REVIEW OF OPTOMETRY
APRIL 2010

Glaucoma Care on the Cutting Edge

Proceedings of the
Eighth Annual Scientific Meeting
of the Optometric Glaucoma Society

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INTRODUCTORY REMARKS

We are excited to bring you the Proceedings from the Eighth Annual Meeting of the Optometric Glaucoma Society (OGS), held in Orlando, FL., November 9 to 11, 2009. In this year's program, we covered a wide range of topics, each focusing on what is new and cutting edge. Topics included a discussion of common clinical tests, such as gonioscopy and optic disc assessment, as well as how laboratory discoveries are translated into clinical uses. Other lecturers discussed whether ethnic differences exist in structural or functional tests and the current status of eye research funding.

The 2009 Honoree, George Spaeth, MD, in his lecture "Gonioscopy and Optic Disc Assessment," addressed the concept of visual disability and how important it is for a clinician to consider this in the overall management of a patient.

In the 2009 President's Lecture, Christopher Girkin, MD, reviewed the concept of "Race and Glaucoma" and why the disease manifests differently among groups.

Peng T. Khaw, MD, PhD, who received the Research Excellence Award, enlightened us on the evolution of glaucoma surgery and how improvements in several areas are making a larger improvement in patient outcomes.

I want to thank the speakers who took time from their busy schedules to share their wisdom with our members and guests. I especially want to thank Brad Fortune, OD, PhD, 2009 OGS Program Chair, as well as Michael Sullivan-Mee, OD, 2008 Meeting Chair. I want to thank John Flanagan, MCOptom, PhD, OGS President for consulting on the development of this supplement as well as his input in planning the meeting.

This supplement, developed by *Review of Optometry* in conjunction with Drs Khaw, Flanagan, Girkin, Patella, Pasquale, Spaeth and myself, includes highlights and key points from their presentations. I want to thank Sean McKinney, who distilled these complex lectures into the articles that follow, and to Alicia Cairns, who handled the graphic design.

Finally, I want to thank Pfizer Ophthalmics for their support of this supplement.

Please visit the OGS Web site, www.optometricglaucomasociety.org, and check out our quarterly e-journal that can be sent to your e-mail account free of charge. I hope you enjoy this supplement and find it useful.

Murray Fingeret, OD
Executive Vice-President, Optometric Glaucoma Society
Editor, Proceedings of the Eighth Annual Scientific Meeting of the Optometric Glaucoma Society

Research Excellence Award Lecture

Repair to Regeneration: Converting Laboratory Discovery to Clinical Advance Translational Research in Glaucoma

BY PENG T. KHAW, MD, PhD

We have been translating laboratory findings into potential breakthroughs for patients, an effort that has been centered on the award of the new National Institute for Health Research Biomedical Research Center in Ophthalmology (NIHRBRC) in the United Kingdom. Our goal is to stimulate innovation and develop new partnerships with industry, and national and international organizations. Our center has achieved many milestones, including the first human retinal gene therapy. Our extensive development of research infrastructure has also involved new psychophysical and functional vision testing facilities, an accredited human stem cell transplant facility, pharmaceutical manufacturing facility, international reading center, and enlarged adult and pediatric clinical research facilities.

Focusing on scarring, repair, and regeneration

My group's primary focus has been on scarring, which plays a role in virtually every major blinding disease and is particularly important after glaucoma filtration surgery. This area is of interest to us because surgery is often the only viable treatment for glaucoma in many parts of the world. The scarring response after filtration surgery is the major determinant of the final intraocular pressure (IOP).

Glaucoma is the most important cause of irreversible blindness in the world and second leading cause of blindness in developed countries. The challenge of working to help these patients is enormous when you consider that glaucoma affects more than 70 million people, causing 7 million to lose their sight.

Sometimes, though, it takes only the inspiration of a single patient to drive home the importance of our mission and keep us focused. A few years ago, a little girl came to us with inflammatory eye disease, and secondary glaucoma and cataract. She was already irretrievably blind in one eye and nearly blind in the other. This article will explore the impact and importance of translational research for patients like her.

Experimenting with fibroblasts

My current research began when I was a PhD student in London and Florida, where I worked with Ian Grierson, Mark Sherwood, and Greg Schultz to look at the effects of various growth factors and other agents on fibroblasts. We found that you could induce long-term growth arrest with very short applications of such inexpensive anti-cancer agents as 5-fluorouracil (5-FU) delivered over a few minutes.[1]

We then developed in vitro and in vivo models of scarring and moved into phase 1 conceptual proof of principle studies with the first human use of intraoperative 5-FU. Our investigations led to large-scale clinical trials in the Far East, Africa, and the UK, showing that a single application of 5-FU could improve the outcome of glaucoma filtration surgery around the world. Although mitomycin C (MMC) is commonly used, surveys, including one completed in the UK and one done recently in Australia and New Zealand, showed that the intraoperative 5-FU treatment was the most common regimen used to prevent scarring after glaucoma surgery.[2]

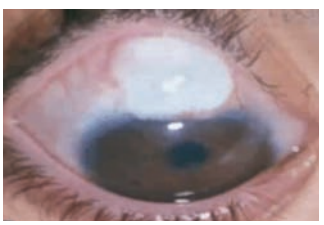
Importance of biomechanics and surgical research

We have also learned that the biomechanics of tissue are very important in glaucoma surgery. For example, we compared a focal bleb – uncomfortable, leaking, prone to infection, and putting the patient at risk for blinding endophthalmitis – to a much more attractive bleb that created a diffuse drainage area. Linking clinical observation with laboratory research, we reconsidered so-called "cystic blebs," which the anti-cancer agents made more prominent and which shared two clinical characteristics:

1. a ring of scar tissue that we named the "ring of steel"
2. consistent presence of anterior limbal aqueous drainage.

We theorized that a ring of uninhibited peripheral fibroblasts was creating a focal, restricted area of fluid flow, resulting in a focal, thin cystic area. If this theory was correct, the advisable treatment was to increase the size of the treatment surface, not decrease it, as clinicians had been doing at the time to attempt to prevent complications. After trying this approach, we saw the morphology of the simple drainage area change dramatically. Even when using larger doses of the anti-cancer agent, we noticed that the blebs, the so-called drainage areas, were much more diffuse. Our complication rates decreased significantly. A 20% incidence of bleb inflammation (plus a few cases of endophthalmitis) went down to 0.5% during the next few years. These and other new techniques have been incorporated into a system of trabeculectomy we call the Moorfields Safer Surgery System.[3]

This success was replicated at Moorfields and other places, such as Miami, by Paul Palmberg, who deserves full credit for making the actual change into clinical practice in the United States and in South America. This was a classic example of research observations in the laboratory and clinic translations into large scale clinical practice, making a significant difference to our patients. Our large-scale, international trials, including the UK "More Flow" Surgery Study, have found significantly increased survival with a single, 5-minute sponge application of 5-FU during surgery, a result that was recently mirrored in a similar large-scale, long-term study with colleagues in Singapore.[4]



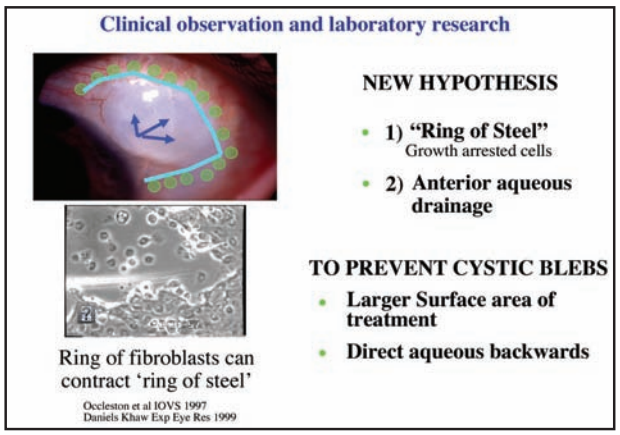
A simple change in biomechanics helps avoid the development of this very focal bleb and associated potential problems, including discomfort, leakage, hypotony, blebitis, and endophthalmitis.

