using dilated clinical examination as a “gold standard” (C-GS).

Results: For group A, using the ETDRS-GS, sensitivities of U-DVRI and D-DVRI were 0.66 and 0.66; specificities of U-DVRI and D-DVRI were 0.66 and 0.86. Using the C-GS, sensitivities of U-DVRI and D-DVRI were 0.79 and 0.80; specificities of U-DVRI and D-DVRI were 0.76 and 0.75; specificities of U-DVRI and D-DVRI were 0.45 and 0.80. Using the C-GS, sensitivities of U-DVRI and D-DVRI were 0.81 and 0.87; specificities of U-DVRI and D-DVRI were 0.45 and 0.69. For both groups, dilation significantly improved specificities.

Conclusions: The 800×600 resolution DVRI system offers an accurate method of detecting diabetic retinopathy, provided there is adequate pupillary dilation and three retinal images are taken. DVRI technology may help facilitate retinal screenings of growing diabetic populations.


INTRODUCTION

Diabetes mellitus is now one of our nation’s top health concerns. In 1999, Eli Lilly and Company built the largest factory dedicated to the production of a single drug in pharmaceutical history. The drug, with 24% year-on-year sales growth, is insulin.1 In 2002, 1.3 million new cases of diabetes mellitus were diagnosed in Americans aged 20 years or older.2 Currently, 18.3% of Americans 60 years and older are diabetic, and 6.3% of the entire US population has the disease.3 Diabetes strikes individuals of all ages and socioeconomic groups. Each year, over 200,000 people die as a result of diabetes and diabetic retinopathy causes 12,000 to 24,000 new cases of blindness.4 The annual cost of diabetes in the United States has been reported to be $132 billion.5 Diabetes mellitus ranks as one of the most deadly, most visually threatening, and most costly diseases known to mankind.

Diabetic retinopathy, a microvascular disease characterized by retinal microaneurysms, hemorrhages, exudates, and vascular proliferation, is a common complication of diabetes mellitus. Twenty years after the onset of diabetes, over 90% of people with type 1 diabetes and over 60% of individuals with type 2 diabetes will have diabetic retinopathy.6,7

The scientific basis for current management of diabetes retinopathy is provided by five large multicenter
clinical trials: the Diabetic Retinopathy Study,5-10 the Early Treatment Diabetic Retinopathy Study (ETDRS),11-35 the Diabetic Retinopathy Vitrectomy Study,36-40 the Diabetes Control and Complications Trial,41,42 and the United Kingdom Prospective Diabetes Study.43-57 Laser photocoagulation has been the mainstay of treatment for diabetic retinopathy for the past quarter century. In 1976, the Diabetic Retinopathy Study Research Group published its preliminary report demonstrating the overwhelming benefit of scatter (panretinal) laser photocoagulation for proliferative retinopathy.58 Nine years later, the ETDRS showed that focal retinal photocoagulation could reduce moderate visual loss from clinically significant macular edema.59 Good glycemic control60 and tight blood pressure control61 have also been shown to retard the progression of retinopathy. Current standards of care can reduce the risk of severe vision loss from diabetic retinopathy to less than 2%.62

Despite treatments of proven efficacy, however, diabetic retinopathy continues to be a major cause of blindness63 and is, in fact, the leading cause of blindness in people under the age of 60 years in industrialized countries, including the United States.64 Delay in treatment is the main reason for the visual loss and is largely preventable with proper screening.65 To detect diabetic retinopathy at an optimal stage for intervention, many professional societies in the United States, including the American College of Physicians, the American Diabetes Association, and the American Academy of Ophthalmology, recommend that patients with diabetes receive an annual dilated fundus examination from a qualified eye care provider.66 The Department of Veterans Affairs has also established performance standards for regular dilated fundus examinations of diabetic patients.67 Annual eye examination of diabetics has been incorporated into the Health Plan Employer Data and Information Set quality guidelines, adopted throughout the managed care industry. Despite these recommendations, reports indicate that only 35% to 50% of managed care patients actually receive the recommended eye examination in a given year.68 Similar low rates of retinal evaluations are reported in Medicare beneficiaries69,70 and the National Health Interview Survey.71 The current challenge is to access and identify all diabetic patients for regular periodic retinal evaluations. Computer modeling studies have suggested that if appropriate screening and optimally timed photocoagulation treatments for diabetic retinopathy were employed, annual health care expenditures could be reduced by $250 to $500 million per year.72-74 In addition, over 1,000,000 person-years of sight could be saved if all diabetics had appropriately timed ophthalmic screening and treatment.75 With such a prevalent and costly disease, and one for which proven treatments exist, there is a critical need for a sensitive and cost-effective screening method.

**Background**

The optimal strategy for detecting diabetic retinopathy in the large diabetic population is unclear. In addition to clinical evaluations by qualified eye care providers, the mainstay of diabetic eye screening in the United States, other modalities including film-based photography programs are in widespread use, especially in Europe. Several new digital imaging systems for detecting diabetic retinopathy also have been recently reported.

In the United States, there is currently no central agency to oversee, regulate, advise, or perform health technology assessment, so research to address issues raised by new health technology is highly variable in quality.76 Indeed, Mason and coworkers77 reviewed the published literature regarding systems for diabetic retinopathy detection and concluded that programs are very difficult to compare on account of inconsistencies and inconclusive evidence. In the United Kingdom, the British Diabetic Association Working Group proposed in 1997 that screening programs should demonstrate a sensitivity of 0.80 when compared to established reference (gold) standards.78 In the United States, however, standards for sensitivities of systems to detect diabetic retinopathy have not been set.

**Detection of Diabetic Retinopathy in the Research Setting: The ETDRS Retinopathy Severity Scale**

The ETDRS Final Retinopathy Severity Scale was developed using ETDRS control data to define severity levels of increasing risk of developing neovascularization. In addition to diabetic retinopathy levels, the ETDRS defined stages for diabetic macular edema, which included a subgroup with “clinically significant macular edema.” Patients in this subgroup showed the greatest benefit from macular (focal) photocoagulation.80

The ETDRS Final Retinopathy Severity Scale involves photographing seven specifically defined fields of each retina with a stereoscopic pair of images (for a total of 14 images of each retina), which are later graded according to strict protocol. ETDRS levels of retinopathy severity were used in the Diabetes Control and Complications Trial.81 Although this protocol is the most accurate at detecting diabetic retinopathy and is widely used in well-funded large trials, the cost of performing this level of diagnostic evaluation for the large diabetic populations of developed countries makes this method impractical for widespread use.

The ETDRS Final Retinopathy Severity Scale is the only validated reference standard for the detection and staging of diabetic retinopathy. For this reason, the ETDRS grading protocol has recently been referred to by Lee82 as the “criterion standard” and several years ago by Singer and coworkers83 as the “gold standard” for the
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Detection of Diabetic Retinopathy in the Clinical Setting

Dilated Clinical Examination. A dilated clinical examination using ophthalmoscopy, performed by a qualified eye care provider, is currently the most widely accepted and readily available method of detecting diabetic retinopathy in the United States today. Clinical examination may involve direct or indirect ophthalmoscopy as well as slit-lamp biomicroscopy, and most studies report this being done after pupillary dilation. In a recent systematic review of English language literature published between 1983 and 1999, Hutchinson and coworkers showed that the reported sensitivity of ophthalmoscopy by health professionals in detecting diabetic retinopathy ranged from 0.13 (by junior hospital physicians detecting proliferative retinopathy) to 0.54 (by an ophthalmologist detecting any retinopathy). Of the six reported studies using ophthalmologists to perform ophthalmoscopy, the mean of the reported sensitivities was only 0.61, well below the British Diabetic Association Working Group proposed sensitivity cut-off of 0.80.

Dilated Film-Based Fundus Photography. Outside the United States, dilated fundus photography is a widely accepted method of detecting diabetic retinopathy in developed countries, mainly in European countries. Most reported studies included in the review of Hutchinson and coworkers used wide-angle (45 degree) retinal photography, with sensitivities exceeding 0.80. Single-field, wide-angle fundus film-based photography, although less costly than standard stereoscopic seven-field ETDRS photography, nonetheless, requires expensive camera equipment, highly trained photography personnel, and pupillary dilation.

Digital Imaging Systems. Several newly introduced digital imaging systems for the retina have been evaluated in the recent literature and may offer advantages over film-based photographic programs. The new digital systems differ widely in technological parameters such as pixel resolution and the ability to perform stereoscopic analysis. They also have wide variation in cost and in the need for pupillary dilation. Several of the recently published evaluations have compared new digital imaging systems to the “gold standard” ETDRS Scale using stereoscopic seven-field photography.

Bursell and coworkers from the Joslin Vision Network Research Team reported moderate agreement ($\kappa = 0.65$) between the clinical level of diabetic retinopathy assessed from undilated stereoscopic digital images and the dilated “gold standard” 35-mm ETDRS photographs. The sensitivity of their system for detecting mild or moderate nonproliferative retinopathy was 0.56, but for detecting severe or very severe nonproliferative retinopathy was only 0.57. The Joslin Vision Network system includes a nonmydriatic fundus camera interfaced to a standard color video camera. Stereo image viewing is achieved using liquid crystal display shuttered goggles.

Fransen and coworkers from the Inoveon Health Research Group recently showed that the DR-3DT digital imaging system had a sensitivity of 0.98 compared to the film-based ETDRS “gold standard.” The system requires pupillary dilation and has a spatial resolution of 1,152 x 1,152. Liquid crystal shutter glasses were used for the stereo viewing of the DR-3DT system. The authors have a financial interest in the Inoveon Corporation.

Lin and associates of the Digital Diabetic Screening Group conducted a study to evaluate a single-field digital monochromatic nonmydriatic system using Ophthalmic Imaging Systems technology compared to ETDRS “gold standard” photographs and to dilated ophthalmoscopy by an ophthalmologist. They showed a sensitivity of 0.78 of the digital imaging system compared to ETDRS standard photography. (Sensitivity of ophthalmoscopy by ophthalmologists was 0.34.) They did not test the imaging system with pupillary dilation. Two of the authors had a financial relationship with Ophthalmic Imaging Systems.

Massin and coworkers recently reported an evaluation of the Topcon TRC-NW6S digital imaging camera with an 800 x 600 resolution. Using five overlapping fields imaged through an undilated pupil, they reported sensitivities ranging from 0.92 to 1.00 for moderately severe to severe retinopathy, using ETDRS photographs as the reference standard.

Numerous other reports have been published recently describing comparisons of digital imaging technology to reference standards that have not been validated, including the clinical examination of various eye care providers. These will not be discussed further because the methodology was inadequate to assess the efficacy of the technology.

Purpose of Study

This study was undertaken to rigorously assess two commercially available “nonmydriatic” digital-video retinal imaging (DVRI) systems for their ability to accurately screen for diabetic retinopathy.

Hypothesis

Based on assessments of clinical accuracy, one or both digital-video retinal imaging (DVRI) systems is an acceptable method of screening for diabetic retinopathy.

METHODS

A prospective, masked, clinical technology assessment was conducted at a tertiary care Veterans Affairs Medical
Center. The primary outcome measure was accuracy (sensitivity, specificity, and predictive values) of each DVRI system compared to standard ETDRS stereoscopic photographs, the “gold standard.” The secondary outcome measure was accuracy compared to dilated clinical examination, the “clinical gold standard.”

**Subjects**
Approval of the Minneapolis Veterans Affairs Medical Center Institutional Review Board was obtained before initiation of the study. Patients with a diagnosis of diabetes mellitus were recruited from the Eye Clinic at the Minneapolis Veterans Affairs Medical Center. The diagnosis of diabetes mellitus was verified in the patient’s medical record. To meet the eligibility requirements for the study, the patient must have had at least one eye without previous retinal laser treatment for diabetic retinopathy (panretinal photocoagulation or focal macular photocoagulation). If the fellow eye had previous laser treatment, only the previously untreated eye was entered into the study. The sampling of patients was nonrandom to ensure a distribution of retinopathy levels broad enough to encompass the full range of retinopathy severities. Demographic and historical data was collected from each patient and/or the patient’s medical record. A unique nonsequential patient identifier number was assigned to each enrolled patient. The patient identification number was the only identifier used on all digital and photographic images. The patient’s date of birth, sex, race, year of diagnosis of diabetes, current diabetic treatment, most recent hemoglobin A1C level, and comorbid ocular conditions were included in the data collection.

**Clinical Protocol**
On the day of the study evaluation, all patients signed a consent form in accordance with Institutional Review Board guidelines. Distance visual acuity with spectacle correction, undilated pupillary measurements, and DVRI through an undilated pupil were performed. After intraocular pressures were measured, the pupils were dilated with 2.5% neosynephrine and 1% tropicamide. A study ophthalmologist then performed an eligibility determination by assessing that the ocular media was clear and that the patient had no prior retinal photocoagulation. The patient then returned to the photographer for digital imaging using the same DVRI camera as was used in the predilatation state. Seven-field stereoscopic ETDRS photographs were taken using the conventional film-based Topcon fundus camera (model TRC-50VT). The patient was then given a complete ophthalmic examination by an ophthalmologist, including the clinical assessment of retinopathy severity for the study.

**Digital Imaging Protocol**
Two DVRI systems were used in the study, the Topcon TRC-NW5SF with a 640×480-pixel resolution and the Topcon TRC-NW6S with an 800×600-pixel resolution. Patients imaged with the low-resolution system were assigned to group A, and patients imaged with the high-resolution system were assigned to group B. The same system was used to take photographs prior to and following pupillary dilation for each patient. All DVRI images were taken at a 45-degree field size, in the color mode, with nonstereoscopic images. The infrared viewing light was set at maximum, and the exposure light was set at minimum, but both could be adjusted as required.

The undilated images were taken in a room where the ambient lighting was reduced to a minimum, and the computer monitor was turned away from the patient’s line of sight. A minimum of 2 minutes was allowed for pupillary dark adaptation, but a longer adaptation period was used if the patient’s pupils were still dilating under observation with the camera’s infrared observing light. After recording the size of the undilated dark-adapted pupils, nonstereoscopic images were obtained in each eligible eye. One photographic field (field B), centered on the fovea as depicted in Figure 1, was taken of each eye. Multiple images could be taken in each eye until the photographer judged that the best-quality image was obtained. Care was taken after each image captured to give the pupil time to maximally dilate before taking another image (usually 2 to 3 minutes). The best-quality image for each eye was chosen for grading at a later time, and the inferior images were deleted.

After dilation, the size of the pupil was recorded. Three nonstereoscopic images were then obtained in each eligible eye (fields A, B, and C), as depicted in Figure 1. As above, multiple images were allowed to be taken of each field in each eye until the photographer judged that the best-quality image had been obtained. The best-quality image for each field was chosen for grading at a later time, and the other images were deleted.

The stereoscopic photographs of the seven standard ETDRS photographic fields were then taken using a conventional film-based Topcon fundus camera (model TRC-50VT). Photographs were taken according to the University of Wisconsin–Madison Fundus Photograph Reading Center’s Fundus Photography Protocol (adapted from the ETDRS Manual of Operations).

**Assessment of Retinopathy Severity by Grading ETDRS Stereoscopic Photographs**
The grading of the stereoscopic seven-field ETDRS photographs was performed by an experienced nonphysician grader, certified by the University of Wisconsin–Madison Fundus Photograph Reading Center.
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All photographs were assessed by the same grader, who was masked as to patient identity. Of patients with both eyes included in the study, each eye was graded independently. The eyes from the same patient were sent to the grader in different batches separated in time.

Assessment of Retinopathy Severity by Dilated Clinical Examination

The examining ophthalmologists were given a seven-choice scale to document retinopathy severity in each eye, ranging from level 1 (no apparent retinopathy) to level 6 (proliferative diabetic retinopathy). The definitions of the different levels of retinopathy are defined on the Fundus Examination Form in the Appendix and are very similar to the recently proposed International Clinical Diabetic Retinopathy Severity Scale.96 A severity level of 7 was given if the level of diabetic retinopathy could not be determined. The examiner was also asked to note the presence of hard exudate within one disk diameter of the center of the macula and/or the presence of macular edema, defined as any retinal thickening within one disk diameter of the center of the macula. The ophthalmologist was able to utilize slit-lamp biomicroscopy or direct or indirect ophthalmoscopy at his or her discretion. A simplified grading scale was used because this is how most clinicians categorize diabetic retinopathy in a clinical setting.

ETDRS grading requires counting lesions in each field, which is difficult and arguably impossible to accomplish in a face-to-face setting.

Assessment of Retinopathy Severity by Grading DVRI Images

The images taken using the two DVRI cameras were graded by ophthalmologists in a fashion analogous to the dilated clinical examination, using the seven-choice scale of retinopathy severity, as well as determination of the presence of hard exudates or macular edema. The digital images were evaluated directly on the computer screen. The grader was allowed to use software tools (image contrast enhancement and imaging sharpening) to enhance the images for grading purposes. It was left to the grader's judgment for each individual image to choose the best combination of enhancements for that particular image grading. For undilated DVRI (U-DVRI), only one image was analyzed per eye (Figure 2). Three images were analyzed per eye for dilated DVRI (D-DVRI). The three images were analyzed together to determine the overall grade for the particular eye (Figure 3). The ophthalmologists performing the DVRI evaluations were masked to any previous grading of that same eye. A reading queue was established so that no ophthalmologist performed the clinical ophthalmoscopic examination in the same week as a digital image set from the same patient was evaluated. Different identification numbers were given for the dilated and the undilated image sets, which were reviewed independently.

Criteria for Presence or Absence of Diabetic Retinopathy

As new pharmacologic treatments for diabetic retinopathy are being developed, including antioxidants,97 protein kinase C inhibitors,98,99 and advanced glycation end products100 that are designed to block the development of retinopathy, there will be a growing need for earlier diagnosis. Methods that detect only the more severe levels of diabetic retinopathy will become obsolete. For this reason, we established criteria for the distinction between "disease" and "no disease" at low levels for all methods of evaluation. For the "gold standard" ETDRS photographic levels, "disease" was defined as an ETDRS level ≥20 or the presence of clinically significant macular edema. "No disease" was defined as an ETDRS level <20 and no clinically significant macular edema.

For the dilated clinical retinal examination, "disease" was defined as retinopathy severity levels 2 through 7 or the presence of retinal thickening or hard exudates within one disk diameter of the center of the macula. "No disease" was defined as retinopathy severity level 1 (no retinopathy) and no hard exudates.
The definitions for “referral” and “no referral” for DVRI were analogous to the clinical examination “disease” and “no disease.” An eye that was above the threshold for “referral” was defined as retinopathy severity levels 2 through 7 or the presence of hard exudates. “No referral” was defined as retinopathy severity level 1 (no retinopathy) and no hard exudates.

**Statistical Analysis**

The study was planned with adequate number of patients to yield expected 95% confidence intervals of 10% or less around the observed measures of accuracy. The variable, “referral” versus “no referral,” was dichotomous. The photographic ETDRS severity levels were used to identify true “disease” and true “nondisease” and served as the reference or “gold standard.” Dilated clinical examination is the “clinical standard” for the detection of diabetic retinopathy in the United States. Although there have been no validation studies to confirm its use, the assessment of diabetic retinopathy severity via dilated clinical examination was used as a “clinical gold standard” for calculating measures of accuracy.

Accuracy was assessed using several measures: sensitivity, specificity, false-positive rate, and false-negative rate. In addition, positive predictive value, negative predictive value, and efficiency were calculated for each method. The primary statistical focus was on sensitivity, because of the desire to minimize the number of false negatives. Effective screening programs should have high sensitivity (low false-negative rates), especially at the more severe levels of disease. Another measure of accuracy, given considerable attention, was specificity, because of its ability to describe the false-positive rate. Screening programs with low specificity (high false-positive rates)
have low economic utility, because they lead to more, often expensive, testing.

Patients were divided into two groups, depending on the DVRI camera with which they were imaged. Group A was imaged with the 640 × 480 resolution DVRI camera, and group B was imaged with the 800 × 600 resolution camera. Accuracy measures were calculated for each of the three methods of retinal evaluation: DVRI through an undilated pupil using one imaging field (U-DVRI), DVRI through a dilated pupil using three imaging fields (D-DVRI), and dilated clinical examination.

Because a large proportion (400/489) of total eyes used in the statistical analysis were paired (in the same patient), it was determined that there may be some dependency in the data. That is, each eye was not truly independent. If dependent data exist, then the use of linear models (eg, standard linear regression) may lead to biased estimates of variance, which could lead to misleading comparisons. Generalized estimating equations, which account for any dependency between paired eyes, were used to obtain the standard errors in order to compute the confidence intervals for all measures of accuracy. Generalized estimating equations were also used to determine the statistical significance of the difference between two measures (via odds ratios).

RESULTS

Between August 4, 1999, and February 23, 2001, 254 diabetic patients with at least one eligible eye were enrolled into the study. Table 1 summarizes the demographic data for all enrolled study participants as well as the demographics for both DVRI camera resolution groups A (151 patients) and B (103 patients). The overall mean age of this predominantly male population was 67.5 years. Caucasian Americans accounted for 93.7% of the study patients, African Americans 3.5%, Hispanic Americans 2%, and Native Americans 0.8%. Other ethnic groups were not represented. This demographic profile is typical of the population served by the Minneapolis Veterans Affairs Medical Center. The demographic data for groups A and B were similar.

The mean duration of diabetes in the study population was 12.4 years (range, 0 to 58). Table 2, summarizing the pertinent diabetic history of study patients, shows the distribution of diabetes duration by intervals. Oral hypoglycemic medication alone was used by 47.4%, insulin alone by 28.9%, oral agents combined with insulin by 20.9%, and diet by only 2.8%. The mean level of the most recent glycosylated hemoglobin was 9.76 (range, 5.8 to 18.5). The diabetic histories of groups A and B were similar.

Table 3 describes the ocular characteristics of the study patients. Spectacle correction was worn by 50.6% of patients. Using the patient’s spectacle correction if present, a visual acuity of 20/30 or better was measured in 67.6% of all eyes. A visual acuity of 20/40 or worse was measured in 32.4% of study eyes. The mean intraocular pressure was 15.4 mm Hg. The mean undilated pupil size was 3.9 mm; after dilation it was 7.3 mm. The ocular characteristics of groups A and B were similar.

Of the 508 eligible study eyes, 489 (96.3%) were able to be graded using the standard stereoscopic seven-field ETDRS photographs. The distribution of eyes by disease severity, as determined by the grading of the ETDRS photographs, is shown in Table 4. An ETDRS level of 10 or 14/15, indicating absent or questionable diabetic retinopathy, was detected in 43.1% of eyes. An ETDRS level of 20 or greater, indicating definite diabetic retinopathy, was detected in 53.1%. Nineteen eyes (3.7%) were unable to be graded.

Tables 5A and 5B show the distribution of disease severity for groups A and B, respectively, by individual patient. The disease severity recorded for a patient equals the maximum severity present in either eye. For group A, 41.0% of patients had absent or questionable diabetic retinopathy and 58.3% had an ETDRS level of 20 or greater in their most severe eye. For group B, 28.1% had absent or questionable retinopathy and 71.8% had an ETDRS level of 20 or greater in their worse eye. There is some evidence that the group B patients presented, on average, with greater severity of diabetic retinopathy than the group A patients. Therefore, the differences in each of the accuracy measures between the two groups were adjusted for disease severity, and these adjusted differences were tested for significance. The increase in sensitivity from the low- to the high-resolution group was borderline significant (P = .09); however, none of the other measures exhibit a significant change.

Table 6 shows the participation data of all enrolled patients. Because the ETDRS photographs served as the primary “gold standard,” the DVRI images taken in the 19 patients with ungradable ETDRS photographs (even if the quality was adequate) were not used in the accuracy or comparison analyses. One patient’s pupils were dilated prior to undilated DVRI, so 488 eyes were included in the undilated DVRI analyses. Approximately 60% of eyes were imaged with the lower-pixel-resolution (640 × 480) DVRI camera (group A), the remaining 40% with the higher-pixel-resolution (800 × 600) camera (group B).

To compare the “clinical gold standard” to the previously validated ETDRS “gold standard,” estimates of accuracy of the dilated clinical examination were calculated as shown in Table 7. For all eyes studied, including both groups A and B, the dilated clinical examination had a sensitivity of 0.73 and a specificity of 0.91. The positive
### TABLE 1. DEMOGRAPHIC DATA FOR STUDY PATIENTS

<table>
<thead>
<tr>
<th>DEMOGRAPHICS</th>
<th>OVERALL STUDY (n = 254)</th>
<th>GROUP A (LOW-RESOLUTION DVRI) (n = 151)</th>
<th>GROUP B (LOW-RESOLUTION DVRI) (n = 103)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
</tr>
<tr>
<td>&lt;50</td>
<td>17 (6.7)</td>
<td>11 (7.3)</td>
<td>6 (5.8)</td>
</tr>
<tr>
<td>50-59</td>
<td>50 (19.7)</td>
<td>25 (16.6)</td>
<td>25 (24.3)</td>
</tr>
<tr>
<td>60-69</td>
<td>78 (30.7)</td>
<td>46 (30.4)</td>
<td>32 (31.1)</td>
</tr>
<tr>
<td>70-79</td>
<td>82 (32.3)</td>
<td>49 (32.4)</td>
<td>33 (32.0)</td>
</tr>
<tr>
<td>80-89</td>
<td>27 (10.6)</td>
<td>20 (13.3)</td>
<td>7 (6.8)</td>
</tr>
<tr>
<td>Mean age (yr)</td>
<td>67.5</td>
<td>67.9</td>
<td>66.6</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>250 (98.4)</td>
<td>149 (98.7)</td>
<td>101 (98.1)</td>
</tr>
<tr>
<td>Female</td>
<td>4 (1.6)</td>
<td>2 (1.3)</td>
<td>2 (1.9)</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>9 (3.5)</td>
<td>6 (4.0)</td>
<td>3 (2.9)</td>
</tr>
<tr>
<td>Native American</td>
<td>2 (0.8)</td>
<td>1 (0.7)</td>
<td>1 (1.0)</td>
</tr>
<tr>
<td>Asian American</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Caucasian American</td>
<td>238 (93.7)</td>
<td>141 (93.4)</td>
<td>97 (94.2)</td>
</tr>
<tr>
<td>Hispanic American</td>
<td>5 (2.0)</td>
<td>3 (2.0)</td>
<td>2 (1.9)</td>
</tr>
<tr>
<td>Other</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
</tbody>
</table>

DVRI, digital-video retinal imaging.

### TABLE 2. DIABETIC MEDICAL HISTORY OF STUDY PATIENTS

<table>
<thead>
<tr>
<th>HISTORY ITEM</th>
<th>OVERALL STUDY (n = 241)*</th>
<th>GROUP A (LOW-RESOLUTION DVRI) (n = 141)</th>
<th>GROUP B (HIGH-RESOLUTION DVRI) (n = 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of diabetes</td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
</tr>
<tr>
<td>&lt;5</td>
<td>70 (29.0)</td>
<td>51 (36.2)</td>
<td>19 (19.0)</td>
</tr>
<tr>
<td>5-10</td>
<td>56 (23.2)</td>
<td>35 (24.8)</td>
<td>21 (21.0)</td>
</tr>
<tr>
<td>10-15</td>
<td>46 (19.1)</td>
<td>19 (13.5)</td>
<td>27 (27.0)</td>
</tr>
<tr>
<td>15-20</td>
<td>31 (12.9)</td>
<td>19 (13.5)</td>
<td>12 (12.0)</td>
</tr>
<tr>
<td>20-25</td>
<td>18 (7.5)</td>
<td>9 (6.4)</td>
<td>9 (9.0)</td>
</tr>
<tr>
<td>25-30</td>
<td>8 (3.3)</td>
<td>3 (2.1)</td>
<td>5 (5.0)</td>
</tr>
<tr>
<td>&gt;30</td>
<td>12 (5.0)</td>
<td>5 (3.6)</td>
<td>7 (7.0)</td>
</tr>
<tr>
<td>Mean duration (yr)</td>
<td>12.4</td>
<td>11.2</td>
<td>14.0</td>
</tr>
<tr>
<td>Range duration (yr)</td>
<td>(0 - 58)</td>
<td>(0 - 58)</td>
<td>(1 - 51)</td>
</tr>
<tr>
<td>Type of diabetes treatment</td>
<td>(n = 249)†</td>
<td>(n = 146)</td>
<td>(n = 103)</td>
</tr>
<tr>
<td>Diet controlled</td>
<td>7 (2.8)</td>
<td>3 (2.1)</td>
<td>4 (3.9)</td>
</tr>
<tr>
<td>Oral agents</td>
<td>115 (47.4)</td>
<td>79 (54.1)</td>
<td>39 (37.9)</td>
</tr>
<tr>
<td>Insulin</td>
<td>72 (28.9)</td>
<td>31 (21.2)</td>
<td>41 (39.8)</td>
</tr>
<tr>
<td>Oral agents and insulin</td>
<td>52 (20.9)</td>
<td>33 (22.6)</td>
<td>19 (18.4)</td>
</tr>
<tr>
<td>Most recent glycosylated hemoglobin</td>
<td>(n = 235)†</td>
<td>(n = 142)</td>
<td>(n = 93)</td>
</tr>
<tr>
<td>Mean</td>
<td>9.76</td>
<td>9.6</td>
<td>10.0</td>
</tr>
<tr>
<td>Range</td>
<td>(5.8 - 18.5)</td>
<td>(5.8 - 18.5)</td>
<td>(6.1 - 17.8)</td>
</tr>
</tbody>
</table>

DVRI, digital-video retinal imaging.

*Data unavailable for 13 patients.
†Data unavailable for 5 patients.
‡Data unavailable for 10 patients.
### Table 3. Ocular Characteristics of Study Patients (by Eye)

<table>
<thead>
<tr>
<th>CHARACTERISTIC</th>
<th>OVERALL STUDY</th>
<th>GROUP A (LOW-RESOLUTION DVRI)</th>
<th>GROUP B (HIGH-RESOLUTION DVRI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spectacle correction* (by patient)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>200 (80.6)</td>
<td>121 (83.4)</td>
<td>79 (76.7)</td>
</tr>
<tr>
<td>No</td>
<td>48 (19.4)</td>
<td>24 (16.6)</td>
<td>24 (23.3)</td>
</tr>
<tr>
<td>Visual acuity†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OD: 20/30 or better</td>
<td>168 (67.5)</td>
<td>103 (68.7)</td>
<td>65 (65.7)</td>
</tr>
<tr>
<td>20/40 or worse</td>
<td>81 (32.5)</td>
<td>47 (31.3)</td>
<td>34 (34.3)</td>
</tr>
<tr>
<td>OS: 20/30 or better</td>
<td>168 (67.7)</td>
<td>101 (67.3)</td>
<td>67 (68.4)</td>
</tr>
<tr>
<td>20/40 or worse</td>
<td>80 (32.3)</td>
<td>49 (32.7)</td>
<td>31 (31.6)</td>
</tr>
<tr>
<td>Intraocular pressure‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (mm Hg)</td>
<td>15.4 mm Hg</td>
<td>15.4 mm Hg</td>
<td>15.2 mm Hg</td>
</tr>
<tr>
<td>Range (0 - 27)</td>
<td>(0 - 27)</td>
<td>(0 - 27)</td>
<td>(3 - 25)</td>
</tr>
<tr>
<td>Pupil size (scotopic)§</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (mm)</td>
<td>3.9 mm</td>
<td>3.9 mm</td>
<td>3.85 mm</td>
</tr>
<tr>
<td>Range (2.0 - 6.0)</td>
<td>(2.0 - 6.0)</td>
<td>(2.0 - 6.0)</td>
<td>(2.0 - 6.0)</td>
</tr>
<tr>
<td>Pupil size (dilated)¶</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>7.3 mm</td>
<td>7.8 mm</td>
<td>6.6 mm</td>
</tr>
<tr>
<td>Range (4.0 - 9.0)</td>
<td>(4.0 - 9.0)</td>
<td>(4.0 - 9.0)</td>
<td>(4.0 - 8.0)</td>
</tr>
</tbody>
</table>

DVRI, digital-video retinal imaging.

*Data unavailable for 6 patients.
†Data unavailable for 5 patients.
‡Data unavailable for 9 eyes.
§Data unavailable for 38 eyes.
¶Data unavailable for 42 eyes.

### Table 4. ETDRS Retinopathy Severity Scale for Overall Study (Individual Eyes)

<table>
<thead>
<tr>
<th>LEVEL</th>
<th>SEVERITY</th>
<th>NO.</th>
<th>PERCENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>DR absent</td>
<td>190</td>
<td>37.4</td>
</tr>
<tr>
<td>14/15</td>
<td>DR questionable</td>
<td>29</td>
<td>5.7</td>
</tr>
<tr>
<td>20</td>
<td>Microaneurysms only</td>
<td>56</td>
<td>11.0</td>
</tr>
<tr>
<td>35</td>
<td>Mild NPDR</td>
<td>119</td>
<td>23.4</td>
</tr>
<tr>
<td>43</td>
<td>Moderate NPDR</td>
<td>53</td>
<td>10.4</td>
</tr>
<tr>
<td>47</td>
<td>Moderately severe NPDR</td>
<td>17</td>
<td>3.3</td>
</tr>
<tr>
<td>53</td>
<td>Severe NPDR</td>
<td>6</td>
<td>1.2</td>
</tr>
<tr>
<td>60</td>
<td>Mild PDR</td>
<td>5</td>
<td>1.0</td>
</tr>
<tr>
<td>66</td>
<td>Moderate PDR</td>
<td>9</td>
<td>1.8</td>
</tr>
<tr>
<td>70</td>
<td>High-risk PDR</td>
<td>5</td>
<td>1.0</td>
</tr>
<tr>
<td>90</td>
<td>Cannot determine</td>
<td>19</td>
<td>3.7</td>
</tr>
</tbody>
</table>

Total: 508 (100%)

ETDRS, Early Treatment Diabetic Retinopathy Study; DR, diabetic retinopathy; NPDR, nonproliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy.
predictive value, the proportion of positive test results
that were correct (true positives), was 0.91.

The estimates of the accuracy of DVRI, including
sensitivity, false-negative rate, specificity, false-positive
rate, positive predictive value, negative predictive value,
and overall efficiency using the ETDRS photography as
the “gold standard” are presented in Table 8. A compari-
son of accuracy by dilation status shows that for most
measures, including specificity, dilation significantly
improved the accuracy for both groups A and B. The
sensitivities of group A (low-resolution) and group B
(high-resolution) DVRI, for both undilated and dilated
eyes, are shown graphically in Figure 4. Using the
ETDRS “gold standard,” the only method of retinopathy
evaluation with a sensitivity above 0.8 (the recommended
minimum sensitivity level by the British Diabetic
Association Working Group) was the high-resolution
DVRI through a dilated pupil. This method also demon-

### Table 5A. ETDRS Retinopathy Severity Scale for Group A (Individual Patients*)

<table>
<thead>
<tr>
<th>LEVEL</th>
<th>SEVERITY</th>
<th>NO.</th>
<th>PERCENT</th>
<th>NO.</th>
<th>PERCENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>DR absent</td>
<td>47</td>
<td>31.1</td>
<td>62</td>
<td>41.0</td>
</tr>
<tr>
<td>14/15</td>
<td>DR questionable</td>
<td>15</td>
<td>9.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Microaneurysms only</td>
<td>21</td>
<td>13.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>Mild NPDR</td>
<td>32</td>
<td>21.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>43</td>
<td>Moderate NPDR</td>
<td>18</td>
<td>11.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>47</td>
<td>Moderately severe NPDR</td>
<td>8</td>
<td>5.3</td>
<td>88</td>
<td>58.3</td>
</tr>
<tr>
<td>53</td>
<td>Severe NPDR</td>
<td>1</td>
<td>0.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>Mild PDR</td>
<td>2</td>
<td>1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>66</td>
<td>Moderate PDR</td>
<td>4</td>
<td>2.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>High-risk PDR</td>
<td>2</td>
<td>1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>Cannot determine</td>
<td>1</td>
<td>0.7</td>
<td>1</td>
<td>0.7</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>151</td>
<td>100</td>
<td>151</td>
<td>100</td>
</tr>
</tbody>
</table>

ETDRS, Early Treatment Diabetic Retinopathy Study; DR, diabetic retinopathy; NPDR, nonproliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy.

*The disease severity recorded for a patient equals the maximum severity present in either eye.

### Table 5B. ETDRS Retinopathy Severity Scale for Group B (Individual Patients*)

<table>
<thead>
<tr>
<th>LEVEL</th>
<th>SEVERITY</th>
<th>NO.</th>
<th>PERCENT</th>
<th>NO.</th>
<th>PERCENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>DR absent</td>
<td>26</td>
<td>25.2</td>
<td>29</td>
<td>28.1</td>
</tr>
<tr>
<td>14/15</td>
<td>DR questionable</td>
<td>3</td>
<td>2.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Microaneurysms only</td>
<td>9</td>
<td>8.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>Mild NPDR</td>
<td>37</td>
<td>35.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>43</td>
<td>Moderate NPDR</td>
<td>16</td>
<td>15.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>47</td>
<td>Moderately severe NPDR</td>
<td>3</td>
<td>2.9</td>
<td>74</td>
<td>71.8</td>
</tr>
<tr>
<td>53</td>
<td>Severe NPDR</td>
<td>3</td>
<td>2.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>Mild PDR</td>
<td>2</td>
<td>1.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>66</td>
<td>Moderate PDR</td>
<td>2</td>
<td>1.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>High-risk PDR</td>
<td>2</td>
<td>1.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>Cannot determine</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>103</td>
<td>100</td>
<td>103</td>
<td>100</td>
</tr>
</tbody>
</table>

ETDRS, Early Treatment Diabetic Retinopathy Study; DR, diabetic retinopathy; NPDR, nonproliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy.

*The disease severity recorded for a patient equals the maximum severity present in either eye.
### TABLE 6. PARTICIPATION DATA OF ENROLLED PATIENTS

<table>
<thead>
<tr>
<th>STEPS IN STUDY DESIGN</th>
<th>NO. OF PATIENTS</th>
<th>% OF POPULATION</th>
<th>NO. OF EYES</th>
<th>% OF EYES</th>
<th>% OF EYES WITH GRADABLE ETDRS PHOTOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eligible participants with completed informed consent</td>
<td>254</td>
<td>100</td>
<td>508</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Participants with gradable stereoscopic 7-field ETDRS color photography</td>
<td>253</td>
<td>99.6</td>
<td>489</td>
<td>96.3</td>
<td>100</td>
</tr>
<tr>
<td>Participants with completed dilated clinical examination and gradable ETDRS photos</td>
<td>253</td>
<td>99.6</td>
<td>489</td>
<td>96.3</td>
<td>100</td>
</tr>
<tr>
<td>Participants with undilated DVRI and gradable ETDRS photos</td>
<td>253</td>
<td>99.6</td>
<td>488</td>
<td>96.1</td>
<td>99</td>
</tr>
<tr>
<td>Participants with dilated DVRI and gradable ETDRS photos</td>
<td>253</td>
<td>99.6</td>
<td>489</td>
<td>96.3</td>
<td>100</td>
</tr>
<tr>
<td>Participants with low-resolution DVRI and gradable ETDRS photos (group A)</td>
<td>157</td>
<td>61.8</td>
<td>292</td>
<td>57.5</td>
<td>59.7</td>
</tr>
<tr>
<td>Participants with high-resolution DVRI and gradable ETDRS photos (group B)</td>
<td>96</td>
<td>37.8</td>
<td>197</td>
<td>38.8</td>
<td>40.3</td>
</tr>
</tbody>
</table>

ETDRS, Early Treatment Diabetic Retinopathy Study; DVRI, digital-video retinal imaging.

### TABLE 7. ESTIMATES OF ACCURACY OF DCE USING ETDRS-P AS “GOLD STANDARD”

<table>
<thead>
<tr>
<th>METHOD</th>
<th>SENS</th>
<th>FALSE-</th>
<th>SPEC</th>
<th>FALSE+</th>
<th>PPV</th>
<th>NPV</th>
<th>EFF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilated clinical examination</td>
<td>0.73</td>
<td>0.27</td>
<td>0.91</td>
<td>0.09</td>
<td>0.91</td>
<td>0.73</td>
<td>0.81</td>
</tr>
</tbody>
</table>

DCE, dilated clinical examination; ETDRS-P, Early Treatment Diabetic Retinopathy Study Photography; Sens, sensitivity; False-, false-negative rate; Spec, specificity; False+, false-positive rate; PPV, positive predictive value; NPV, negative predictive value; EFF, efficiency.

### TABLE 8. ESTIMATES OF ACCURACY OF DVRI SCREENING USING ETDRS-P AS “GOLD STANDARD” AND COMPARISON OF ACCURACY BY PUPILLARY DILATION STATUS

<table>
<thead>
<tr>
<th>METHOD</th>
<th>SENS</th>
<th>FALSE-</th>
<th>SPEC</th>
<th>FALSE+</th>
<th>PPV</th>
<th>NPV</th>
<th>EFF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (low-resolution DVRI)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Undilated</td>
<td>0.66</td>
<td>0.34</td>
<td>0.66</td>
<td>0.34</td>
<td>0.66</td>
<td>0.66</td>
<td>0.66</td>
</tr>
<tr>
<td>Dilated</td>
<td>0.66</td>
<td>0.34</td>
<td>0.86</td>
<td>0.14</td>
<td>0.82</td>
<td>0.71</td>
<td>0.76</td>
</tr>
<tr>
<td>P value</td>
<td>NS</td>
<td>NS</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Group B (high-resolution DVRI)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Undilated</td>
<td>0.76</td>
<td>0.24</td>
<td>0.45</td>
<td>0.55</td>
<td>0.72</td>
<td>0.51</td>
<td>0.65</td>
</tr>
<tr>
<td>Dilated</td>
<td>0.85</td>
<td>0.15</td>
<td>0.81</td>
<td>0.20</td>
<td>0.89</td>
<td>0.75</td>
<td>0.83</td>
</tr>
<tr>
<td>P value</td>
<td>.04</td>
<td>.04</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
</tr>
</tbody>
</table>

DVRI, digital-video retinal imaging; ETDRS-P, Early Treatment Diabetic Retinopathy Study Photography; Sens, sensitivity; False-, false-negative rate; Spec, specificity; False+, false-positive rate; PPV, positive predictive value; NPV, negative predictive value; EFF, efficiency.
Lawrence demonstrated the highest efficiency (0.83), a general measure of overall accuracy.

Table 9 shows the estimates of the accuracy, using the dilated clinical examination as the “clinical gold standard.” A comparison of accuracy by dilation status also shows that for most measures, including specificity, positive predictive value, and efficiency, dilation significantly improved the accuracy for both groups A and B. Using the “clinical gold standard,” only high-resolution DVRI (group B), through both dilated and undilated pupils, demonstrated a sensitivity level above 0.8. Group A, however, approached this level (0.79 and 0.80 for undilated and dilated eyes, respectively).

Generalized estimating equations linear regression models to compare sensitivities and efficiencies of DRVI groups A and B, to the dilated clinical examination, using the ETDRS photography as the “gold standard,” are summarized in Table 10. Images from the undilated pupils for both groups A and B performed significantly worse than a clinical examination by an ophthalmologist with respect to efficiency (P < .01). In other words, a dilated clinical examination was more accurate at detecting diabetic retinopathy than nonmydriatic DVRI of high or low resolution. The accuracy of evaluation of dilated images of group B (dilated, low-resolution DVRI) was similar to (or perhaps slightly worse than) that of a clinical examination (P values = NS) with respect to sensitivity and efficiency. Group B (high-resolution DVRI) through dilated pupils showed an absolute sensitivity score greater than the clinical examination, but the difference did not reach significance. These comparisons show that dilated DVRI performs as well as, or slightly better than, a clinical examination at detecting patients with diabetic retinopathy.

Table 11 and Figure 5 show the sensitivity estimates by increasing levels of disease severity. As the diabetic retinopathy severity increases, the sensitivities of both DVRI systems as well as the dilated clinical examination improve. All screening methods had excellent levels of sensitivity (sens > 0.9) for the more severe (sight-threatening) levels of retinopathy. Dilated group B (high-resolution imaging) performed best at all levels of severity and, in fact, reached a sensitivity of 1.0 for all ETDRS levels ≥43.