Combined, the data from the control and treated groups showed that 24% of patients whose IOP exceeded 21 mm Hg progressed, as defined by visual field or disc progression, at this 18-month mark after surgery. Setting the pressure limits prospectively, we found the following at 80 months:

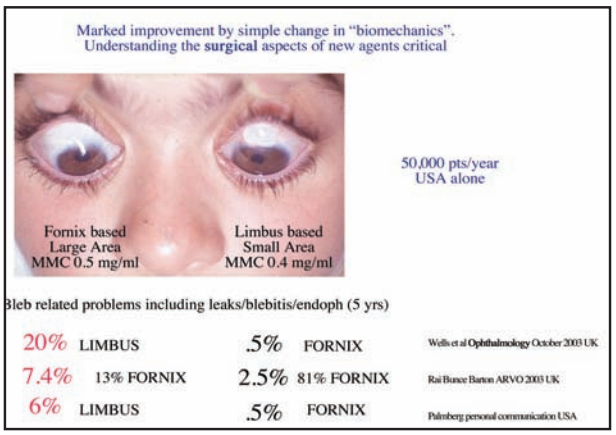
- 17% of patients progressed if they did not not exceed 21 mm Hg.
- 12% of patients progressed if they did not exceed 17 mm Hg.
- No patients progressed if they did not exceed 14 mm Hg.

As you can see, a clear relationship existed between IOP and outcome. However, the real question was whether the desired IOP level was an achievable target. We only attained this level in approximately 20% of the controls and in 30% of controls in the More Flow study.

The other important issue is that we were treating individuals, not



Clinical observation and laboratory studies identified the "ring of steel" and anterior aqueous drainage that characterized cystic blebs. Therefore, a larger surface treatment area was created and drainage was directed backward.



Improvements were dramatic utilizing simple changes in surgical biomechanics.

groups. For example, some clinicians believe intraoperative 5-FU works only on Caucasians of European descent. By randomly, prospectively dividing patients into ethnic groups, we found that patients of African descent achieved a much lower success rate of approximately 50% but this increased to 60% (compared to 90% for Caucasians), a statistically significant level of efficacy that routinely supports the licensing of drugs. This emphasizes the need to understand more so that we can individualize care for different patients.

Improvements in wound-healing research techniques

Because of the benefits of modern biology, we have access to astonishing views of intrinsic cellular processes, such as a cell contracting a single collagen fiber, astrocytes moving like fibroblasts, and cells beginning to activate. A machine called a culture force monitor can measure forces that cells exert or forces that are exerted on cells, facilitating profound changes in the genes that cells express. We have learned that different cell types exhibit varying profiles throughout the eye. By understanding these issues, we can manipulate important activity to prevent scarring. We hope to create a more regenerative environment for wound healing, such as the process we see in the fetus. The fetus demonstrates a remarkable regenerative — rather than repair-oriented—healing process.

For example, you can repair a cleft palate in utero and the newborn will emerge from the womb scarless. Features of fetal scarless healing include low levels of certain growth factors, differences in matrix remodeling, and minimal inflammation. New literature indicates that inflammation is important to every process we see in scarring. Fetal tissue contains low levels of TGF-ß, which is the most potent cytokine that stimulates scarring-related fibroblast function in many ways. Besides low TGF-ß levels, fetal tissue matrix remodeling is different to that found in adult tissues.

Inhibiting scarring by modulating growth factors

The first attempt to create a more specific anti-scarring effect involved the use of the recombinant human TGFß2 antibody (lerdelimumab, Trabio). Cambridge Antibody Technology pioneered this use with its antibody and approached my group, leading to very promising early results with the use of lerdelimumab. Cell cultures and experimental model studies showed tissue that looked much more normal than tissue altered by anti-metabolites. We based our short four-dose clinical regimen on the initial experimental results from Cordeiro.[5] However, subsequent studies by Mead in our laboratory showed an extended survival rate with a different prolonged treatment regimen.[6] Despite these results, it was not possible to change the original treatment regimen because trials had already begun. The clinical trials did not show a statistically significant improvement in clinical outcome, possibly because the dosing regimen was wrong.[7] We learned several lessons – but particularly that in wound healing, longer term delivery was even more important than we had thought.

Another study from Siriwardena in our group has suggested the need for extended treatment to prevent scarring after surgery.[8] Prolonged raised flare meter readings from proteins in the anterior chamber after cataract extraction alone were found to be significantly higher than flare readings associated with trabeculectomy alone, up until the end of the eighth post-operative month. These results may explain why some patients who have combined cataract and glaucoma surgery do so much worse than patients who undergo only glaucoma surgery. The findings also demonstrate that some patients experience prolonged intraocular blood aqueous barrier breakdown after surgery and that they may require long-term scarring inhibition to negate the effects of these proteins passing through the drainage site.

Developing new therapies

While all of this research continues, we are pursuing novel anti-scarring therapies. Pharmaceutical development is moving from the use of small molecules to recombinant proteins and antibodies, cell and tissue therapy, and gene therapy. Further developments include the delivery of old drugs in new ways, creating profound new therapeutic effects.

Here is an example, involving dendrimer nanotechnology. We have revisited inflammation, which Crowston and Chang have shown to be a critical component of failure after drainage surgery.[9,10] Corticosteroids have been successfully used to partially inhibit scarring in glaucoma surgery. However, there is the need for better agents to reduce inflammation and hence scarring after glaucoma surgery.

Working with the school of pharmacy and Imperial College, we compared the effect of a traditional model of drug delivery, including single-cell

binding, to the effect created when glucosamine and glucosamine sulfate were added to a nanomolecule.[11] Glucosamine is a very weak binder to toll-like receptors (TLRs), which facilitate a very potent anti-inflammatory response. However, when you put glucosamine and glucosamine sulfate on a nanomolecule, the new combination assumes Velcro-like qualities. Binding activity increases by a logarithmic factor. The result is one of the most potent inhibitors of TLRs. We found that this chemical alteration significantly inhibited scarring in our model of glaucoma filtration surgery. We have not been able to achieve this result in the past with intensive steroids. This suggests that if we can control inflammation better with a new generation of agents, scarring could be reduced further in humans.

Matrix metalloproteinases

A critical upregulated component of scarring and healing are the matrix metalloproteinases (MMPs), ubiquitous enzymes capable of remodeling soft tissue structures throughout the human body. When we identified the importance of MMP activity during healing, we wanted to find out if we could modulate MMPs and downregulate the contractile process in the conjunctiva, retina, and lens capsule.

We tested lens capsules and found profound contraction in controls and minimal contraction when MMP inhibitors were applied.[12] We subsequently carried out experimental glaucoma filtering surgery.[13] The control eyes, stained with picrosirius red dye, showed very significant scarring in the subconjunctival space and a completely flat drainage area where a tube was inserted into the eyes. By comparison, a very diffuse bleb with minimal scarring and minimally cystic appearance was found in eyes treated with multiple injections of the MMP inhibitor.

The survival curve of the MMP inhibitor injections was almost equivalent to that of mitomycin, which causes massive cell death and apoptosis. The MMP inhibitor histology was much closer to normal. The challenge in using an MMP inhibitor, however, is that you have to deliver it with multiple injections, very frequently, because of the pharmokinetics involved in the formulation.

MMP inhibitors, designed primarily for cancer and arthritis, were envisioned as agents that could combat the manifestation of MMPs in malignancy and cartilage breakdown. At this point, however, no MMP inhibitor is licensed as a drug, despite the expenditure of more than $5 billion in research and development. The large barrier to approval has been a continuing struggle with significant systemic adverse effects.

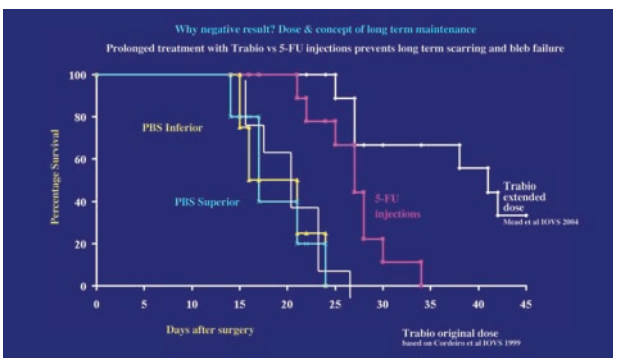
Working with pharmacists and bioengineers, we have synthesized a slow-release tablet, now 1 mm in diameter, that is designed to be placed in the subconjunctival space after glaucoma filtering surgery. The tablet releases the MMP inhibitor at therapeutic levels for up to 30 days. The tablet did not appear to have significant adverse effects, and the drug was not detectable in blood levels. The slow-release tablet can potentially provide results that are equivalent or better than results produced by a MMC sponge treatment - without mitomycin-related tissue damage. If replicated in humans, this finding could offer the prospect of maximal scarring control in 70% to 80% of patients at intraocular pressures associated with minimal glaucoma progression.