Pupillary size also contributed to the ability to grade the undilated DVRI images. Table 12 shows that in all images taken through an undilated pupil, there was a statistically significant difference between the size of the

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**TABLE 9. ESTIMATES OF ACCURACY OF DVRI SCREENING USING DCE AS “GOLD STANDARD” AND COMPARISON OF ACCURACY BY PUPILARY DILATION STATUS**

<table>
<thead>
<tr>
<th>METHOD</th>
<th>SENS</th>
<th>FALSE-</th>
<th>SPEC</th>
<th>FALSE+</th>
<th>PPV</th>
<th>NPV</th>
<th>EFF</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group A (low-resolution DVRI)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Undilated</td>
<td>0.79</td>
<td>0.21</td>
<td>0.68</td>
<td>0.32</td>
<td>0.61</td>
<td>0.84</td>
<td>0.72</td>
</tr>
<tr>
<td>Dilated</td>
<td>0.80</td>
<td>0.20</td>
<td>0.55</td>
<td>0.15</td>
<td>0.78</td>
<td>0.57</td>
<td>0.83</td>
</tr>
<tr>
<td><em>P value</em></td>
<td>NS</td>
<td>NS</td>
<td>.001</td>
<td>.001</td>
<td>.001</td>
<td>NS</td>
<td>.001</td>
</tr>
<tr>
<td><strong>Group B (high-resolution DVRI)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Undilated</td>
<td>0.81</td>
<td>0.19</td>
<td>0.45</td>
<td>0.55</td>
<td>0.66</td>
<td>0.66</td>
<td>0.65</td>
</tr>
<tr>
<td>Dilated</td>
<td>0.87</td>
<td>0.13</td>
<td>0.69</td>
<td>0.31</td>
<td>0.78</td>
<td>0.81</td>
<td>0.79</td>
</tr>
<tr>
<td><em>P value</em></td>
<td>NS</td>
<td>NS</td>
<td>.001</td>
<td>.001</td>
<td>.005</td>
<td>.012</td>
<td>.001</td>
</tr>
</tbody>
</table>

DVRI, digital-video retinal imaging; DCE, dilated clinical examination; Sens, sensitivity; False-, false-negative rate; Spec, specificity; False+, false-positive rate; PPV, positive predictive value; NPV, negative predictive value; EFF, efficiency.
pupil in gradable versus ungradable DVRI images. The mean pupil size was 4.14 mm in diameter for gradable images, and 3.28 mm for ungradable images ($P < .0001$). As expected, this effect was not seen in the images taken through dilated pupils, because the pupil was of adequate size.

Comparisons of accuracy by low- versus high-resolution DVRI systems (group A versus group B) showed that for dilated eyes, high-resolution DVRI (group B) was significantly more sensitive than the low-resolution DVRI (group A). All other estimates of accuracy, as shown in Table 13, did not show a statistically significant difference.

Table 14 shows the results of an analysis to ascertain whether there was a difference over time in the sensitivity estimates. As time, and the number of patients, progressed (each successive tertile), there was a concomitant increase in the sensitivity of DVRI to detect retinopathy. This is likely due to a “learning effect” experienced by the photographer as well as the ophthalmologist readers.

Thirteen ophthalmologists participated in the DVRI...
Lawrence

evaluations using the computer reading station, including two retina specialists, six board certified staff ophthalmologists, and five PGY-4 resident physicians in ophthalmology. Estimates of accuracy, including sensitivity, specificity, positive predictive value, negative predictive value, and efficiency, were calculated for each of the three groups of physicians. Bonferroni 95% confidence intervals were computed for each of the measures. None of the measures differed significantly by physician status. Color contrast enhancement was used by the ophthalmologist to aid in the on-screen evaluation for 83.5% of the eyes.

**DISCUSSION**

The ETDRS Final Retinopathy Severity Scale is the only validated reference standard for the detection and staging of diabetic retinopathy. For this reason, the ETDRS grading protocol has been referred to by several investigators as the “gold standard” for the accurate detection of diabetic retinopathy. The clinical application of ETDRS photographs for periodic retinal evaluations of the diabetic population, however, is impractical, and clinical examination of the retina through a dilated pupil is the mainstay of screening in the United States.

It is important to compare new imaging technology to a validated “gold standard,” but it is also of value to compare it to “standard clinical practice,” hence the use of the “clinical gold standard” in this analysis. Furthermore, the dichotomous grading system used herein (“disease” versus “no disease”) does not require the level of analysis required for the full ETDRS Final Retinopathy Severity Scale. For the determination of presence or absence of disease, it seems justified to use an ophthalmologist’s examination, which can evaluate the entire retina, as a reasonable “clinical gold standard.” (ETDRS photographic protocol misses some retina nasal to the optic disk.)

Because ETDRS photography has been validated in many studies, the comparative analyses performed for this data used the accuracy estimates derived using ETDRS photographic evaluation as the “gold standard.” Sensitivities and negative predictive values were slightly higher for all methods using the “clinical gold standard” instead of the ETDRS “gold standard,” but all estimates of accuracy were similar, suggesting that the “clinical gold standard” is an appropriate comparison.

The results of this study suggest that in the presence of an adequately sized pupil, DVRI is an accurate method for detecting diabetic retinopathy. Most, if not all, commercially available DVRI systems, however, are marketed as “nonmydriatic.” An exhaustive review of the published literature revealed no peer-reviewed report of a “nonmydriatic” DVRI system that achieved a sensitivity of >0.80, compared to the accepted ETDRS photography “gold standard,” across all levels of diabetic retinopathy.

The inverse relationship between age and pupil diameter is also a consideration because almost 29% of the population 60 years and older in the United States has diabetes. The mean age in this study was 67.5 years, significantly older than patients reported in other studies of nonmydriatic systems (48 years in the Bursell study; 50 to 59 years in the Lin study). This study suggests that, given current technology, it may be necessary to routinely dilate patients when using a “nonmydriatic” DVRI system, especially in older populations.

The data herein also suggest that the higher-resolution imaging system may increase the accuracy of screening, especially the sensitivity estimates. It should be noted that the “higher resolution” system used in the reported study is still relatively low in resolution, compared to some systems. Fransen and colleagues report an 1,152×1,152-pixel resolution imaging system. “More” may not be “better,” however, when it is applied to digital screening techniques. Transmitting, storing, and archiving large amounts of digital data have associated costs. It will be important for future studies to determine the optimal pixel resolution to maximize accuracy in the detection of disease and minimize data management issues.

Stereoscopic digital imaging technology has been evaluated by Bursell and coworkers and Fransen and coworkers. It is unclear whether the additional stereoscopic capabilities are helpful in increasing the accuracy of the systems. Bursell and coworkers reported that the sensitivity of the Joslin Vision Network (JVN) system in
detecting any macular edema (using stereoscopic image pairs) was 0.62 and in detecting clinically significant macular edema was only 0.27. Sensitivities in detecting diabetic retinopathy ranged from 0.40 for severe nonproliferative retinopathy to 0.89 for proliferative retinopathy. Fransen and coworkers (also using stereoscopic image pairs) reported a 0.88 sensitivity of the Inoveon DR-3DT system in the detection of macular edema and an overall sensitivity of 0.98 in detecting any retinopathy, including macular edema.

The DVRI systems studied here did not use stereoscopic image pairs. Any suspected macular edema (i.e., presence of hard exudate, its surrogate marker) was accounted for because it qualified the eye as being in the “disease detected” category. With the use of the higher-resolution system through a dilated pupil, the sensitivity of detecting disease in our study (0.85 using the ETDRS-P “gold standard,” 0.87 using the dilated clinical examination “clinical gold standard”) favors comparably with sensitivities reported by Bursell and coworkers, but was less than those reported by Fransen and coworkers.

Only two imaging systems were evaluated in this study, both manufactured by the same vendor. The systems were chosen based on cost, ease of use, pixel resolution, DICOM compliance, potential digital interface compatibility with Veterans Affairs computerized medical record system, and vendor approvals with the Department of Veterans Affairs. Clinical accuracy using
instruments manufactured by other vendors with equivalent pixel resolution may vary due to optical parameters of each system and software characteristics. Variations that might impact clinical accuracy include differences in optical capture performance with respect to pupillary diameter and ambient lighting.

The literature documents that there are many flaws in methodology, as well as inconsistencies in the use of reference (gold) standards and in the reporting of statistical tests of accuracy. All of this calls into question the usefulness of the existing literature in guiding clinical practice. The study described herein indicates that DVRI is clinically effective, but the data are still too sparse to reliably answer questions about pupil size, optimal resolution, and stereoscopic imaging.

An even greater concern is whether DVRI systems are cost-effective. DVRI has great potential advantages over traditional clinical examination and traditional film-based photographic techniques. These include lower cost, ease of use of the equipment, ability for providers to share data, greater efficiency of physician time, ease of integration into computerized medical records, and maybe most importantly, the potential to access patients remotely. These potential advantages are alluring but should not deter rigorous assessment of new technology.

CONCLUSIONS

Based on assessments of clinical accuracy, the Topcon TRC-NW6S, 500×600-pixel-resolution digital-video retinal imaging system offers an accurate method of screening for diabetic retinopathy, provided there is adequate pupillary dilation and three imaging fields are analyzed. For an elderly population, the Topcon TRC-NW5SF, 640×480-pixel-resolution digital-video imaging system may be inadequate for accurate diabetic retinopathy screening. Digital-video retinal imaging, because of its ability to utilize telemedicine technology, may help facilitate the implementation of widespread programs, which will result in improved retinal screening and less visual impairment in diabetic populations.

ACKNOWLEDGMENTS

George Bresnick, MD, contributed to the study design and spearheaded the preparation of the manual of operations for the project; Hannah Rubin, MD, and Dave Nelson, PhD, contributed to study design and methodology; Gary Michalec, BS, CRA, coordinated the project and performed the photography; Sean Nugent, BS, entered and verified the data and helped run the statistical analyses; Joe Grill, MS, managed the data and performed most of the statistical analyses; Travis Hanstad typed the manuscript; and Donald Doughman, MD, provided advice, support, and encouragement. The following ophthalmologists provided the clinical examinations and evaluated the DVRI images: A. Ali, MD, P. Arny, MD, A. Bhavsar, MD, J. Dvorak, MD, D. Eilers, MD, J. Foley, MD, S. Murali, MD, D. Park, MD, A. Pathak, MD, P. Rath, MD, J. Rice, MD, M. Szczepanski, MD, J. Sinclair, MD, C. Skolnick, MD, J. Stephens, MD, D. Tani, MD, S. Uttley, MD, and S. Wang, MD.

REFERENCES

The Accuracy of Digital-Video Retinal Imaging to Screen for Diabetic Retinopathy


The Accuracy of Digital-Video Retinal Imaging to Screen for Diabetic Retinopathy


PHOTOREFRACTIVE KERATECTOMY FOR ANISOMETROPIC AMBLYOPIA IN CHILDREN

BY Evelyn A. Paysse MD

ABSTRACT

Purpose: To assess the safety and efficacy of photorefractive keratectomy (PRK) in children with anisometropic amblyopia and to define the characteristics of children who may be candidates for PRK.

Methods: This thesis comprises four parts: (1) a retrospective analysis of risk factors predictive of amblyopia treatment failure in 104 children, (2) a prospective study of pachymetry in 198 eyes of 108 children, (3) development and implementation of a protocol to perform PRK under general anesthesia, and (4) a prospective interventional case-comparison study of PRK in 11 noncompliant children with anisometric amblyopia to evaluate safety and long-term outcomes. Compliant and noncompliant children with anisometric amblyopia were analyzed as controls.

Results: Factors associated with conventional anisometric amblyopia treatment failure were poor compliance ($P = .004$), age 6 years or older ($P = .01$), astigmatism $\geq 1.5$ diopters ($P = .0002$), and initial visual acuity of 20/200 or worse ($P = .02$). Central and paracentral pachymetry measurements were similar to published adult values. The general anesthesia protocol was efficient, and the laser functioned properly in all cases. All children did well with no anesthesia-related or treatment-related complications. Two years following PRK, the mean reduction in refractive error was 9.7 $\pm$ 2.6 diopters for myopes ($P = .0001$) and 3.4 $\pm$ 1.3 diopters for hyperopes ($P = .001$). The cycloplegic refractive error in 9 of 11 treated eyes was within 3 diopters of that in the fellow eye. Uncorrected visual acuity in the amblyopic eye improved by $\geq 2$ lines in seven of nine children; best-corrected visual acuity improved by $\geq 2$ lines in six of nine children. Stereopsis improved in five of nine children. The mean visual acuity of the PRK patients at last follow-up was significantly better than that of noncompliant controls ($P = .003$). The safety and efficacy indices for PRK in this study were 1.24 and 1.12, respectively.

Conclusions: Photorefractive keratectomy can be safely performed in children with anisometric amblyopia. Visual acuity and stereopsis improved in most eyes, even in older children. Photorefractive keratectomy may have an important role in the management of anisometric amblyopia in noncompliant children.


HYPOTHESIS

Photorefractive keratectomy for anisometric amblyopia in children can be safely performed and results in better uncorrected and best-corrected visual acuity and stereopsis in children who are poorly compliant with standard refractive correction and other amblyopia treatment measures.

INTRODUCTION

Amblyopia

The word “amblyopia,” derived from Greek, literally means “dullness of vision.” Ophthalmologic examination demonstrates reduced visual acuity that is not fully explained by obvious aberrations of the retina or optic nerve. Von Graefe stated over a century ago that amblyopia was the condition in which the observer sees nothing and the patient sees very little.1

Amblyopia affects approximately 2% to 5% of the American population2-4 and is the most frequent cause of unilateral visual impairment in children and young adults in the United States and Western Europe.7,8 Vision screening is recommended between the ages of 3 and 5 years and is usually done in schools or by primary care physicians.14 Amblyopia is most often detected during this routine vision screening.14 Despite these facts, adequate screening is believed to occur in only 21% of preschool children in the United States.15,16 Treatment of amblyopia is less likely to be successful in children older than 6 years of age.17,18

Anisometropia is the most common cause of amblyopia and occurs because of uncorrected unequal refrac-
though uncommon, the risk of microbial keratitis, higher correction in place are common following lens loss. And Significant lapses of time without proper refractive remove, loss is frequent, and the costs are relatively high. Contact lenses are often difficult for parents to insert and issue of aniseikonia for most patients. Unfortunately, anisometropia. Contact lenses essentially eliminate the appearance of the eyes through such spectacles. Children often complain of a noticeable size difference in the hyperopic or myopic lens, respectively. Parents and chil-

tive error between fellow eyes. Uncorrected anisometropia produces image blur in one eye (form vision deprivation) and/or abnormal binocular interaction by producing dissimilar images on the fovea of each eye. Anisometropic amblyopia is often detected later than other forms of amblyopia because vision is generally good in the fellow eye, the eyes are typically orthotropic, and the child functions well with the use of the sound eye. The level of anisometropia required to cause amblyopia has been well studied. In general, anisonyopia of more than 2 diopters, anisohyperopia of more than 1 diopter, and anisoastigmatism of more than 1.5 diopters may result in amblyopia. A direct relationship between the degree of anisometropia and the severity of amblyopia has been reported. Studies of anisometropic amblyopia indicate a prevalence of amblyopia of 100% in hyperopes with 4.0 diopters of uncorrected anisometropia and in myopes with 6.0 diopters of uncorrected anisometropia. Anisometropia of more than about 4 diopters is also believed to portend a worse prognosis for successful visual outcome with traditional amblyopia therapy.

**Treatment of Anisometropic Amblyopia**

Traditional therapy for anisometropic amblyopia includes refractive correction with spectacles or contact lenses, minimization of aniseikonia with contact lenses, and amblyopia management with occlusion therapy and/or pharmacologic and/or optical penalization of the sound eye. Despite this seemingly simple treatment strategy, traditional treatment is often problematic and unsuccessful. Spectacle correction of significant anisometropia produces aniseikonia. Aniseikonia of more than 5% to 6% (typically present with 3 or more diopters of anisometropia) cannot be readily fused. Suppression of the amblyopic eye occurs, often limiting the effectiveness of the amblyopia therapy. An occasional child will experience diplopia due to the aniseikonia. Thus, glasses for moderate to severe anisometropia are commonly not well tolerated. Spectacles for anisometropia of more than 2 to 3 diopters are also cosmetically problematic because of the differential magnification or minification effect of the hyperopic or myopic lens, respectively. Parents and children often complain of a noticeable size difference in the appearance of the eyes through such spectacles.

Contact lenses are an alternative treatment for anisometropia. Contact lenses essentially eliminate the issue of aniseikonia for most patients. Unfortunately, contact lens use in children is difficult for other reasons. Contact lenses are often difficult for parents to insert and remove, loss is frequent, and the costs are relatively high. Significant lapses of time without proper refractive correction in place are common following lens loss. And though uncommon, the risk of microbial keratitis, higher in contact lens wearers, may put the sound eye at risk. Children, who are usually less hygienic than adults, may be at higher risk for this complication than adult contact lens wearers.

Although refractive correction is sometimes all that is needed to correct anisometropic amblyopia, additional amblyopia treatment is frequently required. Occlusion therapy, pharmacologic penalization with atropine or other cycloplegic agents, optical penalization, or all of these in combination are used in cases where refractive correction alone fails to normalize the visual acuity. Noncompliance with these treatment measures is common, especially with occlusion therapy. Disadvantages of atropine penalization include photosensitivity, anticholinergic side effects, and inability to rapidly titrate treatment. Optical penalization using a lens to blur the vision in the sound eye is an accepted treatment alternative. However, it is successful only in willing patients; uncooperative children simply remove or look around their spectacles to avoid the penalizing lens.

Significant psychosocial stress related to amblyopia therapy has been reported by amblyopic children and the families of amblyopic children during the treatment period. Even adults with a history of amblyopia treatment in childhood continue to have psychosocial difficulties related to the previous amblyopia therapy that adversely affect self-image, work, school, and friendships.

Certain neurotransmitters have been implicated in neuronal plasticity. Based on this finding, levodopa/carbidopa and citicoline, which act to enhance dopaminergic neurotransmission in the brain, have been experimentally used to treat amblyopia in adults and children. Both have been associated with some mild improvement of visual acuity that unfortunately was not sustained after discontinuing the medication.

Successful treatment of anisometropic amblyopia with traditional therapy has been reported in 48% to 82% of children. The success rate varies widely among studies, depending on the definition of success, parameters at initiation of treatment, and other factors. Flynn and associates conducted a meta-analysis of 23 studies of therapy for amblyopia that were published from 1965 to 1994; the investigators calculated an overall success rate of 67% (defined as visual acuity of 20/40 or better) for the anisometropic amblyopia subgroup treated with traditional therapy. They also found an inverse relationship between the degree of anisometropia and the final visual acuity. The greater the anisometropia, the more likely a poor visual outcome was the result. Successfully treated patients typically had less than 4 diopters of anisometropia. A direct relationship between initial and final visual acuity has also been reported.

Amblyopia will remain a major public health problem.
until new and improved treatment modalities are developed. Despite all efforts to date to treat anisometropic amblyopia, up to one third of treated children with this condition will not achieve a visual acuity of 20/40 or better (the level of acuity required to obtain an unrestricted driver's license in most states [www.lowvisioncare.com]) with available treatment. A report from the United Kingdom even questioned the efficacy of amblyopia therapy, because no controlled studies had been done in which the control group did not receive treatment. In response to this report, a recent study that included a “no treatment” control group reported that amblyopia treatment is worthwhile in children with visual acuity of less than 20/40 in the amblyopic eye. Additionally, there is a higher incidence of traumatic vision loss in the sound eye of individuals who have only one normally sighted eye, putting amblyopic patients at higher risk for bilateral visual impairment.

Amblyopia treatment is economically sound. Membreno and coworkers reported on the incremental cost-effectiveness of therapy for amblyopia and calculated a savings of $2,281 per quality-adjusted life year with amblyopia treatment. They concluded that when compared to healthcare interventions for other medical conditions, amblyopia care is highly cost-effective.

Poor compliance with treatment is commonly associated with amblyopia treatment failure. The Pediatric Eye Disease Investigator Group recently reported better compliance with atropine penalization than with occlusion therapy, though compliance remained a problem for both treatment groups. Patient compliance with any medical treatment is notoriously suboptimal. Even patients with life-threatening disorders such as asthma and organ transplant frequently fail to comply with treatment recommendations. Poor compliance may be even more severe when the patient is a child who cannot comprehend the reasons for the treatment, as is the case with amblyopia.

Given the known problems with treatment compliance, the long-lasting psychosocial issues associated with standard amblyopia therapy, and the high percentage of treatment failures with standard therapy, consideration of nontraditional treatment options for anisometropic amblyopia that are less dependent on long-term compliance is justified. Refractive surgery is a reasonable alternative to consider. Photorefractive keratectomy (PRK) and laser in situ keratomileusis (LASIK) have both been well received by adults with refractive errors. Refractive procedures that may have utility in children include PRK, LASIK, laser epithelial keratomileusis (LASEK), and possibly others.

**Refractive Surgery**

Excimer laser refractive surgery has been successfully used in the treatment of myopia, hyperopia, and astigmatism in adults. Most adult patients who undergo PRK or LASIK are satisfied with the outcome. PRK and LASIK have been the most extensively studied of the excimer laser procedures. Photorefractive keratectomy involves removing the corneal epithelium, either with the excimer laser or manually, followed by computer-guided ablation of the underlying Bowman's membrane and anterior corneal stroma. Laser in situ keratomileusis involves creating a central corneal flap composed of epithelium, Bowman's membrane, and anterior stroma. Computer-guided excimer laser ablation of the posterior corneal stroma is then performed, followed by repositioning of the corneal flap.

Advantages of LASIK over PRK include less postoperative discomfort, faster visual recovery, and maintenance of an intact Bowman's membrane. Advantages of PRK include avoidance of several serious potential complications associated with LASIK, including corneal flap loss, tear or striae, and keratectasia. An important risk of PRK reported in adult patients is temporary or permanent corneal haze. The implications of persistent or even temporary corneal haze for a child are vastly different from those for the adult because of the child's immature visual system and the risk of worsening the amblyopia from form vision deprivation. Fortunately, postoperative corneal haze typically has been mild in the few children treated with PRK thus far, provided the recommended postoperative topical steroid regimen was followed.

**Refractive Surgery in Children**

Refractive surgery in children, to date, has been applied in a haphazard fashion, without preliminary work to establish which children are most likely to benefit from treatment and to determine if there are unique characteristics of the pediatric cornea that could alter PRK treatment nomograms, intraoperative techniques, postoperative management, or all of these. Experience with other pediatric ophthalmic surgical procedures dictates that children cannot be treated merely as small adults. For example, experience with pediatric corneal transplantation, cataract surgery, and intraocular lens implantation has revealed important, often vision-threatening, differences in pediatric response to surgery compared with adult patients undergoing the same procedures. Surgical techniques for children have often required modification due to issues such as differences in corneal and scleral rigidity, elasticity of the lens capsule, and lens/vitreous characteristics. Anticipation of future eye growth must also be considered when planning and implementing eye surgery in young children. Additionally, postoperative care of the child does not usually parallel that of the adult because of...
differences in healing time, inflammatory response, cooperation, and childhood behaviors that may place the newly operated eye at increased risk for trauma. To avoid repeating serious mistakes of the past when attempting to translate accepted adult procedures to children, careful scientific evaluation of refractive surgery in children is of paramount importance.

When considering performing a procedure on a child that has been performed only on adults, one must be ever cognizant of potential complications that could occur immediately or many years after the procedure. Informed consent for pediatric PRK from the parent and assent of the child (if old enough) must include discussion and understanding of the fact that no data are available on immediately or many years after the procedure. Informed consent for pediatric PRK from the parent and assent of the child (if old enough) must include discussion and understanding of the fact that no data are available on extremely long-term outcomes in excimer laser–treated eyes. This is particularly important for a child who has potentially 70 to 80 more years to live.

Several uncontrolled studies have been published regarding PRK and LASIK in children. In total, 118 children have been included in publications of pediatric excimer refractive procedures. Most studies had fewer than seven children in them; the largest study had 27. Only one study has reported long-term results, and most studies were conducted outside of the United States. With the exception of three children in one study, all previous studies have reported only on PRK or LASIK for the treatment of anisometropic myopia or bilateral high myopia. Most studies have included only children older than 7 years, an age often considered to be less responsive to amblyopia treatment because of closure of the sensitive period of visual development. Only one study has provided data on stereopsis, and none have included a control group. More important, all previous studies have apparently been conducted without preliminary investigation of potential issues related to the pediatric eye that might alter or even eliminate refractive procedures as an option for children.

Anisometropic Amblyopia Failure Risk Factors
Knowledge about risk factors for anisometropic amblyopia treatment failure could be useful in the early identification of children who are most likely to fail conventional amblyopia therapy. More aggressive treatment and closer follow-up might be warranted to improve the chance of a successful outcome in these children. Early utilization of nonconventional treatments, including refractive surgery, might also be warranted in selected children with identifiable risk factors for failure.

Central and Paracentral Corneal Thickness in Children
Both PRK and LASIK are subtraction refractive procedures, resulting in permanent reduction in the thickness of the cornea. Current US Food and Drug Administration guidelines for LASIK limit treatment parameters to ensure that the cornea maintains a minimum thickness of at least 410 to 430 µm (250 µm in posterior stromal bed plus 160 to 180 µm in cap) to protect against potential keratectasia. Very little is known about normative values for corneal thickness (pachymetry) in the pediatric population.

Corneal thickness in premature and neonatal subjects has been reported. In addition to the age-limited information available from these studies, minimal ethnically diverse information has been included. Variation in adult corneal thickness by race has been well documented, with the central corneal thickness in African Americans being significantly thinner than in Caucasians. The previous studies on infant and newborn corneal thickness have reported only central and limbal corneal thickness measurements, which are thicker than those of adults. Paracentral pachymetry data are unavailable for pediatric patients. Both PRK and LASIK ablate tissue in the paracentral region of the cornea; thus knowledge about corneal thickness in this region is important. Only one study to date has evaluated corneal thickness in children older than the neonatal age group. The investigators reported only central measurements and used optical pachymetry, an older technology that is known to be less accurate than modern ultrasound pachymetry. Establishing normative corneal thickness values for children is essential if refractive surgery is to play a role in pediatric ophthalmology. If corneal thickness in children is found to be significantly different than in adults, treatment nomograms may need to be altered for best visual and refractive outcomes.

Practical Issues Regarding Refractive Surgery in Children
Refractive surgery is often considered impractical in young children because of poor cooperation, the need for general anesthesia, and the need for postanesthesia monitoring. Unfortunately, anisometropic amblyopia is best managed early in life during the time the visual system is most responsive to treatment. Photorefractive keratectomy in adults is performed under topical anesthesia in an office setting. Voluntary immobilization of the eye is required during the procedure. Young children, however, are usually not cooperative, even for a detailed biomicroscopy examination, much less ophthalmic surgery. Therefore, general anesthesia will be required in most cases if refractive surgery is to be done in children under 10 or 11 years of age. Most of the published literature on pediatric refractive surgery for anisometropia has included only children old enough to cooperate for surgery under topical anesthesia.
probable in practice, serious application of pediatric refractive surgery for anisometropic amblyopia must include younger children well within the sensitive period of visual development if it is to be maximally effective.

The requirement for general anesthesia creates a host of important practical problems. The excimer laser is not typically housed in a site that is safe for administration of general anesthesia, and most lasers are not easily portable. Inhalational anesthetic agents can alter excimer laser function and even cause laser shutdown. Operational, procedural, and organizational hurdles must be overcome to safely and reliably apply refractive surgery under general anesthesia.

Healing of the corneal epithelial defect following PRK in adults typically occurs over a period of approximately 5 days. Postoperative pain is an important drawback of PRK in adult patients. No published reports have described the rate of corneal healing and the degree of postoperative pain in children treated with PRK. These are important practical issues that pertain to the feasibility and public acceptance of this procedure for children.

Children with severe anisometropic amblyopia who are noncompliant with traditional therapy typically will have permanent, significant visual impairment. Refractive surgery could play an important role in treating this difficult subset of patients. The purpose of this series of studies was to systematically investigate the mechanics, safety, efficacy, and appropriate application of PRK in children with anisometropic amblyopia noncompliant with traditional therapy.

METHODS

This study on patient selection, mechanics, safety, and efficacy of PRK in children with anisometropia consists of four parts: (1) retrospective evaluation of the records of children with anisometropic amblyopia to identify characteristics of children most likely to fail standard treatment, (2) prospective evaluation of central and paracentral corneal thickness in a pediatric population to ensure the feasibility of refractive surgery in children and to make initial judgments regarding the potential need to modify PRK treatment parameters for children, (3) development and implementation of a standardized general anesthesia protocol for PRK in children, and (4) performance of PRK and follow-up of a group of children with anisometropic amblyopia who were noncompliant with conventional anisometropic amblyopia therapy. In this group, we analyzed corneal healing, postoperative discomfort, visual acuity, refractive response, stereopsis, corneal clarity, and complications over a 2-year period. Visual acuity gains and refractive errors were compared to those of two control groups: (1) children with anisometropic amblyopia who were either diagnosed late (after 6 years of age) or were noncompliant with amblyopia therapy (noncompliant group), and (2) children with anisometropic amblyopia who were diagnosed before 6 years of age and were compliant with amblyopia therapy (compliant group). The studies that make up this report were all approved by our institutional review board.

Anisometropic Amblyopia Treatment Failure Risk Factors

In an effort to identify characteristics of children most likely to fail standard therapy for anisometropic amblyopia, a retrospective review was performed of the records of 104 children with anisometropic amblyopia we had treated with refractive correction and occlusion and/or atropine penalization of the sound eye. Inclusion criteria included (1) age 3 to 8 years at the time of treatment initiation, (2) ability to perform Snellen or HOTV visual acuity testing, (3) an initial difference in visual acuity between fellow eyes of at least 3 lines of logMAR acuity, (4) anisometropia of at least 1 diopter, (5) visual acuity in the amblyopic eye of 20/50 or worse, (6) absence of structural ocular abnormalities in either eye, and (7) at least 1 year follow-up or follow-up to successful “functional outcome” (visual acuity of at least 20/40 in the amblyopic eye), whichever came first.

The data analyzed included age at initiation of treatment, male or female sex, initial and final best-corrected visual acuity, initial cycloplegic refraction, presence of manifest strabismus, treatment modality, and treatment compliance by parental report at the first follow-up examination. Visual acuity was obtained using either Snellen or HOTV charts. Compliance was determined from the physician’s assessment in the medical record based on the parental report. Lack of response to treatment was defined prior to data collection in two ways: (1) relative failure was defined as failure of visual acuity to improve by at least 3 lines of logMAR visual acuity, regardless of the final vision, and (2) functional failure was defined as a final visual acuity of less than 20/40 in the amblyopic eye. This level of visual acuity was chosen as the definition of functional failure because 20/40 is the minimum monocular visual acuity required to obtain an unrestricted driver’s license in most states (www.lowvisioncare.com, Vision and Driving: State Rules/ Regulations/ Policies). Visual acuities were converted to logMAR acuities for analysis. They were then converted back to the more familiar Snellen values to facilitate review of the data.

For analysis of age at presentation, we grouped our patients into two groups: 3 to 5 years and 6 years or older. For analysis of the effect of the degree of anisometropia, we grouped the children into those with less than 4 diopters of anisometropia and those with anisometropia of
4 diopters or more. To test the effect of compliance with treatment, we categorized the children into two groups, those with good compliance and those with suboptimal compliance by parental report at first follow-up examination. For analysis of the effect of refractive error in the amblyopic eye, we divided patients into those with spherical equivalent refractive error of greater than or equal to 3 diopters and those with spherical equivalent refractive error of less than 3 diopters. We also categorized patients having astigmatism into those with astigmatic error of 1.5 diopters or more and those with astigmatic error of less than 1.5 diopters.

Statistical analysis was performed using Intercooled Stata, version 7.0 (Stata Corp, College Station, Texas). Logistic regression models were constructed for each of the outcomes to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for each characteristic. An OR greater than 1 indicates an increased effect of the characteristic on treatment failure. The Hosmer-Lemeshow goodness-of-fit statistic was computed for visual acuity. A P value of .05 was chosen for significance.

Central and Paracentral Corneal Thickness
A prospective investigation to determine the normative values for corneal thickness in children aged 6 months to 14 years was conducted. Written parental informed consent was obtained for all participants. Pachymetry measurements were performed on 198 eyes of 108 children undergoing routine strabismus surgery under general anesthesia, using an ultrasound pachymeter (DGH-2000, DGH Technology, Inc, Frazer, Pennsylvania) with a sound velocity of 1,640 meters per second. Any patient with history of a corneal anomaly, cataract, or glaucoma was excluded.

Following induction of general anesthesia, a wire eyelid speculum was placed in the eye. A pre-inked, standard, 6 mm single-ended ring marker with cross hairs (Duckworth & Kent, St Louis, Missouri) was applied to identify the center and four standard paracentral sites 3 mm from the center of the cornea at the 3-, 6-, 9-, and 12-o’clock positions. Next, three pachymetry measurements were recorded at each of these five sites. If a value was greater than 5% different from the other recordings at that site, an additional measurement was taken. The lowest (thinnest) value at each site was used for analysis, because this represented the most perpendicular path through the cornea. The cornea was moistened during the procedure with balanced salt solution.

Statistical analysis was conducted in this part of the study using Microsoft Excel 2000 (Microsoft Corp, Redmond, Washington). The subjects were stratified into the following age groups prior to data collection: less than 2 years, 2 to 4 years, 5 to 9 years, and 10 to 18 years. The two-tailed t test was used for comparison of the continuous means for values of corneal thickness. Analysis of variance (ANOVA) was performed to determine differences among age groups and among different ethnic groups (Caucasian, Hispanic, African American, other). Values are reported as the mean corneal thickness in microns (± standard deviation). Right and left eyes of each patient were analyzed separately.

General Anesthesia Photorefractive Keratectomy Protocol
This general anesthesia PRK protocol has been previously published as briefly reviewed below.132 Nine children (aged 2 to 9 years) treated with PRK in this study required general anesthesia because of inability to cooperate for the procedure under local anesthesia. Idiosyncrasies of the excimer laser were addressed prior to performing an excimer laser procedure under general anesthesia to reduce the risk of unexpected refractive results and/or malfunction of the laser during treatment. The purpose of this component of the study was to develop and implement a standardized, reproducible, effective, and efficient means of conducting excimer laser surgery on children under general anesthesia and to report on the efficiency of the procedure and intraoperative and postoperative complications.

The anesthesia procedure from induction to anesthesia recovery was as follows. General anesthesia was induced in a separate induction room using halothane and nitrous oxide by mask inhalation. An intravenous line was placed after the child was asleep, and a laryngeal mask airway was inserted into the posterior pharynx. Several patients also received small doses of propofol to deepen anesthesia. An adhesive, nonporous drape was placed over the laryngeal mask airway to minimize escape of the inhalational anesthetic agents. The child was then transported to a nearby operating room fully monitored and breathing oxygen and halothane through a Jackson-Rees circuit. Before entering the operating room, the halothane was discontinued. In the operating room, the laryngeal mask airway was connected to a standard semiclosed-circle system through which the patient received 70% nitrous oxide in oxygen. Nitrous oxide given through the semiclosed circuit was administered throughout the remainder of the case. Additional boluses of propofol were administered as needed. The PRK then proceeded as described in the next section.

The time intervals between cases, intraoperative laser function, and intraoperative and postoperative complications were analyzed.

Photorefractive Keratectomy: Safety and Impact on Refractive Error, Visual Acuity, and Stereopsis
A prospective case-comparison study was conducted of
PRK in children. Written parental informed consent (and verbal assent from the children old enough to understand) was obtained for all participants. Eleven children between 2 and 11 years of age were treated with PRK for severe anisometropia with amblyopia. In this study, PRK was investigated rather than LASIK because we felt PRK had a better risk profile for children, with less risk of serious postoperative complications, such as flap loss and keratectasia.133-135 Inclusion criteria were (1) anisomyopia of at least 6 diopters or anisohyperopia of at least 4 diopters, (2) poor compliance with spectacles and/or contact lenses and occlusion therapy based on parental report, and (3) moderate to severe amblyopia of the eye with the highest refractive error, defined as a best-corrected visual acuity in the amblyopic eye that was at least 3 logMAR lines lower than the sound eye or a strong fixation preference for the fellow eye in preverbal children. Children with an abnormality of the cornea, lens, or fovea were excluded.

Each child underwent a comprehensive ophthalmologic examination that included uncorrected and best spectacle-corrected visual acuity, stereoaucuity testing (Titmus stereo fly test, Stereo Optical Co, Chicago, Illinois), pupillary examination, ocular motility, tactile tonometry, biomicroscopy, funduscopy, and cycloplegic refraction. Visual acuity testing was done with the most sophisticated standard visual acuity test the child could comprehend and perform. Visual behavior was tested in younger children using the fixation and following response and the vertical prism test for fixation preference.133 Quantitative visual acuity testing was done as soon as patient comprehension permitted. The Titmus stereo fly test was chosen to test stereoaucuity because of its ease of use and reproducibility in young children. Ultrasound pachymetry and keratometry were performed during the preoperative examination in cooperative children and under general anesthesia prior to the procedure in uncooperative children.

The refractive goal for each child was to reduce the anisometropia to 3 diopters or less, up to a maximum myopic treatment of 11.50 diopters and a maximum hyperopic treatment of 5.25 diopters. Reducing anisometropia to less than or equal to 3 diopters eliminates or greatly reduces the spectacle-induced aniseikonia to the point where fusion is possible, making the condition more amenable to treatment with spectacles. Myopic treatment was limited to no more than 11.50 diopters even though some of our patients had higher levels of myopia, because extensive corneal haze with PRK for higher levels of myopia has been reported in adults.134-137

Photorefractive keratectomy was performed as follows. The supine child’s head was fixated in the desired position with the plane of the iris perpendicular to the laser beam. For cooperative children, topical anesthesia was used for the PRK. These cooperative children then fixated on the fixation light of the excimer laser machine (Visx Star S2, San Jose, California), and the PRK proceeded in the standard fashion. For the children requiring general anesthesia, the surgeon fixated the eye manually with forceps, taking care to avoid globe compression. The laser aiming beam was centered on the entrance pupil. For myopic PRK, laser scrape was used to remove the epithelium, with any residual epithelium being removed manually with a spatula. For hyperopic PRK, the entire epithelium was removed manually. The desired refractive correction was then programmed into the excimer laser, and the PRK was performed. During the entire procedure under general anesthesia, two observers positioned on either side of the patient continually monitored eye position to ensure that the iris plane remained perpendicular to the laser beam. The size of the optical zone was 6.5 mm for all myopic PRKs and 9.0 mm for all hyperopic PRKs.

After the procedure was completed, topical atropine 1%, ketorolac 0.5% (Acular, Allergan, Irvine, California), and gentamicin were placed in the treated eye and a disposable contact lens (SureVue, Johnson and Johnson, Jacksonville, Florida) was placed on the cornea. Collagen plugs were inserted into the upper and lower puncta to maximize the tear film during the initial healing phase, and a soft patch was placed over the eye. Since escape of the inhalational anesthetic in the operating room could potentially affect the function of the excimer laser on subsequent patients, removal of the laryngeal mask airway was deferred until the patient was in the recovery room. The eye patch was removed when the patient was awake in the recovery room.

Postoperative medications included topical ofloxacin (Ocuflax, Allergan, Irvine, California) and loteprednol 0.5% (Lotemax, Bausch and Lomb, Rochester, New York), four times a day in the treated eye until the corneal epithelium healed. Topical ketorolac was prescribed up to four times a day as needed for discomfort for the first 2 postoperative days. Hydrocodone oral elixir was also prescribed as needed for severe discomfort for the first few days. Ofloxacin and loteprednol were discontinued after 1 week, and fluoromethalone 0.25% (FML Forte, Allergan, Irvine, California) was prescribed four times a day for 1 month, followed by a slow taper over the next 5 months.

The children were examined postoperatively at the same time each day until the corneal epithelial defect had healed, at which time the contact lens was removed. The size of the corneal epithelial defect was measured horizontally and followed to determine the rate of corneal healing. The residual epithelial defect size was recorded as the ratio between the diameter of the defect and the...
horizontal diameter of the cornea.

Each day, including the day of surgery, ocular discomfort was assessed using a two-part pain assessment index consisting of a facial expression scale and a digital analog scale. These findings on corneal healing and discomfort following PRK in children have been previously published. For the facial expression scale, a sheet of paper with six faces was presented to the parent and child. The six faces had different facial expressions with the happiest face rated “0” and the saddest rated “10” (Figure 1A). The parent and all children who could cooperate were asked to identify the face that they felt best represented the degree of discomfort felt by the child. On the digital analog scale, a line with the numbers 0 to 10 was presented to all parents and to children 5 years and older (Figure 1B). The parent alone for the younger children or the parent and child together for the children 5 years and older were asked to choose the number that best described the child’s discomfort. The number “0” represented no pain and the number “10” represented the worst pain imaginable. The child was examined daily until the corneal epithelium was fully healed and both scales were rated as “0.”

Thereafter, the children were examined 1 month after the procedure and then every 3 months for 12 months and again at 24 months following the surgery. Cycloplegic refractive correction was prescribed as needed at the 1-month examination and updated as needed thereafter. Occlusion therapy was recommended up to 8 hours per day for the sound eye based on the child’s age and visual deficit. Compliance was assessed at each follow-up visit. “Excellent” compliance was defined as parental reporting of compliance with treatment recommendations 76% or more of the time. “Good” compliance meant that the parent reported compliance 51% to 75% of the recommended time, “fair” that parent reported compliance 25% to 50% of the recommended time, and “poor” that the parent reported compliance less than 25% of the recommended time.

Data analyzed from each comprehensive follow-up examination included uncorrected and best spectacle-corrected visual acuity, stereoacuity, ocular motility, degree of corneal haze, and cycloplegic refraction. Postoperative subepithelial corneal haze was graded on a scale of 0 to 4+ (0 = clear cornea; 1+ = trace haze, only detectable with tangential illumination; 2+ = mild, discrete haze visible with difficulty by focal illumination; 3+ = moderately dense opacity partially obscuring iris detail; 4+ = dense opacity obscuring details of intraocular structures).

Postoperative corneal topography (Humphrey Atlas, version A11.2, Dublin, California) was performed as patient cooperation allowed to assess for centration. Using tangential maps (standardized scale) from the Humphrey Atlas, centration was determined according to the method previously described by Lin and coworkers. The edges of the ablation in the X-axis and Y-axis were marked, and the center of the ablation was estimated to be the intersection of the X and Y axes. With the computer cursor positioned at this point, the legend on the topographic map indicated the distance to the nearest 0.01 mm and the angle (semimeridian in degrees) of the ablation zone relative to the pupillary center.

The best spectacle-corrected visual acuities for the PRK study group at the 24-month examination (or last follow-up visit for one child who was lost to follow-up after 6 months) were compared to those of two control groups: (1) anisometropic children who either were diagnosed after age 6 years or were noncompliant with amblyopia therapy (noncompliant control group), and (2) anisometropic amblyopic children who were diagnosed before age 6 and were compliant with therapy (compliant control group). The best-corrected visual acuity at the last visit in the control group was used for comparison. Control group patients were identified retrospectively by medical records review because we felt it would have been unethical to randomize children prospectively to a “no treatment” group in order to obtain these comparison visual acuity data. Control patients came from my practice and from a database of amblyopia patients from multiple pediatric ophthalmology practices. The control children from my practice were consecutively identified using a computer search for “anisometropia.” All control patients had at least 4 diopters of anisometropia and at least 1 year follow-up. Strabismus was the only other eye abnormality the control patients were allowed to have. We believe that the visual

![Figure 1A](image1a.png)

Facial expression scale. Note the gradual change in emotion in each face progressing from left to right. The parent and children who could cooperate chose the face that best represented how the child felt.