Another promising anti-cancer agent that could help with glaucoma filtering surgery is bevacizumab (Avastin). In experiments involving filtering surgery, the bevacizumab solution has been absorbed in less than 2 hours. However, when formulated in a slow-release format, the drug lasts 70 times longer. These developments give us hope that we can succeed more often with filtering surgery, achieving the endpoints of minimal (<5%) visual field loss and disc damage

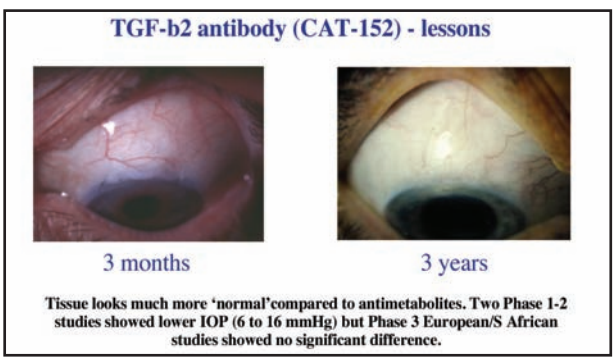
From repair to regeneration - tapping into stem cells

One of the other projects we have worked on involves a series of stem cells, most notably the Müller multipotent progenitor cell. Initial laboratory work has been done on chickens and zebrafish. One recent finding from Raymond Lab has shown that the Müller glial cell is primarily responsible for the remarkable regenerative ability of the zebrafish's retina, which is very complex and similar to the human retina. Limb and colleagues have recently identified the equivalent cell in the human adult eye. One of the challenges is trying to get to the cell to integrate and differentiate in the retina. For this to occur, the retina has to be made "permissive" to regeneration, not unlike the situation in the fetus. By applying different combinations of anti-scarring treatments, we have been able to achieve multifaceted integration of the cells into the retina.[14] There is now early evidence that some function can be restored.[15]

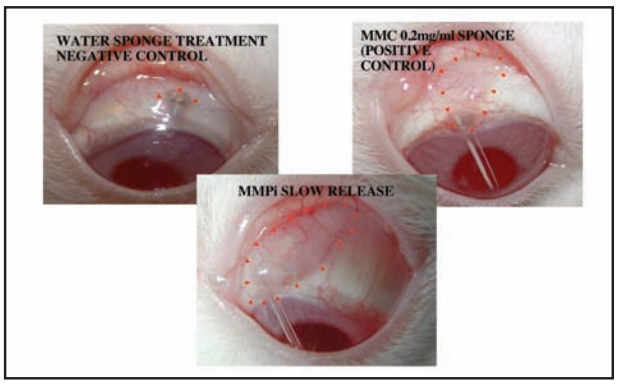
Although these are just early experiments, they show the potential benefits of modulating the scarring process and combining this with multipotent stem cells. It means that support of remaining function or even some restoration of function can potentially occur in end-stage glaucoma. This

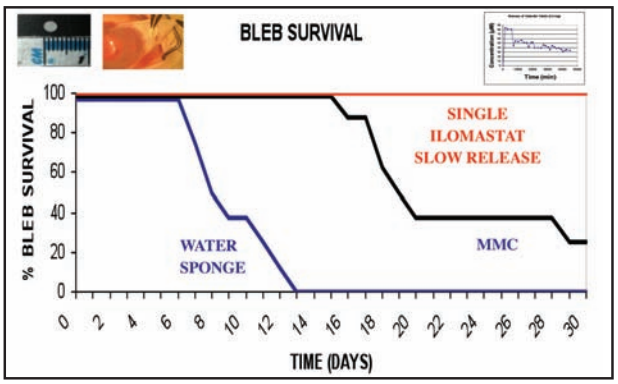


Survival curves of experimental glaucoma filtration surgery showing original data on which the clinical studies were based and the later data showing a much improved bleb survival using a different dose.



Tissue treated with TGF-ß2 looks much more "normal" when compared to tissue treated with antimetabolites. However, phase 3 European\South Africa studies showed no significant difference.





The eyes presented above on the left show the effects of treatment with a water sponge (negative), mitomycin, and the experimental MMP inhibitor treatment after glaucoma filtering surgery. On the right are the survival curves associated with each treatment.

goal, in my view, is more realistic than trying to re-grow optic nerve tissue.

Therefore, our ability to learn though research about the processes involved in repair and regeneration is bringing us closer to several therapeutic end points that would make a significant difference to glaucoma patients around the world, including:

- IOP after surgery of about 10 mm Hg, which evidence shows will eliminate progression in the majority of cases
- turning back the clock by reversing even small percentages of nerve loss that visually cripple so many patients, preventing them from leading independent daily lives.

We could achieve much success by creating first-generation stem cells and enabling an environment that recovers a small amount of function, helping these patients to become semi-independent again. Just a little more progress in this area could be transformative to the lives of our patients.

Stirring passions and giving hope for the future

Developments like these are what stir our passion and give us hope for the future. We can tell our severely impaired patients about the research that is being carried out and see a noticeable lift in their spirits. Research is making a difference in their lives, providing inspiration and hope that they need so much.

Because of this visible progress benefitting patients, ophthalmology is now at the frontline of translational medical research in the UK. And now we have been fortunate to help put a lovely little, beaming face on our progress. Earlier, I mentioned the young girl who came to us with severe inflammatory disease, glaucoma, and cataract. Research helped us develop the surgical techniques that restored her vision. Her story inspired not only us but an extensive network of philanthropic fundraisers, literally raising the new Children's Eye Hospital, which filled the gap between the UCL Institute of Ophthalmology and Moorfields Eye Hospital and iconically provided the first physical connection between the research institute and the hospital. Appropriately, she laid the foundation stone for the facility, which includes the world's largest eye research center for children.

This story illustrates what we have been trying to achieve – the translation of research into real benefits for our patients. This is the best time for translational research and the extraordinary developments in biomedical research. I believe we will see even greater progress in glaucoma care for our patients in the years ahead due to research.

http://www.nihr.ac.uk/about/Pages/about_transforming_health_research_video.aspx.

1. Khaw PT, Sherwood MB, MacKay SL; Rossi MJ, Schultz G. Five-minute treatments with fluorouracil, floxuridine, and mitomycin have long-term effects on human Tenon's capsule fibroblasts. Arch Ophthalmol 1992, 110(8) p1150-4
2. Liu L, Siriwardena D, Khaw PT. Australia and New Zealand survey of antimetabolite and steroid use in trabeculectomy surgery. J Glaucoma. 2008 Sep;17(6):423-30.
3. Khaw PT, Chiang M, Shah P. Glaucoma Filtration Surgery. Textbook of Ophthalmology. Jakobiec and Blodi Elsevier 2008.
4. Wong TTL, Khaw PT, Tin A, Foster PJ, Oen FTS, Gazzard A, Husain R, Devereux JG, Chew PTK, Seah SKL. Trabeculectomy with Intraoperative 5 Fluorouracil in Singapore – Three Year Follow-up on Effects on Intraocular Pressure Control and Disease Progression – a Prospective Randomised Double Masked Clinical Trial. Ophthalmology 2009; 116: 175-184.
5. Cordeiro MF, Gay J, Khaw PT. Human anti-transforming growth factor beta antibody: a new glaucoma anti-scarring agent Invest Ophthalmol Vis Sci 1999; 10: 2225-2234.
6. Mead AL, Wong, TTL Cordiero MF, Anderson IK, Khaw PT. Evaluation of anti-TGF-beta2 antibody as a new postoperative anti-scarring agent in glaucoma surgery. Invest Ophthalmol Vis Sci 2003; 44: 3394-3401.
7. CAT-152 0102. Trabeculectomy Study Group, Khaw PT, Grehn F, Holló G, Overton B, Wilson R, Vogel R, Smith Z. A pha. A phase III study of subconjunctival human anti-transforming growth factor beta(2) monoclonal antibody (CAT-152) to prevent scarring after first-time trabeculectomy. Ophthalmology. 2007 Oct;114(10):1822-30.
8. Siriwardena D, Kotecha A, Minassian D, Dart JKG, Khaw PT. Anterior chamber flare after trabeculectomy or phacoemulsification. Br J Ophthalmol 2000; 84:1056-1057.
9. Chang L, Crowston JG, Sabin CA, Khaw PT, Akbar AN. Human tenon's fibroblast-produced ifnbeta and the prevention of t-cell apoptosis. Invest Ophthalmol Vis Sci. 2001 Jun;42(7):1531-8.
10. Crowston JG Constable PH Daniels JT Chang L Akbar AN Khaw PT. Apoptosis gene expression and death receptor signaling in mitomycin-C-treated human tenon capsule fibroblasts. Invest Ophthalmol Vis Sci 2002; 43: 692-699.
11. Shaunak S Thomas S Gianasi E Godwin A Jones E Duncan R Mireskandari K Luthert P Patterson S Khaw PT Brocchini S. Polyvalent dendrimer glucosamine conjugates prevent scar tissue formation. Nature Biotechnology 2004: 22: 977-984.
12. Daniels JT, Cambrey A, Occleston N, L Garrett Q, Tarnuzzer R, Schultz GS, Khaw PT. Matrix metalloproteinase inhibition modulates fibroblast-mediated contraction and collagen production in vitro. Invest Ophthalmol Vis Sci 2003; 44:1104-1110
13. Wong TTL Mead AM Khaw PT. Long-term anti-scarring effects of Ilomastat following experimental glaucoma filtration surgery: a comparison with Mitomycin-C. Invest Ophthalmol Vis Sci 2005; 46: 2018-2022
14. Limb GA, Singhal S, Lawrence JM, Bhatia B, Ellis JS, Kwan AS, Macneil A, Luthert PJ, Fawcett JW, Perez MT, Khaw PT. Chondroitin sulfate proteoglycans and microglia prevent migration and integration of grafted Müller stem cells into degenerating retina. Stem Cells. Stem Cells. 2008 Apr;26(4):1074-82. Epub 2008 Jan 24.
15. Lawrence JM, Keegan DJ, Reh TA, Coffey PJ, Luthert PJ, Khaw PT, Limb GA. MIO-M1 cells and similar Müller glial cell lines derived from adult human retina exhibit neural stem cell characteristics. Stem Cells. 2007; 25:2033-43