![Figure 1B](image1b.png)

Digital analog scale. “0” represented no pain and “10” represented the worst pain imaginable. The parent and children who could cooperate were asked to choose the number that most accurately represented the child’s discomfort.
photorefractive keratectomy for anisometric amblyopia in children

acuities in the noncompliant control group were comparable to visual acuities that our treated children might have had had prk not been performed, and the visual acuities in the compliant group were comparable to visual acuities that our treated children might have had they been compliant with standard amblyopia therapy.

visual acuities in the prk group and the control groups were converted to logmar acuities for the analyses because of linearity. they were then converted back to the more familiar snellen values to facilitate review of the data. statistical calculations were performed using intercooled stata, version 7.0 (stata corp, college station, texas). continuous data were compared between prk cases and control groups using the student t test.

ordinal data were analyzed using logistic regression. refractive and corneal haze results were analyzed throughout the 24-month follow-up period. visual acuity outcomes were analyzed at the 12- and 24-month follow-up visits.

safety of prk in children with anisometric amblyopia was assessed using a previously published refractive surgery safety index (safety index = postoperative best-corrected visual acuity ÷ preoperative best-corrected visual acuity).107,108 efficacy was assessed using a previously published refractive surgery efficacy index (efficacy index = postoperative uncorrected visual acuity ÷ preoperative best-corrected visual acuity).107,108

results

anisometric amblyopia treatment failure risk factors

one hundred and four children were included. the mean age at initiation of amblyopia treatment was 4.8 ± 1.5 years. thirty children (29%) were more than 6 years old, and 59 (57%) were male. seventy-one (68%) were caucasian, 16 (15%) were hispanic, 9 (9%) were african american, and 8 (8%) were of mixed origin. amblyopia affected the right eye of 46 patients (44%), and strabismus was present in 66 (64%). the mean duration of follow-up was 17 months (range, 3 to 95 months).

the absolute value of the mean difference in spherical equivalent refraction between the two eyes was 5.00 diopters (range, 1.00 to 13.00). the mean spherical equivalent refraction in the amblyopic eye was +4.30 diopters (range, +0.75 to +11.00 d) in the hyperopic group and −5.40 diopters (range, −1.50 to −13.00) in the myopic group. the initial best-corrected visual acuity of the amblyopic eye was 20/60 or better in 27 (26%), 20/70 to 20/100 in 31 (30%), 20/125 to 20/200 in 18 (17%), and worse than 20/200 in 27 (26%). the mean best-corrected logmar acuity in the amblyopic eye was 0.9 (20/160) (range, 0.4 to 2 [20/50 to 20/2000]). the mean logmar visual acuity in the sound eye was 0.2 (20/30). the mean difference in the logmar acuity between fellow eyes was 5 lines (range, 3 to 8). eighty-six patients (83%) were treated with occlusion, and 18 (17%) used atropine penalization of the sound eye.

table 1 summarizes the patient demographics. table 2 summarizes the relative and functional failure rates for each suspected risk factor. the unadjusted and adjusted ors and p values for each risk factor for relative and functional failures are presented in tables 3 and 4, respectively. the correlations of the outcome of treatment to age at initiation of treatment, compliance with the treatment regimen, and amount of astigmatism in the amblyopic eye are presented in table 5. overall, 78 patients (75%) experienced relative success (improvement by at least 3 lines of logmar acuity in the amblyopic eye), and 57 patients (55%) experienced functional success (20/40 or better visual acuity of the amblyopic eye). each suspected risk factor is explored in detail below.

age

twenty-five children (24%) were 6 years of age or older. of these, 17 (68%) achieved relative success and 7 (28%) achieved functional success. of the 79 patients below 6 years of age, 60 (76%) achieved relative success and 49 (62%) achieved functional success. table 5 shows the dose-response relationship between the age and the risk of amblyopia treatment failure. the risk of relative and functional failure increased as age increased. age of 6 years or more at the onset of treatment was a statistically significant risk factor for functional failure (or = 4.69 [1.55, 14.2]; P = .01) (tables 4 and 5) (figures 2 and 3).

<table>
<thead>
<tr>
<th>characteristic</th>
<th>metric</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean age ± sd</td>
<td>4.8 ± 1.5 years</td>
</tr>
<tr>
<td>male:female</td>
<td>59:45</td>
</tr>
<tr>
<td>median duration of follow-up (range)</td>
<td>17 months (3 to 95 months)</td>
</tr>
<tr>
<td>absolute value of the mean difference in sere (range)</td>
<td>5.00 d (1.00 to 13.00 d)</td>
</tr>
<tr>
<td>mean best-corrected logmar acuity in the amblyopic eye (range)</td>
<td>0.9 (0.4 to 2)</td>
</tr>
<tr>
<td>mean difference in the logmar acuity between the two eyes (range)</td>
<td>5 lines (3 to 8 lines)</td>
</tr>
</tbody>
</table>

sd, standard deviation; sere, spherical equivalent refractive error.
Degree of Anisometropia
Twenty-two patients (21%) had anisometropia of 4 diopters or more. Of these, 17 (77%) achieved relative success and 10 (45%) achieved functional success. Of the 82 patients who had anisometropia of less than 4 diopters, 59 (72%) achieved relative success and 44 (54%) achieved functional success. The degree of anisometropia was not found to be a statistically significant risk factor for treatment failure (Tables 3 and 4).

Compliance With Treatment
Suboptimal compliance with treatment was reported in 23 patients (22%). Of these, 11 (48%) achieved relative success and 9 (39%) achieved functional success. Among the 81 patients with good compliance, 67 (83%) achieved relative success and 48 (59%) achieved functional success. Table 5 shows the dose-response relationship between compliance and the risk of amblyopia treatment failure. The risk of relative and functional failure increased as compliance with therapy decreased. Poor compliance with treatment was found to be a statistically significant risk factor for relative failure (OR = 5.47 [2.00, 15.03]; P = .004) (Table 5, Figures 2 and 3).
### TABLE 4. MULTIVARIATE REGRESSION ANALYSIS OF SUSPECTED RISK FACTORS FOR FUNCTIONAL FAILURE OF ANISOMETROPIC AMBLYOPIA TREATMENT (FINAL VISUAL ACUITY OF LESS THAN 20/40 IN THE AMBLYOPIC EYE)

<table>
<thead>
<tr>
<th>CHARACTERISTIC</th>
<th>UNADJUSTED OR (95% CI)</th>
<th>P VALUE</th>
<th>ADJUSTED OR (95% CI)</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age ≥6 years</td>
<td>2.84 (1.18, 6.83)</td>
<td>.02</td>
<td>4.69 (1.55, 14.2)</td>
<td>.01</td>
</tr>
<tr>
<td>Concurrent strabismus</td>
<td>2.45 (1.06, 5.65)</td>
<td>.04</td>
<td>2.41 (0.79, 7.31)</td>
<td>.12</td>
</tr>
<tr>
<td>SERE amblyopic eye ≥3.00 D</td>
<td>0.88 (0.39, 1.98)</td>
<td>.76</td>
<td>1.08 (0.37, 3.20)</td>
<td>.89</td>
</tr>
<tr>
<td>Cylinder of amblyopic eye ≥1.50 D</td>
<td>1.63 (0.61, 4.35)</td>
<td>.33</td>
<td>1.10 (0.29, 4.21)</td>
<td>.89</td>
</tr>
<tr>
<td>Intercocular SERE difference of ≥4.00 D</td>
<td>1.61 (0.57, 4.60)</td>
<td>.32</td>
<td>1.40 (0.78, 2.50)</td>
<td>.29</td>
</tr>
<tr>
<td>Initial visual acuity in amblyopic eye of 20/200 or worse</td>
<td>2.61 (1.05, 6.46)</td>
<td>.04</td>
<td>3.79 (1.28, 11.2)</td>
<td>.02</td>
</tr>
<tr>
<td>Suboptimal amblyopia treatment compliance</td>
<td>2.07 (0.84, 5.09)</td>
<td>.11</td>
<td>2.43 (0.86, 6.85)</td>
<td>.09</td>
</tr>
<tr>
<td>Myopia</td>
<td>1.29 (0.47, 3.53)</td>
<td>.58</td>
<td>1.11 (0.7, 2.75)</td>
<td>.76</td>
</tr>
</tbody>
</table>

D, diopters; CI, confidence interval; OR, odds ratio; SERE, spherical equivalent refractive error.

### TABLE 5. DOSE-RESPONSE RELATIONSHIP BETWEEN (A) AGE, (B) COMPLIANCE WITH THE TREATMENT, AND (C) CYLINDER IN THE AMBLYOPIC EYE AT THE ONSET OF TREATMENT AND THE OUTCOME OF ANISOMETROPIC AMBLYOPIA THERAPY

#### A.

<table>
<thead>
<tr>
<th>AGE AT ONSET OF TREATMENT</th>
<th>RELATIVE FAILURE*</th>
<th>FUNCTIONAL FAILURE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UNADJUSTED OR (95% CI)</td>
<td>P VALUE</td>
</tr>
<tr>
<td>≤4 years</td>
<td>1.00 (referent)</td>
<td>–</td>
</tr>
<tr>
<td>4-5 years</td>
<td>1.74 (0.40, 7.46)</td>
<td>.40</td>
</tr>
<tr>
<td>≥6 years</td>
<td>2.82 (0.72, 11.20)</td>
<td>.08</td>
</tr>
</tbody>
</table>

#### B.

<table>
<thead>
<tr>
<th>AMBLYOPIA TREATMENT COMPLIANCE</th>
<th>RELATIVE FAILURE</th>
<th>FUNCTIONAL FAILURE*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UNADJUSTED OR (95% CI)</td>
<td>P VALUE</td>
</tr>
<tr>
<td>Good</td>
<td>1.00 (referent)</td>
<td>–</td>
</tr>
<tr>
<td>Fair</td>
<td>6.65 (1.58, 28.0)</td>
<td>.01</td>
</tr>
<tr>
<td>Poor</td>
<td>12.0 (2.16, 66.2)</td>
<td>.004</td>
</tr>
</tbody>
</table>

#### C.

<table>
<thead>
<tr>
<th>CYLINDER OF AMBLYOPIC EYE</th>
<th>RELATIVE FAILURE</th>
<th>FUNCTIONAL FAILURE*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UNADJUSTED OR (95% CI)</td>
<td>P VALUE</td>
</tr>
<tr>
<td>&lt;1.00 D</td>
<td>1.00 (referent)</td>
<td>–</td>
</tr>
<tr>
<td>1.00 to 1.50 D</td>
<td>2.2 (0.53, 9.06)</td>
<td>.20</td>
</tr>
<tr>
<td>≥1.5 D</td>
<td>6.6 (2.00, 22.3)</td>
<td>.0002</td>
</tr>
</tbody>
</table>

*Adjusted for age, concurrent strabismus, high cylinder, and poor initial vision.
D, diopters; CI, confidence interval; OR, odds ratio.
Visual Acuity
Thirty-five patients (34%) had initial acuity of 20/200 or worse. Of these, 28 (80%) achieved relative success and 10 (29%) achieved functional success. Of 69 patients with visual acuity better than 20/200, 50 (73%) achieved relative success and 47 (68%) achieved functional success. The Hosmer-Lemeshow goodness-of-fit statistic showed good fit for the models for lines of acuity gained (P = .84) and for best visual acuity obtained (P = .29) (Figures 2 and 3). Visual acuity of 20/200 or worse in the amblyopic eye was found to be a statistically significant risk factor for functional failure (OR = 3.79 [1.28, 11.2]; P = .01).

Concurrent Strabismus
Sixty-six patients (63%) had concurrent strabismus. Of these, 46 (70%) achieved relative success and 32 (49%) achieved functional success. Of the 38 patients who did not have strabismus, 32 (84%) achieved relative success and 25 (66%) achieved functional success. The association of strabismus with anisometropia was a risk factor for both relative failure (OR = 3.30 [1.13, 9.63]; P = .03) and functional failure (OR = 2.45 [1.06, 5.65]; P = .04). When the results were adjusted for the other risk factors, however, strabismus was not found to be a statistically significant independent risk factor for treatment failure.

Type of Refractive Error
Twenty-three (22%) of the patients were myopic. Of these, 14 (61%) achieved relative success and 11 (48%) achieved functional success. Of the 81 hyperopic patients, 63 (78%) had relative success and 46 (57%) had functional success. Although the risk for failure was slightly higher in myopes, this difference was not statistically significant (Tables 3 and 4).

Spherical Equivalent
Seventy patients (67%) had a spherical equivalent refractive error of more than 3.00 diopters. Of these, 52 (74%) achieved relative success and 40 (57%) achieved functional success. Of the 34 patients with spherical equivalent refractive error of less than 3.00 diopters, 26 (77%) achieved relative success and 17 (50%) achieved functional success. Spherical equivalent refractive error of more than 3.00 diopters in the amblyopic eye was not found to be a statistically significant risk factor for treatment failure (Tables 3 and 4).

Astigmatism
Thirty patients (29%) had astigmatism of 1.5 diopters or more in the amblyopic eye. Of these, 17 (57%) achieved relative success and 11 (37%) achieved functional success. Of the 74 patients with astigmatism of less than 1.5 diopters, 61 (83%) achieved relative success and 46 (62%) achieved functional success. Table 5 shows the dose-response relationship between the amount of astigmatism in the amblyopic eye and the risk of amblyopia treatment failure. The risk of failure increased as the degree of astigmatism in the amblyopic eye increased. Astigmatism of 1.5 diopters or more in the amblyopic eye was found to be a statistically significant risk factor for relative failure (OR = 5.78 [1.27, 26.5]; P = .02) (Figure 2).

Summary of Risk Factors for Anisometropic Amblyopia Treatment
The following risk factors were significantly associated with conventional treatment failure of anisometropic amblyopia: (1) poor compliance with treatment recommendations (relative failure), (2) age 6 years or greater at initiation of treatment (relative failure), (3) astigmatism of 1.5 diopters or more (functional failure), and (4) initial visual acuity of 20/200 or worse (functional failure).

Corneal Thickness
We prospectively examined 198 eyes of 108 children. Fifty-seven patients (53%) were male. The eyes examined were divided equally between the right and left eyes (99 eyes each). One hundred ten eyes (56%) belonged to Caucasian patients, 64 (32%) to Hispanic patients, 12 (6%) to African Americans, and 12 (6%) eyes to patients of multiracial origin.

The mean central corneal thickness ± standard deviation (SD) was 544 ± 46 μm. The mean paracentral corneal thickness values ± SD measured at 3 mm from the corneal center were as follows: superior, 575 ± 52 μm; nasal, 568 ± 30 μm; inferior, 568 ± 51 μm; and temporal, 574 ± 47 μm. The mean central corneal thickness values were significantly thinner than at each of the mean paracentral thicknesses (P < .05 for each comparison, paired t test). The paracentral corneal thickness measurements demonstrated no significant differences between locations (P > .05, ANOVA). The mean central corneal thickness values for the right and the left eyes were 548 μm and 550 μm, respectively, which were not significantly different.

Patients ranged in age from 7 months to 14.7 years old. The number of eyes in each age group was as follows: younger than 2 years old, 68; 2 to 4 years, 62; 5 to 9 years, 50; and 10 to 18 years, 18. The mean central corneal thickness ± SD for each age group was as follows: 6 to 23 months, 538 ± 40 μm; 2 to 4 years, 546 ± 41 μm; 5 to 9 years, 565 ± 48 μm; and 10 to 18 years, 555 ± 35 μm (Figure 4). ANOVA performed on the central pachymetry measurement yielded a significant difference between age groups (P = .008). The two-tailed t test performed in the different age subgroups showed that the central cornea was significantly thicker in the group of children aged 5 to 9 years when compared with either the younger-than-2-

Paysse
years age group or the 2- to 4-year-old age group. The difference in the mean central corneal thickness in the other age groups was not statistically significant. Trends of the central corneal thickness among age groups were similar to those of the paracentral locations (Figure 5).

The data were subdivided by ethnic group. Mean central corneal thickness measurements ± SD for each ethnic group were as follows: Caucasian, 551 ± 48 µm; Hispanic, 550 ± 34 µm; African American, 532 ± 48 µm; and other, 542 ± 41 µm (Figure 6). ANOVA performed on central pachymetry values demonstrated no significant differences among racial subgroups overall (ANOVA, $P = .48$) and when divided into the different age subgroups (ANOVA, $P = .79$) (Figure 7).

**General Anesthesia Protocol**

Nine (82%) of the 11 children who underwent PRK in this study required general anesthesia for the procedure. The mean age of this subgroup was 5.5 years (range, 2 to 9 years). Two were female. None suffered anesthesia-related or treatment-related complications. The mean duration from induction of one case to induction of the next was 31 minutes (22 to 44 minutes). The excimer laser functioned normally with no unexpected refractive results. All patients were discharged home after the standard recovery room observation period of 1 hour. No postoperative complications occurred.

**Photorefractive Keratectomy: Safety and Impact on Refractive Error, Visual Acuity, and Stereopsis**

The mean age of the 11 treated children was 6.1 years (range, 2 to 11 years). Nine children (82%) were male and 10 (91%) of the treated eyes were right eyes. Eight children were treated for anisomyopia and three for anisohyperopia. Eight children (73%) were Caucasian, one (10%) was Hispanic, and two (18%) were African American. Mean follow-up time was 22 ± 9.4 months (Table 6).

**Corneal Healing and Discomfort**

The corneal epithelial defect healed steadily each day in all patients. The mean epithelial defect size (mean percentage of the horizontal corneal diameter) was 43 ± 19% on the first postoperative day, 26 ± 15% on the second postoperative day, 20 ± 6% on the third postoperative day, and 2 ± 2% on the fourth postoperative day (Figure 8). All were healed by the fifth postoperative day. The mean time for complete healing of the corneal defect was 3.5 days (range, 3 to 5 days). The corneal epithelium healed completely in 3 days in six patients (60%), in 4 days in three patients (30%), and in 5 days in one patient (10%). The mean healing time for myopic PRK was 2.8 days and for hyperopic PRK was 4.5 days.

Seven (70%) of the children, aged 6 to 10 years, were able to understand and were willing to evaluate their own discomfort using the facial expression and digital analog scales. The parents of three other children, aged 2 to 5 years, solely evaluated their children’s discomfort. Postoperatively, patients/parents reported mild to moderate discomfort on the day of surgery with a mean facial expression rating of 4.8 (range, 2 to 10) and a mean digital analog rating of 2.3 (range, 1 to 7) (Figure 9). On the first postoperative day, patients/parents reported mild postoperative discomfort, with a mean facial expression score of 3.6 (range, 2 to 10) and a mean digital analog score of 2.0 (range, 0 to 7). On the second postoperative day, the patients/parents reported minimal discomfort, with a mean facial expression score of 2.0 (range, 0 to 4) and a mean digital score of 0.3 (range, 0 to 2). After the second postoperative day, all reported no pain or other discomfort. Five children (50%) used topical ketorolac for discomfort once or twice on the first postoperative day and none thereafter. Three children (30%) used the hydrocodone oral elixir analgesic on the first postoperative day, and none used it thereafter.

**Refractive Error**

**Myopia Group.** Table 6 demonstrates complete refractive results. Table 7 shows the preoperative and 24-month postoperative results of the individual patients. The mean preoperative spherical equivalent in the myopic group was –13.70 ± 3.77 diopeters; the mean interocular spherical equivalent difference was 11.07 ± 4.02 diopeters. The maximum refractive spherical equivalent treatment dose was 11.50 diopeters. The mean final target spherical equivalent was –3.50 ± 3.70 diopeters. The mean target refractive error reduction was 10.10 ± 1.39 diopters. The mean spherical equivalent refractive error reductions at 12 and 24 months were 10.56 ± 3.00 diopeters and 9.70 ± 2.80 diopeters, respectively. The mean 12-month and 24-month postoperative myopic spherical equivalents were –3.20 ± 2.50 diopeters and –3.30 ± 2.54 diopeters, respectively (Table 6, Figure 10).

The mean spherical equivalent difference between the 12-month target and 12-month achieved refractive change after myopic PRK was 0.20 ± 2.67 diopeters of overresponse. No patient had an overresponse producing hyperopia. At the 24-month follow-up visit, the cycloplegic refractive error of the treated eye was within 3 diopters of that of the fellow eye in six of eight eyes. At this same visit, three of eight myopes were within 1 diopter of target refractive spherical equivalent and six of eight were within 2 diopters (Table 6 and Figure 10). Two patients who were highly myopic preoperatively achieved a greater degree of correction than targeted. Patient 7, with a preoperative spherical equivalent refractive error of –21.00 diopters, had a refractive target reduction of
11.50 diopters but at 12 months achieved a refractive reduction of 16.75 diopters and a spherical equivalent result of –4.75 diopters. Patient 3, with a preoperative spherical equivalent refractive error of –13.75 diopters, had a refractive target reduction of 10.50 diopters and at 12 months achieved a final refractive reduction of 13.25 diopters and a spherical equivalent result of –0.50 diopters. The spherical equivalent refractive errors on
these two patients at 24 months were –5.9 diopters and –2.50 diopters, respectively, demonstrating some regression of effect.

Refractive error stability over the 24-month follow-up period is illustrated in Figure 11. Our myopic group had moderate refractive regression over the first 12-month follow-up period with a mean spherical equivalent regression of 2.50 ± 2.23 diopters, which stabilized over the next 12 months with minimal further regression of 0.50 ± 1.07 diopters.

Hyperopia Group

Table 6 demonstrates complete refractive results. Table 7 shows the preoperative and 24-month postoperative results of the individual patients. The mean preoperative spherical equivalent in the hyperopic group was +4.75 ± 0.50 diopters; the mean interocular spherical equivalent difference was 4.38 ± 0.45 diopters. The maximum refractive spherical equivalent treatment dose was 5.25 diopters. The mean final target spherical equivalent was plano, and the mean target refractive error reduction was 4.75 diopters ± 0.50 diopters. The mean refractive error reductions at 12 and 24 months were +4.08 ± 0.80 diopters and 2.80 ± 1.00 diopters, and the mean 12-month and 24-month postoperative hyperopic spherical equivalent refractive errors were +0.67 ± 0.50 diopters and +1.78 ± 1.40 diopters, respectively (Table 6, Figure 10). The mean spherical equivalent difference between the 12-month target and 12-month achieved refractive change after hyperopic PRK was 0.96 ± 0.68 diopters of underscr. At the 24-month follow-up visit, the cycloplegic refractive error of the treated eye in both children who returned for follow-up was within 3 diopters of the fellow eye. At this same visit, one hyperope was within 1 diopter of target spherical equivalent. The other, who had developed late-onset peripheral anterior corneal stromal haze, was within 2 diopters of target. The last child did not return for follow-up (Figure 10).

Refractive error stability over the 24-month follow-up period is demonstrated in Figure 11. Over the first 12-month follow-up interval, our hyperopic group showed mild refractive regression with a mean spherical equivalent regression of 1.10 ± 1.6 diopters. Between 12 and 24 months follow-up, further regression of 0.90 ± 0.84 diopters occurred.

Corneal Haze and Topography

The mean postoperative corneal haze measurement at 12 months was 0.5– (range, 0 to 2+) (Figure 12). All but one child with residual corneal haze were myopic. Only one patient (age 4 at treatment) had mild to moderate corneal haze (2+) at 12 months. This child had not been compli-

---

**Table 6. Patient Demographics and Refractive Results of the Children Who Underwent Photorefractive Keratectomy for Anisometropia**

<table>
<thead>
<tr>
<th>CHARACTERISTIC</th>
<th>MYOPIC GROUP</th>
<th>HYPEROPIC GROUP</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>Mean age in years (range)</td>
<td>4 (2 to 8)</td>
<td>9 (8 to 11)</td>
</tr>
<tr>
<td>Mean preop K readings ± SD (D)</td>
<td>44.80 ± 1.54</td>
<td>42.30 ± 1.06</td>
</tr>
<tr>
<td>Mean preop corneal thickness ± SD (µm)</td>
<td>521 ± 43.4</td>
<td>536 ± 42.4</td>
</tr>
<tr>
<td>Mean preop SERE ± SD (D)</td>
<td>-1.37 ± 3.77</td>
<td>4.75 ± 0.50</td>
</tr>
<tr>
<td>Mean interocular SERE difference ± SD (D)</td>
<td>11.07 ± 4.02</td>
<td>4.38 ± 0.45</td>
</tr>
<tr>
<td>Maximum refractive SERE dose (D)</td>
<td>-11.50</td>
<td>+5.25</td>
</tr>
<tr>
<td>Mean target SERE ± SD (D)</td>
<td>-3.50 ± 3.70</td>
<td>plano</td>
</tr>
<tr>
<td>Mean target SERE reduction ± SD (D)</td>
<td>10.10 ± 1.39</td>
<td>4.75 ± 0.5</td>
</tr>
<tr>
<td>Mean 12-mo SERE reduction ± SD (D)</td>
<td>10.56 ± 3.0</td>
<td>4.08 ± 0.50</td>
</tr>
<tr>
<td>Mean 24-mo SERE reduction ± SD (D)</td>
<td>9.70 ± 2.50</td>
<td>2.50 ± 1.00</td>
</tr>
<tr>
<td>Mean 12-mo postop SERE ± SD (D)</td>
<td>-3.20 ± 2.50</td>
<td>+0.67 ± 0.50</td>
</tr>
<tr>
<td>Mean 24-mo postop SERE ± SD (D)</td>
<td>-3.30 ± 2.54</td>
<td>+1.78 ± 0.40</td>
</tr>
<tr>
<td>Mean SERE 12-mo regression ± SD (D)</td>
<td>2.50 ± 2.23</td>
<td>1.10 ± 1.60</td>
</tr>
<tr>
<td>Mean SERE 12- to 24-mo regression ± SD (D)</td>
<td>0.8 ± 1.27</td>
<td>0.90 ± 0.54</td>
</tr>
<tr>
<td>No. of pts within 1 D of target at 24 months</td>
<td>3 of 8</td>
<td>1 of 2</td>
</tr>
<tr>
<td>No. of pts within 2 D of target at 24 months</td>
<td>6 of 8</td>
<td>2 of 2</td>
</tr>
<tr>
<td>% reduction in RE at 24 months</td>
<td>76%</td>
<td>63%</td>
</tr>
</tbody>
</table>

D, diopters; K, keratometry; preop, preoperative; postop, postoperative; mo, month; SD, standard deviation; SERE, spherical equivalent refractive error.
Mean healing of the corneal epithelial defect following photorefractive keratectomy. The defect size is expressed as a percentage of the corneal size. Note the rapid decrease in the size of the epithelial defect.

The mean degree of discomfort after PRK as graded using the digital analog scale (DAS) and the facial expression scale (FES). Note the rapid decrease in discomfort in the first 2 days.

Target refractive treatment change compared to the 12-month and 24-month results in the myopic and hyperopic groups treated with PRK. Note that the points above the line represent overresponse from target and those below the line represent underresponse from target.

Refractive error stability over time in the myopic and hyperopic subgroups of children treated with PRK. The mean refraction is the spherical equivalent refraction.

Corneal haze at 1, 12, and 24 months after PRK in 11 children.
ant with the postoperative treatment protocol. He did not return for follow-up after the 1-month examination until the 12-month examination and discontinued the fluromethalone drops 1 month after the surgery. At 24 months after treatment, the haze in this child has decreased to 1+. The remainder of the patients had only minimal or no haze throughout the entire follow-up period. At the 24-month follow-up visit, the mean corneal haze measurement was 0.25+.

The mean treatment decentration on the cornea of the nine patients cooperative enough to undergo corneal topography was 0.68 ± 0.43 mm (Table 8, Figure 13). The child with the largest decentration was 7 years old at the time of the procedure, had a preoperative spherical equivalent refractive error of −21.00, and had a visual acuity of 5/200 preoperatively and postoperatively with eccentric fixation in this eye. The other outlier with 1.05 mm of decentration was 8 years old at the time of the procedure and had undergone hyperopic PRK under general anesthesia. Her postoperative uncorrected and best spectacle-corrected visual acuities in this eye at the 24-month examination were 20/60 and 20/40, respectively, compared with

<table>
<thead>
<tr>
<th>TABLE 7. PREOPERATIVE AND POSTOPERATIVE RESULTS OF ALL PATIENTS WITH PHOTOREFRACTIVE KERATECTOMY</th>
<th>PREOPERATIVE DATA</th>
<th>2-YEAR POSTOPERATIVE DATA</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT</td>
<td>AGE (YR)</td>
<td>SE (D)</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>-15.75</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>-10.00</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>-10.00</td>
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<tr>
<td>4</td>
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<td>-15.75</td>
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<tr>
<td>5</td>
<td>10</td>
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<td>6</td>
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<td>4</td>
<td>-11.75</td>
</tr>
<tr>
<td>10</td>
<td>13</td>
<td>+5.25</td>
</tr>
<tr>
<td>11</td>
<td>8</td>
<td>+4.75</td>
</tr>
</tbody>
</table>

*Follow-up 12 months or less.
BSCVA, best spectacle-corrected visual acuity; diff, difference; F&F, fix and follow; NA, not able; PD, prism diopters; SE, spherical equivalent; UCVA, uncorrected visual acuity.

<table>
<thead>
<tr>
<th>TABLE 8. CORNEAL TOPOGRAPHY FOR DECENTRATION OF PHOTOREFRACTIVE KERATECTOMY (PRK) TREATMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>PATIENT</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>6</td>
</tr>
<tr>
<td>7</td>
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<tr>
<td>8</td>
</tr>
<tr>
<td>9</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>11</td>
</tr>
</tbody>
</table>

Mean decentration was 0.68 ± 0.43 mm (SD).
Visual Acuity

Nine patients were able to perform quantitative acuity tests preoperatively and postoperatively. At last follow-up (mean, 22 months), the uncorrected visual acuity at the 24-month visit had improved by 2 or more Snellen lines from the preoperative acuity in seven of nine eyes, with a maximum improvement of 7 lines (Figure 14A). In this same group, best spectacle-corrected visual acuity improved at 24 months by 2 or more logMAR lines in six of nine eyes and remained within 1 line of the preoperative visual acuity in two eyes (Figure 14B). Three children who experienced an improvement in visual acuity improved to the point that the amblyopic eye was no longer considered legally blind.

Case-Control Refractive and Visual Acuity Comparison

Tables 9 and 10 show data comparing our PRK cases to the control groups of compliant children (compliant group, n = 13) and noncompliant/late diagnosis children (noncompliant group, n = 10). The two control groups had similar initial best spectacle-corrected visual acuity, and all control patients had anisometropia of at least 4 diopters. The mean spherical equivalent interocular differences in the myopic PRK and control groups were 12.1 ± 3.2 diopters and 11.1 ± 4.0 diopters, respectively (P = .58). The mean spherical equivalent interocular differences in the hyperopic PRK and control groups were 4.4 ± 0.4 diopters and 5.5 ± 1.2 diopters, respectively (P = .15).

For the myopic subgroup, comparing our PRK patients (cases) to controls, final spherical equivalent refractive error (P = .007) and difference between initial and final spherical equivalent refractive error (P = .0001) were significantly different. For the hyperopic subgroup, comparing our PRK cases to controls, initial best spectacle-corrected visual acuity (P = .02), final spherical equivalent refractive error (P < .0001), and final difference between spherical equivalent refractive error (P = .001) were significantly different. The mean posttreatment best spectacle-corrected visual acuity of the compliant control group (both myopes and hyperopes) was 20/40, whereas that of the noncompliant control group was 20/270 (P = .002). Six of nine PRK children experienced improved best spectacle-corrected visual acuity by 2 or more logMAR lines with a maximum improvement of 7 lines. In contrast, none of the 10 noncompliant control patients achieved ≥2 logMAR lines of acuity improvement (P = .003) (Table 10, Figure 15).

The safety index was 1.24 (>1 means the best-corrected visual acuity improved postoperatively and vice versa), and the efficacy index was 1.12 (>1 means the postoperative uncorrected visual acuity was better than the preoperative best-corrected visual acuity and vice versa).

Stereoacuity, Ocular Alignment, and Amblyopia Therapy Compliance

Stereopsis was testable preoperatively and postoperatively in nine orthotropic children. Four (aged 4, 4, 7, and 8 years at treatment) had no measurable stereopsis before or after the treatment. Five patients realized an improvement in stereopsis. These children were 4, 6, 8, 10, and 11 years old at the time of the PRK procedure. The best response was in a 10-year-old child at the time of PRK, who improved from no measurable stereopsis to 60 seconds of arc (Figure 16).

Ocular alignment did not change postoperatively in most subjects. One patient had a small decrease in his esotropia. Another changed from a small esotropia to a small exotropia (Table 7). Compliance with amblyopia occlusion therapy did not improve postoperatively in any patient. All continued to have poor compliance. Patching attempts were discontinued after 6 to 9 months of effort.

### Table 9. Summary Comparisons of PRK Case and Control Baseline and Final Refractive and Visual Outcomes

<table>
<thead>
<tr>
<th>CHARACTERISTIC</th>
<th>MYOPIC GROUP</th>
<th>HYPEROPIC GROUP</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CASES (n = 9)</td>
<td>CONTROLS (n = 9)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>4.8 ± 2.1</td>
<td>5.1 ± 1.9</td>
<td>.74</td>
</tr>
<tr>
<td>Mean follow-up ± SD (months)</td>
<td>24 ± 10.2</td>
<td>19 ± 5.6</td>
<td>.09</td>
</tr>
<tr>
<td>Interocular SERE difference ± SD (D)</td>
<td>12.1 ± 3.2</td>
<td>11.1 ± 4.0</td>
<td>.58</td>
</tr>
<tr>
<td>Final SERE ± SD (D)</td>
<td>-3.3 ± 2.5</td>
<td>-11.3 ± 4.3</td>
<td>.0007</td>
</tr>
<tr>
<td>Difference between initial and final SERE ± SD (D)</td>
<td>-9.9 ± 2.7</td>
<td>+0.2 ± 0.8</td>
<td>&lt;.00001</td>
</tr>
<tr>
<td>Initial BCVA (Snellen)</td>
<td>20/400</td>
<td>20/350</td>
<td>.87</td>
</tr>
</tbody>
</table>

BCVA, best corrected visual acuity; D, diopters; SD, standard deviation; SERE, spherical equivalent refractive error; UCVA, uncorrected visual acuity.
DISCUSSION

The prevalence of amblyopia in the American population is estimated to be 2% to 5%, affecting more than 14 million people.2-5 Amblyopia is the most frequent cause of unilateral visual impairment in children and young adults in the United States and Western Europe.7-11 It is even more prevalent elsewhere in the world.144 If the amblyopia is severe and bilateral, an affected person can be functionally blind from the condition. Tommila and Tarkkanen145 reported that the risk of vision loss in the fellow eye is markedly higher in people with amblyopia. The incidence of loss of vision in the healthy eye of amblyopes was 1.75 per 1,000 compared to an incidence of 0.11 per 1,000 in the general population. In more than 50% of the cases of visual loss in this study, the cause was traumatic.

Anisometropic amblyopia is the most common form of amblyopia, and because the affected child is usually asymptomatic, late diagnosis is common.7-11 Problems with traditional treatments for anisometropia amblyopia are frequent and include poor compliance and long-lasting psychosocial stress for families and patients that continues even into adulthood.40,44 Treatment success for anisometropic amblyopia is achieved in only about two thirds of cases.10-12,18,23,27,29,55-58 Consideration of alternative treatments less dependent on patient/family compliance, such as refractive surgery, is a reasonable consideration.

Currently, most refractive surgeons use the following as upper limits of treatment dose for both PRK and LASIK: 12 diopters for myopia, 4 to 5 diopters for hyperopia, and 4 to 5 diopters for astigmatism.59 Although treating extremely high myopia (greater than 12 diopters) with PRK and LASIK has been reported in adults, the results have been less predictable and the risk for postoperative corneal haze higher.157,146,147 We chose to study PRK in children rather than LASIK because refractive outcomes are equivalent and we felt PRK had a better risk profile for children.

### Table 10. Comparison of Improvement of Visual Acuity of Photorefractive Keratectomy Cases and Noncompliant and Compliant Controls

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cases</th>
<th>Controls</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients with ≥2 lines BCVA after treatment</td>
<td>6 of 9</td>
<td>0 of 10</td>
<td>.003</td>
</tr>
<tr>
<td></td>
<td>12 of 13</td>
<td>(compliant group)</td>
<td>.26</td>
</tr>
</tbody>
</table>

BCVA, best-corrected visual acuity.

### Anisometropic Amblyopia Treatment Failure Risk Factors

Several studies have reported on factors adversely affecting the outcome of treatment for anisometropic amblyopia.17,19,23,57,131,148,149 The severity of the amblyopia has been found to be the most important factor. We explored risk factors that we believed could potentially predict failure of amblyopia treatment in patients with anisometropic amblyopia with or without concurrent strabismus in an attempt to better identify a clinical profile of children who were most likely to fail treatment. Identification of such children might alter the treatment approach, including the potential future application of refractive surgery. Although functional success (visual acuity of at least 20/40) is the more important outcome because of its implications for driving and other activities of daily living, we elected to use a secondary outcome measure (relative success) of at least 3 logMAR lines of acuity improvement as well. This was done because children with extremely poor vision in the amblyopic eye were not excluded. A secondary outcome measure was felt to be necessary to detect useful response to therapy in these children because it was unlikely that they would achieve a visual

**Figure 13**

Decentration measurements of the children treated with PRK who were cooperative enough for corneal topography. Mean decentration was 0.68 ± 0.43 mm. The one extreme outlier had a preoperative spherical equivalent refractive error of -21.00 and visual acuity of 5/200 preoperatively and postoperatively with eccentric fixation in this eye. The other outlier with decentration of more than 1 mm had had a hyperopic PRK.
Suboptimal compliance with treatment by parental report at the first follow-up examination was the most important predictive factor for both relative and functional failure in our cohort. The worse the reported treatment compliance, the higher was the risk for failure. These results are consistent with other studies showing that compliance with treatment was a factor predicting acuity of 20/40 or better.

Comparison of preoperative, 12-month, and 24-month postoperative uncorrected visual acuities. Points below the line represent improved postoperative visual acuity, and points above the line represent reduced postoperative acuity. Seven of nine children able to perform psychophysical visual acuity testing preoperatively and postoperatively had at least 2 lines of improved uncorrected visual acuity.

Comparison of preoperative, 12-month, and 24-month postoperative best spectacle-corrected visual acuities. Points below the line represent improved postoperative visual acuity, and points above the line represent reduced postoperative acuity. Six of nine children able to perform psychophysical visual acuity testing preoperatively and postoperatively had at least 2 lines of improved best spectacle-corrected visual acuity.
the success or failure of treatment. There are, however, contradictory
reports in the literature regarding the role of age at treat-
ment initiation to treatment failure. Oliver and
Nawrätzki\(^{29}\) found that good therapeutic results were
obtained in all age groups up to age 6 years. Lithander and
Sjostrand\(^{121}\) also found that age was not critical for a
successful treatment up to age 7 years. The Pediatric Eye
Disease Investigator Group demonstrated a favorable
response to treatment at 4 months in children with
moderate and severe anisometropic amblyopia in children
up to 6 years of age with no substantial differences in
response from 3 to 6 years.\(^{10,11,142}\) In contrast, Flynn and
Cassady\(^{20}\) demonstrated a lower success rate and the need
for longer duration of therapy with initiation of therapy
after 5 years of age. A clear threshold age above which
treatment began to fail could not be demonstrated in this
retrospective review, though a statistically significant
increasing risk for functional failure after 6 years of age
was demonstrated.

The risk of functional failure (acuity worse than
20/40) was higher when astigmatism greater than or equal
to 1.5 diopters was present in the amblyopic eye. We also
demonstrated that the amount of astigmatism was directly
proportional to the risk of failure. Weakley\(^{21,22}\) showed that
1.5 diopters of cylindrical anisometropia, regardless of
whether the eyes were myopic or hyperopic, was amblyo-
genic and resulted in decreased binocular function.
Somer and coworkers\(^{151}\) concluded that against-the-rule
astigmatism had an unfavorable impact on the outcome of
amblyopia treatment. A possible explanation for this lack
of treatment response in the face of astigmatism is that
these children experience image blur at all distances,\(^{152}\)
unlike myopic patients who can see clearly at their ante-
rior focal point and hyperopic patients who can partially or
completely focus an image through accommodation, lead-
ing to more time without a focused image with anisoastig-
matism. An alternative explanation is that cylindrical
refraction can be more difficult to accurately determine in
children, who are often uncooperative for examination,
potentially reducing the beneficial effect of spectacle
treatment.

It has been reported that initial visual acuity was
worse in amblyopic children with both strabismus and
anisometropia as compared to those with pure
anisometropic amblyopia. The combination of
anisometropia and strabismus has also been reported to
have a lower treatment success rate when compared with
pure anisometropia.\(^{17,18,23,36,37}\) In a large, well-controlled
prospective study, however, the Pediatric Eye Disease
Investigator Group\(^{10,11,142}\) did not find a difference in the
initial visual acuity or response to amblyopia treatment in
children with concurrent strabismus and anisometropic
amblyopia. We, likewise, found that concurrent strabis-
mus was not an independent risk factor for treatment fail-
ure in anisometropic amblyopic children.

The relationship between the degree of
anisometropia and the depth of the amblyopia is contro-
versial. Although some clinicians have reported no corre-
lation between the degree of anisometropia and the depth
of amblyopia, others found that higher degrees of
anisometropia were associated with more severe ambly-
opia. Flynn\(^{16}\) in his meta-analysis of 23 studies,
found that successful treatment was associated with less
than 4 diopters of anisometropia. Prior to analysis of our
data, our clinical gestalt was that we would also find a
positive association between the level of anisometropia and
the severity of amblyopia. Interestingly, this was not
the case. The number of children, however, in our cohort
with extreme anisometropia (ie, 6 diopters or more) was
small, comprising only 11% of the retrospective cohort.
Perhaps our results would have been different if this
subgroup had been larger.

Previous studies have demonstrated that poor initial
visual acuity in the amblyopic eye was more often associ-
ated with a poor outcome.\(^{18,23,37}\) The Pediatric Eye Disease
Investigator Group demonstrated, in three well-
controlled studies, a good response to treatment in chil-
dren with moderate or severe amblyopia up to 20/400
acuity, though visual acuity did not reach 20/40 in 22% to
25%.\(^{10,11,36}\) In this study, initial visual acuity of 20/200 or
worse was not a risk factor for relative failure but was a
risk factor for functional failure, the more important
outcome, because this level of acuity (20/40) is required to
procure an unrestricted driver’s license in most states in
the United States. Patients with very poor initial vision in
the amblyopic eye can experience improvement of their
vision with treatment, though they may not achieve a final
visual acuity of 20/40 or better.

We have demonstrated in this study that the major
risk factors predicting failure of traditional therapy for
anisometropic amblyopia include poor reported treat-
ment compliance (relative failure), age of 6 years or more
at treatment initiation (relative failure), the presence of
1.5 diopters or more of astigmatism (functional failure),
and an initial visual acuity of 20/200 or worse (functional
failure). It is our belief that such patients have the great-
est potential to benefit from refractive surgery.

**Corneal Thickness**

Knowledge about the thickness of the cornea in children
is critical for surgical planning and results of subtraction
refractive surgery, such as PRK. The thickness of the
cornea limits the degree of correction of refractive errors,
because there is a relatively fixed amount of refractive
Both central and peripheral corneal thicknesses have been well studied and reported in adults. In contrast, very little information has been reported regarding corneal thickness in children, especially children older than 1 year of age. This is particularly true regarding the paracentral cornea, an area included in the treatment zone of most refractive surgical procedures. Such information is essential before embarking on excimer refractive procedures in children. If the pediatric corneal thickness measurement were found to be significantly different from the corneal thickness measurement in adults, PRK treatment nomogram modification may be necessary for best visual and refractive outcomes.

Autzen and Bjornstrom found that the mean central corneal thickness in premature infants was 654 mm, significantly thicker than that of the adult. Other studies on corneal thickness in the full-term newborn have likewise reported that the central cornea was thicker than that of the adult, averaging between 573 mm and 583 mm. Ehlers and coworkers published the only study that evaluated the central corneal thickness in the age range between birth and 14 years, even though only 60% of the 61 subjects were older than 1 year of age. They also used optical pachymetry, a technology that is less accurate than modern ultrasound pachymetry. The mean thickness of the central cornea from Ehlers' study was 541 mm for infants and toddlers between 0 and 2 years of age and 520 mm for children in all three groups, 2 to 4 years, 5 to 9 years, and 10 to 14 years of age. Ehlers and coworkers believed that adult corneal thickness was attained by about 3 years of age. Although the report of Ehlers and coworkers gives some good information, more accurate data using modern ultrasound pachymetry on more children between the ages of 2 and 10 years are desirable.

The mean pediatric central and paracentral corneal thicknesses in our pediatric cohort measured with ultrasonic pachymetry were 544 mm and 571 mm, respectively. This central corneal thickness result was not different from the mean adult central corneal thickness. Furthermore, the trend of a thicker cornea paracentrally in our cohort was similar to that reported in adults. The thickness of the paracentral cornea 3 mm from the center in our children fell midway between the mean thicknesses of the paracentral cornea in adults as measured at 2 mm and 4 mm from the center (Figure 17).

Combining our data with the previously published information from premature babies and full-term infants, it appears that the central cornea is thicker at birth. From the data from our cohort and Ehler and coworkers, it then rapidly decreases during the first few months of life followed by a slow increase over the next 9 years. The rapid decrease in the thickness of the cornea occurring in the first few months of life may be explained by regulatory mechanisms that control hydration, evaporation, and transparency.

Contrary to what was reported by Ehlers and coworkers, who found no difference in the thickness of the cornea in different age groups of children older than 2 years, our results showed a slight increase in the thickness of the cornea with age during the first 9 years. This might be explained by the slight increase in the thickness of Descemet's membrane with age.