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Translational Research in Glaucoma

Proteomics of Optic Nerve Head Astrocytes and Laminar Cribrosa Cells

BY JOHN FLANAGAN, PHD, MCOptom, FAAO

Proteomics helps us look at the regulation of proteins and, when applied to the study of optic nerve cells, can tell us much about how the cells respond to mechanical strain. The mechanical strain that we use in our experiments replicates the conditions found in the normal human lamina cribrosa when intraocular pressure (IOP) is raised.

Working with Ross Ethier, PhD, and his team, including Ian Sigal, we have been able to model the biomechanical effects in response to raised IOP. An increase from 15 mm Hg to 50 mm Hg was shown to cause strain of up to 15% at the level of the lamina cribrosa. The work I will discuss here is an extension of this research, which we hope will provide insight into the early mechanisms of glaucoma and ultimately lead to effective new treatments.

Focusing on the trouble spots

We know that glaucoma is associated with damage at the level of the lamina cribrosa, leading to activation of the optic nerve head astrocytes and the lamina cribrosa cells. These cells activate in what is believed to be a wound-healing type response in the early stages of disease, leading to an apoptotic cascade causing the death of the retinal ganglion cells.[1]

The fundamental research questions that continue: Does mechanical stress have an impact on the cellular function of human optic nerve head astrocytes and lamina cribrosa cells in vitro? Are human astrocytes and lamina cribrosa cells involved in the early pathogenesis of glaucoma?

To find answers, we have been dissecting the lamina cribrosa of disease-free human eyes from the Eye Bank of Canada in Toronto. We grow the cells on flexible silastic plates. Once the cells have grown to confluence they are subjected to either 3% or 12% equi-axial stretch for 2 or 24 hours. Prior to stretching, the cells are carefully differentiated and characterized. The optic nerve head astrocytes stain positively for glial fibrillary acidic protein (GFAP) and neural cell adhesion molecule (NCAM), and negatively for alpha-smooth muscle actin (SMA) and PAX2. The lamina cribrosa cells are negative for GFAP and positive for SMA and PAX2. Despite some past controversy over their role – and whether they were even a separate cell type – the lamina cribrosa cells have a distinct appearance and properties, many of which are similar to that of trabecular meshwork cells. They are also morphologically distinct from the optic nerve head astrocytes and scleral fibroblasts.

Growing cells

Once we have differentiated the astrocytes and lamina cribrosa cells, we grow them in specific types of media for several months. Then we seed them onto collagen-coated, deformable silastic plates. We deform these plates using vacuum and therefore stretch the cells by a controlled amount. Once the cells have been stretched using 1 Hz cycles of 3% or 12% stretch for 2 or 24 hours, the cells are harvested and ready for protein analysis. The proteomics are performed in collaboration with the Ontario Cancer Biomarker Network, using the new iTRAQ system (Isobaric Tag for Relative and Absolute Quantitation). Samples are labeled with different carrier proteins to enable the identification of upregulated and downregulated proteins simultaneously.

Making sense of the results can become complicated, however. We use a software called the Protein Pilot. We look for downregulation by 0.67 or upregulation by at least 1.5 times. We also use receiving operator characteristic (ROC) curves, much like the type used when considering sensitivity and specificity, to establish internal control and validity. In this way, we have identified more than 520 proteins per cell line with a 95th percentile confidence level and a few less, 410 and 467, at the 99th percentile confidence level.

What do the results mean?

After stretching cells by 3% and 12% for 2 hours, we found that 69 and 80 proteins, respectively, were significantly differentially regulated when compared to the control and that 50 of those overlapped. After 24 hours, 3% and 12% strain produced 113 and 126 differentially regulated proteins. In this set, 50 also overlapped. The results were similar for the astrocytes, although the number of overlapping proteins were lower (16 for the 2-hour group and 38 for the 24-hour group).

We could look at networks of related proteins and identify specific mechanisms that were affected. After 2 hours of 3% strain, we started to see some regulation of protein. The regulation increased at 3% strain after 24 hours and at 12% strain after 2 and 24 hours. After 12% strain for 24 hours cell death was clearly occurring, reflected by the appearance of markers of apoptosis, along with MMPs.

Biomarkers worth noting

We have identified activity in a few biomarkers that are worth noting. These include PA15, the astrocytic phosphoprotein associated with anti-apoptosis and negative regulation of glucose import. Acidic leucine-rich nuclear phosphoprotein, an anti-apoptotic protein, functions as a caspase-3 inhibitor involved in cell cycle progression and cell survival. Of course, at this early stage, we cannot say what this means. I like to think it has potential as an anti-apoptotic factor involved in signal transduction.

Implications of lamina cribrosa cells

In the realm of the lamina cibrosra cells, we find BAG-family proteins that act as molecular chaperones and are associated with apoptosis, protein-folding, binding, and regulation. The proteins are also related to the heat shock proteins that have often been associated with glaucoma. Also involved is the thioredoxin domain containing protein, a stress response protein associated with neurodegenerative disease, and nuclear protein 66, which has been involved in protein binding and has been linked to Alzheimer's disease. It is not a great stretch to hold out hope that these proteins will be of some interest to us in glaucoma.

The next step will be to take our candidate proteins and analyze the mechanisms associated with them in great detail.

Key cellular pathways

We have identified several cellular pathways that are implicated in the regulation of the human optic nerve head astrocytes and lamina cribrosa cells when they are undergoing biomechanical stress. Both cell types demonstrated a common primary protein hub around transforming

growth factor beta 1, tumor necrosis factor, and tumor protein 53. All of these have been associated in the past with activation of astrocytes and with glaucoma.

The primary pathways of the lamina cribrosa cells are related to cellular movement, cell-to-cell signaling, cell morphology, cell development, and protein folding. Optic nerve head astrocytes are associated with cell death, apoptotic cascade, cellular movement, the cell's assembly and organization, and some DNA replication and recombination and repair. When all of this is fed through the networks, the principal disorders that the software flags are ophthalmic and neurological diseases, which is somewhat comforting.

The important task now is to look at the early functions of these biomarkers and determine if they are common across different types of strain and hypoxia.

1. Tezel G; Fourth ARVO/Pfizer Ophthalmics Research Institute Conference Working Group. The role of glia, mitochondria, and the immune system in glaucoma. Invest Ophthalmol Vis Sci. 2009 Mar;50(3):1001-12.

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OGS President's Lecture:

Understanding Race and Glaucoma

BY CHRISTOPHER GIRKIN, MD

Primary open-angle glaucoma is the leading cause of irreversible blindness in African-Americans. It appears earlier, progresses more rapidly, and is associated with a higher rate of conversion in African-American patients with ocular hypertension. Race and glaucoma also have a history of misinterpretation. Although race has biologic merit, it provides a poor summary of human biodiversity when used for patient care.

When race was not an issue?

In the late 1990s, several popular books suggested race was purely a cultural concept. However, even in an early paper examining the genetic diversity of populations,[1] using ancestral informative markers, self-described racial characteristics were identified that remain legitimate for clinical research to this day. In this early paper, self-described race clustered well to genetically described ancestry. The authors concluded that, for clinical research, self-described race could be suitable to substite for ancestry. One of the flaws of the "no race" argument was that it did not consider enough genes. To define a person, such as a criminal who leaves DNA at a crime scene, we need only a few genetic markers. However, as many as 300 genes are needed to define race. The analysis of much more data is needed to discern differences between one race and another.

Specific single nucleotide polymorphisms (SNPs) called ancestry-informative markers (AIMs) function as limited polymorphisms in the genome that are very weakly correlated with ancestral groups. If you identify enough AIMs, you can measure ancestry and geographic clustering fairly accurately. For example, we identified AIMs associated with Sub-Saharan African genetic ancestry in 85% of 50 self-described African-Americans from the African Descent and Glaucoma Evaluation Study (ADAGES).

However, making broad statements about patients based on race is inappropriate, even fraught with potential errors. For example, a patient may or may not have a particular gene which helps determine response to a particular medication. The gene may be found in different frequencies in different racial groups. While the gene might have a higher frequency in one group, this information has little clinical value when applied an individual. In addition, through our work with some members of the ADAGES subgroup, we have glimpsed some of the cultural and socio-economic interests that go beyond genetics. The University of Alabama at Birmingham (UAB) School of Optometry and Department of Ophthalmology have screened patients in nearby Perry and Lowndes counties, where as many as 42% of the residents live below the poverty level.

In a study by Owsley and coworkers at UAB, we found that African-Americans were 40% less likely to receive eye exams.[2] Nearly 45% of participating eye care providers cited accessibility as a major barrier, followed by cost (34%), and trust (17%). Accessibility was identified as a barrier by 75% of patients, but 65% of them cited trust as a major issue. Many practitioners, including me, did not perceive this lack of trust because we were seeing patients who trusted us. The individuals who did not trust us did not show up.

Evaluating risk factors

We have been taking a closer look at the physical characteristics of this population that may be putting them at increased risk, even when they receive eye care. What we know now is that systemic disease cannot account for racial disparity in glaucoma, even though African-Americans are at increased risk for hypertension and diabetes. Ocular factors that encompass all socio-economic strata, such as structure of the nerve, central corneal thickness (CCT), and intraocular pressure (IOP), have been established as more significant issues.

We are using quantitative instruments in a longitudinal cohort study to try to develop detection and progression algorithms. Controversy over IOP continues, but CCT is definitely thinner in African-Americans, as demonstrated in several studies, including the Ocular Hypertension Treatment Study (OHTS).[3]

The multivariate model used in the OHTS largely explains increased risk in terms of distinctive anatomical characteristics, crudely measured by vertical cup-disc ratio and CCT. Race may be a surrogate for these biologic ocular factors.

In the Baltimore Eye Study, individuals of African ancestry had a larger optic nerve, larger cup area, and a similar volume of neural tissue than other individuals.[4] The rim area was the same and no other associations existed, aside from interesting gender differences across racial lines.

Quigley and colleagues found the vertical disc diameter was larger in African-Americans than in Caucasians in post-mortem eyes.[5] In addition, they found a thinner nerve fiber layer that was more spread out across larger discs, which is also seen in European ancestry, most commonly in females.

These anatomical variations have implications in the diagnosis of glaucoma. A normal disc with a very large neural canal is probably much more likely to be misdiagnosed as glaucoma. A small amount of cupping on a very small disc can easily be overlooked in early glaucoma.

The differences also have implications in pathogenesis. Bellezza and colleagues used idealized computer models to determine that large, oval-oriented neural canals are strained at any level of IOP.[6] The larger the lamina, the more vulnerable it becomes to glaucomatous injury.

Why ADAGES was established

In response to these studies, researchers established ADAGES, sponsored by the National Eye Institute, in 2002. This collaborative multicenter project includes the Hamilton Glaucoma Center at the University of California, San Diego; UAB; and the New York Eye and Ear Infirmary. The goals are to:

- quantify differences in optic disc, retinal nerve fiber layer structure, visual function, and progression of vision loss between glaucoma patients of African and European descent
- determine multivariate methods to optimally combine information from structural imaging and visual function testing to improve detection of glaucoma and help delineate differences in onset and rate of progression in patients of African and European descent
- determine the role of ancestry as a determinant of glaucoma.

We have enrolled more than 1,500 patients with glaucoma - defined by nerve, visual fields, and IOP - and 500 normal subjects. Complete testing is being used, including optical coherence tomography (OCT) and specialized perimetry. The baseline study design paper was just published in *Archives of Ophthalmology*[7] and the baseline normal structure paper and normal function papers are in press with *Archives of Ophthalmology*. A future paper will consider how to incorporate findings into diagnosis based on normal patients, glaucoma patients, and racial variation.

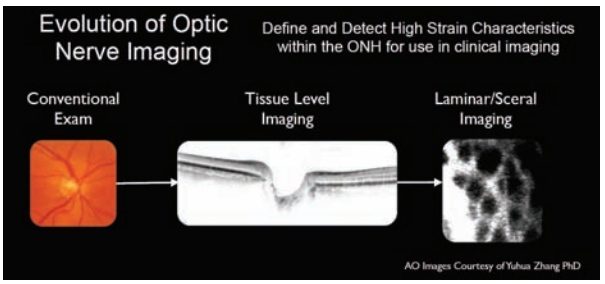
Behind ADAGES

Interestingly, despite differences in vertical cup-disc ratio measured subjectively by the masked photo grading center, similar numbers of patients in ADAGES were inaccurately determined to be glaucomatous in both the African and European ancestral groups. Disc area was probably taken into account more than just cup-disc ratio (CDR).

After adjusting topographic findings for age, disc area, and plane height, we found the only residual difference between ancestral groups was a deeper cup in individuals of African descent. This could have some pathogenic significance. A deeper cup may mean the presence of a thinner lamina that is susceptible to strain and deformation - and possibly a posterior insertion of the lamina. We do not know because Heidelberg Retinal Tomography (HRT) provides only a view of the vitreoretinal interface, which may not correspond to the scleral lamina surface. The HRT view is also confounded by neural tissue and vitreous and blood vessels.

OCT imaging in ADAGES found a larger vertical disc area, larger CDR, and a larger cup area in the African descent group. However, after adjusting for the size of the nerve, these differences disappeared. Most remaining differences in the optic nerve head between African and European ancestral groups were associated with disc size. Residual differences in cup depth were also apparent.

Under the HRT automated GPS classification system, patients of European ancestry tended to have steeper RNFL curvature while patients of African descent had a flatter curvature with a more glaucomatous appearance. What did this tell us? That if we relied solely on GPS modeling, without an ethnically specific normative database, we might misclassify patients of African descent.



Work is underway on defining and detecting high-strain characteristics within the optic nerve head for use in clinical imaging.

When evaluating RNFL with OCT, we saw differences that persisted even after adjustment for the variation in disc area and axial length. Overall, the RNFL was thicker in the African ancestral population, which had a thinner papular-macula bundle and thicker superior and inferior bundles. This finding also corresponded to the macular thickness. The inner macular volume and thickness were reduced in the African ancestral group compared to the European ancestral group.

Diagnostic functions that included the disc area as a parameter, such as Moorfields Regression Analysis (MRA), performed with comparable specificity across the two groups. The GPS performed similarly when we used an ethnic-specific database and carefully avoided misclassifying subjects based on GPS parameters that look more glaucomatous in individuals of African descent. The advantage of MRA is that it accounts for disc area. However, the original MRA had a limited normative database of 100 to 125 patients, all examined in London, all of European ancestry.

Individual differences

In prior studies, we have demonstrated that age-adjusted sensitivity was similar among individuals of African ancestry. In individuals of European ancestry, subjective classification by the masked grading expert was a little bit better than by the Moorfields Regression Classification (MRC). The MRC tended to misclassify patients with larger optic nerve heads.

Since reaching these findings, we have loaded into the database broader-based information and we have created MRC 2 for the HRT3, eliminating misclassification. Similarly, when you adjust for disc areas in multivariate models for OCT, GDx, and HRT, the misclassification also goes away. You can account for some of the differences by using disc area in your quantitative model, just as we do when measuring a disc in clinical practice.

Going forward, we will try to determine if we need normative databases across racial groups or simply parameters that differ between racial groups.

Next phase

We also hope to overcome the limitation of not being able to see the lamina surface. With the introduction of spectral domain OCT (SD-OCT), we are close to discerning the lamina in many patients. At the 2009 Association for Research in Vision and Ophthalmology (ARVO) meeting, Claude F. Burgoyne, MD, demonstrated a delineation of the lamina surface in comparison to the opening of the nerve in 12 non-human primates. Using this new technology and new optics that may capture more precise

images, we want to define ethnic differences and eventually better understand the biomechanical characteristics of a larger and deep cup and whether it plays a significant role in glaucoma.