The central corneal thickness in the adult African American population has been reported to be thinner than that in the Caucasian population. The average thickness of the cornea in our African American subgroup was slightly thinner than in our Hispanic and Caucasian subgroups; however, the number of African American children in our study was too small to yield reliable statistical results.

In summary, pediatric central and paracentral corneal thicknesses in the 2- to 14-year-old age groups were consistent with those of the adult. This information is important when planning pediatric subtraction refractive surgery such as PRK. Based on these findings, special surgical considerations regarding corneal thickness are probably not warranted for PRK in children 2 years and older.

General Anesthesia Protocol
Photorefractive keratectomy in adults is performed under topical anesthesia in an office setting. Use of topical or local anesthesia with facial block has been reported in children. Children younger than 10 years of age, however, typically cannot be treated with either of these approaches because of inability to adequately cooperate. Unfortunately, waiting until children are old enough to cooperate for the PRK under topical anesthesia is less likely to produce favorable visual outcomes because the sensitive period of visual development will have passed. Therefore, a protocol for general anesthesia was developed to enable PRK treatment of younger children.

This protocol included induction of general anesthesia in an induction room, using the laryngeal mask airway with transfer of the child to the operating room, where nitrous oxide was then administered through a semiclosed circuit. This was done to minimize the risk of leakage of volatile gases into the operating room. The 193-nm wavelength of the argon fluoride excimer laser lies within the absorption spectrum of many anesthetic gases, including nitrous oxide. If a volatile gas escapes, attenuation of the
laser beam may occur and the laser will increase voltage in an effort to maintain fluence until the laser malfunctions and an error message appears.\textsuperscript{125} Using my protocol, no laser malfunctions occurred, implying no or insignificant levels of leakage of nitrous oxide, the only volatile gas used during the PRK procedure.

Other reported protocols to anesthetize children for refractive surgery have included sedation with induction using nitrous oxide and halothane without intubation,\textsuperscript{104} sevoflurane 50\% inhalation with a laryngeal mask,\textsuperscript{89} and sedation using intravenous ketamine.\textsuperscript{107} Cook and coworkers\textsuperscript{125} reported laser shutdown during anesthesia induction using nitrous oxide. They, however, performed anesthesia induction in the same room as the laser procedure, which predisposed to leakage of the nitrous oxide and/or halothane into the operating room. This probable leakage of nitrous oxide most likely caused secondary attenuation of the laser beam and an increase in laser voltage until the laser malfunctioned. An important potential problem with the use of ketamine is its tendency to induce nystagmus, which could render treatment difficult and inaccurate.\textsuperscript{107,160}

Because of the inability of anesthetized children to maintain gaze on a fixation target, another issue of concern with use of general anesthesia was the possibility of a decentered ablation. Mild decentration has not been shown to be a significant problem in any published study.\textsuperscript{141} It is generally assumed that decentration of greater than 1 mm is needed in order to cause clinically significant adverse visual effects.\textsuperscript{140} In one pediatric PRK study, one child among the six operated on under general anesthesia had decentration of more than 0.5 mm.\textsuperscript{102} These results are comparable with optical zone centration analysis following PRK in adults with a mean decentration of 0.5 mm.\textsuperscript{170} Deitz and coworkers\textsuperscript{171} reported that 9\% of his adult PRK patients had decentration of 1 mm or more from the pupillary center. Importantly, they noted that this amount of decentration did not adversely affect best-corrected visual acuity or contrast sensitivity. In our series, using manual centration and two observers to monitor eye position, the mean decentration (0.68 mm) was slightly higher than that reported for adults but was still within an acceptable range in seven of nine children able to be tested. The cause of the decentration in my group could certainly have been intraoperative difficulty in detecting tilt of the iris plane; however, it is also possible that some of the error was artifactual because of suboptimal fixation of the children during the topographic measurement, especially in those with significant residual postoperative amblyopia. Furthermore, it is noteworthy that the child with the decentration of 1.05 mm experienced a large improvement in uncorrected and best spectacle-corrected visual acuity postoperatively, with the uncorrected visual acuity improving from 20/200 to 20/60 and the best spectacle-corrected visual acuity improving from 20/60 to 20/40.

**Photorefractive Keratectomy: Safety and Efficacy**

Both PRK and LASIK have been performed in a small number of children.\textsuperscript{96,105-107,109,132,139} Previous studies have reported good short- and moderate-term refractive and vision results. Most were conducted outside of the United States and have predominantly included children aged 7 years and older.\textsuperscript{96,105-107,109,110} By the time a child is 7 years of age, amblyopia is less likely to respond to therapy because the period of visual cortical plasticity (ie, the sensitive period) has passed. Almost all reports in the literature on refractive surgery in children have reported only on myopic PRK in children; only three children treated with hyperopia PRK have been reported.\textsuperscript{106} None of the previous studies of refractive surgery in children have compared their results with those in a control group.

Ideally, amblyopia should be treated as early in life as possible. If refractive surgery is to play an active role in the treatment of anisometric amblyopia, it is likely that it, too, will need to be applied early in life, during the sensitive period of visual development. Very little was known about the pediatric response to refractive surgery, namely, the corneal healing rate, postoperative discomfort, refractive response, long-term corneal status, visual acuity, and stereopsis.

We have now followed 11 children, aged 2 to 11 years, for 2 years who were treated with PRK for anisometric amblyopia, of which six (55\%) were less than 6 years of age at treatment. These are some of the youngest children reported to date to have undergone an excimer laser refractive procedure. We have also compared these PRK patients’ visual gains to a control group. I chose to include only children with extreme amounts of anisometropia (≥26 diopters of myopia or ≥4 diopters of hyperopia) who were noncompliant with traditional therapy in order to be as conservative as possible while exploring the potential benefits or complications of PRK in children. In fact, our study group had a mean spherical equivalent interocular difference of 9.90 diopters, well above the entry criteria minimum. We believe, from our own clinical experience and from my noncompliant control group data, that our study group children were likely to have had worse visual outcomes if they had not undergone the PRK.

Corneal epithelial wound healing following PRK in adults treated for myopia has been shown to take approximately 3 to 5 days.\textsuperscript{126,132} Postoperative epithelial healing is affected by several parameters, with a major factor being the size of the initial epithelial defect. It is generally thought that the corneal epithelium in younger individuals heals faster than in older patients following injury.\textsuperscript{172}
This proved to be the case in our surgically induced epithelial defects for myopia; all but one healed within 3 days. The one who did not heal within 3 days had sustained a postoperative traumatic enlargement of the epithelial defect after being hit in the eye with a ball on the second postoperative day. This postoperative event further supports our decision to study PRK in children because of concerns for postoperative flap disruption from trauma or eye rubbing that could occur with LASIK. The epithelial defect that is created from hyperopic PRK is larger than that created to treat myopia, and healing time is prolonged in adults. This was true in our hyperopic study patients as well, with a mean healing time of 4.5 days in the hyperopic group versus 2.8 days for the myopic group.

It has been demonstrated that after PRK, the status of the corneal epithelium can influence collagen synthesis. An unhealthy epithelium may result in anterior stromal haze and regression of the refractive correction. The rapid and smooth healing of the corneal epithelium after PRK that occurred in our study patients may imply the presence of healthy epithelium, which may in turn have translated to the minimal corneal haze most of these treated children experienced.

Conflicting reports exist regarding the effect of bandage contact lenses on the rate of epithelial healing after PRK. The disposable soft contact lens used in our study may have decreased postoperative discomfort and possibly sped up healing. This, however, may not necessarily be true, because two study children lost their contact lenses at 1 and 2 days after PRK and did not appear to experience increased discomfort. Because they were comfortable, the contact lens was not replaced, and the corneal epithelium healed at the same rate as in those children who retained their contact lenses. Insertion of collagen punctal plugs in the upper and lower puncta of the treated eye may also have contributed to rapid epithelial healing by keeping the corneal surface well hydrated during the early healing period. This may have also aided in reducing the postoperative discomfort.

The refractive goal in our study was to reduce the amount of anisometropia to less than or equal to 3 diopters. The aniseikonia would then be reduced to the point where fusion was possible and amblyopia potentially more amenable to therapy. The full amount of myopia in the patients with more than –11.50 diopters of spherical equivalent myopia was not treated because PRK for these higher levels of myopia has been associated with severe corneal haze, an undesirable situation in an adult and an even more undesirable situation in a child whose visual system is immature. Six of eight myopes and all hyperopes had their anisometropia reduced to 3 diopters or less. Interestingly, two patients with extremely high levels of myopia (spherical equivalent of –21.00 diopters and –13.75 diopters) had larger-than-expected responses to their PRK treatment dose of 11.50 diopters, reducing their final refractive errors at 12 months to –4.75 diopters and –0.50 diopters, respectively. Both results were within 3 diopters of the fellow eye. The basis for this over-response is not known. Williams reported a similar result in adults with refractive errors over –10.00 diopters. Astle and coworkers also noted this same response in their series of myopic children treated with PRK. Reduced scleral rigidity and/or a difference in corneal remodeling in the highly myopic eye may play a role in children and adults with extremely high levels of myopia treated with PRK.

In the myopic subgroup, four of eight had refractions within 1 diopter of the refractive target and five of eight within 2 diopters at the 12-month postoperative visit. At the 24-month follow-up visit (mean, 22 months), three of eight were within 1 diopter of the target and six of eight were within 2 diopters. The mean preoperative refractive error of –13.70 diopters was reduced to a 24-month mean postoperative refractive error of –3.30 diopters. These results are in agreement with those found in previous studies of PRK in myopic children, though our patients were generally younger. These results are also similar to results reported from adults with similar levels of extremely high myopia treated with PRK. In our hyperopic group, two of the three patients were within 1 diopter of the refractive target and all were within 2 diopters at the 12-month postoperative visit. At the 24-month follow-up visit, only two returned for follow-up. One was within 1 diopter and the other was within 2 diopters of target. There are not enough hyperopic children in this study to make any conclusions, but these results are similar to those of the only study that included pediatric hyperopic PRK.

The myopic group had moderate refractive regression over the first 12 months following PRK with a mean spherical equivalent regression of 2.50 diopters, though there was wide variation (Figure 12). This early regression was likely due to corneal healing and is similar to refractive regression reported in extremely high myopic adult patients treated with PRK. Regression thereafter in our myopic subgroup appeared to have stabilized with minimal further change. The small change in refractive error after 12 months was probably due to continued eye growth in this pediatric population. The myopic regression in adults treated with PRK appears to be associated with increased corneal haze and fibrosis. Significant corneal haze was not an issue in our compliant PRK children, so the mechanism for regression in children may be different. Other studies in children treated with PRK for myopia have demonstrated a myopic shift (ie, regression)
ranging from 0.8 diopters to 1.7 diopters over a 12-month follow-up period, though the levels of treated myopia tended to be lower overall than in the current study.\textsuperscript{100-103,106}

The hyperopic group showed mild refractive regression over the initial 12-month follow-up interval, with a mean spherical equivalent regression of 1.10 diopters. This amount of regression is similar to that found in adult hyperopic PRK for similar levels of preoperative hyperopia.\textsuperscript{73,186-187} At the 24-month follow-up, one was stable and the other had experienced further regression. There are too few hyperopic subjects to draw any further conclusions.

Corneal haze in our cohort during the early preoperative period was minimal and not visually important, even in the one child who was noncompliant with the postoperative topical steroid regimen. The haze was at its maximum at the 1-month follow-up visit and was never worse than mild in the group who followed postoperative treatment recommendations. The corneal haze never impacted the retinoscopic reflex during retinoscopy and thus was not felt to be visually important in any patient. Four (36%) of the study patients had a minimal non–visually significant degree of residual corneal haze, and one child had mild to moderate corneal haze 1 year after surgery. All but one of these children were high myopes. All children had no or minimal haze at the 24-month visit.

Higher degrees of corneal haze have been reported in adults with extremely high myopia following PRK.\textsuperscript{134-137,138} Alio and coworkers\textsuperscript{102} reported that corneal haze was the main optical complication following pediatric PRK, but noted that it decreased by 1 year. In the only study that included hyperopic PRK in children, corneal haze was not mentioned.\textsuperscript{106} The low incidence of corneal haze in our study group may have been due to the longer use of topical corticosteroids, good hydration during the early postoperative period, or improvements in excimer laser technology. The VISX Star S2 laser, a third-generation excimer laser, was used in this study. Older studies of pediatric refractive surgery used first-generation broad-beam excimer lasers.\textsuperscript{102,106}

Most children treated with PRK in our study enjoyed mild to moderate improvement in uncorrected and best spectacle-corrected visual acuity at 12 months and 24 months after PRK, despite many having severe amblyopia at presentation. When compared with the noncompliant control group, my PRK group experienced a statistically significant improvement in best-corrected visual acuity. This is the first study of refractive surgery in children that has compared visual outcomes to a control group of comparable children. Efficacy has been demonstrated even in the face of severe preexisting amblyopia in many of the study children. We feel that if refractive surgery were performed at an earlier age, before severe amblyopia has been established, long-term visual outcomes might improve more than was seen in this study group. As an example, the youngest child in this study, who preoperatively at age 2 years could be assessed only by comparing fixation behavior, had a best-corrected visual acuity of 20/50 at the 12-month and the 24-month visits, despite his continued postoperative lack of compliance with spectacle use and amblyopia therapy. Compared with our noncompliant control group, his vision was markedly better than what we might have anticipated otherwise (Table 10). For example, there were two noncompliant control patients with spherical equivalent refractive errors on either side of his spherical equivalent refractive error of –13.75 diopters. One, with a spherical equivalent refractive error of –10.50 diopters, had no visual acuity improvement with a pretreatment and posttreatment visual acuity of 20/200. The other child, with a spherical equivalent refractive error of –17.00 diopters, also had no change in visual acuity with a pretreatment and posttreatment visual acuity of 20/300.

Prior to commencing this study, we were concerned first about the safety and second about efficacy of PRK in children with anisometropic amblyopia. The safety index (1.24) found in our study demonstrated that PRK in noncompliant children with anisometropic amblyopia appears to be safe through 24 months follow-up, though this safety index only evaluates visual acuity as an indicator for safety. The efficacy index (1.12) in our study showed some efficacy, even in this group of children with marked anisometropia and profound amblyopia who had already failed traditional amblyopia therapy. We postulate that performing PRK on younger children identified as having a high risk for amblyopia treatment failure would be significantly more efficacious. Stereoacuity improved in five of nine testable children. This was an encouraging but unexpected finding, since most of our patients were well beyond 2 years of age, the age at which most ophthalmologists feel stereopsis development is largely complete. Longer-term follow-up is planned to evaluate for any late complications.

This study, although important, has some limitations. First and foremost, our case-comparison study is limited by a small sample size. However, this small sample size was intentional. Because refractive surgery in children is a new area for research, with little previously published information, we elected to treat only a small group of noncompliant children with severe anisometropia and follow them for a significant period of time to detect any adverse long-term complications, before subjecting other children to this procedure. Because of small sample size in some of the parts of this study, categorical variables were sometimes used for the analysis. Next, when performing the statistical analysis, we assumed that the
control groups were representative of severe anisometropic amblyopes in the population at large. Selection bias is a potential weakness of all studies; however, we controlled for this bias as much as possible by including all patients that met the inclusion criteria in our practice and the amblyopia database.

At 24 months post-PRK for anisometropic amblyopia in children, the refractive error response appears to be similar to that of adults with comparable refractive errors. More experience is needed with larger numbers of children with extremely high myopia in order to adequately address the possible need to modify treatment nomograms for this subgroup of patients. Postoperative corneal haze was minimal. Improvements in uncorrected and best spectacle-corrected visual acuities and stereopsis were seen in most children. Photorefractive keratectomy, indeed, appears to have potential as a treatment option for anisometropic amblyopia in children noncompliant with traditional therapy.

SUMMARY

We set out to systematically investigate and demonstrate the potential use of PRK for children with anisometropic amblyopia who were noncompliant with conventional therapy. The predictive risk factors for failure of traditional amblyopia therapy were determined and included (1) suboptimal compliance, (2) age 6 years or older, (3) astigmatism of 1.5 diopters or more, and (4) initial visual acuity of 20/200 or worse. We investigated corneal thickness and found that children between the ages of 2 and 14 years have corneal thickness parameters similar to those of adults, suggesting that PRK treatment nomograms probably do not require adjustment for children or adolescents. A protocol to perform PRK under general anesthesia was designed and successfully implemented, with no intraoperative or postoperative complications or laser malfunctions. Finally, we demonstrated that PRK for anisometropic amblyopia in children was safe, well tolerated, and associated with no significant complications through 2 years of follow-up. Photorefractive keratectomy resulted in statistically significant improvement of uncorrected and best spectacle-corrected visual acuity when compared to a noncompliant control group. Lastly, stereopsis improved in a majority of patients, and this improvement did not seem limited by age. Treating children with anisometropic amblyopia well within the sensitive period of visual development, soon after identification of failure risk factors, appears to be a reasonable consideration and may result in better long-term visual outcomes.

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REFERENCES


Photorefractive Keratectomy for Anisometropic Amblyopia in Children


ABSTRACT

Purpose: The clinical and molecular genetic classification of syndromes with congenital limitation of eye movements and evidence of cranial nerve dysgenesis continues to evolve. This monograph details clinical and molecular genetic data on a number of families and isolated patients with congenital fibrosis of the extraocular muscles (CFEOM) and related disorders, and presents an overview of the mechanisms of abnormal patterns of motor and sensory cranial nerve development in these rare syndromes.

Methods: Clinical examination of one patient with CFEOM1, one family with clinical features of CFEOM2, one family with recessive CFEOM3, one family with horizontal gaze palsy and progressive scoliosis (HGPPS), and four patients with various combinations of congenital cranial nerve abnormalities. Genotyping of families with CFEOM and HGPPS for polymorphic markers in the regions of the three known CFEOM loci and in the HGPPS region, and mutation analysis of the ARIX and KIF21A genes in patients with CFEOM were performed according to standard published protocols.

Results: The patient with CFEOM1 had the second most common mutation in KIF21A, a 2861 G>A mutation that resulted in an R954Q substitution. The family with CFEOM2 phenotype did not map to the CFEOM2 locus. The family with recessive CFEOM3 did not map to any of the known loci. The HGPPS family mapped to 11q23-q25. One patient had optic nerve hypoplasia and fifth nerve dysfunction. Two patients had the rare combination of Möbius syndrome and CFEOM. One patient had Möbius syndrome and fifth nerve dysfunction.

Conclusions: There is genetic heterogeneity in CFEOM2 and CFEOM3. Abnormalities in sensory nerves can also accompany abnormalities of motor nerves, further substantiating the effect of individual mutations on developing motor as well as sensory cranial nerve nuclei.

INTRODUCTION

Normal extraocular muscle (EOM) innervation depends on normal cranial nuclear motoneuron formation from neuronal precursors, normal axonal pathfinding from the cranial nuclei to developing EOMs, and the establishment and maintenance of normal connections between mature neurons and their target cells. A number of well-defined syndromes characterized by congenital limitation of eye movements from abnormal innervation or miswiring of the EOMs have recently been grouped as the “congenital cranial dysinnervation disorders,” a term coined for congenital disorders resulting from aberrant innervation of the ocular and facial musculature.1 In the most common of these conditions, Duane retraction syndrome (DRS) type I, there is absence of the motoneurons of the sixth nerve nucleus and abnormal innervation of the lateral rectus muscle by a distal branch of the third nerve. This leads to limited abduction of the affected eye and cocontraction of the medial and lateral rectus muscles on attempted adduction. Less common are the several types of congenital fibrosis of the extraocular muscles (CFEOM), in which there is severe restriction of eye movements and ptosis from abnormal oculomotor and trochlear nerve development, or from abnormalities of EOM innervation. Other conditions involving congenital limitation of eye movements include congenital third nerve palsy, congenital fourth nerve palsy, Möbius syndrome, double elevator palsy, and congenital horizontal gaze palsy. Abnormalities of cranial nerve development have been clearly demonstrated in some of these conditions using magnetic resonance imaging and/or post-
mortal examination.\textsuperscript{2,6}

The past few years have witnessed a significant expansion of our understanding of the genetics of CFEOM, predominantly through the work of Elizabeth Engle, MD, of Boston Children’s Hospital.\textsuperscript{17-20} In less than a decade, her group mapped and cloned the genes for two types of CFEOM and laid the foundation for the understanding of the pathophysiology of this group of disorders. A significant degree of genetic heterogeneity exists for some of the less common types of CFEOM, and work is under way to identify additional genes that cause these and other congenital disorders of ocular motility.

In addition to well-delineated syndromes, there exist a number of rare cases in which DRS or CFEOM is associated with congenital abnormalities of other cranial nerves. Some of these cases present well-defined clinical constellations of abnormalities and appear to result from abnormal development of multiple motor and sensory cranial nerves.\textsuperscript{27-34}

Finally, some congenital cranial neuropathies are occasionally associated with recurrent and consistent patterns of craniofacial or systemic malformations, indicating a more generalized effect of the responsible genetic or teratogenic agent on the developing fetus, and leading to such well-defined conditions as thalidomide embryopathy\textsuperscript{15} and Okihiro syndrome.\textsuperscript{17,36,37}

The purpose of this monograph is to (1) review the current literature on congenital disorders of cranial nerve development; (2) present clinical and molecular genetic data on unreported patients and families with CFEOM, including evidence of genetic heterogeneity, supported by negative mutation analysis and linkage data; (3) describe an unreported family with autosomal recessive congenital horizontal gaze palsy with progressive scoliosis that confirms linkage to the recently identified HGPPS locus; (4) review the clinical data on a few rare patients with clinical findings that bridge some of the well-described syndromes; and (5) present an overview of the mechanisms of abnormal patterns of motor and sensory cranial nerve development that may lead to common and rare clinical syndromes encountered in clinical practice.

**Embryology and Anatomy of Cranial Nerve Nuclei**

**Embryology**

The hindbrain, or rhombencephalon, is composed of the cerebellum, pons, and medulla. With the exception of the oculomotor nuclei, cranial nerve nuclei are derived from rhombencephalic neuronal precursors. In early development, the hindbrain is segmented into five compartments called rhombomeres. Neuronal populations within individual rhombomeres display limited intermixing with neighboring compartments. As a result, the location and position of a neural progenitor during hindbrain segmentation determine its contribution to adult brainstem structures and axonal connections. For example, the trochlear, trigeminal, abducens, and facial neurons are generated in rhombomeres 1, 2-3, 5-6, and 4-5, respectively. Due to their compartmental identity, these neuronal progenitors display programmed migratory behaviors and send axons along defined trajectories toward their peripheral targets. While a neural cell progenitor’s position along the anteroposterior axis determines its identity, the cell’s position along the dorsoventral axis appears to influence its sensory or motor function.

Investigations into the molecular mechanisms of hindbrain development have yielded insights into the potential relationship between human developmental disorders and the molecular signals that determine neuronal identity and axonal guiding in the brainstem.\textsuperscript{38} The sonic hedgehog (SHH) gene appears to be a key player in the process of neuronal class determination along the dorsoventral axis. Graded expression of SHH along this axis appears to generate domains conducive to either motor (ventral) or sensory (dorsal) neuron development. Using knockout mice generated by gene-specific homologous recombination, several families of homeobox transcription factors (Hox, Kreisgler, Nkx, Phox, and Krox20) and tyrosine kinase receptors (Eph) have been shown to play important roles in rhombomeric compartmentalization, neuronal precursor determination, and the establishment of specific brainstem axonal pathways. Other studies on the dorsoventral patterning of the rhombencephalon have implicated the homeobox genes Pax6 and Nkx2.2 in the early divergence of the transcriptional program of hindbrain somatic and visceral motor neuronal differentiation. Pax6 is involved in the differentiation of the hindbrain somatic motoneurons and VI interneurons in the hindbrain and/or spinal cord. In the mouse, Osumi and colleagues\textsuperscript{39} have shown that Pax6 is involved in the specification of hindbrain neurons through the regulation of Islet2 and Wnt7b gene expression patterning. Takahashi and coworkers\textsuperscript{40} demonstrated that Pax6 regulates specification of the ventral neuron subtypes by establishing the correct progenitor domains. Pattyn and colleagues\textsuperscript{41} provided genetic evidence that the paired-like homeodomain protein Phox2b is required for the formation of all branchial and visceral, but not somatic, motor neurons in the hindbrain. They used mice lacking Phox2b in which both the generic and subtype-specific programs of motoneuronal differentiation are disrupted at an early stage. Most motor neuron precursors died inside the neuroepithelium, whereas those that emigrated to the mantle layer failed to switch on early postmitotic markers and to down-regulate neuroepithelial markers.\textsuperscript{42} PHOX2A or ARIX is another gene involved in differentiation of motor nuclei of the hindbrain, especially the nuclei of the third and fourth nerves.\textsuperscript{43} The PHOX2A protein is
Genes expressed in the developing rhombencephalon are good candidates for developmental disorders such as Möbius syndrome, DRS, and the CFEOM syndromes. Teratogenic insults or mutations in these developmental genes may lead to dysgenesis of cranial nerve nuclei, or in the processes involved in establishing normal connections to target muscles or end-organs. If a particular gene is involved and the pattern of dysgenesis consistent, a recurrent constellation of clinical findings appears and leads to development of a “syndrome.” This concept has been identified in a number of clinical syndromes with congenital limitation of eye movements and has been expanded to a more global mechanistic and pathophysiologic scheme that explains common and rare abnormalities of ocular and oculomotor abnormalities and their associated clinical nonocular systemic manifestations.

**Anatomy**

Nuclei containing brainstem motor neurons form two discontinuous columns or sets (Figure 1). One set of motor nuclei is located dorsomedially in the brainstem, close to the ventricle. Its axons exit the brainstem in a ventromedial position. The second set of motor nuclei is located more laterally. Axons of this lateral set exit the brainstem in a lateral position, adjacent to the entrance of sensory fibers. Cranial nerves III in the rostral midbrain, IV in the caudal pons, and XII in the rostral medulla belong to the first set, called somatic motor because they innervate muscles derived from head somites. Their nuclei are located close to the midline and to the surface of the ventricle. Their axons exit the brainstem in a ventral position, close to the pyramidal tract: medial to it in the case of III, lateral to it in the cases of VI and XII. The trochlear, or fourth, nerve, located in the caudal midbrain, is also somatic motor, but its axons exit from the dorsal surface of the midbrain and are crossed, such that the right nucleus supplies the left superior oblique and vice versa. The other set of motor cranial nerves is called branchial motor because they innervate muscles associated with structures derived from branchial arch mesoderm. Their nuclei are located much more laterally. Motor 5, in the rostral pons, is close to the lateral angle of the fourth ventricle. Motor 7, in the caudal pons, is ventral to the descending sensory tract and nucleus of 5. The third nucleus of this set, nucleus ambiguous, contains many motoneurons which send their axons through the tenth nerve to skeletal muscles of the soft palate, pharynx, and larynx, and a smaller contingent, which send axons into the ninth nerve to innervate a single muscle, the stylopharyngeus. The axons of these three nuclei share the property that they exit in a lateral “dorsal root” position, adjacent to entering sensory axons of the same cranial nerves, rather than in a “ventral root” position like somatic motor cranial nerves. Axons of motor 7 travel medially and form a loop around the sixth nerve nucleus, and then travel ventrolaterally to exit the brainstem just medial to a special sensory nerve, the eighth nerve. The last branchial motor nerve, the spinal accessory, behaves partly like a spinal nerve, partly like a cranial nerve. Its motoneurons are in the caudal medulla and in the first five segments of the spinal cord, but the axons exit from the spinal cord in a “branchial motor” position just dorsal to the denticulate ligament, not with the ventral roots of those segments. The nerve then ascends to the cranial cavity, joins the vagus nerve briefly, then leaves it again and descends to the neck to innervate the two neck muscles, trapezius and sternocleidomastoid (see Figure 2 for the sites of exit of cranial nerves).

**Congenital Syndromes With Motor and/or Sensory Cranial Nerve Abnormalities**

It is beyond the scope of this paper to discuss all syndromes with congenital abnormalities of cranial nerve development. Table 1 highlights the salient clinical features of some of these syndromes and lists the identified genetic loci, individual genes, or evidence of genetic etiology or familial occurrence of each syndrome.

**Congenital Abnormalities of EOM Cranial Nerve Nuclei Leads to Specific Syndromes**

Hotchkiss and coworkers described the intracranial and orbital pathology of a clinically documented case of bilat-
eral DRS. Both abducens nuclei and nerves were absent from the brainstem, and the lateral rectus muscles were partially innervated by branches from the oculomotor nerves. Miller and colleagues reported a case of unilateral DRS in which the right side of the brainstem, cavernous sinus, and orbit was completely normal. The left abducens nucleus contained no cell bodies from motor neurons, but contained in its rostral portion several small cell bodies compatible with internuclear neurons. The left abducens nerve was absent. The left lateral rectus muscle was partially innervated by branches from the inferior oculomotor nerve.

Engle and colleagues reported the intracranial and orbital pathology of one and the muscle pathology of two other affected members of a family with CFEOM1. The superior division of the oculomotor nerve and its corresponding alpha motor neurons was absent, and there were abnormalities of the levator palpebrae superioris and the superior rectus, both innervated by the superior division of the oculomotor nerve. They also found increased numbers of internal nuclei and central mitochondrial clumping in other extraocular muscles, suggesting that the muscle pathology extends beyond the muscles innervated by the superior division of cranial nerve III. Their report presented the first tangible evidence that CFEOM results from an abnormality in the development of the extraocular muscle lower motor neuron system.

Radiologic and neuropathologic evidence of brainstem or cranial nerve nuclear abnormalities has also been presented in patients with Möbius syndrome and horizontal gaze palsy.

Congenital Fibrosis of the Extraocular Muscles

One of the earliest descriptions of CFEOM was given by Baumgarten in 1840, but Heuck in 1879 gave the first account of the familial occurrence and the postmortem findings in one patient with this condition. The term “general fibrosis syndrome” was coined by Brown in 1950 to describe patients with fibrosis of three or more extraocular muscles. Brown proposed that strabismus disorders with congenital, nonprogressive ophthalmoplegia and active limitation and passive restriction of globe movement resulted from fibrous changes in the muscles or their tendon sheaths. He categorized them into five syndromes based on the putative muscles involved and the degree of paralysis: (1) typical and atypical (horizontal) retraction syndromes (medial and/or lateral recti), (2) strabismus fixus (medial and/or lateral recti), (3) vertical retraction syndromes (superior and/or inferior recti), (4) superior oblique tendon sheath syndromes (inferior and superior oblique muscles), and (5) general fibrosis syndrome (three or more of the extraocular muscles). We currently refer to Duane syndrome for the horizontal retraction syndromes and Brown syndrome for the superior oblique tendon sheath syndromes. The remaining conditions are globally called CFEOM.

The incidence and prevalence of the fibrosis syndromes is unknown. They have been reported as isolated or familial cases from around the globe. Dominant forms are more common in Western countries, and recessive forms are more commonly encountered in the Middle East. The original clinical classification proposed by Harley and coworkers that divided patients with CFEOM into five categories is now complemented by one that is based on the mode of inheritance and individual gene mutations (Table 2). A correlation between phenotype and individual genetic types appears to exist.

The management of patients with CFEOM has been discussed in other publications and is beyond the scope of this article.

CFEOM1

CFEOM1 is autosomal dominant, and its major determining gene maps to the centromere of chromosome 12 (12p11.2-q12). Engle and colleagues found evidence of genetic heterogeneity among 11 CFEOM1 pedigrees. All demonstrated autosomal dominant inheritance and nine were consistent with linkage to FEOM1. Two small CFEOM1 families were not linked to FEOM1, and both were consistent with linkage to FEOM3. Thus far, no ARIX mutations have been identified in any affected members of CFEOM1 pedigrees or in any sporadic cases of classic CFEOM. Individuals with CFEOM1 have congenital nonprogressive bilateral ptosis and external ophthalmoplegia. The eyes are fixed in 20 to 30 degrees of downgaze, and patients hold their heads in a chin-up position. Horizontal strabismus is common, and residual eye movements are often aberrant, with convergent or diver-

\[\text{FIGURE 2}\]

Sites of exit of cranial nerves from the hindbrain.
gent movements on attempted upgaze. Families with CFEOM1 demonstrate full penetrance of the trait with little or no variability among or between families from around the world. The evaluation of a Turkish family with CFEOM1 demonstrated a greater phenotypic heterogeneity at the FEOM1 locus than previously reported. The phenotype in this family was marked by variable expression and overlap with both classic CFEOM1 and CFEOM3 phenotype (vide infra).

Yamada and coworkers discovered that patients with CFEOM1 harbor heterozygous missense mutations in a kinesin motor protein encoded by KIF21A. They identified six mutations in 44 of 45 probands with CFEOM1. The primary mutational hotspots were in the stalk domain of the protein, highlighting an important new role for KIF21A in the formation of the oculomotor axis. Neurons use kinesin and dynein microtubule-dependent motor proteins to transport essential cellular components along axonal and dendritic microtubules. Kinesins are mechanochemical motors that utilize the energy of ATP hydrolysis to walk along microtubules. Kinesin motors have been implicated in several types of motile processes, including transport of mitochondrial or vesicular cargoes along microtubules, intermicrotubule sliding, and the assembly and motility of mitotic and meiotic spindles. Ubiquitously expressed in all eukaryotic organisms, kinesin family members (KIFs) are defined by the extensive homology they share within a globular motor domain that contains both microtubule- and ATP-binding sites. Marszalek and coworkers identified two mouse kinesins that provided insight into a unique intracellular kinesin-targeting mechanism in neurons. KIF21A and KIF21B share colinear amino acid similarity to each other but not to any previously identified kinesins outside of the motor domain, and localize differently to dendrites and axons. KIF21A protein is localized

<table>
<thead>
<tr>
<th>CRANIAL NERVE</th>
<th>DISORDER</th>
<th>CLINICAL SIGNS</th>
<th>GENE</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Kallman syndrome</td>
<td>Anosmia, hypopituitarism</td>
<td>KAL1, FGFR1</td>
</tr>
<tr>
<td>II</td>
<td>optic nerve hypoplasia</td>
<td>Small optic nerve head, poor vision</td>
<td>None mapped. Familial cases have been reported</td>
</tr>
<tr>
<td>III</td>
<td>Septo-optic dysplasia</td>
<td>Optic nerve hypoplasia, absent septum pellucidum</td>
<td>HESX1, PAX6</td>
</tr>
<tr>
<td>III</td>
<td>Congenital third nerve palsy</td>
<td>Ophthalmoplegia, ptosis</td>
<td>None mapped</td>
</tr>
<tr>
<td>III</td>
<td>Congenital ptosis</td>
<td>Ptosis, isolated</td>
<td>None mapped</td>
</tr>
<tr>
<td>IV</td>
<td>CFEOM1</td>
<td>Ophthalmoplegia, ptosis</td>
<td>KIF21A</td>
</tr>
<tr>
<td>IV</td>
<td>CFEOM3</td>
<td>Ophthalmoplegia, ptosis</td>
<td>Gene maps to 16q24</td>
</tr>
<tr>
<td>V</td>
<td>CFEOM2</td>
<td>Ophthalmoplegia</td>
<td>Two reports of familial cases</td>
</tr>
<tr>
<td>VI</td>
<td>Duane syndrome type I</td>
<td>Ophthalmoplegia, cocontraction</td>
<td>DRSL, mapped to 2q31</td>
</tr>
<tr>
<td>VI</td>
<td>Okhiro syndrome</td>
<td>Duane syndrome with radial ray defects</td>
<td>SALL4</td>
</tr>
<tr>
<td>VI</td>
<td>Duane syndrome and optic nerve hypoplasia</td>
<td>Cocontraction, optic nerve hypoplasia, central incisor</td>
<td>No gene mapped</td>
</tr>
<tr>
<td>VI</td>
<td>Horizontal gaze palsy with progressive scoliosis</td>
<td>Bilateral adduction deficit, progressive scoliosis</td>
<td>One gene mapped to 11q23-25</td>
</tr>
<tr>
<td>VI (+/- VIII)</td>
<td>Duane syndrome variants, includes Wilderwank syndrome</td>
<td>Various ophthalmoplegia patterns, deafness, other systemic malformations</td>
<td>No genes mapped</td>
</tr>
<tr>
<td>VII</td>
<td>Congenital familial facial palsy</td>
<td>Unilateral or bilateral facial weakness</td>
<td>A few families reported</td>
</tr>
<tr>
<td>VI, VII, other</td>
<td>Möbius syndrome</td>
<td>Ophthalmoplegia, facial weakness, other systemic malformations, Poland anomaly, flat foot, microdactyly, polydactyly, paralysis of lower legs with abnormal broad gait. Autism</td>
<td>Several familial instances with autosomal dominant inheritance, One gene localized to 3q61, Another gene mapped to 10q62</td>
</tr>
</tbody>
</table>
throughout neurons, whereas KIF21B protein is highly enriched in dendrites. 70

**CFEOM2**

CFEOM2 is autosomal recessive and its gene is located on chromosome 11q13.1.11 Affected individuals are born with bilateral ptosis and restrictive ophthalmoplegia with eyes partially or completely fixed in an exotropic position. Ability to adduct or depress the globe is absent or severely reduced. Nakano and coworkers15 identified **ARIX**, previously called **PHOX2A**, as the mutated gene in CFEOM2. They reported three mutations in four pedigrees. Yazdani and colleagues24 found a fourth mutation in a recessive Iranian pedigree with two affected siblings who displayed bilateral ptosis and exotropic strabismus fixus. The 439C>T mutation in this last family was the first nonsense mutation to be identified, and confirmed **ARIX**/**PHOX2A** as the autosomal recessive CFEOM2 disease gene.

ARIX is a transcription factor essential for the development of oculomotor and trochlear nuclei in mice and zebra fish.71 It is believed that CFEOM2 results from hypoplasia of the oculomotor and trochlear nerve nuclei as a result of mutations in both copies of **ARIX**.

Traboulsi and coworkers14 reported a Yemenite family with autosomal recessive CFEOM consisting of two affected sisters, four unaffected siblings, and unaffected consanguineous parents. The investigators analyzed the family and excluded linkage to the CFEOM2 locus, as well as to the autosomal dominant CFEOM3 locus on chromosome 16q24. A lod score of 2.0 (the maximum possible, given the family size and structure) was obtained at the CFEOM1 locus. Haplotype analysis showed that the alleles at the CFEOM1 locus reduced to homozygosity in both affected daughters but none of the other children. This data was compatible with genetic heterogeneity in autosomal recessive CFEOM and suggested that a second recessive locus may be allelic to the autosomal dominant CFEOM1 locus at 12cen.14

Since the publication of that paper, and with identification of the CFEOM1 and CFEOM2 genes, mutation analysis of the **ARIX** and the **KIF21A** genes has failed to reveal any mutations in this family (E. Engle, MD, personal communication).

**CFEOM3**

CFEOM3 is autosomal dominant with variable expression and probable incomplete penetrance. The gene maps to markers on 16q24.2-q24.3.12,22 Affected individuals are born with a nonprogressive eye movement disorder characterized by variable ptosis and restrictive external ophthalmoplegia. Severely affected individuals have ptosis, eyes fixed in a downward and exotropic position, and bilateral severe restriction of eye movements. Their phenotype resembles that of CFEOM1. Mildly affected individuals have normally positioned globes with limitation of vertical gaze. Moderately affected individuals have asymmetric involvement with one eye severely and one eye mildly affected. Mackey and colleagues22 reported a second family that maps to the FEOM3 locus in which the phenotype is fairly uniform in 15 affected individuals and involves primarily the vertically acting EOMs, thereby expanding the manifestations of gene mutations at this locus.

**Systemic Abnormalities in Patients With CFEOM**

Patients with fibrosis of the extraocular muscles are generally healthy except for rare ocular or systemic problems in sporadic cases or in single family members of otherwise typical pedigrees (reviewed by Kalpakian and colleagues25). The associated ocular findings include, for example, bilateral optic nerve hypoplasia in one patient,27,29,67 a chorioretinal coloboma in case XXII of Brown’s patients,65 and microphthalmia in one other

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**TABLE 2. CLASSIFICATION OF CONGENITAL FIBROSIS SYNDROMES**

<table>
<thead>
<tr>
<th>TYPE</th>
<th>INHERITANCE</th>
<th>OMIM NO.</th>
<th>GENE/LOCUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFEOM1</td>
<td>Autosomal dominant</td>
<td>135700</td>
<td>KIF21A/12p11-q12</td>
</tr>
<tr>
<td>CFEOM2</td>
<td>Autosomal recessive</td>
<td>602078</td>
<td>ARIX/11q13</td>
</tr>
<tr>
<td>CFEOM3</td>
<td>Autosomal dominant</td>
<td>600638</td>
<td>16q24.2-24.3</td>
</tr>
<tr>
<td>Unclassified</td>
<td>Autosomal recessive, unique phenotype</td>
<td>Family reported by Traboulsi and colleagues14</td>
<td>Possibly at 12p11-q12</td>
</tr>
<tr>
<td>Unclassified</td>
<td>Autosomal recessive with CFEOM2 phenotype</td>
<td>Present paper</td>
<td>No mutations in ARIX or KIF21A</td>
</tr>
<tr>
<td>Unclassified</td>
<td>Autosomal recessive, with CFEOM3 phenotype</td>
<td>Present paper</td>
<td>No mutations in ARIX or KIF21A</td>
</tr>
</tbody>
</table>

OMIM, Online Mendelian Inheritance in Man.

Cryptorchidism, a benign mesenchymoma of the inguinal hernias and unilateral cryptorchidism, as well as bilateral inguinal hernias and unilateral cryptorchidism, indicate a syndrome distinct from classic CFEOM. Systemic associations include bilateral inguinal hernias and unilateral cryptorchidism, and unilateral cryptorchidism, indicating that genetic defects can involve the oculomotor nuclei as well as cerebellar structures.

**Horizontal Gaze Palsy With Progressive Scoliosis**

Congenital horizontal gaze palsy with early-onset progressive scoliosis is a rare autosomal recessive condition that probably results from brainstem maldevelopment and is probably caused by mutations in a gene necessary for the normal development of neurons in the abducens nuclei and caudal longitudinal fascicle. This leads to the development of “horizontal gaze palsy” and kyphoscoliosis. This condition is classified with the congenital cranial dysinnervation syndromes. The gene for this condition has been recently mapped to chromosome 11q23-q25.

Clinical as well as gene mapping and/or mutation analysis data on individual patients or families with typical or variants of CFEOM and with horizontal gaze palsy with progressive scoliosis (HGPPS) is presented in the following sections.

**METHODS**

**Gene Mapping Studies**

**Patient and Pedigree Collection**

Index patients and family members were examined. The Cleveland Clinic and Boston Children’s Hospital institutional review boards approved the study, and all participants signed informed-consent forms. The methods adhere to the Declaration of Helsinki for research involving human subjects. Clinical information was recorded and pedigrees were drawn using the program Cyrillic (CyrillicSoftware, Reading, United Kingdom).

**DNA Typing**

Blood samples were collected from participating family members, and lymphocyte DNA was extracted using the Puregene kit (Genta, Research Triangle Park, North Carolina). Linkage studies were conducted using polymorphic DNA microsatellite markers (di-, tri-, and tetranucleotides). To assess linkage to the dominant CFEOM1 region,7 markers D12S61, D12S87, D12S1584, D12S1621, D12S1648, D12S345, D12S1692, GATA63D01, AFM114yh2, D12S1029, D12S1048, and D12S1668 were analyzed. To assess linkage to the dominant CFEOM3 region,12 markers D16S498, D16S486, D16S3963, D16S689, D16S413, D16S3026, D16S303, and D16S671 were analyzed. To assess linkage to the recessive CFEOM2 region,11 markers D11S1337, D11S4139, D11S4196, D11S4162, D11S1314, D11S4184, D11S1369, D11S4207, D11S916, and D11S2371 were analyzed. To assess linkage to the dominant HGPPS region,57 markers D11S908, D11S925, D11S4464, D11S328, D11S1896, and D11S415 were analyzed. All primer sequences are available from either the Genome Data Base (http://gdbwww.gdb.org) or the publications cited above. Primers were purchased from Research Genetics and Genosys Biotechnologies (http://www.genosys.com). Amplification and analysis of each repeat polymorphism were performed as reported elsewhere.7,11,22

**Linkage Analysis**

Haplotype analysis was done by inspecting the combination of alleles in any particular locus. If there was evidence of segregation of individual haplotypes with the disease trait, lod scores were calculated with the Fastlink version 3.0 package of programs29 with the assumption of autosomal recessive or dominant inheritance with complete penetrance. Absence of cosegregation of a given haplotype with the disease trait was considered evidence of no linkage. Calculations involving the CFEOM3 haplotype were done with the assumption of autosomal dominant inheritance and reduced penetrance. Data on the population incidence of CFEOM mutations are not available; for purposes of lod-score calculations, a disease incidence of 1/1,000,000 births and 10 marker alleles of equal frequency was used.7,11 Using standard convention, linkage was excluded based on a lod score of –2 or less across the entire critical region of a disease gene locus, and linkage was established based on a lod score of 3 or greater for marker(s) within the critical region. A lod score of –2 corresponds to an odds ratio of 1:100 that the disease gene and the marker are linked versus not linked, and a lod score of 3 corresponds to an...
odds ratio of 1,000:1 that the disease gene and the marker are linked versus not linked.

**Mutation Analysis**

*KIF21A* mutation analysis was conducted by polymerase chain reaction amplification of the 38 *KIF21A* exons and flanking intron-exon boundaries from genomic DNA of each proband. The amplicons were subjected to analysis by denaturing high-performance liquid chromatography using the WAVE Nucleic Acid Fragment Analysis System (Transgenomic, Inc, Omaha, Nebraska) and/or to direct DNA sequencing on an ABI 377 DNA sequencer (PE-Applied Biosystems, Foster City, California) as previously described.25 This was done in Dr Engle’s laboratory.

The 3 *PHOX2A* (ARIX) exons and flanking intron-exon boundaries were similarly amplified using published primer sets,15 and these amplicons were directly sequenced. Results were compared to normal control individuals. If a mutation was detected in a proband, the participating family members were subsequently screened for the mutation as well.

**RESULTS**

**Representative CFEOM1 Case**

A 32-year-old woman presented for genetic counseling about her strabismus syndrome, diagnosed as CFEOM at about age 3 years, and the risk of having an affected child. She was born with bilateral ptosis, severely restricted eye movements (Figure 3), and right Marcus-Gunn jaw winking.

She underwent three eye muscle surgeries at ages 3, 4, and 5 years and left frontalis suspension at age 6. Her visual acuity was 20/40 OD and 20/100 OS. She had a mild chin-up head position. There was almost no movement of her left eye; she could adduct and abduct her right eye about five degrees. There was no family history of a similar condition. Although her clinical features were most compatible with autosomal dominant CFEOM1, the possibility of a recessive variant of CFEOM3 could not be ruled out. She was told that she had a maximal risk of 50% of having an affected child. At the time of her first visit, the gene for CFEOM1 had not been identified. She contributed a blood sample to the research on CFEOM. She went on to have an unaffected daughter. Mutation analysis of the *KIF21A* gene in Dr Engle’s laboratory revealed a 2861G>A mutation in the 21st exon of the *KIF21A* gene, resulting in an R954Q substitution (Figure 4).

**Genetic Heterogeneity in CFEOM2**

Traboulsi and colleagues studied an Iranian family (Figure 5, pedigree 869) with a phenotype identical to that of the family reported by Yazdani and colleagues.24 There were two affected brothers, one affected sister, and two unaffected siblings. The parents were consanguineous. All three patients had bilateral ptosis and bilateral exotropia strabismus fixus (Figure 6).

Mutation analysis of *ARIX* and *KIF21A* failed to reveal any mutations, and haplotypes analysis at the CFEOM2 locus failed to show evidence of linkage (data not shown), indicating further genetic heterogeneity in CFEOM2.

**A Presumed New Recessive Form of CFEOM3**

A previously unreported Lebanese family (pedigree 205) with what appears to be a recessive form of CFEOM3 was examined. Clinical data was obtained and blood specimens were collected after informed consent. Two siblings and a cousin had unilateral EOM fibrosis and ptosis (Figure 7). Computed tomographic scanning in one of the patients revealed typical thinning of the EOMs on the affected side. The three children underwent a number of surgical procedures with moderate improvement in ocular alignment and ptosis. None of the parents were affected. Consanguinity was denied.

The family was studied in conjunction with Dr Engle’s group for linkage to the CFEOM1, CFEOM2, and CFEOM3 loci and for mutations in the *ARIX* and *KIF21A* genes as outlined in the “Methods” section. Haplotype analysis of multiple markers in the three loci failed to suggest linkage to any of the three regions (Figure 8, A through C). Furthermore, there were no mutations in *ARIX* or *KIF21A*.

**A New Family With Horizontal Gaze Palsy and Progressive Scoliosis**

Five siblings of a United Arab Emirates family ranging in age from 4 to 17 years had an ocular motility disorder characterized by congenital bilateral esotropia and bilateral abduction deficits. Vertical eye movements were normal. All patients had convergence-like eye movements on attempted abduction. Visual acuity was normal in all patients. Pupils reacted normally to near viewing. Forcedduction testing under general anesthesia revealed moderate tightness of the medial rectus muscles in all patients. All affected children had scoliosis (Figure 9).

Bilateral medial rectus recession resulted in good ocular alignment in primary position of gaze without any alteration of the abnormal ocular movement pattern. A clinical diagnosis of autosomal recessive horizontal gaze palsy with scoliosis (HGPPS) was made. Haplotype analysis revealed homozygosity for markers at the 11q23-q25 locus in affected individuals, and heterozygosity of the markers in parents or unaffected carrier siblings (Figure 10).

Since this thesis was accepted, the *ROBO3* gene responsible for HGPPS was discovered (Jen JC, et al, Science 2004;304:1509-1513). *ROBO3* is important in axonal guidance and required for hindbrain axon midline
crossing. Mutations in ROBO3 result in uncrossed motor and sensory projections in patients with HGPPS, including sixth nerve fibers, resulting in bilateral gaze palsy. The present family has an IVS13 +1 G>A mutation in exon 13 of ROBO3 that results in abnormal splicing (Jen

Patient with CFEOM1 preoperatively at age 3. There is bilateral ptosis and the eyes are fixed in downgaze.

Pedigree of family with CFEOM2. The parents are consanguineous. There are two affected boys (solid black squares) and one affected girl (solid circle). The proband is indicated by an arrow. Clear symbols indicate unaffected individuals.

Two siblings (CE08 and CE11) and their cousin (CE17) with unilateral ptosis and ophthalmoplegia. Symbols with crosses indicate individuals with congenital cataracts. Solid symbols indicate individuals with CFEOM. Clear symbols indicate unaffected individuals.

DNA sequence around codon 954. There is a G>A substitution at position 2861. Bottom sequence shows heterozygosity (G and A) for mutation (arrowhead), compared to homozygosity for a G in the same position in normal top sequence (arrow).

Three siblings with CFEOM2, ptosis, and exotropia.


Patients With Rare Combinations of Congenital Cranial Nerve Abnormalities

Optic Nerve Hypoplasia and Fifth Nerve Dysfunction

A 2-year-old boy was brought in because of a self-induced scratch of his left eye. He had been poking himself in the left eye for a number of months and also had a habit of inserting foreign objects under his lid. Examination showed a central corneal abrasion of the left eye and ipsilateral optic nerve hypoplasia (Figure 11). His ocular movements were full. Corneal sensation was normal on the right but absent on the left. There was no developmental delay and no other medical problems or congenital malformations. The corneal abrasion was treated with
patching and antibiotic ointment applications. The patient returned several times over the next 3 years with similar episodes. Vision was hand movements in the left eye, and he developed a large esotropia (Figure 12). The fundus examination remained unchanged.

Optic Nerve Hypoplasia and Duane Retraction Syndrome
Traboulsi and colleagues examined a patient who was born with a tracheo-esophageal fistula, tetralogy of Fallot with partial arteriovenous canal cardiac defect, bilateral type III DRS, and bilateral optic nerve hypoplasia. He was referred for neuroendocrinologic evaluation but failed to keep up with his appointments.

Fifth, Sixth, and Seventh Nerve Dysfunction
A 14-month-old boy presented with left congenital facial nerve palsy and an abduction deficit with esotropia of his left eye (Figure 13). There was mild developmental delay. The child had some difficulties with swallowing and was fed through a gastrostomy tube. There were no malformations

FIGURE 8
Genotyping data with markers on chromosomes 11 (A), 12 (B), and 16 (C) fails to show segregation of any specific haplotypes with the disease trait, indicating absence of linkage to the CFEOM1, 2, and 3 loci.
of the extremities. Vision was central, steady, and maintained OD, and he was able to fix and follow poorly OS. There was a hypermetropia of +5.00 diopters OU. The left cornea was anesthetic, and there were epithelial corneal defects and subepithelial scars. A diagnosis of Möbius syndrome with multiple cranial nerve involvement was made. Lubrication of the left eye was instituted, as well as patching for his amblyopia. He later underwent eye muscle surgery for his esotropia. On his latest visit at age 7 years, vision was 20/40 OD and 20/50 OS. His facial palsy, left sixth nerve palsy, and anesthetic and scarred cornea persisted. He continues to receive lubrication to his left eye.

**Third, Seventh, and Eighth Nerve Dysfunction**

**Case 1.** A 9-year-old patient with congenital bilateral facial nerve palsy, ptosis, and severe limitation of movements of both eyes was examined. He had been diagnosed with Möbius syndrome and strabismus. He had significant feeding difficulties as an infant and failed to thrive. He had bilateral hernia repair and was discovered to have bilateral mild hearing loss.

His visual acuity was 20/60 OU. There was latent nystagmus. He had bilateral moderate ptosis. Bell’s phenomenon was present bilaterally. The eyes were positioned in 20 degrees of downgaze (Figure 14), with converging eye movements on attempted upgaze and an exotropia on attempted downgaze. He had mild limitation to abduction of both eyes. Ophthalmoscopy showed anomalous, slightly hypoplastic optic nerve heads. Strabismus and ptosis repair were refused. He was re-examined 4 years later, and there were no changes in vision, ptosis, or ocular motility.

**Case 2.** A 6-year-old girl with congenital bilateral ptosis, exotropia, and facial palsy had extremely limited ocular movements, and her eyes were fixed in a hypotropic position. Vision was 20/160 OU with a myopic correction of –20.00 + 2.00 × 180 bilaterally. Her fundi were severely myopic with tilted optic nerve heads but no macular degenerative changes.

**DISCUSSION**

The initial hypotheses that DRS and CFEOM are due to
Abnormalities in extraocular muscle development have been overturned by several lines of evidence of neurogenic etiologies. Abnormalities of cranial nerve nuclear development have been established for conditions such as Möbius syndrome and its variants and for familial horizontal gaze palsy. To date, there appear to be two main pathways through which congenital cranial nerve dysfunction occurs in patients with congenital ocular movement disorders. The first involves failure of the cranial nerve nuclei to develop normally and their motoneuron component cells to differentiate, aggregate, and establish proper neuronal connections; this could occur as a result of genetic factors or of teratogenic insults. Classic examples include the absence of the sixth nerve nucleus in DRS, the presumed absence of the third nerve and fourth nerve nuclei in CFEOM2, and the generalized abnormalities of midbrain, pons, and medulla in patients with Möbius syndrome and its variants. The second mechanism involves genetic defects that lead to abnormal axonal transport of molecules necessary for normal extraocular muscle function and development. This mechanism appears to operate in CFEOM1, in which a kinesin-related defect of axonal transport is caused by mutations in KIF21A. The histopathologic studies in CFEOM1
showing absence of the superior division of the oculomotor nerve and its corresponding alpha motor neurons, as well as abnormalities of the levator palpebrae superioris and rectus superior (the muscles innervated by the superior division of the oculomotor nerve), and suggesting that CFEOM1 results from an abnormality in the development of the extraocular muscle lower motor neuron system, have to be reconciled with the more recent molecular genetic studies that favor an axonal transport defect. Because of the occasional presence of mild facial weakness, hypotonia, gross motor delay, and nonspecific abnormalities in quadriceps biopsies from affected CFEOM1 family members, Engle and coworkers also proposed that the normal CFEOM protein plays at least a transient role in normal skeletal muscle development.

Additional evidence for a neurogenic etiology of the congenital cranial dysinnervation syndromes comes from the wide variety of cases in which CFEOM or DRS has been associated with other congenital abnormalities of motor and sensory cranial nerve development. Brodsky and associates reported a 5-month-old boy with generalized CFEOM, oculocutaneous albinism, and neural misdirection resulting in synergistic divergence and Marcus-Gunn jaw winking phenomenon. They suggested that their patient’s abnormalities provide evidence for a primary developmental defect precluding the establishment of normal neuronal connections in CFEOM, a theory certainly in line with current findings. Brodsky later presented three additional patients with an identical constellation of clinical findings. All displayed a variant of synergistic divergence characterized by simultaneous abduction with intorsion and depression of the synkinetically abducting eye. Three of the four patients had a variant of Marcus-Gunn jaw winking characterized by elevation of the ptotic eyelid during mouth opening. Brodsky

**FIGURE 14**
Bilateral facial nerve palsy and total ophthalmoplegia in 9-year-old boy.

**FIGURE 15**
Six-year-old girl with bilateral facial palsy, ptosis, and ophthalmoplegia with the eyes fixed in a hypotropic and exotropic position.

**FIGURE 16**
Diagram summarizing conditions with evidence of congenital cranial dysinnervation and their causative genes.
suggested that the patterns of neuronal misdirection implicated a regional innervational disturbance involving cranial nerves III through VI as the underlying cause of the generalized ophthalmoplegia in these patients. Another patient with unilateral fibrosis, enophthalmos, retraction, and Marcus-Gunn jaw winking phenomenon was included in the series of 24 patients reported by Traboulsi and coworkers,65 suggesting that this phenomenon is present in about 5% of patients with CFEOM.

A patient was previously reported with de Morsier syndrome (septo-optic dysplasia), a developmental malformation complex characterized by optic nerve hypoplasia, dysgenesis of the septum pellucidum, hypothalamic-pituitary dysfunction, and DRS.27 The patient had classic right Duane syndrome type I and bilateral optic nerve hypoplasia with double ring sign. He also had a superior central incisor, a finding usually associated with midline brain defects, an undescended testicle, and pituitary insufficiency with growth hormone deficiency and diabetes insipidus. It was postulated that DRS and de Morsier syndromes in this patient were due to an underlying genetic disturbance of neuronal development.27 Parentin and coworkers49 reported a 4-year-old Italian child with the association of a solitary median maxillary central incisor, growth hormone deficiency, DRS, and a duplicated thumb phalanx. The report did not mention the presence of optic nerve hypoplasia. Personal written communication with the authors revealed that a detailed ocular examination had been done and that optic nerve hypoplasia had been excluded. DRS and optic nerve hypoplasia can also occur in patients with fetal alcohol syndrome.54 There was no history or evidence of exposure to teratogens in the two cases examined, suggesting a probable genetic etiology.

The second most common mutation in KIF21A was found in one patient with CFEOM1. This mutation has been identified in three other probands.21 Five other mutations (M356T, M947V, M947R, R954W, and I1010T) also alter conserved amino acid residues within the KIF21A protein stalk region and are proposed to interfere with KIF21A dimerization, hence with the ability of KIF21A to carry its unidentified cargo from the oculomotor nucleus motoneurons toward the developing neuromuscular junction of the extraocular muscle.25 These six mutations account for 98% of cases of CFEOM1.21

Traboulsi

The present paper offers additional evidence of genetic heterogeneity in autosomal recessive CFEOM. One family with a clear-cut CFEOM2 phenotype did not link to the FEOM2 locus on 11q13 and did not have a mutation in PHOX2A/ARIX or in KIF21A. Genetic heterogeneity in CFEOM3 is also postulated in the previously unreported family in which two siblings and their cousin had a CFEOM3 phenotype, yet does not map to the three known CFEOM loci, nor has mutations in PHOX2A/ARIX or in KIF21A. The molecular genetic data suggests that this family has a third, yet-undescribed form of recessive CFEOM with clinical features that resemble those of CFEOM3 and are characterized by unilateral ptosis and severe restriction of extraocular movements. A genome-wide screen is under way to localize the responsible gene in these last two families.

In this paper, the localization of the HGPS gene to 11q23-q25 in a family with five affected siblings and consanguineous parents was confirmed. The search for the responsible gene is under way in the family described in this thesis and in several others. It is hoped that discovery of the gene will be reported in the near future.

The occurrence of total external ophthalmoplegia in patients with Möbius syndrome, as observed in two cases in the present paper, has been previously reported in a few patients. Verzijl and coworkers66 investigated the variable clinical picture of Möbius syndrome to further understand the pathogenesis of the disorder. They used a standardized questionnaire and examined 37 Dutch patients with Möbius syndrome. All patients underwent standardized neurologic examination with special attention to cranial nerve functions, motor skills, and facial and limb anomalies. All had facial paresis, and 97% had bilateral and 3% had unilateral ocular abduction weakness. Further analysis showed isolated abducens nerve palsy in 9%, a conjugated horizontal gaze paresis in 48%, features of DRS in 34%, and congenital fibrosis of the extraocular muscles in 9%. Other signs included lingual involvement (77%), dysfunction of palate and pharynx (56%), general motility disability (88%), poor coordination (83%), and respiratory abnormalities (19%). The presence of gaze palsies, DRS, feeding and respiratory problems, and poor motor development led these investigators to suggest that Möbius syndrome was the result of abnormal regional rhombencephalic development, involving predominantly motor nuclei and axons, as well as traversing long tracts.50

Although some cases of Möbius syndrome have been attributed to brainstem ischemia, genetic causes are most likely operative in others. Cytogenetic abnormalities in some patients have suggested genetic loci at 1p22 and 13q12.2-13.46,56 Dutch investigators mapped two loci for dominant bilateral facial palsy, one on chromosome 3q21-q22 and the other on the long arm of chromosome 10.46 The families in which these loci were mapped were considered to have Möbius syndrome, even though abducens palsy was not invariably present.

It has become evident that mutations in genes such as ARIX, SALL4, and KIF21A that are important in either motoneuronal development or in the integrity and normal function of cranial nerves can result either in well-delineated abnormalities of ocular motility or in complex syndromes in which multiple cranial nerves are dysfunctional from birth. These syndromes can combine abnor-
neural malformations of sensory as well as motor nerves and can be associated with other neurologic abnormalities or other malformations. The description of additional cases similar to those in this report is needed to better define individual rare syndromes and to establish their molecular etiology.

Teratogenic insults to the fetal brain at the time of cranial nerve development have clearly been shown to cause well-defined clinical entities such as DBS, or complex neurologic and malformative syndromes with cranial nerve dysfunction. A detailed maternal gestational history should be obtained in patients with congenital ocular motility defects, and teratogenic causes should be excluded before a genetic etiology is presumed.

Figure 16 illustrates the isolated as well as the complex syndromes that involve cranial nerves II through VIII. With time, additional genes will undoubtedly be identified that cause less common individual syndromes, and more cases with overlapping phenotypes will be described, allowing a better delineation of phenotype/genotype correlations.

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ANTERIOR LENS CAPSULE MANAGEMENT IN PEDIATRIC CATARACT SURGERY

BY M. Edward Wilson Jr MD

ABSTRACT

Purpose: To describe and analyze pediatric anterior capsulotomy techniques and make recommendations.

Methods: Five anterior capsulotomy techniques were compared using a porcine model. Extensibility was measured by calculating the mean stretch-to-rupture circumference of each capsulotomy (20 eyes per technique) as a percentage of its circumference at rest. Edge characteristics were reviewed using scanning electron microscopy. A 10-year review of consecutive pediatric cataract surgeries performed by the author focused on the anterior capsulotomy results. A worldwide survey was used to determine current practice patterns.

Results: Manual continuous curvilinear capsulorrhexis (CCC) produced the most extensible porcine capsulotomy (185%) with the most regular edge and is preferred by surgeons for patients aged 2 years and older. In the pseudophakic clinical cases reviewed, a radial tear developed in 3 (6.5%) of 46 manual CCC cases. Vitrectorhexis (porcine extensibility, 161%) is preferred by surgeons during the first 2 years of life. A radial tear developed in 16 (7.7%) of 208 vitrectorhexis pseudophakic eyes (29 tears in 284 pseudophakic eyes [10.2%] overall). The Kloti diathermy unit, Fugo plasma blade, and “can-opener” technique produced porcine capsulotomies of 145%, 170%, and 149% extensibility, respectively, and radial tears numbering 4 (21%) of 19, 5 of 8, and 1 of 2, respectively, in the clinical series.

Conclusions: All five capsulotomy techniques are recommendable for children. Only the vitrectorhexis and manual CCC are commonly used today. Vitrectorhexis is well suited for use in infants and young children; manual CCC is best used beyond infancy, and it produces the most stable edge.

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INTRODUCTION

Childhood blindness occurs at a rate of one child every minute. It is estimated that there are 1.5 million blind (defined as best-corrected visual acuity less than 20/400 in the better eye) children in the world. Of those, 75% have treatable or preventable causes of blindness. The control of blindness in children was given a high priority within the World Health Organization (WHO) VISION 2020—The Right To Sight program.

Globally, an estimated 200,000 children are bilaterally blind from cataract. This is despite the many technical advances that have been made in the surgical removal of adult cataracts. The overall incidence of clinically significant cataract (unilateral or bilateral) in childhood is unknown, but it has been estimated to be as high as 1 in 250 (0.4%). WHO VISION 2020 targets for the control of blindness in children contained the following as a specific disease-control measure: “Provide appropriate surgery to all children with cataract, with immediate and effective optical correction, in suitably equipped specialist centers.”

Achieving this goal has been a challenge even within the developed world. However, dramatic advances have occurred in the treatment of childhood cataracts in the last 10 years. Primary implantation of an intraocular lens (IOL) after cataract removal in children has now become commonplace. Unique features of the child’s eye, however, have created the need for variations in adult procedures, and at times, completely different techniques have been needed to ensure safe and effective cataract removal and stable central fixation of the IOL in the child.

One of the most marked differences between adult and pediatric cataract surgery is the behavior of the anterior lens capsule during the procedure. The anterior capsulotomy shape, size, and edge integrity are now recognized as very important to the long-term centration of a capsular-fixated IOL. A surgeon who applies adult cataract surgery maneuvers to the pediatric anterior capsule may be surprised and disappointed at the
outcome. Furthermore, the vast majority of surgeons operating on children perform less than 10 pediatric cataract procedures per year. For these reasons, an analysis of the pediatric lens capsule and an investigation of the currently available methods for performing an anterior capsulotomy in young eyes will help guide surgeons faced with the important task of removing a cataract from a child.

Opening the anterior lens capsule (anterior capsulotomy) allows access to the lens nucleus and cortex. After cataract removal, it allows for the placement of an IOL within the capsule remnant. The anterior capsulotomy must be done in a manner that does not compromise the structure and stability of the remaining lens capsule. A compromise in the capsule may affect the centration of an IOL and its separation from uveal tissue. An ideal anterior capsulotomy technique for children would be one that is both easy to perform and predictable in size and centration. In addition, it would create a strong edge that would not tear during removal of the lens substance or during implantation of the IOL. Manual continuous curvilinear capsulorrhexis (CCC) is a popular anterior capsulotomy technique for adult cataract surgery because it is relatively easy to perform and yields a low rate of radial tears. However, the anterior lens capsule in children is very elastic and requires the application of more force before tearing begins. Manual CCC is, therefore, more difficult to perform and control in young eyes. This has led researchers and surgeons to search for alternative methods to open the anterior capsule in children. The alternatives currently available include the use of the vitrector handpiece, a bent needle for multipuncture, diathermy, and plasma energy. Also, variations in the adult manual CCC technique have been suggested to help avoid the “runaway rhexis” that can occur when standard CCC methodology is applied to young elastic capsules.

Therefore, the goal of this thesis is to describe and analyze the techniques currently being utilized to open the anterior lens capsule in a child and establish which techniques are recommendable. To establish this goal, the anatomic and physiologic differences between the pediatric and adult anterior lens capsule will be described. A historical account of various pediatric capsulotomy techniques that have been utilized will be provided. A laboratory comparison of those techniques will be profiled using an animal lens capsule model that has been shown by our laboratory to exhibit similar elastic characteristics to the pediatric human lens capsule. Additionally, a clinical review of the anterior capsulotomy outcomes in a 10-year consecutive series of pediatric surgical cataract cases performed by the author will be reported. Cases with complications will be reviewed in limited detail to help shape the recommendat...
Anterior Lens Capsule Management in Pediatric Cataract Surgery

In the early 1980s, Parks and Taylor were among the first to utilize for pediatric cataract surgery in the late 1970s. Mechanical suction and cutting instruments began to be used for IOL insertion through capsulotomy sizes more than adequate resistance to unwanted capsule tears when used for IOL insertion through capsulotomy sizes currently used in clinical practice.

The original name, “mechanized anterior capsulotomy,” emphasized the mechanized nature of the vitrectorhandpiece and the fact that a portion of the capsule was removed (capsulotom) rather than merely opened (capsulotomy). Later, the term “vitrectorhexis” came into common use. The vitrectorhexis name emphasizes the fact that it is a substitute for capsulorhexis performed using the vitrector. However, the term “vitrectorhexis” is, in reality, a misnomer because “rhesis” and 30 mm near the lens equator. Krag and coworkers analyzed 67 human eyes from age 7 months to 98 years and found anterior capsule thickness increased from 11 to 33 mm. The association of thickness with age fit a straight line from birth to age 75 years, after which it changed slope and thinned slightly.

The neonatal anterior capsule surface contains threadlike fibers about 100 Å in thickness and 1.5 Å long. They penetrate about 0.6 µm into the capsular matrix. These become more numerous near the lens equator and disappear completely between ages 6 and 17 years. Numerous parallel laminae are repeated regularly at intervals of about 700 Å. Capsular laminations are lost with age, most markedly after age 50 years. Electron-dense formed elements or inclusions, thought to be excretion products from the most metabolically active epithelial cells, are found only after age 17 years and increase markedly with old age. Although the inclusions are most common near the equatorial anterior capsule, they are found to occur closer and closer to the anterior pole of the lens as aging progresses. In conjunction with these anatomic changes, the anterior capsule’s biomechanical properties are altered, and age. The young anterior lens capsule is strong and very elastic. The elderly anterior lens capsule is, by comparison, weak and inelastic. Krag and coworkers found that anterior lens capsule extensibility was maximal in infancy and decreased about 0.5% per year throughout life (measured range, 105% to 40%).

In summary, aging of the human anterior lens capsule leads to a progressive loss of mechanical strength. Overall tensile strength decreases by a factor of five during the life span, and the extensibility decreases by at least a factor of two. In surgery, the young capsule is highly elastic and difficult to puncture. Much more force is required before tearing begins. In contrast, the capsule of the elderly is much less extensible, easier to open, and tears with much less force.

Techniques for Pediatric Anterior Capsulotomy

For purposes of this introduction, the techniques will be presented in the order they will appear in the “Methods” and “Results” sections. Later (in the “Discussion” section), a more detailed historical account of the development of these techniques will be presented using a chronologic order.

Vitrector-Cut Capsulotomy

Mechanical suction and cutting instruments began to be utilized for pediatric cataract surgery in the late 1970s and early 1980s. Parks and Taylor were among the first to advocate for the performance of a primary mechanized posterior capsulotomy and anterior vitrectomy during pediatric cataract surgery. Taylor also described removal of the anterior capsule mechanically.

In 1994, Wilson and coworkers performed a laboratory comparison of mechanized anterior capsulotomy (later named vitrectorhexis) and manual CCC. Because of difficulty with manual CCC on the elastic capsules of young children, Wilson began utilizing the vitrector handpiece to perform the anterior capsulotomy during pediatric cataract surgery and IOL implantation. In the laboratory, 18 pairs of pediatric eyes obtained postmortem were operated on by Wilson within 24 hours of enucleation. The age of the donors ranged from 4 days to 16 years. A mechanized anterior capsulotomy was performed on one eye of each pair, and a CCC was performed on the fellow eye. The integrity of the anterior capsulotomy edge was assessed after capsulotomy completion, after lens removal, and again after IOL implantation. Radial tears were noted and described.

A radial tear developed in only one of the 18 pediatric eyes in which a mechanized anterior capsulotomy was performed. This occurred in one of the oldest pediatric eyes (age 16 years), where a single radial tear extended from an angled capsulotomy edge during IOL insertion. In contrast, no radial tears occurred in any of the fellow eyes in which a manual CCC was performed. However, in six of the fellow eyes, the manual CCC edge extended out to the lens equator rather than continuing in a circular fashion. All six of these errant capsulotomies were from eyes of children younger than 5 years of age. Wilson and coworkers subsequently published a prospective clinical series containing data from 20 eyes from 17 children after performance of a mechanized anterior capsulotomy at the time of cataract surgery with IOL implantation. In all patients the capsulotomy was round, centered, and accurately sized. Two patients, both aged 11 years, were noted to develop radial tears in the anterior capsule during IOL insertion. None of the children younger than age 11 years developed radial tears.

Andreo, Wilson, and Apple subsequently reported that whereas the CCC offered greater resistance to capsule tearing in a laboratory study utilizing a porcine model, the mechanized anterior capsulotomy displayed more than adequate resistance to unwanted capsule tears when used for IOL insertion through capsulotomy sizes currently used in clinical practice.

The original name, “mechanized anterior capsulotomy,” emphasized the mechanized nature of the vitrector handpiece and the fact that a portion of the capsule was removed (capsulotomy) rather than merely opened (capsulotomy). Later, the term “vitrectorhexis” came into common use. The vitrectorhexis name emphasizes the fact that it is a substitute for capsulorhexis performed using the vitrector. However, the term “vitrectorhexis” is, in reality, a misnomer because “rhesis”
means to tear rather than to cut. Nonetheless, the commonly used “vitrectorhexis” label will be used in this thesis.

**Manual Continuous Curvilinear Capsulorrhexis Capsulotomy**

Gimbel and Neuhann, developers of the manual CCC capsulotomy method, have stated that without this technique, the potential of IOL implantation in children might not have been realized. Ideally, the technique provides for any size of smooth, circular, capsular opening with a strong capsular rim that resists tearing even when stretched during lens material removal or IOL implantation. The development of CCC occurred simultaneously in Canada (by Gimbel) and in Germany (by Neuhann). Both investigators presented their new technique in 1985. By September of 1987, Gimbel had used CCC in 33 children older than age 2 years with good results. However, Gimbel noted a greater tendency in children for the tear to extend peripherally. When this occurred, he recommended releasing some of the anterior zonular fibers in the area of the tear and using an elongated forceps to pull the capsulotomy edge toward the center.

The increased elasticity of the pediatric capsule had created the tendency for the capsulotomy edge to extend peripherally. In addition, reduced scleral rigidity in children produces posterior vitreous “upthrust” when the eye is entered. This vitreous “pressure” pushes the lens anteriorly and keeps the anterior capsule domed, convex, and taut. This also contributes to the tendency for a so-called runaway rhexis. Performing a CCC in infants proved to be even more challenging than in older children. Vasavada and Chauhan reported a success rate of less than 20% in 21 infant eyes.

Despite the difficulty of performing a CCC in a child, once completed, the edge integrity was reported to be excellent, easily withstanding the manipulations of lens substance removal and subsequent IOL implantation.

**Multipuncture (Can-opener) Capsulotomy**

To avoid the difficulties with CCC in children, some surgeons have returned to the can-opener style capsulotomy when operating on children. Others advocate using the can-opener technique in children with an intumescent lens. Wood and Schelonka compared the strength and safety of a CCC with a can-opener capsulotomy in a porcine model that closely resembles the high elasticity of the human pediatric lens capsule. A CCC or can-opener capsulotomy was performed inside the anterior chamber of fresh pig eyes. Any uncontrolled tears were noted. According to these authors, the porcine capsule is more reliably opened with fewer uncontrolled tears by a can-opener capsulotomy than by a CCC.

**Kloti Bipolar Radiofrequency Diathermy Capsulotomy**

Radiofrequency diathermy capsulotomy, developed by Kloti and colleagues in 1984, has been used as an alternative to CCC for cataract surgery in children as well as intumescent adult cataracts. The Kloti device (Oertli Instruments, Berneck, Switzerland) consists of a curved cannula housing an active electrode tip. It cuts the anterior capsule with a platinum-alloy-tipped probe using high-frequency current (500 kHz). The probe tip is heated to about 160°C and produces a thermal capsulotomy as it is moved in a circular path across the anterior capsule. Small gas bubbles are formed while the tip is active. Gentle pressure must be maintained on the capsule as the tip moves either clockwise or counterclockwise. Even when performed perfectly, a diathermy-cut capsulotomy can be seen to have coagulated capsular debris along the circular edge. In addition, this edge has been shown experimentally to be less elastic than a comparable CCC edge.

Since the stretching force needed to break the edge of a diathermy-cut capsulotomy is reduced compared to a CCC edge, surgical manipulations needed to remove a cataract and place an IOL may result in more radial tears when the diathermy is used. However, these observations were made using adult autopsy globes. It is well known that the pediatric capsule responds differently than the adult lens capsule. In fact, Comer and coworkers reported no radial tears when using the Kloti radiofrequency diathermy instrument to perform the anterior capsulotomy in 14 eyes of seven children whose mean age was 23 months.

**Fugo Plasma Blade Capsulotomy**

The Fugo blade has also been recently introduced as a plasma knife that can be used to perform an anterior capsulotomy. The Fugo blade unit is a portable electronic system that operates on rechargeable batteries. It requires no red reflex for visualization of the capsulotomy edge during the cutting procedure. The unit also allows the surgeon to easily revise the size of the capsulotomy opening. This new instrument has not yet been tested in children. It is hoped that it will work well with the elastic capsule found in children.

**METHODS**

**Porcine Ocular Model**

Abattoir globes were purchased (Visiontech, Inc, Mesquite, Texas) from two porcine age groups: young pigs approximately 6 months of age and weighing 200 to 250 pounds, and adult pigs 2 to 3 years of age and weighing 1,000 to 1,500 pounds. Globes were collected within 4 hours following death, packed on ice, and transported to the laboratory for use. Globes were rinsed with normal...
saline, trimmed, and stored at 5°C until used (24 to 48 hours). Only globes with a clear cornea were used for each closed chamber technique. Anterior lamellar corneal dissection or lidocaine injection was used as needed, to aid corneal clarity.36

Regardless of the intended surgical technique, each globe was placed on a gauze pad in a Styrofoam head-model to approximate the customary positioning of the eye for surgery.

Five anterior capsulotomy techniques were completed using the porcine model described above. Analysis was made of 20 eyes per group (i.e., 10 young eyes, 10 adult eyes). Anterior capsulotomy techniques were assigned to the following groups:

Group 1: Vitrectorhexis, using an Accurus vitrector (Alcon Laboratories, Fort Worth, Texas)

Group 2: Manual CCC, using a 26-gauge cystotome (Alcon Laboratories, Fort Worth, Texas) and Utrata capsulorrhexis forceps (Ellis Ophthalmic Technologies, Inc, Jamaica, New York)

Group 3: Multipuncture can-opener, using a 26-gauge cystotome (Alcon Laboratories, Fort Worth, Texas)

Group 4: Kloti radiofrequency diathermy, using a Kloti unit (Oertli Instruments, Berneck, Switzerland)

Group 5: Fugo plasma blade, using the electrosurgical base unit (Medisurg Research and Management Co, Norristown, Pennsylvania) attached to a Fugo blade tip (Medisurg Research and Management Co, Norristown, Pennsylvania)

Two additional porcine globes were included in each group: one served as a procedure “pilot” and was not analyzed; the other was implanted with a poly(methylmethacrylate) MC52BM intraocular lens (Alcon Laboratories, Fort Worth, Texas) and submitted for scanning electron microscopy (SEM) evaluation.

Eight eyes were excluded from analysis of stretching capacity due to tears in the capsule edge that occurred either during the capsulotomy procedure (manual CCC, 1; Kloti radiofrequency, 3; Fugo plasma blade, 2) or during lens substance removal (manual CCC, 2). Additional eyes were operated on until 20 completed and intact capsulotomy openings were available for analysis of extensibility from each capsulotomy technique group.

The project was registered with the institutional animal care and use committee. Additional peer-reviewed approval was not required for use of these abattoir globes.

**Lens Substance Removal**

Hydrodissection followed each anterior capsulotomy in all groups. Lens substance of young pig eyes was aspirated with a syringe, and the lens substance of adult pig eyes was removed using phacoemulsification. Following removal of the lens substance, Healon-GV (Pharmacia, Inc, Peapack, New Jersey) was used to fill the empty capsular bag.

**Anterior Capsule Opening Measurements—Unstretched and Stretched**

For all capsulotomy techniques, unstretched and stretched measurements were made after the lens substance had been completely removed.

Prior to recording any ocular measurements, three eyes were used to determine that the caliper edge itself would not cause a capsule tear. Unstretched and stretched measurements of the capsule opening were then performed in each group using electronic digital vernier calipers (Chicago Brand Industrial, Inc, Fremont, California). The caliper jaws were placed inside the capsule opening, and the unstretched diameter of the capsule opening was measured (in millimeters) three times in the vertical direction and three times in the horizontal direction; the mean of these six measurements determined the unstretched diameter of each eye. Stretching of the anterior capsule opening was measured next. The caliper jaws were again placed inside the capsule opening whereupon each capsule was slowly stretched until it tore. When the capsule tore, the caliper reading was recorded in millimeters as the length of the stretched capsule opening.

Data regarding the five anterior capsulotomy techniques were compiled using the following equations:37:

1. The unstretched capsulotomy diameter (Dun) was recorded as the average of six measurements per eye.
2. The circumference of the unstretched (Cun) capsulotomy opening was calculated for each eye using the formula: Cun = π(Dun)
3. The stretched length to breaking (Ls) of the capsule opening was recorded per eye, and the diameter of the caliper tips (Dtips) was considered to be negligible or zero.
4. The circumference of the stretched (Cs) capsulotomy opening was calculated for each eye using the formula: Cs = π(Dtips) + 2(Ls)
5. The percentage comparison (R) of the stretched to unstretched circumference was calculated for each eye using the formula: R = (Cs/Cun) × 100%

**Statistical Evaluation**

Compilations were stored and analyzed using Microsoft Excel 2000 (Redmond, Washington) spreadsheets and analytical tools, and SPSS for Windows 2001 (Chicago, Illinois). The five anterior capsulotomy techniques were analyzed per technique and compared. Statistical analyses included the Student t test and analysis of variance...
(ANOVA). The level of probability accepted as significant was \( P < .05 \).

**Limitations**
The number of eyes required for this study negated the use of pediatric autopsy globes. Therefore, porcine necropsy globes were chosen.

### Scanning Electron Micrography of the Capsulotomy

Eyes prepared for SEM were placed in freshly prepared 2% cacodylate glutaraldehyde for 24 hours. The eyes were then rinsed in 0.1 mol/L cacodylate buffer with 7% sucrose, postfixed in 2% aqueous osmium tetroxide, and dehydrated through a series of graded ethyl alcohol concentrations (50%, 70%, 95%, and 100%). Each sample was dried using the EMS850 Critical Point Dryer (Fort Washington, Pennsylvania) then mounted onto a stub and coated with 20 nm of gold/palladium using the Polaron SC7640 Sputter Coater (Agawam, Massachusetts). The sample was viewed in the JEOL 5410LV Scanning Electron Microscope (Peabody, Massachusetts). The microscope was operated at 5 kV.

### Comparative Evaluation

Representative digital images from each of the five capsulotomy techniques were collected for comparison of the capsulotomy opening and the edge.

### Limitations

Scanning electron microscopy was chosen for surface comparison. Intracellular detail using transmission electron micrography was not pursued.

### Pediatric Cataract Surgery Database

Between January 1, 1994, and December 31, 2003, all pediatric cataract surgery operative dictations from the practice of the author contained a description of the anterior capsulotomy and an assessment of whether any radial tears were present at the completion of each major step in the procedure (ie, anterior capsulotomy, cataractous lens removal, IOL insertion, hydrodissection, or ocular visco-surgical device removal). When radial tears occurred, the stage of the procedure and any other pertinent details about the causation of the tear were dictated as well. These consecutive patient data were entered into a computerized database, which continues to be maintained. The data from each case were entered by a trained physician after each dictated operative note was completed and signed. For purposes of this project, all surgeries performed by a clinical fellow or resident-in-training were excluded.

Retrospective database analysis of consecutive pediatric cataract surgery cases performed by the author revealed 379 consecutive surgeries on patients between the ages of birth and 18 years of age between January 1, 1994, and December 31, 2003. Surgeries to perform secondary IOL implantation (94 eyes) were not included in the surgery total listed above, since simultaneous cataract extraction was not performed and thus an anterior capsulotomy was not always needed at the time of surgery. Additionally, surgeries to remove various forms of opacification of the visual axis after cataract surgery, such as lens cortex proliferation, posterior capsule opacification not treatable with laser, or pupillary membranes, were not included in the above total. Likewise, surgeries to reposition, exchange, or remove an IOL were also not a part of the surgery total.

Consecutive pediatric cataract surgery cases to be analyzed were divided into the following postsurgery categories: (1) pseudophakic eyes (eyes undergoing cataract extraction with placement of an IOL at the primary surgery); (2) aphakic eyes (eyes undergoing cataract extraction without placement of an IOL at the primary surgery); and (3) eyes with traumatic or spontaneous anterior capsule rupture prior to surgery.

The following data were collected: (1) date of birth; (2) date of surgery; (3) eye location; (4) capsulotomy technique used; (5) IOL placement (ie, primary and where); (6) preoperative anterior capsule rupture; and (7) anterior capsule radial tear occurring during cataract surgery, when the tear occurred, and if the tear interfered with capsular fixation of an IOL.

The project received peer-reviewed approval by the institutional human investigation review board for the collection of these data and their analysis for purposes of research publication.

### Statistical Evaluation

Compilations were stored and analyzed using Microsoft Excel 2000 (Redmond, Washington) spreadsheets and analytical tools and SPSS for Windows 2001 (Chicago, Illinois). Statistical analyses utilized the Student \( t \) test and ANOVA. The level of probability accepted as significant was \( P < .05 \).

### Limitations

Data from consecutive cases were entered into a database. Therefore, no effort could be made to control the number of cases per age or surgical technique.

### Worldwide Survey of Pediatric Ophthalmic Surgeons

In July 2003, a four-question survey of demographics and anterior capsulotomy preferences was mailed to 996 physicians representing the membership of the American Association for Pediatric Ophthalmology and Strabismus.
Anterior Lens Capsule Management in Pediatric Cataract Surgery

(AAPOS) (816 domestic and 180 international mailings) (see Appendix). Respondents were asked to mail or fax their completed survey to the indicated Service Center. Responses were accepted for analysis through September 2003.

Statistical Evaluation
Responses were compiled using Microsoft Excel 2000 (Redmond, Washington) spreadsheets and analytical tools and SPSS for Windows 2001 (Chicago, Illinois). Statistical analyses utilized the Student t test and ANOVA. The level of probability accepted as significant was $P < .05$.

Limitations
A single reminder email was sent to all member physicians 2 months after the initial survey distribution. No additional reminder was sent.

RESULTS

Capsulotomy Techniques
Standardization of the surgical techniques included the following criteria: (1) all capsulotomies were performed by the author; (2) a 5-mm central, circular anterior capsule opening was the surgical goal in each eye; (3) closed chamber technique was used for manual CCC (group 2), and the cornea and iris were removed before performing the anterior capsulotomy in groups 1 and 3 through 5. The laboratory accommodations for these techniques are shown in Figure 1. Healon GV was the ophthalmic viscoelastic device (OVD) for each technique.

Each anterior capsulotomy technique is described per group:

Group 1: Vitrectorhexis capsulotomy was performed using an Accurus vitrector and a cutting rate of 150 cuts per minute (Figure 2). A central capsular puncture was made with the vitrector. The vitrector probe was then positioned in contact with the center of the anterior capsule, its cutting port positioned posteriorly. The cutter was turned on and suction was gradually increased using the foot pedal, until the capsule edge was engaged. The cutting port was then moved in a spiral fashion with the cutting edge positioned posteriorly, until the desired size and shape were achieved. Care was taken to avoid leaving any right-angled edges.

Group 2: Manual CCC was performed using a closed-chamber technique (Figure 3). Healon GV was used. Capsulorhexis was initiated using a 26-gauge cystotome and completed with Utrata capsulorhexis forceps. Centripetal force and frequent regrasping maneuvers were used.

Group 3: Multipuncture can-opener capsulotomy was accomplished with a 26-gauge cystotome (Figure 4). An initial puncture was made at the 6-o’clock position, 2.5 mm from the center of the capsule. The next puncture was made 1 mm from the initial puncture in a counterclockwise direction. The cystotome punctured the capsule with a downward (posterior) movement. At the moment of puncture the cystotome was pulled toward the center of the pupil. Additional punctures were made within the 1-mm spacing, if needed, to connect the capsular opening with the one made from the adjacent puncture. This was continued until a completed circular capsulotomy of approximately 5-mm diameter was made.