1. Rosenberg NA, Pritchard JK, Weber JL et al. Genetic structure of human populations. Science. 2002;298:2381-5.
2. Owsley C, McGwin G, Scilley K, Girkin CA, Phillips JM, Searcey K. Perceived barriers to care and attitudes about vision and eye care: focus groups with older African Americans and eye care providers. Invest Ophthalmol Vis Sci. 2006 Jul;47(7):2797-802.
3. Gordon MO, Beiser JA, Brandt JD, Heuer DK, Higginbotham EJ, Johnson CA, Keltner JL, Miller JP, Parrish RK 2nd, Wilson MR, Kass MA. The Ocular Hypertension Treatment Study: baseline factors that predict the onset of primary open-angle glaucoma. Arch Ophthalmol. 2002 Jun;120(6):714-20; discussion 829-30.
4. Varma R, Tielsch JM, Quigley HA, Hilton SC, Katz J, Spaeth GL, Sommer A. Race-, age-, gender-, and refractive error-related differences in the normal optic disc. Arch Ophthalmol. 1994 Aug;112(8):1068-76.
5. Quigley HA, Brown AE, Morrison JD, Drance SM. The size and shape of the optic disc in normal human eyes. Arch Ophthalmol. 1990 Jan;108(1):51-7.
6. Bellezza AJ, Hart RT, Burgoyne CF. The optic nerve head as a biomechanical structure: initial finite element modeling. Invest Ophthalmol Vis Sci. 2000 Sep;41(10):2991-3000.
7. Sample PA, Girkin CA, Zangwill LM, Jain S, Racette L, Becerra LM, Weinreb RN, Medeiros FA, Wilson MR, De León-Ortega J, Tello C, Bowd C, Liebmann JM; African Descent and Glaucoma Evaluation Study Group. The African Descent and Glaucoma Evaluation Study (ADAGES): design and baseline data. Arch Ophthalmol. 2009 Sep;127(9):1136-45.

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Race And Glaucoma

Is a Race-Specific Normative Database Important for Imaging in Glaucoma?

BY MURRAY FINGERET, OD, FAAO

Carl Zeiss Meditec has released a normative database for its Cirrus optical coherence tomography (OCT) unit that does not offer the race-specific data found in Heidelberg Engineering Tomography (HRT) and Optovue's RTVue. This raises fundamental questions: Is a race-specific normative database important for imaging in glaucoma? If so, why?

Will race-specific databases improve the ability of imaging instruments to detect glaucomatous loss? Here, I will review some studies to try to help address these important questions.

Evaluating fiber layer and optic disc

One study compared the abilities of the scanning laser polarimeter with variable corneal concentration (GDx VCC), Heidelberg Retinal Tomograph II (HRT II), and Stratus OCT to discriminate between healthy eyes and eyes with glaucomatous visual field loss.[1] Sensitivity was found to frequently be in the 60% range and specificity was reduced as well. This and other studies illustrated that sensitivity is not where it needs to be for any of these instruments.

Two companion studies provided insights on ethnic implications. In the first one, we looked at 1,081 individuals enrolled at 15 clinics around the world. Our goal was to determine if Fourier-domain OCT could demonstrate ethnic differences in RNFL thickness and optic disc area in healthy eyes.[2]

Besides OCT readings, the following were documented as part of a standard exam: standard achromatic perimetry (Humphrey field analyzer [HFA] using the Swedish Interactive Thresholding Algorithm [SITA], standard 24-2), intraocular pressure (IOP), central corneal thickness, axial length, refraction, and optic disc photographs. We applied a backward elimination multivariate linear model that considered ethnicity, age, optic disc area, and signal strength. Exclusion criteria included visual field loss, known disease, high refractive error, elevated IOP, and unacceptable scan quality.

The members of the ethnic groups, by number, included African-Americans (103), Caucasians (204), Chinese (246), Hispanic (99), Indians (243), and Japanese (149), all matched as best as possible at a mean age of 49 years.

The Caucasian group had the thinnest RNFL by far, at 102.69 µm. The average thickness for the other groups: Japanese, 105.66 µm; Indian, 106.57 µm; African American, 106.80 µm; Hispanic, 112.64; and Chinese, 113.84 µm. Results were similar in the temporal, superior or nasal and inferior locations. Not surprisingly, African-Americans had the largest optic disc size (2.15 mm^2). Caucasians had the smallest, at 1.82 mm^2. The Hispanic (2.06 mm^2), Chinese (2.02 mm^2), Indian (1.93 mm^2), and Japanese (1.92 mm^2) patients were in the middle.

We also found relationships between RNFL layer thickness and age (growing thinner with age) and between RNFL thickness and disc area (thicker layer associated with larger disc area). As expected, no relationships existed between disc area and age.

Our conclusion? Ethnic differences were found with RNFL thickness when accounting for the effects of age, disc area, or signal strength. Further, correlations were found between average RNFL thickness and each of the following: age, disc size, and signal strength, in that order.

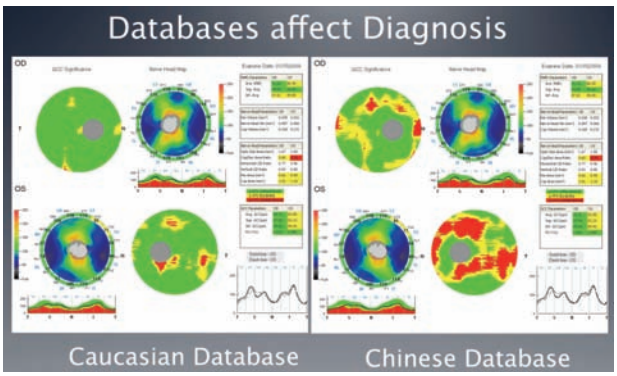
Effect on ganglion cell complex

The second study that considered the ethnic backgrounds of patients focused on the ganglion cell complex (GCC) within the macula.[3] Optovue has created a test with its OCT that will evaluate the thickness of the ganglion cell complex within the macula and compare the findings to a normative database. The concept is that reduced thickness may be associated with glaucoma.

We collected data on 1,065 eyes at 15 clinical sites worldwide. Participants, an average of 49 years old, ranged from 18 to 82 years of age. They were free of known ocular pathology, had IOP below 22 mm Hg, and had a normal visual field test based on the Humphrey 24-2 SITA Standard perimetry. The appearance of the optic disc was not used as an exclusion criterion because it could have introduced bias in the database. All images had acceptable image quality (SSI > 35). A multivariate general linear model was used to test significance of ethnicity and age.

The results in this study were similar to that of the first. As was the case when evaluating RNFL thickness and optic disc area, we found ethnicity to have an effect on GCC thickness. What was slightly different, however, was that the macula in Chinese eyes (103.30µm) was by far the thickest. The African-American macular area was quite a bit thinner (95.90 µm) than we had seen within the nerve fiber layer. The remaining values: Caucasian, 95.20 µm; Japanese, 96.30 µm; Indian, 97.20 µm; and Hispanic, 100.30 µm.

When comparing groups with similar ages and different ethnicities (Caucasian vs. Japanese or Chinese vs. Indian), we found apparent ethnic-based differences in the superior and inferior GCC. These findings, at least to a point, are not new. Results of studies conducted by Dr. Christopher Girkin and Rohit Varma, MD, MPH, at the University of Southern California have shown ethnic variations.[4,5,6] Statistically significant differences between African-Americans and Caucasians have also been found within



Above are two Fourier domain OCT scan printouts from the same patient. The difference between the two is determined by separate normative databases – one for Caucasians on the left and the other for Chinese on the right.

the macula of healthy eyes when using the Stratus OCT.[7]

Improving sensitivity at what cost?

Another study looked at the performance of Moorfields regression analysis with the HRT after it was updated with race-specific results. In essence, sensitivity improved for whites and blacks but, surprisingly, specificity decreased slightly for blacks.[8] The study urged careful scrutiny before using new software and data based on ethnicity in clinical practice.

Dr. Girkin has also described problems with the use of race. So how should we proceed? How should we use data to categorize individuals? While ethnicity replaces the concept of race, it is still ambiguous and difficult to understand, especially in the context of these new databases.

Evidence of ethnic differences in the disc, nerve fiber layer - and perhaps even in the macular region – suggests that the continuing development of ethnic-specific databases may improve the sensitivity and specificity of imaging instruments. However, the question is how to put these databases into clinical use.