Group 4: Radiofrequency diathermy utilized a Kloti unit to perform the capsulotomy (Figure 5). The upper limits of current intensity (amperes) and potential (volts) were preselected and fixed by the manufacturer. The diathermy capsulotomy was performed using a continuous, circular, uninterrupted movement. Gentle pressure was maintained on the capsule by the instrument tip as it moved in a circular fashion, beginning in the center of the capsule.

Group 5: Fugo plasma-blade capsulotomy was accomplished using the electrosurgical base unit attached to a Fugo blade tip (Figure 6). The “cut power” was set to “medium” and the “cut intensity” was set to “level 5,” as per the instruction of the manufacturer. With the foot-petal engaged, the Fugo blade filament was placed in contact with the capsule and the tip was moved in a circle while maintaining contact with the capsule. An attempt was made to avoid creating any right-angled edges.

Biomechanical Characteristics of the Porcine Anterior Capsule
The calipers used to measure the capsulotomy opening...
FIGURE 2
Vitrector equipment needed for the vitrectorhexis capsulotomy technique.

FIGURE 3
Surgical equipment needed for the manual continuous curvilinear capsulorhexis (CCC) capsulotomy technique.

FIGURE 4
Surgical equipment needed for the manual multipuncture “can-opener” capsulotomy technique.

FIGURE 5
Kloti equipment needed for the radiofrequency diathermy capsulotomy technique.
are shown in Figure 7A. Panel B shows the caliper tips inserted in the unstretched capsulotomy, and panel C shows that the stretch-to-break occurred elsewhere (arrow) and not at the point where the caliper tips met the edge of the capsulotomy opening; in this case the tear occurred at about the 2-o’clock position.

The unstretched capsulotomy diameter (Dun) per capsulotomy technique is shown in Table 1 (young pigs) and Table 2 (adult pigs). No significant difference (\( P > .05 \)) was shown between these measurements per procedure, or between the young and adult eyes (\( P > .05 \)) of each procedure. Likewise, there was no significant difference between the stretched length-to-breaking (Ls) measurements (Tables 1 and 2) per procedure (\( P > .05 \)), or between the young and adult pig eyes (\( P > .05 \)).

The unstretched and stretched circumference of the young and adult pig eyes are presented in Tables 3 and 4, respectively. The stretched to unstretched circumference per anterior capsulotomy technique is shown in Figure 8.

The mean percentage of stretched to unstretched circumference of young lens capsules, each presented as a mean value in the order of animal used for each of the five anterior capsulotomy technologies, and similarly for the adult lens capsules, showed that in this population there was no significant difference (\( P > .05 \)) between the anterior capsule stretch of the young eyes compared to the adult eyes.

### Scanning Electron Micrography of Porcine Lens Capsule

One porcine globe per each of the five anterior capsulotomy techniques was implanted with a PMMA MC52BM intraocular lens (Alcon Laboratories, Fort Worth, Texas) following lens removal and submitted for SEM evaluation.

Group 1, using the vitrectorhexis anterior capsulotomy technique, is shown in Figure 9. Scanning electron micrography

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**FIGURE 6**

Fugo equipment needed for the plasma-blade capsulotomy technique.

**FIGURE 7**

Calipers were used to measure the unstretched and stretched capsulotomy opening. Left, the caliper tips are placed in the unstretched capsulotomy opening (white arrows indicate the caliper tips). Right, The same stretched capsulotomy opening as appears at left, showing where this opening was torn when stretched (white arrow).
microscopy images showed the capsular edge of the vitrectorhexis cut was a typical scalloped edge with the whole edge rolled-over, presenting a smooth surface toward the inside of the capsulotomy (panel A). High-magnification SEM shows a slightly less regular edge (panel B) than that achieved with the manual technique (Figure 10).

Group 2, using the manual CCC anterior capsulotomy technique, is shown in Figure 10. Scanning electron microscopy images revealed that the edge of the manual capsulorrhexis is smooth and exhibits no obvious defects (panel A). High-power SEM shows a smooth, regular edge (panel B).

Group 3, multipuncture can-opener anterior capsulotomy technique, is shown in Figure 11.

Group 4, using the Kloti radiofrequency diathermy anterior capsulotomy technique, is shown in Figure 12. Scanning electron microscopy images showed the ragged capsular edge of the diathermy cut (panel A). High-magnification SEM shows a rough irregular edge (panel...
Group 5, using the Fugo plasma-blade anterior capsulotomy technique, is shown in Figure 13. Scanning electron microscopy images showed the capsular edge of the plasma blade cut (panel A). High-magnification SEM shows a slightly rough edge (panel B) when compared to the manual technique (Figure 10).

Ten-Year Review of Pediatric Cataract Surgery Cases
Retrospective analysis of 379 consecutive pediatric cataract surgeries performed between January 1, 1994, and December 31, 2003, was undertaken (Figure 14). All surgery was performed by the same surgeon. Emphasis was placed on the integrity of the edge of the anterior capsulotomy during cataract surgery. The analysis revealed that the anterior capsulotomy withstood the stresses of surgical manipulation without tearing in 91.9% (329 of 358) of cases. These cases included 74 eyes left aphakic and 284 pseudophakic eyes. The remaining 21 cataract cases had preoperative rupture of the anterior capsule from trauma (19 eyes) or spontaneously (2 eyes with Alport syndrome). These groups were analyzed separately. The five different anterior capsulotomy techniques used in these surgical cases were vitrectorhexis, manual CCC, multipuncture can-opener, Kloti radiofrequency diathermy, and the Fugo plasma blade.

Twenty-nine anterior capsular tears were recorded
during the 284 pediatric cataract surgeries where an IOL was implanted (Figure 15). Distribution of the capsular tears was not associated with placement in time \((P > .05)\); that is, there were statistically no more tears occurring between 1994 and 1998 (91 cases; 10 tears) than between 1999 and 2003 (193 cases; 19 tears). The surgical step during which each tear occurred is also shown in Figure 15: 4 (13.8%) of 29 tears occurred during the capsulotomy, 7 (24.1%) during cataract removal, 13 (44.8%) during IOL insertion, 4 (13.8%) during hydrodissection, and 1 (3.4%) during OVD removal. The tears occurring during IOL insertion were not statistically different from the incidence of the other tears \((P > .05)\).

The age of the patients ranged from birth to 234 months. The mean age of all patients was 53.5 months \(\pm 50.4\) (median, 44 months). Capsular tears in those aged 0 to 72 months were not statistically different from those 72 to 234 months \((P > .05)\). Of all pediatric cataract surgeries where an IOL was implanted (284), 198 (69.7%) were from patients \(\leq 72\) months of age; tears occurred in 19 (9.6%) of these 198 eyes. Table 5 presents the mean distribution of patient age, with or without tear, per capsulotomy technique. Age was not related to tear incidence \((P > .05)\). The surgical step during which the tear occurred is shown in Figure 16, but was not statistically limited to any age group \((P > .05)\).

Each of the capsulotomy techniques is presented per patient age, beginning with the vitrectorhexis technique. Vitrectorhexis was the capsulotomy technique of choice for younger patients (Figure 17). There were 16 capsule tears (7.7%) during the 208 cases using the vitrectorhexis technique. One hundred sixty-four (78.8%) of the 208 cases were \(\leq 72\) months of age, and 9 (56.3%) of the tears were from patients \(\leq 72\) months of age. The surgical step during which the tear occurred is also shown in Figure 17.

There were 3 capsule tears (6.4%) during the 47 cases using the manual CCC technique (Figure 18). Fourteen (29.8%) of the 47 cases were \(\leq 72\) months of age, and 2 (66.7%) of the tears were from patients who were \(\leq 72\) months of age. The surgical step during which the tear occurred is also shown in Figure 18.

The multipuncture can-opener capsulotomy technique did not have sufficient use in this clinical series to be statistically compared to the other techniques (Figure
19). There was one capsule tear during the two cases using the can-opener capsulotomy technique. Both patients were ≤72 months of age. The surgical step during which the tear occurred is also shown in Figure 19.

Kloti radiofrequency diathermy was used less frequently in this series when compared to manual CCC (Figure 18) and vitrectorhexis (Figure 20). There were 4 capsule tears (21.1%) during the 19 cases using the Kloti radiofrequency diathermy technique. Twelve (63.2%) of the 19 cases were ≤72 months of age, and 3 (75.0%) of the tears were from patients who were ≤72 months of age. The surgical step during which the tear occurred is also shown in Figure 20.

The Fugo plasma blade capsulotomy technique did not have sufficient use in this clinical series to be statistically compared to the other techniques (Figure 21). There were 5 capsule tears (62.5%) during the 8 cases using the Fugo plasma blade capsulotomy technique. Six (75.0%) of the 8 cases were ≤72 months of age, and 4 (80%) of the tears were from patients who were ≤72 months of age. The surgical step during which the tear occurred is also shown in Figure 21.

Overall (Figure 22), 15 capsule tears (10.1%) occurred in the 148 cataractous right eyes (mean age, 53.2 months ± 50.1; range, birth to 234 months; median, 44 months). One hundred and two (68.9%) of the 148 cases were ≤72 months of age, and 11 (73.3%) of the tears were from these 102 patients. There were 14 capsule tears (10.3%) in left eyes during these 136 surgeries (mean age, 53.9 months ± 50.8; range, birth to 229 months; median, 43.7 months). Ninety-six (70.6%) of the 136 cases were ≤72 months of age, and 8 (57.1%) of the tears were from these 96 patients. The surgical step during which the tear occurred is also shown in Figure 22 but was not statistically attributable to either eye (P > .05).

Seventy-four cases were left aphakic. The vitrectorhexis technique was utilized 73 times and the Kloti radiofrequency diathermy used once. No radial tears developed in this group. These were young patients (mean age, 16.2 months; range, birth to 57 months; median, 2 months).

Twenty-one eyes had preoperative rupture of the anterior capsule from trauma (19), or spontaneously (2; in association with Alport syndrome) (mean age, 85.2
Wilson

In these eyes, the vitrectorhexis technique was used to complete the capsulotomy in 16 eyes, the Fugo plasma blade capsulotomy technique was used in 1, manual CCC was used in 3, and in 1 eye the ruptured anterior capsule was not enlarged at all. No additional tears occurred during these surgeries.

Pediatric Case Details For Eyes With Radial Tears

Vitrectorhexis. There were 16 anterior capsule tears in the vitrectorhexis group. Nine of these occurred during IOL insertion. Six capsule tears occurred nasally in the right eye or temporally in the left eye at the location where the trailing haptic of the IOL was exerting pressure as it was being dialed into the capsular bag. Of the remaining 3 tears, 2 occurred when three-piece acrylic lenses were unfolded too anteriorly, with the leading haptic inadvertently in the ciliary sulcus. Extensive dialing of the lens was needed to secure the IOL completely in the capsular bag. During this maneuver, a tear formed nasally in a left eye and temporally in a right eye. The remaining IOL insertion-related tear occurred superiorly as forceps were being used to place the trailing haptic. All 9 of these tears occurred during manipulation of either a PMMA (3 of 9) or a three-piece foldable IOL (6 of 9).

In 5 of 9 tears, the terminal edge of the tear was easily seen and the tear did not continue to extend toward the lens equator. In all 9, the IOL was successfully positioned completely within the capsular bag with the IOL haptics 90 degrees away from the radial tear.

Three of the vitrectorhexis tears (3 of 16, 18.7%) occurred during removal of lens cortex. Two were superior (subincisional) tears and one was an inferior tear, all at recognized right-angled edges in the capsulotomy. Two vitrectorhexis tears (2 of 16, 12.5%) occurred while the capsulotomy was being performed. In both cases, the

TABLE 5. MEAN AGES OF 284 PATIENTS RECEIVING VARIOUS CAPSULOTOMY TECHNIQUES DURING PEDIATRIC CATARACT SURGERY WITHOUT COMPLICATION, OR WITH AN ANTERIOR CAPSULE TEAR DURING SURGERY

<table>
<thead>
<tr>
<th>CAPSULOTOMY TECHNIQUE</th>
<th>EYES WITHOUT CAPSULAR TEARS</th>
<th>EYES WITH CAPSULAR TEARS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MEAN AGE (±SD)</td>
<td>RANGE</td>
</tr>
<tr>
<td>Vitrectorhexis</td>
<td>38.0 (±38.7) mo</td>
<td>(0 - 181 mo)</td>
</tr>
<tr>
<td>Manual CCC</td>
<td>108.9 (±57.5) mo</td>
<td>(7 - 234 mo)</td>
</tr>
<tr>
<td>Can-opener</td>
<td>1.0 mo</td>
<td>NA</td>
</tr>
<tr>
<td>Klotti radiofrequency</td>
<td>77.0 (±55.3) mo</td>
<td>(6 - 156 mo)</td>
</tr>
<tr>
<td>Fugo plasma blade</td>
<td>54.2 (±33.0) mo</td>
<td>(21 - 87 mo)</td>
</tr>
</tbody>
</table>

CCC, continuous curvilinear capsulorrhexis.
wounds were too large and fluid leak caused anterior chamber instability. One vitrectorhexis tear occurred during hydrodissection as the lens substance forcibly moved anteriorly. This tear, however, did not extend after the fluid was decompressed from the capsular bag. It remained stable for the remainder of the case. One vitrectorhexis tear occurred after Healon GV removal as the bimanual irrigation and aspiration instruments were withdrawn from the eye. The anterior chamber became shallow and the IOL pressed against the edge of the capsulotomy. The tear occurred at a previously recognized right-angled edge, nasally.

**Manual CCC.** There were 3 tears in the manual continuous curvilinear capsulorrhexis group. One tear occurred during lens cortex removal, one tear occurred during IOL insertion, and one peripheral extension occurred during the capsulotomy itself. Additionally, two manual CCC capsulotomies that were extending toward the lens equator were aborted before radial tears formed. These were converted into a circular capsulotomy using the radiofrequency diathermy unit and are thus not classified as radial tears.

The manual CCC that tore during lens cortex removal had been complicated by poor visualization from liquefied cortex that escaped the capsular bag upon initiation of the capsulotomy. A capsular flap superiorly was left and not recognized until after the tear had developed in this same area. Another manual CCC radial tear occurred at a site where the keratome used to enter the eye had inadvertently pierced the capsule. Despite
manipulating the CCC to incorporate this keratome puncture site, a tear emanated from this area when stressed by IOL insertion. The third manual CCC tear occurred as a peripheral extension during the capsulotomy procedure itself. This capsulotomy was completed using the vitrector. In all three of these cases, the IOL was successfully implanted into the capsular bag with the IOL haptics placed 90 degrees from the tear.

**Multipuncture Can-opener.** There was one capsule tear in the multipuncture can-opener group. It was recorded during the IOL insertion. Two eyes of one infant were operated on using the can-opener technique. The capsule was difficult to puncture using the needle tip. Although the capsulotomy was reasonably round, one of the two capsulotomies tore when the unfolding haptic of a three-piece acrylic IOL came in contact with the capsulotomy edge.

**Kloti Radiofrequency Diathermy.** There were four capsule tears in the Kloti radiofrequency diathermy group. One tear was recorded each during anterior capsulotomy, hydrodissection, lens cortex removal, and IOL insertion. In one case, a tear occurred at the completion of the capsulotomy when the instrument tip remained adherent to the capsular edge as it was withdrawn from the eye. In another case, at the completion of the capsulotomy, the wound was enlarged slightly to facilitate removal of the capsular cap. Bimanual irrigation and aspiration handpieces were used to remove the lens substance. The enlarged opening allowed fluid leakage around the instruments, making the anterior chamber unstable. Coincident with the shallowing of the anterior chamber, a radial tear occurred in the capsulotomy edge beneath the superior wound. The edge remained visible, however, and did not extend to the lens equator. In all of the radiofrequency diathermy tear cases, the IOL was successfully placed into the capsular bag with the IOL haptics placed 90 degrees from the tear.

**Fugo Plasma Blade.** This group had five anterior capsule tears when using the Fugo plasma blade capsulotomy technique. Among the nine eyes (8 in pseudophakic...
group) where the Fugo plasma blade was used, a tear developed in 5 (55.5%). One eye had a preoperative rupture of the capsule from trauma. This capsule opening was completed using the Fugo blade without additional tears occurring. Among the primary IOL cases without preoperative trauma, radial tears occurred in 5 of 8 eyes (62.5%) where the Fugo blade was used to perform the anterior capsulotomy. The tears occurred during hydrodissection (2 eyes), IOL insertion (1 eye), or cataract removal (2 eyes).

Worldwide Anterior Capsulotomy Survey of Pediatric Surgeons
Responses to the 2003 worldwide anterior capsulotomy survey of pediatric surgeon preferences were received from 563 of 996 mailed questionnaires for an overall response rate of 56.5%. The response rate from US surgeons was 57.2% (467 of 816), and the international surgeon response rate was 53.3% (96 of 180). Three retired US respondents and 134 US respondents not practicing pediatric cataract surgery and not implanting intraocular lenses were omitted from further review, leaving 330 returned questionnaires for review and preference analysis. Twenty-eight international respondents not practicing pediatric cataract surgery and not implanting intraocular lenses were omitted from further review, leaving 68 returned questionnaires for review and preference analysis.

Overall, 99.5% (396 of 398) of the respondents whose questionnaires were analyzable were performing pediatric cataract surgery. Thirty-six of the 328 respondents performing pediatric cataract surgery in the United States were not implanting IOLs (11.0%). Four of the 68 international respondents performing pediatric cataract surgery were not implanting IOLs (5.9%).

Of the overall analyzed questionnaires, 82.9% of the respondents (330 of 398) were implanting IOLs in pediatric patients. Two of the 294 US respondents (0.7%), but none of the international respondents that were implanting IOLs, were not also performing pediatric cataract surgery.

Figure 23 shows the number of responses attributed to each of five anterior capsulotomy categories and the pediatric ages each applies to. Vitrectomy and manual capsulotomies were the most popular choices, where preference appears to inversely relate to patient age, particularly during the first 6 years of life, between these two techniques. As would be expected, the preferences per age group for vitrectomy are significantly different from those of manual capsulotomy ($P < .05$). The numbers of responses to the remaining three categories of Figure 23 are too few to compare. Tables 6 and 7 show the same data redistributed as US and international preferences of the five capsulotomy techniques.

DISCUSSION
The human lens forms from an invagination of surface ectoderm. It is because of this invagination that the lens capsule, a true PAS-positive basement membrane, comes to lie external to the cells that secreted it. The lens capsule continues to grow throughout life in conjunction with the volume increases of the crystalline lens. The lens capsule changes physiologically as well. The tensile strength of the anterior lens capsule is five times greater in infancy as compared to old age. The anterior lens capsule of the young eye also has double the extensibility of the aged capsule. These differences between the adult and child must be understood and accounted for when planning for surgery on the pediatric lens. Because modern cataract surgery at all ages takes place almost
### Table 6. Percentage of Total Responses from US Pediatric Surgeons Per Patient Age Group (Percentage) Per Anterior Capsulotomy Technique

<table>
<thead>
<tr>
<th>PATIENT AGE (YR)</th>
<th>NO. OF VITRECTOR AC RESPONSES</th>
<th>NO. OF MANUAL AC RESPONSES</th>
<th>NO. OF CAN OPENER AC RESPONSES</th>
<th>NO. OF RADIO DIATH AC RESPONSES</th>
<th>NO. OF OTHER AC RESPONSES</th>
<th>TOTAL RESPONSES (N PER AGE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>192 (72.5%)</td>
<td>57 (21.5%)</td>
<td>12 (4.5%)</td>
<td>3 (1.1%)</td>
<td>1 (0.4%)</td>
<td>265</td>
</tr>
<tr>
<td>1-2</td>
<td>171 (57.6%)</td>
<td>111 (37.4%)</td>
<td>11 (3.7%)</td>
<td>3 (1.0%)</td>
<td>1 (0.3%)</td>
<td>297</td>
</tr>
<tr>
<td>2-3</td>
<td>143 (45.5%)</td>
<td>155 (49.4%)</td>
<td>12 (3.8%)</td>
<td>3 (1.0%)</td>
<td>1 (0.3%)</td>
<td>314</td>
</tr>
<tr>
<td>3-4</td>
<td>131 (41.5%)</td>
<td>169 (53.5%)</td>
<td>13 (4.1%)</td>
<td>2 (0.6%)</td>
<td>1 (0.3%)</td>
<td>316</td>
</tr>
<tr>
<td>4-5</td>
<td>113 (36.2%)</td>
<td>186 (59.6%)</td>
<td>10 (3.2%)</td>
<td>2 (0.6%)</td>
<td>1 (0.3%)</td>
<td>312</td>
</tr>
<tr>
<td>5-6</td>
<td>100 (32.2%)</td>
<td>200 (64.3%)</td>
<td>9 (2.9%)</td>
<td>1 (0.3%)</td>
<td>1 (0.3%)</td>
<td>311</td>
</tr>
<tr>
<td>6-8</td>
<td>85 (27.5%)</td>
<td>216 (69.9%)</td>
<td>6 (1.9%)</td>
<td>1 (0.3%)</td>
<td>1 (0.3%)</td>
<td>309</td>
</tr>
<tr>
<td>8-10</td>
<td>67 (21.9%)</td>
<td>231 (75.5%)</td>
<td>6 (2.0%)</td>
<td>1 (0.3%)</td>
<td>1 (0.3%)</td>
<td>306</td>
</tr>
<tr>
<td>10-15</td>
<td>51 (16.9%)</td>
<td>241 (80.1%)</td>
<td>6 (2.0%)</td>
<td>1 (0.3%)</td>
<td>2 (0.7%)</td>
<td>301</td>
</tr>
<tr>
<td>15-18</td>
<td>44 (15.1%)</td>
<td>238 (81.8%)</td>
<td>6 (2.1%)</td>
<td>1 (0.3%)</td>
<td>2 (0.7%)</td>
<td>291</td>
</tr>
</tbody>
</table>

AC, anterior capsulotomy; NA, not applicable; radio diath, radiofrequency diathermy.

*Technique preferences of greater than 50% participation are highlighted in grey.

†Three US respondents were retired and 134 US respondents were not practicing pediatric cataract surgery and not implanting intraocular lenses, and thus were omitted from analyses. Multiple responses allowed.

### Table 7. Percentage of Total Responses from Non-US Pediatric Surgeons Per Patient Age Group (Percentage) Per Anterior Capsulotomy Technique

<table>
<thead>
<tr>
<th>PATIENT AGE (YR)</th>
<th>NO. OF VITRECTOR AC RESPONSES</th>
<th>NO. OF MANUAL AC RESPONSES</th>
<th>NO. OF CAN OPENER AC RESPONSES</th>
<th>NO. OF RADIO DIATH AC RESPONSES</th>
<th>NO. OF OTHER AC RESPONSES</th>
<th>TOTAL RESPONSES (N PER AGE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>33 (53.2%)</td>
<td>21 (33.9%)</td>
<td>5 (8.1%)</td>
<td>2 (3.2%)</td>
<td>1 (1.6%)</td>
<td>62</td>
</tr>
<tr>
<td>1-2</td>
<td>25 (36.8%)</td>
<td>35 (51.5%)</td>
<td>5 (7.4%)</td>
<td>2 (2.9%)</td>
<td>1 (1.5%)</td>
<td>68</td>
</tr>
<tr>
<td>2-3</td>
<td>21 (28.8%)</td>
<td>45 (61.6%)</td>
<td>3 (4.1%)</td>
<td>2 (2.7%)</td>
<td>2 (2.7%)</td>
<td>73</td>
</tr>
<tr>
<td>3-4</td>
<td>20 (27.8%)</td>
<td>45 (62.5%)</td>
<td>3 (4.2%)</td>
<td>2 (2.8%)</td>
<td>2 (2.8%)</td>
<td>72</td>
</tr>
<tr>
<td>4-5</td>
<td>18 (26.1%)</td>
<td>46 (66.7%)</td>
<td>1 (1.4%)</td>
<td>2 (2.9%)</td>
<td>2 (2.9%)</td>
<td>69</td>
</tr>
<tr>
<td>5-6</td>
<td>15 (22.1%)</td>
<td>48 (70.6%)</td>
<td>1 (1.5%)</td>
<td>2 (2.9%)</td>
<td>2 (2.9%)</td>
<td>68</td>
</tr>
<tr>
<td>6-8</td>
<td>11 (16.2%)</td>
<td>53 (77.9%)</td>
<td>1 (1.5%)</td>
<td>1 (1.5%)</td>
<td>2 (2.9%)</td>
<td>68</td>
</tr>
<tr>
<td>8-10</td>
<td>4 (6.2%)</td>
<td>57 (87.7%)</td>
<td>1 (1.5%)</td>
<td>1 (1.5%)</td>
<td>2 (3.1%)</td>
<td>65</td>
</tr>
<tr>
<td>10-15</td>
<td>4 (6.3%)</td>
<td>56 (87.5%)</td>
<td>1 (1.6%)</td>
<td>1 (1.6%)</td>
<td>2 (3.1%)</td>
<td>64</td>
</tr>
<tr>
<td>15-18</td>
<td>3 (5.2%)</td>
<td>51 (87.9%)</td>
<td>1 (1.7%)</td>
<td>1 (1.7%)</td>
<td>2 (3.4%)</td>
<td>58</td>
</tr>
</tbody>
</table>

AC, anterior capsulotomy; NA, not applicable; radio diath, radiofrequency diathermy.

*Technique preferences of greater than 50% participation are highlighted in grey.

†Twenty-eight non-US respondents were not practicing pediatric cataract surgery and not implanting intraocular lenses, and thus were omitted from analyses. Multiple responses allowed.
entirely within the capsule of the lens, knowledge of the mechanical properties of the lens capsule is needed to help the surgeon achieve the goal of safe and complete cataract removal followed by implantation of an IOL that will remain stable and well supported. Openings into the anterior capsule of the crystalline lens are made to gain access to the nuclear and cortical lens material during cataract surgery. In addition, they allow an IOL to be placed within the capsular bag after cataract removal. The fact that modern cataract surgery has been called “capsular surgery” attests to the importance of the lens capsule and the capsulotomy to the outcome of the procedure.

For purposes of this discussion, anterior capsulotomy techniques will be presented in the chronologic order in which they were developed and first utilized. This will allow a better historical perspective of the evolution of these techniques. The findings of the current study and the recommendations of this author will then be incorporated within this chronologic context.

**Forceps Capsulotomy**

Sir Harold Ridley described the first anterior capsulotomy technique designed to be followed by insertion of an IOL. A complete anterior capsule removal was advocated, using toothed forceps. If anterior capsular flaps or remnants were seen after lens cortex removal, they were grasped with smooth-bladed capsule forceps and cut with scissors. Tearing was avoided for fear of removing the posterior capsule as well. Drews described the classic technique this way: “With the pupil maximally dilated, the forceps was opened and its teeth rested against the lens capsule on either side. The exquisitely sharp teeth were then gently pressed through the capsule and the jaws of the forceps closed. Hopefully and usually, this resulted in the tearing away of an appropriate size flap.” However, if the teeth were not sharp enough, delayed puncture of the capsule would sometimes result in splitting of the posterior capsule when the forceps teeth were brought together. Drews also wrote: “To keep the ... surgeon humble, the anterior capsule is relatively thick and resistant to the surgeon's attempts to produce a uniform and controlled aperture while the posterior lens capsule is thin and easily ruptured.”

**Triangular or “V”-shaped Capsulotomy**

Kelman recommended a triangular anterior capsulotomy for use with his new phacoemulsification technique in 1975. This so-called Christmas tree or fir tree capsulotomy was performed by placing an irrigating cystotome on the capsule 4 mm inferior to the optical center of the lens. The cystotome engaged the capsule gently so as not to puncture it, but merely to tent it. As the cystotome was withdrawn toward the incision, the capsule was opened in a triangular shape. Additional slits were made in the capsule to open it more widely. This technique was used for children as well as adults. However, the capsule did not always tear to produce a triangular flap. The dimensions of the capsulotomy were not always consistent or reproducible. Also, the more elastic pediatric capsule increased the stress that the technique placed on the capsule and the zonules. At times, the capsule would not tear, resulting in zonular rupture. Or, the capsule would tear too far into the lens equator. The V-shaped capsulotomy, popularized for developing world extracapsular adult cataract surgery by the Fred Hollows Foundation, is a variation of the Kelman capsulotomy. It utilizes the side of a sharp straight needle to do the cutting. In this way, the size and shape of the capsulotomy can be more easily controlled. This technique has been utilized for children as well as adults by the Himalayan Cataract Project. The V-shaped capsulotomy has the advantage of not requiring an OVD, thus reducing the cost of the surgery. The straight needle can be manipulated within the anterior chamber without the loss of aqueous humor. The disadvantage is that the angled edges of the “V” shape can tear out to the lens equator or even onto the posterior capsule. To test the stability of a slit or V-shaped capsulotomy in an animal model of the human pediatric anterior lens capsule, we stretched 20 adult pig eye capsulotomies made with a needle and scissors. Each capsulotomy tore very quickly as soon as even minimal stretch was placed perpendicular to one of the ends of the slit (unpublished data, 2003). These data were not part of the formal porcine study described in detail herein.

**Can-opener Capsulotomy**

To reduce stress placed on the zonular fibers and produce a more rounded capsulotomy with fewer tags, the multipuncture or can-opener capsulotomy came into common use. This technique has been ascribed to Pearce and, alternatively, to Little. This technique was performed using a bent 27-gauge needle. A jagged but circular opening in the capsule was formed. Frequently, however, radial extensions of the capsulotomy occurred during lens removal or IOL insertion. Wasserman and coworkers analyzed 250 consecutive postmortem adult eyes operated on for cataracts between September 1978 and June 1989. More than 90% had been operated on using the can-opener anterior capsulotomy technique. Eighty-six percent of the eyes had one to five radial tears. Asymmetric IOL loop fixation and resulting decentration were more common when radial tears were present. Each of the early methods of capsulotomy discussed above allowed access to the contents of the lens, but they often resulted in numerous radial tears emanating from the capsular openings. These tears resulted in uncertainty regarding the stability of the capsular bag and the position of the IOL after implantation.
Rosen lamented in 1990 that the can-opener technique remained the most popular anterior capsulotomy worldwide, in spite of “progressive developments which should confine the technique to history.”

Recently, Wood and Schelonka recommended a return to the can-opener capsulotomy technique for pediatric cataracts. Using a porcine model, Wood and Schelonka compared can-opener capsulotomy to manual CCC capsulotomies. A total of 47 can-opener capsulotomies were compared with 102 manual CCC capsulotomies. The first author, an experienced cataract surgeon, compared his capsulotomy performance to that of the study’s second author, a medical student with only a single capsulotomy training session prior to the study. The first author of the paper performed 20 can-opener capsulotomies using the porcine model without any radial tears. The second author had one radial tear in 27 consecutive can-opener capsulotomies (2.1%). In contrast, the overall failure rate for manual CCC in their study was 22.5%. The experienced surgeon encountered uncontrolled tears in 17 (21.3%) of 80 manual CCC eyes. The inexperienced surgeon experienced uncontrolled tears in 6 (27.3%) of 22 manual CCC capsulotomies. The number of tears compared to the experience of the surgeon was not statistically different (P > .05). The relative risk of an uncontrolled radial tear during the manual CCC in the Wood and Schelonka study was 10.58, as compared to the can-opener capsulotomy. Even more surprising was the finding that the mean maximum strain (stretch-to-rupture) of the can-opener capsulotomies (46.7%) was not statistically different from the mean maximum strain of the manual CCC (47.7%) in their porcine model.

For the animal model portion of the present study, the porcine eye was also utilized. We found that the can-opener capsulotomy stretched to a mean diameter of 149% of the unstretched diameter before breaking (Figure 8). This is very similar to the findings of Wood and Schelonka discussed above. Extensibility in the current study is reported as the percentage of stretched compared to unstretched capsulotomy circumference. Wood and Schelonka reported the percentage of stretch beyond the baseline resting circumference. Thus the two figures, 149% and 46.7%, represent very similar stretch characteristics.

The manual CCC capsulotomy in our porcine study stretched to a mean of 185% compared to the unstretched circumference (Figure 8). This is in contrast to the 47.7% mean stretch-to-rupture reported by Wood and Schelonka. In the current porcine study, the manual CCC (P < .001, one-way ANOVA) and the Fugo plasma blade capsulotomy (P = .004, one-way ANOVA) were significantly more extensible than the can-opener capsulotomy. However, no statistical difference was detected when the mean stretch-to-break percentage of the can-opener capsulotomy was compared to the Kloti radiofrequency diathermy capsulotomy (P > .05) or the vitrectorhexis (P > .05).

The adult porcine capsule has been shown to be a valid model for the pediatric human capsule. For the current study, porcine eyes from young (6 months of age) as well as adult (2 to 3 years of age) pigs were studied. However, there was no significant difference (P > .05) between the percentage stretched to unstretched circumference of the young pig eyes when compared to the adult pig eyes.

As in human pediatric eyes, the capsule of the pig is very elastic and difficult to puncture. These characteristics create a very different opening after multipuncture can-opener capsulotomy in children when compared to adults. Observations during the current study revealed that each puncture of the needle created a small CCC as the needle tip was pulled toward the center of the capsule, not unlike the tearing that occurs when the Auffarth technique for CCC is used in rabbits. The further the needle tip is pulled toward the center of the pupil, the more extensive the small CCC arc-tear will be. When the needle is then reengaged into the capsule adjacent to the previous puncture, another small CCC arc tear occurs. A scalloped edge results, but the tips of each pointed capsular tag are directed toward the center of the pupil and tend to roll outward when OVD is placed in the capsule, thus appearing as a smooth edge. This is in contrast to a can-opener capsulotomy performed on the adult human capsule. With less elasticity and less resistance to tearing, each puncture of the needle results in a small radial tear with the capsular tags pointing outward. These outward-pointing tags have been shown by computer models and a mathematical tool called the finite element method to be areas of high stress that tear toward the lens periphery when minimal stretch is applied. In contrast, inward-pointing capsular flaps, as seen in the can-opener capsulotomy on the pediatric capsule, show no increased stress and no increase in tearing tendency when compared with the remainder of the capsulotomy edge.

Prior to the development of the manual CCC, the can-opener capsulotomy had a long and favorable track record in pediatric cataract surgery. Helveston and Ellis, in their 1984 book entitled Pediatric Ophthalmic Practice, illustrated the use of a can-opener capsulotomy, which had been the standard of practice for pediatric cataract surgery for some time, and remained so in their hands even after they adopted the vitrector handpiece for performing the posterior capsulotomy and anterior vitrectomy. A meta-analysis of published cases from 1983 to 1995 revealed a total of 509 can-opener capsulotomies in children with follow-up ranging from 4 to 18 years. A reoperation was documented in only seven eyes (1.4%). None of the repeated surgeries were attributed to defects or tears in the capsulotomy. However, 26 IOLs had iris/papillary capture, and 324 IOLs (of 509) were believed to have one or both haptics outside of the capsular bag. Basti and
coworkers\textsuperscript{44} reported a total of 169 operated eyes with all “except the last few” having had a can-opener capsulotomy. A radial tear was reported in only four eyes, and in each of these the IOL was still placed easily into the capsular bag.

These rather dramatic differences between the pediatric and adult can-opener capsulotomy make the technique more recommendable for children than for adults. However, it appears that for the past 10 years the can-opener technique has not been utilized often by pediatric surgeons. In a 1994 survey, only 15\% of the responding surgeons reported using a can-opener capsulotomy when operating on children.\textsuperscript{45} The 2003 surgeon preference survey included in this thesis showed an even lower utilization rate of 1.9\% to 5.2\% (Figure 24), depending on the age of the child. For US surgeons, the can-opener capsulotomy technique utilization rate ranged from a high of 4.5\% of surgeons when operating on patients less than 1 year of age to 1.9\% of surgeons when operating on children older than age 6 to 8 years (Table 6). Multiple responses were allowed, so this represents the total number of responding surgeons who reported the technique as one of their preferred options utilized at the indicated ages between <1 and 18 years.

In the porcine model of this study, the capsules were difficult to puncture using even a fresh sharp cystotome. Force was directed posteriorly until the needle tip punctured the capsule, creating a sudden surge of the needle into the substance of the lens. A variable amount of capsular opening would then result. Also, additional capsular punctures were sometimes needed when the adjacent punctures did not connect with one another, leaving a gap of uncut capsule. This created strands of capsule that were frequently engaged by the aspiration handpiece, interfering with the lens removal that followed the capsulotomy. Only two eyes were operated on using the can-opener technique in the clinical series reported herein. These were a pair of infant eyes from one patient. One of the two developed a radial tear during IOL insertion when the unfolding haptic came in contact with the capsulotomy edge. Despite the fact that the movement of the needle was toward the center of the pupil, more zonular stress seemed to be created than with the other techniques. The tear occurred at a right-angled edge that had been inadvertently created.

\section*{Manual CCC}

The manual CCC technique is an outgrowth of the can-opener style capsulotomy developed to address the tendency of the adult can-opener edge to develop numerous radial tears.\textsuperscript{41} The widespread recognition of the need for a better technique resulted in the near simultaneous development of a continuous tear capsulotomy on three different continents. The technique known today as manual CCC was developed simultaneously in North America by Gimbel and in Europe by Neuhann.\textsuperscript{27} A third surgeon, Shimizu, in Asia, also developed a similar technique, which was reported less than 2 years after Gimbel’s first presentation. Since Shimizu had no prior knowledge of the North American or European work (neither had yet been reported in print), he also shares credit for the CCC development.\textsuperscript{27}

Gimbel\textsuperscript{27} recalls watching Gills use a combination of scissor cuts and tears to make capsular openings in 1983. Gimbel then began making a continuous tear superiorly to avoid capsular flaps that would interfere with efficient cortical removal, and gradually began tearing the capsule in a complete circle. After more than 1,000 cases of what he called “continuous tear capsulotomy,” Gimbel made a video presentation for the American Intraocular Implant Society (April 1985, Boston, Massachusetts). This video was also shown at the American Academy of Ophthalmology annual meeting in 1985 (IOLAB booth, San Francisco, California). Ocular Surgery News reported on Gimbel’s technique in its July 1, 1985, issue (pages 20-21). The first peer-reviewed publication on this new type of capsulotomy was authored by Neuhann in 1987.\textsuperscript{28}

In 1990,\textsuperscript{27} a name was suggested for the new capsulotomy technique that would incorporate terms used to describe it by all three of the originators. From Gimbel’s continuous tear capsulotomy, Neuhann’s capsulorrhexis, and Shimizu’s circular capsulotomy came the name “continuous circular capsulorrhexis” and therefore the abbreviation CCC. Later, the term “continuous curvilinear capsulorrhexis” was adopted, because the word “curvilinear” was generally more correct than “circular.”\textsuperscript{28} Gimbel and Neuhann\textsuperscript{28} also stated that CCC makes possible safe and effective IOL implantation in children, because secure posterior chamber in-the-bag implants generally cause no problems during physical maturation. The authors even stated that without the development of CCC, lens implantation in children might not have been realized. Although this appears to be an overstatement, it does emphasize that the development of CCC was important to the evolution of cataract surgery in children.

In the porcine eyes studied herein, the CCC edge was the most resistant to tearing (Figure 8) and also showed the cleanest edge microscopically with no debris or irregularities (Figure 10). The CCC method clearly creates the “gold standard” capsulotomy edge. However, as Gimbel and Neuhann\textsuperscript{27} first noted, it is much more difficult to perform this method in children as compared to adults. The increased fracture toughness and extensibility of the child’s capsule increases the tendency for the capsulotomy to extend peripherally. Also, more force is required before tearing begins. The considerable distention that occurs before propagating the tear has been called “tractional preload” and has been noted to create a pronounced outward-pulling vector force.\textsuperscript{28} In addition, reduced scleral rigidity results in posterior vitreous upthrust when the eye is entered. The vitreous “pressure” pushes the lens contents anteriorly, leading to a taunt and domed anterior
lens capsule. These difficulties are more pronounced with decreasing age of the child. Illustrative is a case series of infantile cataract surgeries reporting inadvertent peripheral extensions in 19 (90.5%) of 21 eyes when a manual CCC was attempted. In a cadaveric study, Wilson and coworkers reported inadvertent peripheral extensions in 6 of 18 pediatric eyes when a CCC was performed. All peripheral extensions were in eyes from children younger than age 5 years. This ex vivo study was criticized for not simulating the closed chamber conditions found in a clinical setting at cataract surgery. As a result, the porcine CCCs in the current study were made in a closed chamber setting without removing the cornea. Only one CCC extended peripherally. Andreo, Wilson, and Apple validated the porcine eye as an appropriate model for the human pediatric eye. The lower rate of peripheral extensions in the current porcine study as compared to the previous cadaveric study may have been due to the closed chamber technique in the current study, which allowed OVD to be utilized to flatten the capsule and prevent vitreous upthrust. It is interesting to note that inadvertent peripheral extensions during the capsulotomy procedure in the current porcine study occurred less often with manual CCC than with the Kloti radiofrequency diathermy, or the Fugo plasma blade technique.

Modifications in adult techniques to facilitate CCC in children are now more widely known and practiced. These include the use of a high-molecular-weight OVD, pulling more toward the center, aiming for a smaller-than-desired capsulotomy opening, and frequent regraping near the site of the advancing tear so that the direction of pull can be readjusted more easily. Some surgeons have altered the adult technique even more substantially in order to facilitate CCC in elastic capsules. Auffarth and colleagues developed a modified CCC technique for use in experiments on eyes of young albino rabbits and suggested that it be used for young human capsules as well. Auffarth and coworkers had discovered that rabbits have very elastic lens capsules reminiscent of the pediatric lens capsule. The technique begins with a puncture of the lens capsule at the superior border of the intended capsulotomy using a 27-gauge needle. Capsulorrhexis forceps are then used to grasp the anterior capsule centrally. The capsular flap is torn toward the 6-o’clock position until a half-circle is completed. The force is then reversed toward 12 o’clock, pulling with equal force to both tearing edges. This technique was used in an experimental study with 16 rabbits (32 eyes). The authors reported a radial tear in only 2 of the 32 eyes.

Nischal described a modification of the Auffarth technique in which two stab incisions are made in the anterior capsule, outlining the desired diameter of the capsulorrhexis. Capsulorrhexis forceps are used to grasp one end of the distal edge of the proximal anterior capsule stab incision. The grasped edge is gently pushed toward the corresponding point of the distal stab incision until the edge reaches halfway to the distal stab. The corresponding end of the proximal edge of the distal stab incision is similarly grasped, but with the capsule pulled gently toward the proximal stab incision. The two tears meet to form the CCC. This is repeated for the other end of each stab incision to complete the entire CCC. Nischal has named this technique the two-incision push-pull, or “TIPP,” capsulorrhexis. The tearing force, using this technique, is always directed toward the center of the pupil.

In the clinical cases reviewed herein, the manual CCC was very stable with only 3 of 47 eyes (6.4%) having a radial tear. No eye with a continuous uninterrupted capsulotomy edge developed a tear during surgery. In one eye, from a child 3 years old with a white complete cataract, liquefied cortex escaped the capsular bag upon initiation of the capsulotomy and obscured the view of a capsular flap. It was not recognized that the capsulotomy had not been completed. The tear occurred at the site of this flap. If liquefied cortex escapes the capsular bag at the beginning of the capsulotomy, it should be gently aspirated before completing and inspecting the capsulotomy. Alternatively, the vitrectorhexis technique can be used. Since the vitrector handpiece has aspiration capability, the escaping cortex can be removed coincident with the making of the capsulotomy. Visualization is, therefore, not compromised.

Another manual CCC that developed a radial tear was in a 2-year-old child. The keratome used to enter the eye had inadvertently pierced the capsule. Despite attempting to manipulate the CCC to incorporate the puncture site, a radial tear formed at this site during IOL insertion. The in-the-bag positioning of the IOL was not compromised. The haptics were oriented 90 degrees away from the tear. The third manual CCC tear occurred during the capsulotomy itself. A peripheral extension occurred in the superior temporal quadrant of the eye of a 10-year-old child. The capsulotomy was then completed using the vitrector. The IOL was placed into the capsular bag with the haptics oriented along the 11-o’clock to 3-o’clock axis. The IOL centered well despite the tear. Two additional manual CCC openings extended toward the lens equator but were rescued (before radial tears formed) by switching to the Kloti radiofrequency diathermy technique. If these two errant capsulotomies are added to the other three, the percentage of unsuccessful manual CCC technique capsulotomies is 10.6%. The vitrectorhexis tear rate, for comparison, was 7.7% (16 of 208) when an IOL was placed and 5.7% (16 of 282) when aphakic patients were also included. Still, no manual CCC edge tore during surgery if it was a completed circular opening. The same cannot be said for any other technique utilized in this study.

A manual CCC that begins to extend peripherally can be rescued by the vitrector or the Kloti radiofrequency diathermy technique.
diathermy device, as in the cases above, or the Fugo plasma blade, or even the can-opener technique. Alternatively, the capsulotomy can be regrasped near the leading edge of the capsulotomy and redirected by pulling toward the center of the pupil. Neuhann⁶⁹ has pointed out that these errant capsulotomies often encounter zonular fibers and tend to extend directly toward the lens periphery, like tearing paper alongside a ruler. He suggests increasing the microscope magnification to identify the responsible zonular fiber or fibers and removing their insertions with a needle or forceps tip. The edge can then usually be brought back toward the center of the pupil. Bluestein and colleagues⁶⁴ have documented the zonular-free zone of the anterior lens capsule in children to average 6.1 mm in infancy and enlarging to 8.0 mm by age 16 years.

The manual CCC was the most commonly utilized anterior capsulotomy technique when operating on children aged 2 years and older (Tables 6 and 7) based on the survey herein of the AAPOS membership. This survey received a 57.2% (467 of 816) response rate from pediatric ophthalmologists in the United States and a 53.3% (96 of 180) response rate from pediatric ophthalmologists internationally. Practice preferences were remarkably similar between the domestic and international respondents. Although more challenging to perform well, the manual CCC remained the 2003 “gold standard” capsulotomy technique for toddlers and up.

**Kloti Radiofrequency Diathermy Capsulotomy**

The Kloti radiofrequency diathermy tip is a specialized instrument designed for anterior capsulotomy. It has been used in children⁵₂,⁶⁷ and was tested herein. Kloti developed a miniature bipolar diathermy unit in 1984 that featured bipolar forceps for use in conjunctival, strabismus, or oculoplastic surgery, as well as a bipolar anterior capsulotomy tip.⁵¹ In 1985, Gassmann and colleagues⁶¹ stated that the anterior capsulotomy was a crucial step in cataract surgery and that the tense capsule was inherently capricious. They advocated using the bipolar radiofrequency endodiathermy probe because it was more precise. Four years later, experimental and clinical data were published on a more refined version of the Kloti radiofrequency capsulotomy instrument. The electronic control device had been improved, and the results of 21 patients compared with 21 controls were reported.⁵¹,⁵₂ Several advantages were listed for the diathermy capsulotomy over other techniques, including a precisely controlled capsulotomy size, an edge that is resistant to mechanical forces (reducing the risk of radial tears), and a well-defined grayish coagulation line that helps define the border of the capsulotomy (making implantation easier).⁵³

Coincident with the initial 1984 Kloti publication, Berger⁶⁴ (also in 1984) introduced a bipolar loop device and showed that the loop could cut a round capsular opening in animal and human cadaver eyes. However, the temperature under the anterior capsule reached 73.5°C during the capsulotomy procedure. The temperature was 36.2°C adjoining the capsulotomy and 27.7°C in the anterior chamber. Hausmann and Richard⁶⁶ noted that the Berger loop could not be placed through the smaller incision that was being used for phacoemulsification and therefore tested the Kloti bipolar tip instead. The Kloti tip was modified to include a concentric cannula ending near the hot tip to be used for irrigation (cooling) and maintenance of the anterior chamber. The overall external diameter of the modified tip was 1.25 mm. Results from 25 adult patients were reported. The capsulotomy procedure took no longer than 20 seconds. Temperature readings were taken using a probe placed inside the anterior chamber at the time of the capsulotomy. Corneal endothelial cell analysis was also done before and after surgery and compared to the fellow eye that had undergone traditional can-opener capsulotomy. These authors showed that room-temperature irrigation fluid provided sufficient cooling of the tip of the instrument to prevent any apparent endothelial cell damage. This lack of corneal endothelial damage was later verified by a prospective randomized study of 55 patients published in 1997.⁶⁶ Other experiments also showed that no electrolysis occurs as a result of the Kloti tip.⁶⁸ The Kloti tip used high-frequency current at 500 kHz. The international norm, set by the International Electrical Commission for medical applications, had defined 300 kHz as the lower limit for high-frequency current without causing electrolysis.⁶⁸

Hausmann and Richard⁶⁶ pointed out, in 1991, that the two commonly used capsulotomy techniques for adult cataract surgery each had disadvantages. The can-opener technique frequently led to radial tears out to the lens equator, and manual capsulorhexis required both “specialized surgical skill” and a good red reflex for effective control. In their patients, the modified Kloti radiofrequency bipolar tip had created a capsulotomy edge that was smooth and did not tear during surgery. Kloti⁶⁶ recommended using a visco-surgical device rather than continuous irrigation to ensure that the anterior chamber was maintained and that the corneal endothelium was protected from heat. Using this technique, other investigators reported, in 1993, 48 consecutive cases with more than 15 months of follow-up. No clinically observable differences were noted between Kloti radiofrequency bipolar capsulotomy and manual capsulorhexis in adult patients in the categories of rigidity or elasticity of the capsulotomy edge, corneal decompensation, posterior synechiae, or IOL decentration.⁶⁷

However, laboratory analyses utilizing adult autopsy eyes (published in 1994, 1996, and 1997) demonstrated that the diathermy capsulotomy edge was mechanically inferior to the edge produced by the manual CCC.⁵³,⁵⁴,⁶⁸
Morgan and coworkers\textsuperscript{31} found that the mean increase in edge length before breakage was 53% using the manual CCC and only 18% in the diathermy group. Luck and colleagues\textsuperscript{84} also noted that on SEM analysis the diathermy caused a loss of the normal lamellar architecture of the capsule with distorted and unrecognizable collagen fibrils and adherent lens fibers. In contrast, the manual CCC edge was completely smooth and free from irregularity with a preserved lamellar organization. Despite these differences, the ability to perform a controlled size capsulotomy independent of the elasticity of the capsule was noted as a potential advantage of diathermy over manual CCC for pediatric use.\textsuperscript{33,34,68} It was noted that despite the reduction from that seen with the manual CCC, the diathermy capsulotomy edge retained considerable elasticity.\textsuperscript{34} Without the need to deliver an intact hard nucleus through the capsulotomy opening, the retained elasticity of the diathermy capsulotomy may be sufficient to meet the needs of pediatric cataract surgery. In fact, in 1997, Comer and colleagues\textsuperscript{68} did compare adult human eyes to adult (5- to 6-month-old) porcine eyes. Others have reported data that validate the adult porcine eye as a model of the human pediatric eye for studies of anterior lens capsule elasticity.\textsuperscript{13} Krag and coworkers\textsuperscript{68} found that the mean extensibility between the manual CCC and the diathermy capsulotomy (mean, 61 mN ± 15) as determined by Krag and coworkers.\textsuperscript{68} The single-piece AcrySof (Alcon, Fort Worth, Texas) currently in use is more flexible and probably generates much less force when the haptics open.

In the clinical portion of the current study, the radial tear rate with the Kloti radiofrequency diathermy capsulotomy technique was 21% (4 of 19). The radial tears were divided among the various steps in the cataract procedure, including the anterior capsulotomy, hydrodissection, lens cortex removal, and IOL insertion. It is my clinical impression that the edge produced by this capsulotomy technique expands to the break point with less force than that needed by other capsulotomy techniques. If force applied to the edge of the capsulotomy can be minimized, the radial tear rate will be small. Care must be taken to make the capsulotomy large enough so that subincisional cortex removal and IOL insertion do not place much force on the capsulotomy edge. Compared to the manual CCC, the diathermy capsulotomy edge does not seem to be as resistant to the force applied by the aspiration handpiece when the surgeon reaches deep into the capsular bag equator to remove cortex. In other words, it feels less stiff and, therefore, may reach its maximum extensibility even when relatively little force is applied. In our cases with tears, however, the tears did not extend out to the lens equator. In each case, the edge was visible and the IOL was successfully placed in-the-bag. This lack of extension into the equator or onto the posterior capsule was true of many of the anterior capsule tears in children regardless of technique used. This implies that the pediatric capsule behaves differently in this regard than the adult capsule.

\textbf{Vitrectorhexis}

Despite the fact that anterior capsulectomy using the vitrector handpiece had been reported as part of the “lensectomy” (with no IOL) approach to pediatric cataracts as early as 1981,\textsuperscript{24} a mechanized (vitrector-cut) anterior capsulotomy technique combined with IOL insertion in children was not reported until the 1990s.\textsuperscript{25,26} Wilson performed a mechanized anterior capsulotomy combined with IOL insertion in eight children initially.\textsuperscript{25} All of the capsular openings were curvilinear and continuous, and no radial tears were seen even after the IOL insertion was complete. The original name, “mechanized anterior capsulectomy,” emphasized the mechanized nature of the...
vitrector handpiece and the fact that a portion of the capsule was removed (capsulectomy) rather than merely opened (capsulotomy). As stated in the “Introduction,” the commonly used term “vitrectorhexis”12,13 emphasizes the fact that it is a substitute for capsulorhexis performed using the vitrector. It is, in reality, a misnomer, because “rhexis” means to tear rather than to cut.27

In 1994, the new technique (called mechanized anterior capsulotomy) was compared to manual CCC using 18 pairs of human pediatric eyes and two pairs of human adult eyes obtained at autopsy.25 The age of the pediatric donors ranged from 4 days to 16 years. The adult eyes were from donors aged 65 years or older. The globes were prepared for study using a modification of a technique devised by Assia and coworkers.40 The integrity of the anterior capsule opening following lens nucleus and cortex removal and several IOL insertions was assessed by direct visual observation. A radial tear developed in only one of the 18 pediatric eyes in which a mechanized anterior capsulotomy was created. This occurred in one of two eyes from a 16-year-old child. The radial tear extended from a “squared-off” or angled capsulotomy edge during IOL insertion. No radial tears occurred in any of the manual CCC eyes. However, in six eyes (33%), all less than age 5 years, the leading edge of the manual CCC extended out to the lens equator rather than continuing in a circular path. Interestingly, a radial tear occurred in both of the elderly adult eyes (during phacoemulsification of the lens contents) in whom the mechanized anterior capsulotomy was performed. The mechanized technique seemed to work best in the youngest eyes, whereas the manual CCC seemed most suited for the oldest eyes.

Subsequently, an additional comparative study between manual CCC and mechanized anterior capsulotomy (referred to as “vitrectorhexis”) using adult pig eyes was performed.13 To validate the use of the pig eyes, CCC toomy (referred to as “vitrectorhexis”) using adult pig eyes was performed. The mechanized technique seemed to the lens equator rather than continuing in a circular path. Interestingly, a radial tear occurred in both of the elderly adult eyes (during phacoemulsification of the lens) in whom the mechanized anterior capsulotomy was performed. The mechanized technique seemed to work best in the youngest eyes, whereas the manual CCC seemed most suited for the oldest eyes.

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Case selection is an additional factor that has lead to a reduction in radial tears in the last 2 years. The vitrectorhexis technique is ideally suited to the very young child with a highly elastic anterior capsule. In these children, the manual CCC is more difficult to complete even with liberal use of a high-molecular-weight OVD. In the current study, when the vitrectorhexis technique was used, the median patient age of these pseudophakic eyes was 24 months. However, the median patient age of those
vitrectorhexis eyes that suffered a radial tear was 61 months. Compare this to the manual CCC, where the median age for these pseudophakic eyes was 104 months and the median patient age of eyes with tears was 47 months. The vitrectorhexis edge is more stable and resistant to tearing in the very young elastic capsules but does not behave as well in the older eyes. This was also evident from the initial vitrectorhexis laboratory study published in 1994. Radial tears developed in a 16-year-old eye and both adult eyes, but in none of the young eyes. The current (2003) practice pattern survey (Figures 23 and 24; Tables 6 and 7) showed that vitrectorhexis is the most commonly utilized capsulotomy technique for the first 2 years of life around the world. As the child gets older, the manual CCC becomes somewhat easier to complete and control. It produces the best edge characteristics and can withstand most of the surgical manipulations needed when operating on small soft eyes.

The other advantage of the vitrectorhexis when operating on small eyes is that the anterior capsulotomy and the lens aspiration can be done sequentially without taking the instruments from the eye. Since the vitrector is most efficient when placed through a tight-fitting wound, small-incision capsulorhexis forceps are recommended for manual CCC in young eyes. The smaller wound (20-gauge or smaller) allows easy conversion to a vitrectorhexis if the manual CCC begins to tear peripherally.

**Fugo Plasma Blade Capsulotomy**

The Fugo plasma blade was recently developed and has been approved by the Food and Drug Administration for capsulotomy use. The Fugo blade uses plasma technology to create a nearly resistance-free incision into the anterior capsule. The anterior capsule is gently applanated with the tip activated, and a continuous circular movement is made to create the capsulotomy. Alternatively, several arcuate incisions on the capsule can be made and connected to form a circular capsulotomy. The capsulotomy is performed under an OVD. Cavitation bubbles are created along the path of the capsulotomy as the Fugo blade cuts it.

In the current porcine study, the Fugo plasma blade made a round capsulotomy with a mean stretch-to-rupture circumference of 170% compared to the circumference at rest. This extensibility was significantly less than that of the capsulotomy made by the manual CCC (P = .039, one-way ANOVA), but greater than that of the capsulotomy made using the can-opener (P = .004, one-way ANOVA) and the Kloti radiofrequency diathermy (P = .001, one-way ANOVA) techniques. The Fugo plasma blade anterior capsulotomy extensibility was not significantly different from the vitrectorhexis anterior capsulotomy extensibility (P > .05).

The porcine capsules were easily cut using the Fugo plasma blade. However, making a complete circular capsulotomy with a single continuous movement was not always achieved. Cutting the first 180 degrees of the capsulotomy was easy due to the stretch and tension of the capsule. However, finishing the remaining 180 degrees was sometimes complicated by folding of the capsular flap that was no longer on stretch. Nonetheless, a circular capsulotomy of the intended size usually resulted. The SEM analysis of the Fugo plasma blade capsulotomy edge revealed a rough edge that did not resemble the manual tear CCC edge.

While no clinical studies have been published using the Fugo plasma blade for pediatric anterior capsulotomy, Singh has reported that he now uses the instrument to perform pediatric capsulotomies. Nine children in the current clinical study had an anterior capsulotomy using the Fugo plasma blade technique. One of these cases had a traumatic rupture of the anterior capsule preoperatively. The rupture was successfully rounded using the Fugo plasma blade. Of the eight eyes in the pseudophakic group, a radial tear developed in five. While the technique did not have sufficient use in this clinical series to be statistically compared to the other techniques, the tear rate was disappointing. Two tears developed during hydrodissection, two during lens aspiration, and one during IOL insertion. In each case, the stress on the capsular edge did not seem excessive. The clinical impression was of an unstable edge that tore easily with minimal stress. This is in contrast to the mean extensibility of 170% recorded for the porcine capsulotomies made with the Fugo plasma blade. Although the force needed to reach the stretch-to-rupture circumference was not measured in the current study, my clinical impression is that, like the Kloti radiofrequency diathermy capsulotomy, the Fugo plasma blade capsulotomy has reduced elastic stiffness. Whether the collagen at the cut edge of the Fugo plasma blade capsulotomy is denatured in some way remains unknown. However, it appears that although the Fugo plasma blade creates a capsulotomy with good extensibility, it may lack the elastic stiffness present in the manual CCC. Additional clinical experience is needed before a meaningful statistical comparison can be made with other techniques used to create a pediatric anterior capsulotomy.

**SUMMARY**

Cataract surgery is more complex when performed on children as compared to adults. The behavior of the anterior lens capsule during surgery is an example of that complexity. The anterior lens capsule in young children will be approximately three times thinner than the capsule in an elderly adult, but with five times the tensile strength.
and double the extensibility. An intact and appropriately sized anterior capsulotomy is needed during pediatric cataract surgery to help ensure the structure and stability of the remaining lens capsule. Capsular fixation of an IOL in children at the time of cataract surgery has become the standard of care. Therefore, more emphasis is being placed on the anterior capsulotomy procedure by pediatric ophthalmic surgeons today as compared to when primary IOL implantation in children was less common.

With a response rate well over 50%, the 2003 practice preference survey indicated that manual CCC was the most common technique for anterior capsulotomy in children above age 2 years. Vitrectorhexis was the most commonly performed technique for infants. The Kloti radiofrequency diathermy, Fugo plasma blade, and can-opener style techniques were used for anterior capsulotomy in children by only a small number of surgeons.

A porcine model was used to compare the five capsulotomy techniques discussed above. All produced a round capsulotomy that was near the size intended. There was no significant difference in the mean unstretched circumference among the five groups (P > .05), and the capsulotomy produced by the techniques studied showed no difference between young pig eyes and adult pig eyes (P > .05). A previous study validated the adult pig eye as a good model for the pediatric human eye for studies on anterior capsule extensibility.

In the current porcine study, extensibility was determined by stretching each capsulotomy until it ruptured. Each group demonstrated a mean expansion of more than 140% of its resting circumference before rupture. This degree of extensibility appears to be sufficient to allow a surgeon to place a foldable IOL through an appropriately sized (approximately 5 mm) capsulotomy created by any of these techniques. Therefore, they are all viable options for the pediatric cataract surgeon to choose from. When the mean change in circumference from unstretched to stretched was analyzed for the five groups, a significant difference was found (P < .001, one-way ANOVA). A post-hoc analysis was done to see which groups were different. The manual CCC technique created the most extensible capsulotomy. The manual CCC extensibility was significantly greater than in each of the other techniques (vitrectorhexis, P = .001; Kloti, P < .001; can-opener, P < .001; Fugo, P = .039; one-way ANOVA). The vitrectorhexis extensibility was significantly less than the manual CCC (P = .001, one-way ANOVA), but greater than the Kloti radiofrequency diathermy (P = .025, one-way ANOVA). The Fugo plasma blade capsulotomy extensibility was significantly less than the manual CCC (P = .039, one-way ANOVA), significantly greater than the can-opener (P = .004, one-way ANOVA) and the Kloti radiofrequency diathermy (P = .001, one-way ANOVA), and showed no difference from the vitrectorhexis (P > .05). The can-opener technique produced a capsulotomy that showed less extensibility than the manual CCC (P < .001, one-way ANOVA) and the Fugo plasma blade capsulotomy (P = .004, one-way ANOVA), but was not different from the vitrectorhexis (P > .05) or the Kloti radiofrequency diathermy capsulotomy (P > .05). Finally, the Kloti radiofrequency diathermy capsulotomy was less extensible than the manual CCC (P < .001, one-way ANOVA), can-opener (P = .004, one-way ANOVA), and Fugo plasma blade (P = .001, one-way ANOVA) but was not different from the vitrectorhexis (P > .05).

A retrospective analysis of 379 consecutive pediatric cataract surgeries done by one surgeon over a 10-year period was conducted. When cases with a ruptured lens capsule prior to surgery were excluded, the anterior capsulotomy was round and intact with no radial tears in 91.9% of the cases (329 of 358 eyes). Twenty-one additional cases had preoperative rupture of the capsule, primarily from trauma. These ruptured capsule openings were rounded out using the vitrector handpiece, the Kloti radiofrequency diathermy unit, or the Fugo plasma blade.

Aphakic eyes had no radial tears (0%, 0 of 74 eyes). Most were very young, with a median age of 2 months. All but one eye had a vitrectorhexis (98.6%, 73 of 74 eyes). Pseudophakic eyes had 29 radial tears (10.2%, of 284 eyes). Thirteen of these tears (44.8%, of 29) occurred during IOL insertion. Seven tears occurred during cataract removal (24.1%, of 29). Four tears occurred during the anterior capsulotomy itself (13.8%, of 29). Four tears occurred during hydrodissection (13.8%, of 29). One tear occurred during removal of the OVD at the conclusion of surgery (3.4%, of 29 tears).

The vitrectorhexis technique was more commonly used in younger eyes (mean, 40.2 months; range, 0 to 181 months; median, 23.5 months). The radial tear rate was 7.7% (16 of 208 eyes) over the course of 10 years. However, only one tear has occurred in the last 50 vitrectorhexis capsulotomies. This recent success is attributed to the use of the softer single-piece AcrySof IOL (Alcon, Fort Worth, Texas), which can be injected into the capsular bag with no dialing of the haptics, and thus less stress on the capsulotomy, and to experience with the technique. Avoiding right-angled edges in the capsulotomy and selecting younger patients with more elastic capsules have helped to minimize peripheral tears when using the vitrectorhexis.

The manual CCC capsulotomy technique was more commonly used in older pediatric eyes (mean, 106.2 months; range, 7 to 234 months; median, 104.0 months). The radial tear rate was 6.4% (3 of 47 eyes) over the course of 10 years. Two additional manual CCC capsulotomies developed peripheral extensions but were salvaged.
by converting to a Kloti radiofrequency diathermy capsulotomy. If these errant capsulotomies are added to the previous total, the rate of unsatisfactory capsulotomies increases to 10.6% (5 of 47 eyes). Unlike the vitrectorhexis, the manual CCC technique was easier to perform and control when it was done on older children. For manual CCC, the mean age of eyes with no tear was 108.9 months (range, 7 to 234 months; median, 104.0 months) compared to a mean age of 66.7 months for eyes with tears (range, 25 to 128 months; median, 47.0 months).

For children in the first 72 months of life, eyes in which a vitrector was used for the anterior capsulotomy had 0.53 times the risk of developing a radial tear compared to when the manual CCC technique was used. In contrast, in patients who were older than 72 months of age, eyes in which a vitrector was utilized for the anterior capsulotomy had a 3.75 times greater risk of developing a radial tear compared to when a manual CCC was performed.

The can-opener, Kloti radiofrequency diathermy, and Fugo plasma blade techniques were used in the current clinical series on 2, 19, and 8 eyes, respectively, within the pseudophakic group. Radial tears were seen in 1, 4, and 5 of these eyes, respectively. The can-opener technique produces a more stable and extensible capsulotomy in children than it does in adults. However, the can-opener technique puts more stress on the zonular fibers and is more likely to leave capsule tags or strands that can interfere with lens aspiration. The Kloti radiofrequency diathermy and Fugo plasma blade techniques can be useful for cutting through fibrotic anterior capsules and do not require a red-reflex for visualization during the capsulotomy. However, these two techniques leave a thin rim of coagulum at the capsulotomy edge, which also appears less smooth on SEM compared to the other techniques. While extensibility remains, elastic stiffness can be reduced if the collagen at the capsulotomy edge has been altered.

**RECOMMENDATIONS**

The development of capsulotomy techniques that consistently preserve the structure and stability of the remaining lens capsule has helped lead to safer pediatric cataract surgery and the emergence of IOL implantation for children of all ages. The thin, strong, and elastic anterior capsule of children requires a unique approach to the anterior capsulotomy. The following recommendations are offered.

Manual CCC produces the most stable capsulotomy edge and should be utilized whenever possible. However, the risk of peripheral extensions is greater when manual CCC is attempted in the very young child. The vitrectorhexis technique has become the most popular anterior capsulotomy for children in the first two years of life and remains a popular choice for patients up through age 6 to 8 years. The vitrectorhexis technique is recommended as the anterior capsulotomy of choice until the age at which the surgeon decides to leave the posterior capsule intact and not perform an anterior vitrectomy at the time of surgery. For many surgeons, a primary posterior capsulotomy and anterior vitrectomy are performed at the time of cataract surgery until the patient is aged 6 to 8 years. Others begin to leave the posterior capsule intact at an earlier age. When the vitrectomy equipment and the vitrector handpiece are to be used later in the procedure to remove vitreous, it is recommended that it also be used for the anterior capsulotomy.

When performing a vitrectorhexis anterior capsulotomy, the following caveats are offered: (1) Use a vitrector supported by a Venturi pump. Peristaltic pump systems will not cut the anterior capsule as easily. (2) Use a separate infusion cannula that matches the gauge of the vitrector handpiece, thus assuring that the incisions into the eye provide for a tight fit while the instruments are in the eye. The anterior chamber of these soft eyes will collapse readily if leakage occurs around the instruments. (3) Separate the incisions for the infusion and vitrector handpieces by at least 4 clock hours. This will enable easy access to lens capsule and lens cortex throughout the anterior chamber. If the infusion cannula and the vitrector handpiece are the same gauge, the instrument positions can be switched to gain access to subincisional lens cortex during aspiration without producing leakage at either wound site. (4) Do not begin the capsulotomy with a bent-needle cystitome. The increased intralenticular pressure and vitreous upthrust of the pediatric eye may cause lens material to spontaneously prolapse through this initial opening and create a radial tear. Instead, place the vitrec- tor, with its cutting port positioned posteriorly, in contact with the intact anterior capsule. Turn the cutter on and increase the suction using the foot pedal until the capsule is engaged and opened. A slow cutting rate of 150 cuts per minute with high infusion rate (raise the bottle to the maximum height for gravity-fed infusion, or use a fluid setting of 50 mm Hg for active fluid pump systems) is recommended when cutting the anterior capsule (as opposed to the high cutting rate with lower infusion used to cut the vitreous). With the cutting port facing down against the capsule, enlarge the round capsulotomy in a spiral fashion until the desired size and shape are achieved. (5) Any lens cortex that escapes into the anterior chamber during the capsulotomy can be easily aspirated without interfering with the capsulotomy technique. (6) Care should be taken to avoid right-angled edges,
which are predisposed to radial tear formation. With experience, a more rounded capsulotomy is made and fewer angled edges are formed. If a right-angled edge is seen during the capsulotomy, it should be rounded out using the vitrector before completion of the capsulotomy. If the capsulotomy needs to be enlarged, the vitrector can be used for this purpose even after the IOL is in place (often coincident with removal of the OVD). 

(7) The vitrectorhexis technique is not recommended for older children (above age 8 years) unless the anterior capsule is fibrotic or already ruptured preoperatively.

The manual CCC is easier to control and complete in older children as opposed to the very young. The manual CCC is the “gold standard” capsulotomy and produces the most stable edge. It is recommended for children above the age of 2 years who will have the posterior capsule left intact and will undergo no anterior vitrectomy. This transition from vitrectorhexis anterior capsulotomy, lens aspiration, IOL implantation, vitrectorhexis posterior capsulotomy, and anterior vitrectomy to manual CCC, lens aspiration, IOL implantation, and an intact posterior capsule will occur with patients aged 6 to 8 years for many surgeons, but will occur at an earlier age for others.

When performing a manual CCC capsulotomy in children, the following caveats are offered. (1) Use a high-molecular-weight OVD to flatten the anterior capsule and deepen the anterior chamber. With the stretch in the anterior capsule, the opening is usually larger at completion than it appears to be during the active tearing. (3) When creating the manual CCC capsulotomy, frequently release the capsular flap and inspect the size, shape, and direction of the tear. Regrasp near the site of the continuous tear and readjust the direction of pull if needed to keep the capsulotomy on the planned course. Often, more pull is needed toward the center of the pupil to avoid an extension of the manual CCC out toward the lens equator. (4) Additional OVD should be added as needed to keep the capsule lax during the tearing. (5) Modifications of the manual CCC technique designed for elastic capsules such as the TIPP technique will be preferred by some surgeons. This technique is recommended if the surgeon finds it easier to control than the standard method described above. The caveats offered above apply to the TIPP technique as well.

The Kloti radiofrequency diathermy and Fugo plasma blade techniques are recommended when fibrotic capsules are encountered, especially if a vitrector handpiece is not available. They are also useful for white cataracts with absence of the red reflex. With practice, a smooth continuous motion can be made with these instruments to create a round capsulotomy in one motion. The Fugo plasma blade is easier to master because it does not require tip contact with the capsule throughout the capsulotomy. Still, visualization of the advancing edge can be difficult as a result of the tip design with an overhanging silicone sleeve and the cavitation bubbles that become suspended within the OVD. The current Fugo plasma blade tip does not fit well through the 20-gauge opening used for the vitrector handpiece. The Kloti radiofrequency diathermy tip fits easily through a 20-gauge opening, which allows the vitrector to be utilized later in the case without sacrificing the tight fit of the wound around the vitrector handpiece. The Kloti radiofrequency diathermy handpiece is reusable but must be placed in fluid immediately after use to avoid coagulated capsule remnants from interfering with the function of the tip on the next application. Gentle but consistent contact with the capsule must be maintained when using the Kloti radiofrequency diathermy handpiece. If contact is too light or movement is too fast, skipped areas will result. If contact is too firm or movement too slow, the tip will burn through the capsule and enter the lens cortex. Subsequent tip movement drags the capsulotomy edge rather than cutting it, which can cause radial tearing.

The can-opener capsulotomy method is seldom used by pediatric surgeons, but when needed, it is safer and more effective in children than in adults. The elastic nature of the pediatric capsule causes each can-opener puncture to convert to a small arc tear similar to a mini manual CCC. For intumescent lenses in children, the use of a can-opener anterior capsulotomy, which is later converted to a manual CCC, as described by Gimbel and DeBroff, is recommended for those surgeons experienced with the technique. An errant manual CCC can sometimes be rescued using a can-opener maneuver, although the vitrectorhexis will be preferred by many for this salvage of a so-called runaway rhexis.

Because the pediatric anterior lens capsule is thinner, stronger, and more elastic than in adults, unique surgical maneuvers are needed when performing an anterior capsulotomy during cataract surgery on a child. The elasticity of the porcine anterior lens capsule closely approximates that of the human childhood anterior lens capsule. The porcine model is recommended as a valuable training aid to help the surgeon master the various techniques discussed in this thesis.

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2003 Survey of Anterior Capsulotomy in Pediatric Cataract Surgery

(Results to be submitted as part of the American Ophthalmology Society thesis of M. Edward Wilson, Jr., M.D.)

1. Location of your current country of clinical practice.
   - [ ] USA
   - [ ] Canada
   - [ ] Other (specify) ________________

2. Are you currently performing pediatric cataract surgery in the <18-year-old patient?
   - [ ] Yes
   - [ ] No

3. Are you currently implanting IOLs in children?
   - [ ] Yes
   - [ ] No

   If you answered "No" to Questions 2 AND 3, please stop here and return your survey to the Service Center.

4. Which anterior capsulotomy do you usually perform in children at each of the following ages? (Check only one per age group). Please check NA if you do NOT implant IOLs in an age group.

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<td>10-15</td>
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<td>15-18</td>
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</tbody>
</table>

* continuous curvilinear capsulotomy

Thank you for completing this survey.

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M. Edward Wilson, Jr., M.D.
MUSC – Storm Eye Institute
167 Ashley Avenue
Charleston, SC 29425-5536
REFERENCES


DISSECTING THE GENETICS OF HUMAN HIGH MYOPIA: A MOLECULAR BIOLOGIC APPROACH

by Terri L. Young, MD

ABSTRACT

Purpose: Despite the plethora of experimental myopia animal studies that demonstrate biochemical factor changes in various eye tissues, and limited human studies utilizing pharmacologic agents to thwart axial elongation, we have little knowledge of the basic physiology that drives myopic development. Identifying the implicated genes for myopia susceptibility will provide a fundamental molecular understanding of how myopia occurs and may lead to directed physiologic (ie, pharmacologic, gene therapy) interventions. The purpose of this proposal is to describe the results of positional candidate gene screening of selected genes within the autosomal dominant high-grade myopia-2 locus (MYP2) on chromosome 18p11.31.

Methods: A physical map of a contracted MYP2 interval was compiled, and gene expression studies in ocular tissues using complementary DNA library screens, microarray matches, and reverse-transcription techniques aided in prioritizing gene selection for screening. The TGIF, EMLIN-2, MLCB, and CLUL1 genes were screened in DNA samples from unrelated controls and in high-myopia affected and unaffected family members from the original seven MYP2 pedigrees. All candidate genes were screened by direct base pair sequence analysis.

Results: Consistent segregation of a gene sequence alteration (polymorphism) with myopia was not demonstrated in any of the seven families. Novel single nucleotide polymorphisms were found.

Conclusion: The positional candidate genes TGIF, EMLIN-2, MLCB, and CLUL1 are not associated with MYP2-linked high-grade myopia. Base change polymorphisms discovered with base sequence screening of these genes were submitted to an Internet database. Other genes that also map within the interval are currently undergoing mutation screening.


INTRODUCTION

The long-term objective of this research project is to uncover the molecular genetic basis of myopia. Myopia occurs when the focused image falls anterior to the retinal photoreceptor layer of the eye. Myopia is the most common human eye disease, and severe cases (high myopia greater than 5 diopters) may lead to blinding disorders such as premature cataracts, glaucoma, retinal detachment, and macular degeneration. Myopia can occur as an isolated finding or as a part of specific genetic syndromes. There is substantive evidence that genetic factors play a significant role in the development of nonsyndromic high myopia. We have identified multiple families with nonsyndromic high myopia and have mapped three autosomal dominant loci by linkage analysis. Myopia-2 locus (MYP2) is localized to chromosome 18p11.31, myopia-3 locus (MYP3) is localized to chromosome 12q23.1-q24, and we recently mapped another locus to chromosome 17q21-q22. The overall goal is to positionally clone the genes responsible for high myopia. Initial studies reviewed in this thesis have been directed at the identification of the MYP2 gene, as we have narrowed the recombinant interval within 18p11.31 to a 2.2 centimorgan (cM) region in which this gene is located. This report discusses initial findings of positional candidate gene base pair screenings for the MYP2 locus.

It is hypothesized that the identification of myopia disease genes such as the MYP2 gene will not only provide insight into the molecular basis of this significant eye disease, but will also identify pathways that are involved in eye growth and development. In addition, this information may implicate other genes as possible myopia disease gene candidates. This effort may lead to effective therapies for the severe forms of this potentially blinding eye disease.

Background and Significance

Public Health Significance

Myopia affects approximately 25% of the population of the United States and is a significant public health prob-
Myopic chorioretinal degeneration is the fourth most frequent cause of blindness leading to registration for visual services and disability, and it accounted for 8.8% of all causes. It has been estimated that 5.6% of blindness among school children in the United States is attributable to myopia. Substantial resources are required for optical correction of myopia with spectacles, contact lenses, and, more recently, surgical procedures such as photorefractive keratectomy. The market for optical aids in the United States was estimated to exceed $8 billion in annual sales in 1990; most dollars were spent for the correction of myopia. The development of methods for preventing the onset, or limiting the progression, of myopia would be of considerable importance.

**Epidemiology and Clinical Characteristics of High Myopia**

**Prevalence Rates.** High myopia (refractive spherical dioptic power of –5.00 or higher) is a major cause of legal blindness in many developed countries. It affects 27% to 33% of all myopic eyes, corresponding to a prevalence of 1.7% to 2% in the general population of the United States. High myopia is especially common in Asia. In Japan, pathologic or high myopia reportedly affects 6% to 18% of the myopic population and 1% to 2% of the general population. Comparative prevalence rates from different countries show considerable variability but confirm that myopia affects a significant proportion of the population in many countries.

**Progression of Myopia and Ocular Refractive Parameters.** Juvenile-onset myopia most often develops and progresses between the ages of 10 and 16 years, whereas pathologic myopia usually begins to develop in the perinatal period and is associated with rapid refractive error myopic shifts before 10 to 12 years of age. The key ocular parameters that determine refractive error are the refractive dioptic power of the cornea and lens, depth of the anterior chamber, and axial eye length (AEL). Several studies have shown that the refractive status of an eye is determined primarily by AEL. The average refractive error at birth is approximately 1 to 2 diopters (D) of hyperopia, and the AEL measures approximately 17 mm. By adulthood, the AEL grows to about 24 mm. This results in little change in refractive error, because the radius of curvature of the cornea increases and the refractive power of the lens decreases. Axial eye lengths of a myopic adult population may show a bimodal distribution with a second peak of increased AEL relating to high myopia (less than –6 D at 24 mm, greater than –6 D at 30 mm) when plotted as a distribution curve. This suggests that myopia of –6 D or greater represents a deviation from the normal distribution of AEL and is not physiologic.

**Ocular Morbidity.** Many investigators have reported on the association of high myopia with cataract, glaucoma, retinal detachment, and posterior staphyloma with retinal degenerative changes. High myopia is associated with progressive and excessive elongation of the globe, which may be accompanied by degenerative changes in the sclera, choroid, Bruch’s membrane, retinal pigment epithelium, and neural retina. Various fundusoscopic changes within the posterior staphyloma develop in highly myopic eyes. These changes include geographic areas of atrophy of the retinal pigment epithelium and choroid, lacquer cracks in Bruch’s membrane, subretinal hemorrhage, and choroidal neovascularization. Among these various fundus lesions, macular choroidal neovascularization is the most common vision-threatening complication of high myopia. Clinical and histopathologic studies have documented choroidal neovascularization in 4% to 11% of highly myopic eyes. Relative to emmetropic eyes, an approximate twofold increased risk of choroidal neovascularization was estimated for eyes with 1 to 2 D of myopia, a fourfold increase with 3 to 4 D, and a ninefold increase with 5 to 6 D. Poor visual outcome following choroidal neovascularization in myopic eyes is not uncommon and often affects relatively young patients.

The risk of retinal detachment is estimated to be three to seven times greater for persons with myopia greater than 5.0 D than for those with myopia of less than 5.0 D. Myopia between 5.0 and 10.0 D was associated with a 15- to 35-fold greater risk of retinal detachment relative to that associated with low levels of hyperopia. The lifetime risk for retinal detachment was estimated to be 1.6% for patients with less than 3 D of myopia and 9.3% for those with more than 5 D. A subgroup with lattice degeneration greater than 5 D of myopia had an estimated lifetime risk of 35.9%. The prevalence of lattice degeneration increases with increasing levels of myopia as measured by AEL.

Glaucoma was observed in 3% of patients with myopia who had AELs of less than 26.5 mm, in 11% with AELs between 26.5 and 33.5 mm, and in 28% of those with longer lengths.

**Role of Environment and Genetics in Myopic Development**

Many studies report a positive correlation between parental myopia and myopia in their children, indicating a hereditary factor in myopia susceptibility. Children with a family history of myopia had on average less hyperopia, deeper anterior chambers, and longer vitreous chambers even before becoming myopic. This implies a strong role for genetics in the initial shape and subsequent growth of the eye in myopia. Assessing the impact of genetic inheritance on myopic development may be
confounded by children adopting their parents’ behavioral traits, such as higher-than-average near-work activities (eg, reading).47

In addition to genetics, moderate myopic development can be influenced by environmental factors. This is exemplified by experimental modulation of refractive error in the developing eyes of several animal models (mammalian and avian)48-50 and the development of myopia in young children with media irregularities that prevent a focused retinal image.31-33 Moreover, the prevalence of myopia in some populations appears to have increased dramatically from one generation to the next in increasingly industrialized settings, or with increased level of educational achievement.54-56 Of course, this is an environmental effect. The identification of myopia genes may therefore provide insight into genetic-environmental interactions.

Consensus opinion regarding common, juvenile-onset myopia of moderate amounts is that its etiology is influenced by both genetic and environmental factors.59

As a multifactorial, common, complex trait, genes or gene loci for this type of myopia have yet to be identified. Susceptibility loci contributing to common, juvenile-onset myopia may be difficult to map by classic linkage analysis because of the limited power to detect genes of intermediate or small effect using independent pedigrees.

There are multiple genetic syndromes with systemic findings that have myopia as a consistent clinical feature. For example, Stickler syndrome is an autosomal dominant connective tissue disorder characterized by ocular, orofacial, and skeletal abnormalities. Associated ocular manifestations include high myopia, glaucoma, cataracts, vitreoretinal degeneration, and retinal detachment.60,61 Marfan syndrome is an autosomal dominant disorder with clinical findings that have myopia as a consistent clinical feature.62-64 The extracellular matrix that provides the structural framework for the eye. As the sclera defines the shape of the eye, it is also likely to determine the AEL. The extracellular matrix of the sclera has been shown to contain collagen fibrils in close association with proteoglycans and glycoproteins.65-67 Alterations in any of these extracellular matrix compo-
nents are likely to lead to changes in eye shape. Recent studies have shown that the scleral extracellular matrix undergoes significant changes during growth and aging, and is dramatically altered during the development of myopia. The sclera of highly myopic human eyes differs considerably from normal sclera in both its physical dimensions and its biomechanical properties. Many of the pathological changes seen in highly myopic human eyes are a consequence of gross scleral thinning, particularly at the posterior pole of the eye.

Genes responsible for several syndromic genetic disorders with myopia as a consistent clinical finding have been identified: collagen 2A1 and 11A1 for Stickler syndromes type 1 and 2, respectively, llsyl-protocollagen hydroxylase for type 4 Ehler-Danlos syndrome, collagen 18A1 for Knobloch syndrome, and fibrillin for Marfan syndrome. Each of these genes is expressed in the sclera, demonstrating how knowledge of gene expression in the scleral wall is critical to our understanding of eye expansion and myopia.

It is the hypothesis of this thesis that the nonsyndromic high myopias result from distinct, but analogous, developmental defects of scleral wall growth control, and that their causative genes may be functionally or structurally related to one another and have parallel functions in the development of the visual axis. To this end, genes expressed by human sclera using both complementary DNA (cDNA) library and microarray techniques have been identified to aid in the selection of candidate genes for high myopia. In addition to the knowledge gained through direct studies of the MYP2 disease gene and its protein product, it is predicted that identification of the MYP2 disease gene will benefit genetic studies of all high-myopia genes.

Preliminary Studies

Several families with a heritable form of high myopia have been identified and ascertained. As described below, linkage analyses have led to the mapping of four high-myopia genetic loci, including MYP1, MYP2, and MYP3, and a chromosome 17q21-22 locus. A physical map was constructed with a bacterial artificial chromosome (BAC) contig (overlapping BACs that carry small DNA sequence segments for that region) spanning the contracted recombinant interval for the MYP2 locus. Multiple candidate genes have been identified within the MYP2 critical region and within the other mapped loci. Some genes have been provisionally excluded based on screening results. This study protocol was approved by the Children’s Hospital of Philadelphia Institutional Review Board on Human Subjects research, and adhered to the tenets of the Declaration of Helsinki.

Clinical and Genetic Classification of Myopia Pedigrees

Myopia Study Population. A large myopia study population provides the foundation for the proposed research. The study population, summarized in Table 1, includes 622 individuals from 95 families with nonsyndromic high myopia and has been ascertained over the past 5 years through the identification of families from clinical practice, and in collaboration with other clinicians. Large families with (a) affected study participants in three or more generations or (b) affected study participants in two generations were initially sought with at least two participating offspring of affected individuals. Affection status was initially defined as a spherical refractive error of 6 D of myopia or more. This was solely based on historical consensus that the “pathologic” myopia criterion boundary began at that spherical diopter power. Studies of myopia ocular morbidity, such as retinal detachment and choroidal neovascularization, suggest that 5 D of myopia or greater is an acceptable cutoff. Using this criterion led to the recent mapping of a novel locus on chromosome 17q21-22 of a large English/Canadian kindred. There was justification in using −5.00 D as the cutoff because of the extreme level of myopic severity in most affected members (average myopia of −13.925 D, with a range from −5.50 to −50 D), with haplotype analysis confirmation. Five diopters of myopia is now used as the cutoff for affection status.

Molecular Genetic Studies of MYP2

Identification of the MYP2 Locus. Linkage analysis using seven of the families described above in the database demonstrated significant linkage to chromosome 18p11.31 with a maximum cumulative likelihood of the odds (LOD) score of 9.59 (an LOD score of 3 or greater is considered significant for linkage). The 7.6 cM recombinant interval was defined distally by marker D18S59 and proximally by marker D18S1138, with recombinants in pedigrees 1, 4, and 5 (Table 2). The genetic boundaries of the MYP2 region are currently defined by linkage analysis of these seven existing MYP2 pedigrees. These seven pedigrees, in addition to any new pedigrees that may be identified, represent the group of MYP2 families to be screened for mutations at the MYP2 locus.