How do we separate ethnic groups? For example, we often use the term "Asian," but Japanese and Chinese eyes are as different from each other as are Caucasian and African-American eyes. Do we need subcategories? What about Korean eyes?

Instrument company representatives tell me that every database costs at least $1 million. Can they afford to do this? Can we afford to not do this, mindful that a bad database is unacceptable? Or is it possible we can use one large heterogeneous database that includes many different groups in sufficient numbers? These are the questions we need to ask ourselves and find a way to answer.

1. Medeiros FA, Zangwill LM, Bowd C, Weinreb RN. Comparison of the GDx VCC scanning laser polarimeter, HRT II confocal scanning laser ophthalmoscope, and stratus OCT optical coherence tomograph for the detection of glaucoma. Arch Ophthalmol. 2004 Jun;122(6):827-37.
2. Sinai MJ, D.F.; Garway-Heath DF; Fingeret M; Varma R, Liebmann JM; Greenfield DS; Girkin CA; Schuman JS; Clinical Affairs, Optovue, Inc; Carlsbad, CA; NIHR BMRC for Ophthalmology, Moorfields Eye Hospital NHS Foundation Trust, and UCL Institute of Ophthalmology, London, United Kingdom; VA NY Health Care System, New York, NY; Ophthalmology, Doheny Eye Institute, Los Angeles; Ophthalmology, Manhattan Eye and Ear Throat Hospital, New York, NY; Ophthalmology, Bascom Palmer Eye Institute, Palm Beach Gardens, FL; Opthalmology, University of Alabama Birmingham, Birmingham, AL; Ophthalmology, Ophthalmology and Visual Science Research Center, UPMC Eye Center, Eye and Ear Institute, Pittsburgh, PA. The Role of Ethnicity on the Retinal Nerve Fiber Layer and Optic Disc Area Measured With Fourier Domain Optical Coherence Tomography. ARVO Supplement, May 2009.
3. Fingeret M; Sinai M; Garway-Heath DF; Greenfield D; Varma R; Liebmann J; Schulman JS; Girkin C; Optovue Normative Database Study Group; Optometry, VA; NY Health Care System, Brooklyn, NY; SUNY Optometry, New York, NY; Clinical Affairs, Optovue, Inc, Freemont, CA; NIHR BMRC for Ophthalmology, Moorfields Eye Hospital NHS Foundation Trust, and UCL Institute of Ophthalmology, London, United Kingdom; Ophthalmology, Bascom Palmer Eye Institute, Palm Beach Gardens, FL; Ophthalmology, Doheny Eye Insitutute, Los Angeles, CA; Ophthalmology, Manhattan Eye and Ear Hospital, New York, NY; Ophthalmology, University of Pittsburgh Medical Center, Pittsburgh, PA; Ophthalmology, University of Alabama in Birmingham, Birmingham, AL.; The Normal Thickness of the Ganglion Cell Complex in the Macula measured with RTVue OCT. ARVO, May 2009.
4. Girkin CA, McGwin G Jr, Xie A, Deleon-Ortega J. Differences in optic disc topography between black and white normal subjects. Ophthalmology. 2005 Jan;112(1):33-9.
5. Racette L, Boden C, Kleinhandler SL, Girkin CA, Liebmann JM, Zangwill LM, Medeiros FA, Bowd C, Weinreb RN, Wilson MR, Sample PA. Differences in visual function and optic nerve structure between healthy eyes of blacks and whites. Arch Ophthalmol. 2005 Nov;123(11):1547-53.
6. Varma R, Tielsch JM, Quigley HA, Hilton SC, Katz J, Spaeth GL, Sommer A. Race-, age-, gender-, and refractive error-related differences in the normal optic disc. Arch Ophthalmol. 1994 Aug;112(8):1068-76.
7. Kelty PJ, Payne JF, Trivedi RH, Kelty J, Bowie EM, Burger BM. Macular thickness assessment in healthy eyes based on ethnicity using Stratus OCT optical coherence tomography. Invest Ophthalmol Vis Sci. 2008 Jun;49(6):2668-72.
8. Zelefsky JR, Harizman N, Mora R, Ilitchev E, Tello C, Ritch R, Liebmann JM. Assessment of a race-specific normative HRT-III database to differentiate glaucomatous from normal eyes. J Glaucoma. 2006 Dec;15(6):548-51.

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Race And Glaucoma

Is a Race-Specific Normative Database Important for Perimetry in Glaucoma?

BY VINCENT MICHAEL PATELLA, OD

Normative limits play an important role in everyday clinical practice and require periodic re-evaluation. Re-evaluation may result in refinements in the limits themselves or in how the limits are being used. For example, normative limits for intraocular pressure were established in Germany in the 1950s.[1] In the 1960s, patients of any race with intraocular pressures exceeding those limits were considered to have glaucoma. Today we know that there are racial differences in normal pressure, and we also know that it is inappropriate to base glaucoma diagnosis solely on IOP.

Early on

In the early days of automated perimetry, we did not worry much about the effects of ethnicity on normative limits. The normative database for the original Humphrey perimeter included African-Americans from the Baltimore Eye Survey, but those results were lumped together with findings from Sweden, Iowa, and California. Other Humphrey normative data packages were later collected from all over the world, but these also were not ethnically stratified.

Looking for differences

Twenty years ago, Professor Yoshi Kitazawa's research group obtained Humphrey threshold fields in 100 normal Japanese subjects and compared them to the Humphrey normative limits The Japanese normal subjects produced a lot fewer significant test points on the commercial Humphrey Statpac probability charts than statistically expected, but we concluded that the differences probably were due to Kitazawa's use of much more stringent inclusion criteria than had been applied in

the original Humphrey normative database.[2]

In work that has spanned more than 20 years, Ron Harweth and colleagues reported only small differences between the subjective Standard Automated Perimetry (SAP) visual fields of monkeys and humans, leading us to conclude that human ethnic differences probably were small.[3,4] Later, we did see site-to-site differences in the Swedish Interactive Thresholding Algorithm (SITA) normals, but, as shown below, those differences did not seem to be ethnically-based.

SITA Normals Sites	Mean MD (dB)	Mean PSD (dB)
Two Japanese Sites	0.46 -0.95	1.60 2.31
Two American Sites	0.17 -0.34	1.77 1.90
One European Site	-0.21	1.70

In the OHTS study, differences in MD between Caucasians and African Americans were only about a quarter dB, an amount that was comparable to the inter-site variability we had seen in our SITA normative data studies.[5]

Recent findings

A 2005 study found no statistically significant SAP or Short Wavelength Automated Perimetry (SWAP) differences between African Americans and Caucasians, but a surprisingly large 1.77 dB difference in MD for Frequency Doubling Technology (FDT).[6] Two recent reports from the African Descent and Glaucoma Evaluation Study (ADAGES) study found that individuals self-identifying as being of African descent produced statistically different SAP, SWAP, and FDT findings, compared to normal subjects who identified themselves as being of European descent.[7,8]

While statistically significant, the SAP differences were too small – only fractions of a decibel – to be diagnostically important. In contrast, reported differences in SWAP (0.42dB) and Matrix (0.96 dB) MDs might be large enough to demand further evaluation for us to fully understand their practical effects. (The Matrix's 5˚-by-5˚ squares are smaller than the stimuli provided by SAP.) SAP, SWAP, and Matrix PSD differences were statistically significant but, again, too small to be of clinical concern.

Looking ahead

We are just beginning to understand the complex relationship between African American and European visual field sensitivities, and we know very little about how other ethnic groups compare. Although currently available information does not justify development of race-specific normative databases for SAP, it may be time to investigate the possible value of normative limits specific to African Americans for FDT and Matrix – and perhaps also for SWAP. Further work is required to understand other ethnicities.