In an effort to contract the MYP2 interval, transmission disequilibrium test (TDT) statistics were obtained with the Statistical Analysis for Genetic Epidemiology Transmission Disequilibrium Test (SAGE-TDT) and the GENEHUNTER2-Transmission Disequilibrium Test (GH2-TDT) programs. TDT analysis was focused on 11 chromosome 18p polymorphic microsatellite DNA markers used for fine-mapping in the original study. Both programs examine each allele separately to look for increased frequency of disequilibrium or nonrecombina-
Dissecting the Genetics of Human High Myopia: A Molecular Biologic Approach

TABLE 1. RESEARCH LABORATORY MYOPIA STUDY POPULATION DATABASE

<table>
<thead>
<tr>
<th>PHENOTYPE (CHROMOSOMAL LOCATION)</th>
<th>NO. OF PEDIGREES</th>
<th>NO. OF PARTICIPATING INDIVIDUALS</th>
<th>NO. AFFECTED</th>
</tr>
</thead>
<tbody>
<tr>
<td>MYP1 (Xq27-28)</td>
<td>2</td>
<td>28</td>
<td>9 (7 carriers)</td>
</tr>
<tr>
<td>MYP2 (18p11.31) (AD)</td>
<td>7</td>
<td>71</td>
<td>37</td>
</tr>
<tr>
<td>MYP3 (12q23-24) (AD)</td>
<td>1</td>
<td>22</td>
<td>14</td>
</tr>
<tr>
<td>MYP4 (17q21-22) (AD)</td>
<td>1</td>
<td>22</td>
<td>12</td>
</tr>
<tr>
<td>Autosomal dominant (other)</td>
<td>82</td>
<td>419</td>
<td>13</td>
</tr>
<tr>
<td>Autosomal recessive</td>
<td>2</td>
<td>60</td>
<td>22</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>95</strong></td>
<td><strong>622</strong></td>
<td><strong>227</strong></td>
</tr>
</tbody>
</table>

TABLE 2. MYP2 LOCUS MARKER RECOMBINANTS AND TRANSMISSION DISEQUILIBRIUM TEST (TDT) ALLELIC ASSOCIATION ANALYSIS*

<table>
<thead>
<tr>
<th>MARKER DISTANCE (cM)</th>
<th>PEDIGREE MARKER</th>
<th>1</th>
<th>4</th>
<th>5</th>
<th>SAGE-TDTEX ( p ) VALUE</th>
<th>GH2-TDT ( p ) VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Telomere</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.036</td>
<td>0.053</td>
</tr>
<tr>
<td>0.1 &lt;</td>
<td>D18S1140</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.5 &lt;</td>
<td>D18S59</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>0.013</td>
<td>0.317</td>
</tr>
<tr>
<td>0.1 &lt;</td>
<td>D18S476</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.007</td>
<td>0.045</td>
</tr>
<tr>
<td>4.5 &lt;</td>
<td>D18S1146</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.227</td>
<td>0.083</td>
</tr>
<tr>
<td>1.4 &lt;</td>
<td>D18S481</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.001</td>
<td>0.108</td>
</tr>
<tr>
<td>0.1 &lt;</td>
<td>D18S63</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.002</td>
<td>0.034</td>
</tr>
<tr>
<td>0.7 &lt;</td>
<td>D18S1138</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>3.9 \times 10^{-4}</td>
<td>0.011</td>
</tr>
<tr>
<td>9.4 &lt;</td>
<td>D18S52</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>1.79 \times 10^{-6}</td>
<td>0.007</td>
</tr>
<tr>
<td>18.6 &lt;</td>
<td>D18S62</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>0.141</td>
<td>0.479</td>
</tr>
<tr>
<td>4.1 &lt;</td>
<td>D18S1150</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0.018</td>
<td>0.096</td>
</tr>
<tr>
<td><strong>Centromere</strong></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0.214</td>
<td>0.683</td>
</tr>
</tbody>
</table>

*TDT analysis was performed using two different statistical programs, Statistical Analysis for Genetic Epidemiology-TDT (SAGE-TDTEX) and GeneHunter 2 (GH2-TDT). The + sign denotes a recombinant marker; the - sign denotes a nonrecombinant marker. The dark shaded area highlights the region excluded by recombinants. The boxed lighter shaded area highlights the most significant marker associations with the myopia phenotype by TDT analysis.

tion events on disease-bearing chromosomes over normal chromosomes using a standard one-sided test (Fisher’s exact test). The SAGE program also calculates a summary \( \chi^2 \) for each marker, as it examines the degree of linkage disequilibrium at the marker. The significance values determined by both programs are listed in Table 2 for each marker locus in marker order for the chromosome 18p11.31 region. Markers D18S52 and D18S1138 show
the strongest statistical association with the disease phenotype. It is noted that one pedigree (No. 5) appears to exclude marker D18S52 from the initial haplotyped region. Possible explanations for this include marker order imprecision (despite cross-referencing of several databases as well as the use of CEPH [a commercial sample of standard DNA] family DNA for marker analysis); ethnic variation between families; possible pheno-copy issue; possible second locus cosegregation at 18p11; and/or allele assignment error.

Critically important are the recent independent confirmations of the MYP2 locus with an Italian patient population with autosomal dominant high myopia by Heath and colleagues79 and six families of Hong Kong Chinese descent by Lam and colleagues. Their findings support directing further gene identification efforts to the centromeric region of the initial 7.6-cM recombinant interval. This combined data suggests that the MYP2 gene is likely within a 2.2-cM interval between D18S52 and D18S481. These results provide a basis for focused positional candidate gene analysis at the MYP2 locus, as the interval of interest has likely contracted significantly from the initial 7.6 cM.

Construction of a Physical Map Spanning the MYP2 Critical Region. By taking advantage of the multiple data-bases available in conjunction with the Human Genome Project, a physical BAC contig (overlapping BAC sequences which cover the interval) map across the MYP2 critical region was constructed, shown in Figure 1. Integration was obtained by mapping markers of different types (monomorphic, polymorphic, genes, and expressed sequence tags [ESTs]) from different public and private database sources (eg, National Center for Biotechnical Information [NCBI], Genethon, Whitehead Institute, University of California Santa Cruz [UCSC] “Golden Path,” Celera). The core region extends from marker D18S481 to D18S52. It ranges in depth from one to nine BACs, with an average depth of 4 BACs, and requires 19 overlapping BACs, averaging 150 to 200 kilobases (kb), to span the MYP2 region. The MYP2 critical region on the p arm is now almost a single contig; it contains one gap just centromeric to D18S481, which according to the Celera database is spanned by the myomesin (MYOM1) gene. Because of both the overlap and the gaps, it is difficult to estimate the true physical size of the region, but 1.2 megabases (Mb) would be the upper size limit. By adding up all sequence fragment lengths and estimating gap size, using the UCSC Web site, a 960-kb interval size is calculated. At this juncture, there are six known and 20 hypothetical genes that map within the interval. Other institutions, such as the genome centers at the Riken Institute in Yokahama City, Japan, the Whitehead Institute for Biomedical Research/MIT, Cambridge, Massachusetts, and the Genome Sequencing Center, Washington University in St Louis, are sequencing chromosome 18 BACs (http://www.ncbi.nlm.nih.gov/genome/seq/chr.cgi?CHR=18&SRT=ppos&MIN=0& ORG=Hs for integrated Web site information). BACs are placed on the map as they become available. Over half of the sequences, which are available from NCBI (http://www.ncbi.nlm.nih.gov/), are still phase 1 or “working draft” sequence.

Current Genes Within the MYP2 Critical Region. The direct analysis of sequence within a critical region can be the most accurate, precise, and efficient approach to disease gene identification. This is particularly true for instances where the “perfect” candidate gene (based on function or expression) does not exist within a defined critical region. It is also true for a disorder such as myopia, in which the temporal and spatial expression of the disease gene is not known and could be restricted to early development and to any eye component. All genes that map within the MYP2 critical region are candidate disease genes based on position. However, a gene triage bias was developed to prioritize study of extracellular matrix genes, because the high myopia phenotype uniformly involves scleral wall rearrangement with increased AEL. Moreover, a subset of affected patient participants (roughly 25%) have nonspecific connective tissue disorder–type findings, such as fallen arches, localized joint hypermobility, pectus excavatum, and nonsignificant heart murmur.

Initially, a possible role for the alpha subunit of laminin (LAMA) as a MYP2 candidate disease gene was discussed. LAMA is a component of an extracellular matrix protein that binds microfibrils to collagen fibrils. LAMA was excluded, because it mapped outside of the initial 7.6 cM critical region by radiation hybrid analysis. Figure 2 shows reverse transcription–polymerase chain reaction (RT-PCR) transcripts for MYP2 candidate genes in eye and nonocular tissues. As described below in the “Methods” section, bidirectional sequencing of four additional genes was performed, transcription genes clusterin-like 1 (CLUL1), elastin-microfibril located interface-protein (EMLIN-2), and 5’-TG-3’ interacting factor homobox protein (TGF). CLUL1 mapped within the larger 7.6 cM MYP2 region. It was not tested by RT-PCR, because it is known to be predominantly expressed in the retina. All other genes, except MLCB, show eye tissue expression by RT-PCR analysis using extracted RNA from human donor eyes in the laboratory (Table 3). TGF is transcribed in two variant spliced isoforms, revealing an alternative transcript in retina, optic nerve, and brain by RT-PCR analysis. Interestingly, and providing greater urgency to screen this gene, TGF has recently been implicated as the MYP2-causative gene by single nucleotide polymorphism (SNP) association studies, but
Dissecting the Genetics of Human High Myopia: A Molecular Biologic Approach

**FIGURE 1**
Physical map of the chromosome 18p11.31 critical region. The horizontal scale in megabases (M) is at the top. Polymorphic microsatellite DNA markers are labeled above the scale in red. Below the scale, finished (phase 3) bacterial artificial clones (BAC) clones are labeled in blue, working draft (phase 2 or 1) BAC clones are in black, known genes are in dark blue, *insilico* predicted genes by the public databases GENSCAN (http://genes.mit.edu/GENSCAN.html) and OTTO* (http://cds.celera.com/biolib/info) are in light blue and green, respectively. The gene myomesin 1 (MYOM1) has been mapped to two different positions. In the NCBI database, MYOM1 maps distally with overlap just outside of the critical region. The Celera assembly (*) shows that MYOM1 spans the gap between BAC clones AP001024 and AP002471 in a more centromeric position.94

**FIGURE 2**
Polymerase chain reaction amplicons of MYP2 candidate gene complementary DNA (cDNA) from reverse-transcribed RNA from human ocular tissues and commercially available RNA from various human tissue types (Ambion). 1-sclera, 2-cornea, 3-optic nerve, 4-retina, 5-lung, 6-skeletal muscle, 7-heart, 8-trachea, 9-kidney, 10-brain. Expected amplicon size based on primer selection encompassing exonic sequence is shown. Note that TGIF transcripts show two variant spliced isoforms. The commercial DNA ladder has standard-sized DNA molecular weights spaced at 100 base pairs apart and is used to determine the approximate molecular weights of the test amplicons.
not by DNA sequencing.\textsuperscript{101} TGIF mutations are associated with holoprosencephaly.\textsuperscript{98-100}

**Molecular Genetic Studies of MYP3**

Significant linkage of autosomal dominant high myopia to a second locus on chromosome 12q21-q23 in a large German/Italian family was determined recently.\textsuperscript{77} The average age at diagnosis of myopia was 5.9 years (range, 4 to 8). The average spherical component refractive error for the affected individuals was $-9.47$ D (range, $-6.25$ to $-15.00$ D). A representative AEL of 30.06 mm was noted in individual 5. Corneal curvature was normal. The maximum LOD scores with pairwise linkage analysis were 3.85 for markers D12S1706 and D12S327. Recombination events identified markers D12S1654 and D12S1605 as flanking markers, defining a 30.1-cM interval on chromosome 12q21-23. The coding sequences of the chromosome 12q21-23 recombinant interval candidate proteoglycans lumican, decorin, and dermatan sulfate proteoglycan-type 3 were screened for mutations first by heteroduplex analysis, and then by bidirectional sequencing. No mutations were found.\textsuperscript{102}

**Molecular Genetic Studies of a Newly Identified Locus on Chromosome 17q21-q22**

One large multigenerational English/Canadian family with autosomal dominant high myopia (family MYO-68) was ascertained and genotyped (Figure 3).\textsuperscript{78} The average age at diagnosis of myopia for affected individuals was 8.9 years (range, 2 to 11), and the average spherical component refractive error for the affected individuals was $-13.925$ D (range, $-5.50$ to $-50.00$ D). The representative average AEL of 35.28 mm, measured only for affected individuals 9 and 10, was significantly longer compared to adult normal values. All candidate gene loci were excluded. After a genome screen and fine-point mapping, a maximum pairwise LOD score of 3.17 with marker D17S1604 was obtained. Haplotype analysis revealed recombinant events that narrowed the critical region containing the gene to 7.71 cM, between markers D17S787 and D17S1811.

The proband (individual 9) has the highest documented level of myopic refractive error in our clinical experience, varying between $-50$ D and $-60$ D. Despite the severe myopia most affected members exhibited, there were two carriers of the putative disease haplotype, with high but less severe myopia ($-4.50$ to $-5.50$ D) — individuals 12 and 20 — reflecting variability in the phenotype and possible modifying factors (individual 12 was classified as unaffected). The phenotypic variability and somewhat arbitrary assignment of affection status underscore the difficulty in mapping analyses when applying a dichotomous phenotype model to a quantitative trait. The extracellular matrix proteins collagen 1A1 (COL1A1) and proteoglycan chondroadherin (CHAD) were screened as the most promising candidate genes.\textsuperscript{103,104} Both CHAD and COL1A1 are expressed in human sclera by RT-PCR. Sequencing of the coding regions of both genes revealed no disease-causing mutations.

| Table 3. List of Known Genes that Map Within the MYP2 Candidate Interval as Depicted in the Physical Map\textsuperscript{a} |
|-----------------------------------------|----------------|----------------|-----------------|----------------|
| **GENE** | **SYMBOL** | **OMIM** | **ACCESSION NO.** | **TRANSCRIPT SIZE (NO. OF EXONS)** | **EYE EXPRESSION** |
| Elastin microfibril interphase located protein | EMLIN-2 | NM_032048 | 4009 bp (8) | Yes (S,C,ON,R) |
| Myomesin 1 | MYOM1 | 603508 | NM_003803 | 4949 bp (20) | Yes (S,C,ON,R) |
| Lipodystrophy nuclear protein | LPIN-2 | 605519 | NM_014646 | 6221 bp (20) | Yes (S,C,ON,R) |
| Myosin, light polypeptide, regulatory | MLCB | NM_006471 | 944 bp (4) | No |
| TG interacting factor (TALE family homeobox) | TGIF | 602630 | NM_003244 | 2214 bp (2) | Yes: 125hp-(R, ON); 361hp- (S,C,ON, R) |
| Disks, large (Drosophila) homolog-associated | Dlgap1 | 605445 | NM_004746 | 1591 bp (4) | Not studied |

\textsuperscript{a}These genes will be screened first for sequence mutations. The gene name and symbol, the reference number from the Online Mendelian Inheritance of Man (OMIM) database, the standard database gene accession number, the gene size in base pairs (bp) and number of exons (coding sequence), and the expression in eye tissue type by reverse transcription-polymerase chain reaction of human donor eye tissue in our laboratory is presented. The Ensembl database (http://www.ensembl.org/Homo_sapiens/geneview) was accessed to obtain gene transcript information and accession numbers. Ocular tissues tested for gene expression include human sclera, cornea, optic nerve, and retina.

S. sclera; C, cornea; ON, optic nerve; R, retina.
Molecular Genetic Studies of MYP1
The Bornholm eye disease consists of X-linked high myopia, high cylinder, optic nerve hypoplasia, reduced electroretinographic flicker with abnormal photopic responses, and deuteranopia. The disease was the first designated high-grade myopia locus (MYP1). The Bornholm eye disease family phenotype had a provisional assignment to the distal part of the X chromosome at Xq28 using a limited number of markers. We studied a family from Minnesota with a similar X-linked phenotype, also of Danish descent. All affected males had protanopia instead of deuteranopia, high myopia, abnormal photopic electroretinographic responses, and peripapillary temporal conus. The families originated from neighboring Denmark villages; thus we explored the degree of identity in genotype and haplotype. DNA from six members of the original Bornholm eye disease family was obtained to compare haplotypes and to determine if mutations in the red or green cone pigment genes could be responsible for color vision defects and cone dysfunction. Of the 22 individuals of the Minnesota family, eight affected males and five carrier females were studied. Significant maximum LOD scores of 3.38 and 3.11 were obtained with microsatellite markers DXS8106 and DXYS154, respectively. Haplotype analysis defined a 37.8-cM interval at chromosome Xq27.3-Xq28 in the Minnesota family. Analysis of the Bornholm eye disease DNA reduced the interval to 15.3 cM. Affected Minnesota males had a red-green hybrid cone pigment gene in the first position of the cone pigment gene array consistent with a protan defect by directing sequencing and single-strand conformation polymorphism analysis. The Bornholm eye disease subjects had a first-position green-red hybrid cone pigment gene array sequence, consistent with a deutan defect. Cytogenetic and Southern blotting analysis showed no deletion abnormalities. Both families appear to have a novel form of cone dysfunction with associated myopia, and not simple high myopia. The hybrid gene mutations cosegregate with the cone dystrophy, but are common and not associated with high myopia.
**Human Scleral Gene Expression Experiments**

Several gene products show varied levels of expression in the sclera during the development of experimentally induced myopia in animal models. The synthesis and accumulation of the scleral proteoglycans decorin, biglycan, and aggrecan, the matrix metalloproteinase, gelatinase A, inhibitors of metalloproteinases TIMP-1 and TIMP-2, and collagen have all shown alterations in sclera during form deprivation myopia development in chicks, tree shrews, and primates. Moreover, the expression levels of various proteoglycans, gelatinase A, and TIMP-2 normalize following restoration of normal (unrestricted) vision, indicating a direct relationship between the expression of these extracellular matrix constituents and the rate of ocular elongation. These are considered candidate genes for myopia. It is unclear, however, how experimental myopia relates to human physiological myopia and how human differs from animal sclera.

**Human Scleral Complementary DNA Library.** To identify positional candidate genes for human scleral disorders that may include myopia, we constructed a human scleral cDNA library from which ESTs (smaller segments of DNA known to be expressed in cellular tissues because the DNA sequence was derived from messenger RNA sequence) were generated after single-pass sequencing and characterizing randomly isolated clones. The cDNA library was constructed from RNA isolated from sclera of human donor eyes, with known nonmyopic refractive history. Human donor eyes were obtained from the Lions Eye Bank of Minnesota and treated by submersion in RNA Later Solution (Ambion, Austin, Texas) within 12 hours postmortem. Extracted RNA was submitted to Stratagene for commercial preparation of a pCMV-PCR vector cDNA library. We examined the insert sequences for similarities to genes and ESTs using the GenBank database of expressed genes, http://www.ncbi.nlm.gov/GenBank/. This was accomplished using the "basic local alignment search tool" program (BLASTN), available through the National Center for Biotechnology Information, Bethesda, Maryland, http://www.ncbi.nlm.gov/BLAST/, /GenBank/, and /dbEST/.

DNA sequences were obtained from 609 clones. With noted redundancy, 337 scleral EST sequences matched 228 known human genes. Four scleral ESTs showed sequence homology to four nonhuman genes. Of the remaining 268 scleral ESTs, 252 showed significant homology to ESTs from other cDNA libraries in GenBank. Sixteen transcripts did not match any sequences in GenBank (nonredundant database, human and mouse EST databases, mitochondrial database) and are possibly novel genes. The EST sequences were submitted to GenBank. The most abundant connective tissue–related genes were α-A crystalline (CRYAA), Xα-1 collagen, and β-5 integrin. Other extracellular matrix gene matches were biglycan, syndecan, decorin, fibromodulin, proline arginine-rich and leucine-rich repeat protein, transgelin, TIMP-1, and fibulin 1. Human scleral expression of all but decorin and biglycan has not previously been reported.

Five genes mapped to high myopia loci. The genes identified were biglycan (BGN), which maps to the MYP1 locus at chromosome Xq28; myosin regulatory light chain 2 (MYL2) and decorin, which map to the MYP3 locus; and keratin 13 (KRT 13) and transducer 1 of avian erythroblastic leukemia viral oncogene homolog 2 (ERBB2) (TOB 1), which map to the chromosome 17p21-22 locus. No gene matches were found for the MYP2 locus at chromosome 18p11.31.

<table>
<thead>
<tr>
<th>AFFYMETRIX PROBE NO.</th>
<th>CYTOGENETIC LOCS</th>
<th>UNIGENE NO.</th>
<th>GENE NAME</th>
<th>GENE SYMBOL</th>
</tr>
</thead>
<tbody>
<tr>
<td>36628_at</td>
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<td>rαA binding protein 1</td>
<td>RALBP1</td>
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<td>34893_at</td>
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<td>Hs.51299</td>
<td>NADH dehydrogenase (ubiquinone) flavoprotein 2</td>
<td>NDUVF2</td>
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<td>35137_at</td>
<td>18p11.3-11p32</td>
<td>Hs.2504</td>
<td>myomesin 1 (skelemi)</td>
<td>MYOM1</td>
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<td>18p11.3-11p21</td>
<td>Hs.194148</td>
<td>v-yes-1 Yamaguchi sarcoma viral oncogene homolog 1</td>
<td>YES1</td>
</tr>
<tr>
<td>33371_s_at</td>
<td>18p11.3</td>
<td>Hs.223025</td>
<td>member RAS oncogene family</td>
<td>RAB31</td>
</tr>
<tr>
<td>38805_at</td>
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<td>Hs.90077</td>
<td>TGFB-induced factor (TALE family homeobox)</td>
<td>TGF1</td>
</tr>
<tr>
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<td>Hs.180224</td>
<td>myosin regulatory light chain</td>
<td>MLCB</td>
</tr>
<tr>
<td>37893_at</td>
<td>18p11.3-11p21</td>
<td>Hs.82829</td>
<td>protein tyrosine phosphatase, nonreceptor type 2</td>
<td>PTPN2</td>
</tr>
</tbody>
</table>

*Detection P values for all data points were <.005. The Affymetrix probe No. is the position on the Affymetrix chip that the gene occupies, cytogenetic locus is the band location on chromosome 18p11, and the Unigene No. is the standard identification number of the gene in the Unigene database (www.ncbi.nlm.nih.gov/UniGene/Hs.Home.html).
Hypomethylated 18p11.31 or the chromosome 7q36 locus. Eleven EST-BAC matches mapped specifically to the MYP3 locus at chromosome 12q23-24.

Human Scleral Microarray Analysis. We performed preliminary scleral RNA microarray absolute expression studies as a second strategy to identify myopia candidate genes. Purified poly (A) mRNA was isolated from six donor sources of human sclerae, and reverse transcribed into cDNA. An in vitro transcription reaction was performed to produce biotin-labeled cRNA from the cDNA. Each cRNA was fragmented and mixed in a cocktail with probe array controls before a 16-hour incubation/hybridization to oligonucleotide probes (representing 12,625 human genes) on six Affymetrix U95A chips. The chips were scanned using GeneChip software. Array analyses were carried out with Microarray Suite, version 5.0 (Affymetrix), using the expression analysis algorithm to run an absolute analysis after cell intensities were computed. All arrays were normalized to the same target intensity using all probe sets. There were 3,789 genes with “present” calls assigned independently to all six human scleral samples. Eight genes mapped to chromosome 18p11.31 (Table 4), three of which are placed on our physical map—myomesin 1 (MYOM1), TGFB-induced factor (TALE family homeobox) (TGIF), and myosin regulatory light chain (MLCB).

These studies have identified expressed human scleral proteins that may be important in the maintenance of biochemical and biomechanical properties of the sclera. The collection of genes expressed in these studies is not indicative of all genes expressed in the sclera. Indeed, expression of genes such as collagen type I and elastin—known constituents of the sclera—was not identified in our library screening procedure. This most likely reflects the incomplete screening for all expressed genes in a cDNA library, reverse transcription bias, differences in the developmental expression of various transcripts, and the limitations of a predesigned chip with a finite number of genes (eg, candidate genes EMLIN 2, LIPIN 2, and DLGAP are all on version 2 of the U95A chip). (“The absence of proof is not the proof of absence.”) The expression studies by these two methods are, to our knowledge, the first effort to establish a comprehensive list of genes involved in human scleral composition and physiology and are potentially useful for directed myopia candidate gene screening.

Hypothesis and Rationale

The hypothesis is that the MYP2 gene is most likely a gene involved in scleral formation or regulation. We have provisionally narrowed the candidate interval on chromosome 18p11.31 to 2.2 cM (1 to 1.2 Mb). We continually review the genes known to be located in the region and have selected the most biologically relevant gene(s) for further analyses. We have screened for gene mutations in the MYP2 families, looking for mutations that segregate with affected subjects only. This positional candidate approach has proven feasible due to the Human Genome Project. It relies on the identification of a critical region for a disease (by the presence of a translocation, deletions, or by linkage analysis) and a search of genes known to map within or near this region. It has simplified the search efforts for the disease gene in a number of disorders such as X-linked familial exudative vitreoretinopathy,111 spastic paraplegia,112 and congenital fibrosis of the extraocular muscle type 2.113 To our knowledge, there are no reports of cytogenetic breakpoints due to deletions or translocations associated with high myopia as the sole phenotype. Therefore, mapping studies establishing linkage are the only avenue to initiate positional candidate screening. The flow chart (Figure 4) summarizes the steps necessary to identify the bona fide MYP2 transcript within a recombinant interval.

Methods to Identify Transcripts Within the 2.2-cM Recombinant Interval Using Available Databases

The genomic and EST/cDNA annotations at the MYP2 locus were analyzed using various databases in combination such as UniGene (http://www.ncbi.nlm.nih.gov/UniGene/), GeneMap ’99 (http://www.ncbi.nlm.nih.gov/gene.map99/), UCSC Human Genome Project Working Draft (University of California at Santa Cruz; http://genome.ucsc.edu), Ensembl (http://www.ensembl.org), Celera, and eGenome. This keeps us abreast of new transcripts as they are mapped to the MYP2 region, new sequence that provides the 3’ or 5’ extension of known transcripts, any new BACs that map to the region, and the continual refinement of the BAC sequences and fragment order. This effort has been significantly enhanced by the construction of a map of the whole human genome to enable the selection of clones for sequencing and for the accurate assembly of the genome sequence by the International Human Genome Mapping Consortium.114 It is important to take advantage of the continuously and rapidly updated genomic and cDNA sequence as part of the Human Genome Project, and of the power of annotation programs to identify the transcripts within the MYP2 region.

The order of transcript analysis was based on a balance between the availability of a transcript’s cDNA and genomic sequence and the strength of its functional and structural characteristics as a candidate for MYP2. Clearly, the more that is known or can be deduced about a transcript’s corresponding cDNA and genomic structure, the greater the saving of time and resources. Therefore, based on information from publications and database analysis using OMIM, eGenome, and NCBI,
Young each MYP2 transcript is ranked with regard to available sequence as follows: (1) known cDNA and defined or inferred genomic structure; (2) known cDNA but inadequate genomic sequence to infer genomic structure; (3) partial cDNA or EST cluster with more than one genomic exon in the region; (4) EST cluster that maps to a single genomic location; (5) a predicted gene based on homology to known proteins; or (6) a predicted gene based on computational programs such as GENSCAN and GENIE. In addition, refinement of the MYP2 region genomic sequence will result in the continuous upgrading of a transcript’s category.

Additional candidates have been added as they are mapped to the interval or identified from genomic sequence. Potential open reading frames (ORFs) will be identified using exon prediction programs (OTTO, GENSCAN, NCBI’s ORF FINDER and BLAST, Metagene [http://rqd.mcw.edu/METAGENE/]). The ORF sequences will be compared to the EST, UniGene, and other databases using BLAST or FASTA, to identify known transcripts and assist in recognition of additional exons of the ORF. Previously unmapped transcripts will be added to the list of candidates and analyzed for paralogy to other human genes or coding sequences as well as orthology to coding sequences from other organisms, especially those with complete or emerging sequences (e.g., mouse, rat, Drosophila, zebra fish).

There are 20 predicted genes within the interval that are partial cDNA/EST clusters represented by two or more hits within the same region of genomic sequence. Such a pattern of hits suggests the presence of multiple exons with intervening intronic sequence and classifies these transcripts as likely positional candidates. Most have start codons, and from one to 14 exons. There are also nine hypothetical genes with protein homologies to human or other species, indicating possible duplications to known genes in the interval, or alternative splice variants. There are 11 genes for which there are no corresponding cDNA sequences present in the databases. The strategy is to simultaneously use four different approaches to validate a gene: ab initio (looking at the sequence itself using algorithms such as GenScan), homology/similarity (such as BLAST), evidence of expression (such as alignment with ESTs), and comparative genomics (comparing homologies on the genomic level with genome sequence of mouse, rat, zebra fish, etc). Only genes verified by the majority of gene prediction programs, by the identification of promoters, CpG islands, poly A signals, by correlation with ESTs, and by orthology comparisons will be screened, first to confirm expression by RT-PCR in the eye and other tissues, and then by sequencing.

Alternative splicing posed another challenge for mutation screening, as was shown with the candidate gene TGIF in Figure 3. There are two forms of syndromic high myopia, the Wagner and Knobloch syndromes, with implicated isoform mutations of collagens 2A1 and 18A1, respectively. The strategy is to identify ESTs that come from the same gene and look for differences between them that are consistent with alternative splicing, such as a large insertion or deletion in one EST. Each splice form was further assessed by aligning the ESTs exactly to their gene sequence in the draft genome. This reveals candidate exons separated by candidate splices. As intronic sequences at splice junctions are highly conserved (99.24% of introns have a GT-AG at their 5′ and 3′ ends, respectively), they can be used to verify candidate splices. One way to determine if a particular splice form is worth further investigation is to note it in multiple ESTs from different libraries, which suggests that it is unlikely to be a low-frequency error product. Using large-scale EST analysis, it has been determined that the amount of alternative splicing is comparable between humans and other animals. Cross-comparisons of splice variants in humans with other species also enhances validity, and we will make such comparisons with nonhuman genomes. There are a variety of alternative splicing databases for use (ASDB http://cbcg.nersc.gov/ASDB, AsMAMDB http://166.111.30.65/ASMAMDB.html, ISIS http://isis.bit.uq.edu.au/). Alternative transcript expression in human eye tissues by RT-PCR relative to other organ tissue types can be confirmed.

Positional Candidate Genes Were Triaged Based on Existing Functional and Tissue Expression Data

To glean a given transcript’s functional characteristics, a database and literature review of its sequence was performed to learn about DNA identity and similarity, protein sequence identity and similarity, protein structural identity and similarity, protein interactions, and protein domain identification. Transcripts were also ranked from
highest to lowest priority based on their pattern of expression. Transcripts whose protein products play a functional role in eye development and structure will be analyzed with higher priority. However, because it is surprising how many diseases appear to be caused by defects in genes that are ubiquitously expressed, prioritizing candidate genes will be done cautiously. The MYP2 gene is ultimately not required to have expression restricted to affected tissues. Expression data can be collected from sources such as the NEI Bank and UniGene Web sites (www.ncbi.nlm.nih.gov/UniGene/Hs.Home.html), as well as from the human eye tissue RT-PCR, our human scleral and other eye tissue cDNA libraries, and microarray analyses. Additionally, the MYP2 disease gene may be developmentally regulated or expressed only upon induction in specific tissues, and therefore the candidate gene may not have been shown to be expressed in adult or immature tissues, or might be missed in our adult human eye tissue RT-PCR experiments.

As discussed in the preliminary data section, there is a bias toward analyzing genes with an extracellular matrix–associated function. Obvious structural genes that map to the candidate region will be screened, such as collagens or proteoglycans. Genes found to be important for constituent organization and maintenance of connective tissue function will also be given priority. For example, a strong candidate, EMLIN-2 (elastin microfibril located interface protein), is an elastic fiber–associated glycoprotein found at the interface between amorphous elastin and microfibrils and regulates elastic fiber formation. It is expressed in eye tissues based on the RT-PCR studies (see Figure 2). The exonic structure and designed PCR primers that overlap intron-exon sequence for EMLIN-2 screening are provided in Figure 5. Transforming growth β-inducing factor (TGIF) is also a strong candidate, because it showed ocular expression in both the microarray analysis of human sclera and by RT-PCR studies of eye tissue. These genes were the first to be screened.

The candidate gene may also be expressed in the retina and influence scleral growth. This retinal hypothesis emanates mainly from animal studies of experimental myopia. The induction of myopia in juvenile animals by deprivation of form vision demonstrates a visual feedback mechanism in eye growth control. Experimental work indicates that this neural control mechanism is at least partly localized to the retina itself, but how retinal signals directly control the growth of the outer coats of the eye is presently unknown. Genes which map to the interval will be ranked using information from retinal gene expression databases such as Ret Net (http://www.sph.uth.tmc.edu/RetNet/) and publications of retinal gene expression (cDNA library and microarray analyses).
To establish that a candidate gene is the MYP2 disease gene, pathogenic mutations must be consistently identified in affected individuals. Disease-causing mutations can then be distinguished from benign polymorphisms by screening (1) dbSNP database (http://www.ncbi.nlm.nih.gov/SNP/) for known polymorphisms, (2) a panel of 100 normal unaffected and unrelated individuals of diverse geographic and ethnic background, and (3) when possible, a panel of 50 normal unaffected individuals of similar ethnic background to the affected individual. Once the MYP2 gene has been identified, we will search for mutations in all families linked to the MYP2 locus. We will also screen sporadic MYP cases and small, unlinked MYP families.

Because of the number of MYP2 families, their ethnic diversity, and the lack of evidence of a founder mutation, we believe that our collection of MYP2 pedigrees reflects mutation events that have arisen independently and thus may represent multiple different pathological events. Structure-function analysis of these mutations should highlight important regions of the gene and its protein product and may provide insight into phenotype-genotype correlations.

All MYP2 mutations were mapped to the cDNA of the MYP2 gene and to its predicted protein structure. Changes also found in normal control individuals were considered polymorphisms, reflecting nonpathogenic base pair changes. Changes found in affected MYP2 individuals may include deletions, nonsense mutations, and missense mutations. Clustered missense mutations may implicate a particular functional domain that is essential to the protein product’s direct interaction with itself or with another protein. Alternatively, missense mutations may cause abnormal folding of the protein and not reflect a primary disturbance of a direct interaction.

**Patients Studied.** Probands and affected subject representatives of the 7 MYP2 families with an autosomal dominant form of high myopia were studied (Table 5). Each of the affected individuals had high myopia of –6.00 diopters sphere or more with elongated axial lengths. Clinical details regarding the complete pedigrees were published previously.

Controls were obtained from family marry-ins, nonmyopic family members, and unrelated subjects. Table 5 displays the family and member number of each individual, as well as controls with refractive error. A total of 20 patient samples were studied, of which 10 had high myopia and 10 were nonmyopic.

Total genomic DNA was extracted from 10 to 15 mL of venous blood from all participants after informed consent was obtained. DNA was purified from lymphocyte pellets according to standard procedures using the PUREGENE kit (Gentra Systems, Minneapolis, Minnesota) or phenol-chloroform extraction method.

Polymerase chain reactions were performed on 150-ng genomic DNA using standard methods. Amplified products were separated by agarose gel electrophoresis and visualized by staining with ethidium bromide. Amplicons were purified using QIAquick purification columns (Qiagen, Valencia, California), and sequenced using BigDye Terminator v3.1 on an ABI 3700 Genetic Analyzer (Applied Biosystems, Foster City, California).

The genes EMLIN-2, TGIF, CLUL1, and MLCB were screened first because of known expression in ocular tissues. The recent report by Lam and associates...
described a TGIF sequence variation study of the 3-exon transcript variant 4 using conformation specific gel-electrophoresis. One consideration is that the TGIF genetic structure studied by Lam and colleagues had 3 exons; the current sequence build is a 10-exon gene structure. Exons 1, 2, and 3 are now exons 5, 9, and 10, respectively, according to the reference sequence build 33 (http://www.ncbi.nlm.nih.gov/genome/guide/human/HsStats.html) of TGIF, which corresponds to transcript variant 4. They found 25 SNPs on exon 3 (exon 10 in our study). Six SNPs showed significant high myopia association with univariate analysis, and one showed significance with multivariate analysis. They did not sequence the full TGIF gene.

RESULTS

EMLIN-2
Using NCBI BLAST, the EMLIN 2 cDNA was aligned against the BACs that contained sequence similarity, and its genomic structure was electronically determined. The ~40 kilobase human EMLIN-2 gene is encoded by eight exons (Figure 5). Primers were designed to amplify the exons and their adjacent splice sites using Oligo 6.6, a software program that searches for and selects oligonucleotides from a sequence file for PCR, sequencing, and other applications. We designed 12 primer pairs to span intron-exon boundaries, and to overlap each other in exonic sequence. The gene was analyzed by direct sequencing.

Eleven single nucleotide polymorphisms were found after screening; all were novel and in intronic sequence or 3′ or 5′ untranslated regions (Table 6). The novel SNPs were submitted to the dbSNP database (http://www.ncbi.nlm.nih.gov/SNP/).

CLUL1
The ~53 kb human CLUL1 gene is encoded by 11 exons (Figure 6). We designed 13 primer pairs to span intron-exon boundaries and to overlap each other in exonic sequence (Table 7). The gene was analyzed by direct sequencing. No polymorphisms were identified.

MLCB
The ~8.7 kb human MLCB gene is encoded by four exons (Figure 7). We designed four primer pairs to span intron-
The genomic structure of TGIF, as reported in MapViewer (build 33) of the reference human genome sequence, is outlined below. The genomic structure for TGIF contains 10 exons spanning ~47.6 kb and has eight transcript variants encoding four proteins of 402 residues (variant 1), 287 residues (variant 2), 273 residues (variants 3 and 4), and 253 residues (variants 5 through 8) (Figure 8). All participant DNA samples were screened for sequence variants on exon 7, although it has no continuous open reading frame with the conserved region of exons 9 and 10, and only one known corresponding EST. Fourteen oligonucleotide primer pairs were designed to amplify the exonic sequences with 50 to 200 base pairs extensions beyond the intron-exon boundary (Table 9).

A total of 21 polymorphisms were found in the 10 exons screened for TGIF (Table 10). Of these, three were missense variances, two were silent, 10 were not translated, four were intronic, and two were homozygous deletions. The three missense allelic variants were observed at exon 10 at positions 236C→T (Pro→Leu), 244C→T(Pro→Ser), and 245C→T(Pro→Leu). Silent mutations were observed on exon 10 at positions 177A→G and 333C→T. The two deletions causing frameshift mutations were observed in exon 6 at positions 3442216 and 3442223 on NT_010859.13. Both deletions are predicted to cause early termination, yielding proteins of 132 and 141 residues, respectively. Ten polymorphisms were novel and have been submitted to the dbSNP database. Eleven polymorphisms corresponded with previously reported SNPs in public databases. None of the sequence variants cosegregated with the affected myopia phenotype. Specifically, there were no heterozygous or homozygous polymorphisms observed only in affected individuals in any MYP2 pedigree.

**DISCUSSION**

We sequenced the full coding regions of EMLIN2, CLUL1, MLCB, and TGIF positional candidate genes in our patient samples of individuals from pedigrees with MYP2-associated high myopia. No DNA sequence variants were noted that implicated any as the causative gene. We were especially interested in the TGIF candidate gene because of its published association with MYP2 by SNP association studies. TGIF exon 10 (exon 3 in the initial build of this gene) did not show the same level of polymorphic variants in our cohort, because we observed eight variants rather than the 25 reported by Lam and colleagues. This may be due to the ethnic differences in

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**TABLE 6. POLYMORPHISMS DETECTED WITH DIRECT DNA SEQUENCING OF GENOMIC DNA USING EMLIN-2 PRIMERS**

<table>
<thead>
<tr>
<th>mRNA POSITION</th>
<th>OBSERVED BASE PAIR CHANGE</th>
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<td>573</td>
<td>T</td>
<td>C</td>
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<tr>
<td>1200</td>
<td>A</td>
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<td>1512</td>
<td>T</td>
<td>C</td>
</tr>
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<td>1881</td>
<td>Heterozygous T/C for all affected, and one control</td>
<td>C</td>
</tr>
<tr>
<td>2130</td>
<td>A</td>
<td>G</td>
</tr>
<tr>
<td>2508</td>
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</tbody>
</table>
Dissecting the Genetics of Human High Myopia: A Molecular Biologic Approach

our two sample sets, although family 1 of the MYP2 pedigrees studied was of Chinese descent. All other families were of Northern European descent. The TGIF gene was not fully screened based on the methods described in their publication, and therefore the SNP association is most likely due to a nearby gene or regulatory element. The fact that TGIF mutations cause holoprosencephaly also reduces the likelihood that it is directly associated with simplex myopia.

Information derived from this effort will be useful for submissions to the ever-growing SNP database and to other researchers also exploring candidate genes in this region, whether it is for a myopia-related project or others. Other researchers screening for myopia candidate genes in this interval may wish to avoid repeated screening of those genes that have been excluded. The molecular study of any of these genes requires PCR primers that have been optimized for the gene exonic and intronic area of interest.

Nonsyndromic high myopia is a common, complex disorder that is likely to result from alterations of multiple

### TABLE 7. PRIMERS DESIGNED FOR SEQUENCE ANALYSIS OF THE CLUL1 GENE

<table>
<thead>
<tr>
<th>PRIMER NAME</th>
<th>mRNA POSITION</th>
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<th>AMPLICON SIZE (bp)</th>
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</table>

bp, base pair.

**FIGURE 7**

Genomic structure of the 4-exon MLBC gene.
Indeed, several loci have been mapped for nonsyndromic high myopia. We will continue our efforts to determine the gene alterations involved for the MYP2 locus and for the other known high-grade simplex myopia loci.

**CONCLUSION**

Mutation analysis of four encoded positional candidate genes shown to be expressed in ocular tissues for MYP2 autosomal dominant high myopia did not identify sequence alterations associated with the disease phenotype. Further studies of MYP2 candidate genes are needed to determine the gene causative for this potentially blinding disorder. Mutation screening of other genes that also map to this interval is in progress.

Considerations for further study and future directions point to two other features with disease gene identification:

1. Are we ascertaining the right families? The genes that contribute to complex or multifactorial disease—those such as diabetes, asthma, cancer, heart disease, and psychiatric illness—are notoriously difficult to identify as they typically exert small effects on disease risks. The magnitude of their effects is likely to be modified by other unrelated genes as well as environmental factors. We recognize the problem of genetic heterogeneity, phenocopy, and shared environmental factors. Multiplex families with unilinear transmission of the affected phenotype provide the most unambiguous information for detecting linkage, especially for complex traits. Selectively ascertaining such pedigrees with high disease severity and early age of onset biases toward a strong genetic etiology, minimizing environmental influences to the disease trait. This has

<table>
<thead>
<tr>
<th>PRIMER NAME</th>
<th>mRNA POSITION (NT_010859)</th>
<th>PRIMER SEQUENCE 5'-3'</th>
<th>PRIMER SIZE (BASE PAIR)</th>
<th>AMPLICON SIZE (BASE PAIR)</th>
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</table>

**TABLE 8. PRIMERS DESIGNED FOR SEQUENCE ANALYSIS OF THE MLCB GENE**

Genomic structure of the 10-exon TGIF gene. The associated ~47.6kb region of NT_010859 on chromosome 18p11.31 of TGIF, showing 10 exons with alternative start sites and splicing that generates eight transcript variants. The exons are represented as boxes, initiation codons are represented by a vertical line with arrow, and stop codons are represented by a vertical line with a black square.
been our ascertainment strategy for our mapping studies.

2. Are there other genotyping techniques that may be utilized? While our mapping studies are based on polymorphic microsatellite marker allele variations, we recognize that the use of SNPs for haplotype-based association studies may offer advantages over the use of conventional markers.\(^\text{126}\) Genomic regions can be tested for association without requiring the discovery of the functional variants. SNPs are more densely distributed and abundant, occurring roughly every 1,000 bp along the human genome. SNPs are binary, and thus well suited to automated, high-throughput genotyping. Finally, in contrast to more mutable markers, such as microsatellites, SNPs have a low rate of recurrent mutation, making them stable indicators of human history. We will consider and test emerging SNP-based technologies as they become available in our institution, and if they practically improve our genotyping efforts in terms of both cost and efficiency. Irrespective of this, we can use selected SNPs as supplemental polymorphic markers for fine-point mapping after a genome-wide scan implicates a linked region. High-resolution, fully integrated maps of SNPs are publicly available at the SNP database (dbSNP, http://www.ncbi.nlm.nih.gov/SNP/).

**REFERENCES**

**TABLE 10. LIST OF OBSERVED SEQUENCE POLYMORPHISMS FOUND IN THE TGIF GENE LABELED IN BASE PAIRS**

<table>
<thead>
<tr>
<th>NT_010859.13 POSITION</th>
<th>WILD-TYPE CHANGE OBSERVED</th>
<th>SNP rs NO.†</th>
<th>ORIGINAL MYP2 FAMILY NO.-INDIVIDUAL NO.</th>
<th>EXON POSITION</th>
<th>AMINO ACID CHANGE</th>
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<td>3402167</td>
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bp, base pair; C, control; UTR, untranslated region.

*The wild-type sequence is derived from the scaffold sequence NT_010859 of chromosome 18p. Amino acid changes are for relevant splice variants. Affected individual sample numbers are in bold type.

†rs No. is the public reference SNP number from the dbSNP database (http://www.ncbi.nlm.nih.gov/SNP/).


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