1. Leydhecker W, Akiyama K, Neumann HG. Klin Monatsblatter Augenheilkd Augenarztl Fortbild. 1958;133(5):662-70.
2. Iwase A, Shirai J, Ido T, Shimizu U, Kitazawa Y, Patella M. The analysis of normal fields with the Humphrey Statpac. Proceedings of the International Perimetric Society, 1988.
3. RS Harwerth RS, EL Smith EL 3rd, DeSantis L. Behavioral perimetry in monkeys. Invest Ophthalmol & Vis Sci. 1993 Vol 34:31-40.
4. Harwerth RS, Vilupuru AS, Rangaswamy NV, Smith EL 3rd. The relationship between nerve fiber layer and perimetry measurements. Invest Ophthalmol Vis Sci. 2007 Feb;48(2):763-73.
5. Gordon MO, Kass MA. The Ocular Hypertension Treatment Study: design and baseline description of the participants. Arch Ophthalmol. 1999 May;117(5):573-83.
6. Racette L, Boden C, Kleinhandler SL, Girkin CA, Liebmann JM, Zangwill LM, Medeiros FA, Bowd C, Weinreb RN, Wilson MR, Sample PA. Differences in visual function and optic nerve structure between healthy eyes of blacks and whites. Arch Ophthalmol. 2005 Nov;123(11):1547-53.
7. Sample PA, Girkin CA, Zangwill LM, Jain S, Racette L, Becerra LM, Weinreb RN, Medeiros FA, Wilson MR, De León-Ortega J, Tello C, Bowd C, Liebmann JM; African Descent and Glaucoma Evaluation Study Group. The African Descent and Glaucoma Evaluation Study (ADAGES): design and baseline data. Arch Ophthalmol. 2009 Sep;127(9):1136-45.
8. L. Racette, J.M. Liebmann, C.A. Girkin, L.M. Zangwill, S. Jain, F.A. Medeiros, C. Bowd, R.N. Weinreb, P.A. Sample, ADAGES Study Group. Racial Differences in Visual Function in Healthy Eyes in the African Descent and Glaucoma Evaluation Study (ADAGES). Presentation #6198, ARVO 2009 Annual Meeting.

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Clinical Testing; New Ideas And Implications

Disc Hemorrhages: Red Flags or Red Herrings?

BY LOUIS R. PASQUALE, MD

Since the mid-1970s, we have believed, based on our clinical observations, that a patient with glaucoma is likely to progress when disc hemorrhages occur. As Budenz and colleagues later confirmed, disc hemorrhages are a risk factor for converting from ocular hypertension to primary open-angle glaucoma when you control for intraocular pressure (IOP) and central corneal thickness (CCT).[1]

On the other hand, some might say hemorrhages could function as red herrings that distract us from more important signs of glaucoma, which, as we know, provides us with few tell-tale clues about extent of involvement. Here, I will answer a central question: Are disc hemorrhages diverting us from pressure-related deterioration of the nerve or do they merit closer scrutiny because of an important role in contributing to the drop-out of retinal nerve fiber layer tissue?

Disc hemorrhage overview

Bjerrum described disc hemorrhages in glaucoma patients in 1887. Through the years, we have seen them in posterior vitreous detachment, hypertensive retinopathy (similar in appearance to glaucomatous disc hemorrhages), diabetic retinopathy (blot-like and distinctly different from disc hemorrhages), and optic disc neuropathies. Despite Bjerrum's writings, most of us did not see disc hemorrhages in glaucoma patients until the mid 1970s, when we began dilating the pupil and limiting our use of pilocarpine. Disc hemorrhages related to glaucoma are typically found straddling the disc margin. They are fleeting and easily missed if you are not looking for them.

Based on the Budenz data from the Ocular Hypertension Treatment Study (OHTS), we now know that disc hemorrhages can occur early in glaucoma. However, fluorescein angiography studies have not confirmed their source, nor do we know why they occur. Are they a secondary phenomenon or a primary phenomenon, an upstream event in pathogenesis? If they are important, how might they relate to disc damage?

Key questions to consider

Here are the important questions that persist to this day.

- *How common are disc hemorrhages in glaucoma patients?*

The Budenz study indicated they occur, at most, about 5% of the time. However, in 1981, Bengtsson and colleagues looked at 51 patients very frequently over a long period and found that most of them developed disc hemorrhages sooner or later.[2] Disc hemorrhages usually last about 6 to 8 weeks, meaning we are likely to miss many if we only see patients every 4 months.

The Early Manifest Glaucoma Trial (EMGT), which called for careful ophthalmoscopy every 3 months and disc photographs every 6 months, found that 55% of patients developed disc hemorrhages during a median follow-up of 8 years.[3] The observations that Bengtsson made in the 1981 were born out in a standardized, randomized clinical trial that was completed two decades later.

• ***Are disc hemorrhages more common in normal-tension glaucoma?*** The evidence suggests that, in open-angle glaucoma, you will see more disc hemorrhages at a normal pressure level than at a high pressure level. In 1998, among cases discovered in a cross sectional, population-based analysis, the Blue Mountain Eye Study found a prevalence of 25% in normal-tension glaucoma and 8% in high-tension open-angle glaucoma.

None of 99 patients with chronic angle-closure glaucoma were found to have disc hemorrhages in one study in Taiwan.[4] In another study of Asian patients, disc hemorrhages were found in 4 of 90 of those with open-angle glaucoma (4.4%) and in 1 of 69 of those with angle-closure glaucoma (1.4%).[5]

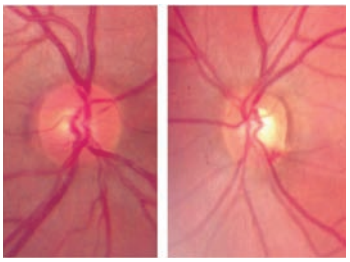
More recent publications in Asia suggest that disc hemorrhages in angle-closure glaucoma are more common than 1%. Therefore, we still need to remain open to the possibility of a phenomenon that is secondary to elevated intraocular pressure (IOP). Remember, too, that pressures often trend to the upper limit and are the most difficult to control in normal-tension glaucoma.

• ***Does lowering IOP retard disease progression in patients with open-angle glaucoma who have disc hemorrhages?*** EMGT findings suggest the answer to this question is no.[3] The percentage of patients who were treated and had disc hemorrhages (51%) roughly equaled the percentage of patients who were not treated and had disc hemorrhages (45%). Treated patients received a beta-blocker and underwent laser trabeculoplasty. No attempt was made to achieve a target IOP, so perhaps pressure was not lowered enough to retard the onset of disc hemorrhages.

Two studies contradicted the EMGT, finding that lowering IOP does reduce the frequency of disc hemorrhages. Henrickx found this to be the case only in high-tension patients, not normal-tension patients.[6] A study by Miyakie and colleagues out of Japan suggested that performing a trabeculectomy reduced disc hemorrhage frequency in open-angle glaucoma.[7]

Disc hemorrhages in a 39-year-old woman

We do not know if disc hemorrhages are the first step in the glaucoma disease process, but we do know they occur early—more frequently in open-angle than in angle-closure glaucoma—and that we should pay attention to them. Randomized clinical trials strongly indicate that they are a marker of optic nerve damage that is independent of IOP. Consider the case of a 39-year-old woman who was referred to me in 1999 because of disc hemorrhages in her left eye. A history revealed that her father, also my patient, had a history of elevated IOP and advanced glaucomatous optic neuropathy.



A 39-year-old woman had disc hemorrhages in the left eye despite otherwise normal findings, including an IOP of 18 mm Hg, normal slit lamp exam findings in both eyes, and CCT readings of 540 µm, OD, and 530 µm, OS.

She had an IOP of 18 mm Hg and normal slit lamp exam findings in both eyes. Her CCT readings were 540 µm, OD, and 530 µm, OS. A visual field deficit in her left eye was associated with the hemorrhage, indicating a superior paracentral scotoma that corresponded to a papillomacular bundle below the horizontal meridian. This was an amazing finding, considering she was otherwise healthy, without thin corneas and with reasonable pressures.

Seven years later, in 2006, I found that her right eye had remained disease-free but that her left eye was progressing. Besides the paracentral scotoma, she now had a superior nasal step. This was a pattern of open-angle glaucoma I had been puzzling over in about 5% of my patients through the years. Back in 1999, I had wondered if the same mechanism that defies gravity, ensuring that we maintain blood supply in our brains when we rise from bed, was malfunctioning when these patients reclined at night. Faulty retinal vascular autoregulation had been hypothesized as a possible cause of glaucoma since the early 1900s.

To put the hypothesis to the test in 1999, after she had turned 40, we had used a Cannon Doppler Blood Flowmeter to conduct a retinal hemodynamic study. The device uses a fundus camera, Doppler capability, and a device that measures the diameter of a discreet vascular segment. By knowing the Doppler signal and the retinal vascular diameter, we can measure flow in discreet segments of the retinal vasculature.

We measured her flow in the infratemporal branch of the retinal arterial of her left eye, where she had experienced the nerve damage. We conducted the measurements while she was seated, with the compensatory mechanisms of her body presumably working against gravity, and while she was lying down, with her heart at the same level as her retina. We found that the blood flow to her retina increased by 60% when she was lying down, demonstrating remarkable hyperperfusion at rest. Meanwhile, a 40-year-old control patient showed consistent flow, also in the intratemporal branch of the retinal artery of the left eye, while lying down and sitting up.

How much of a factor is hyperperfusion?

Can transient hyperperfusion of the retina contribute to disc hemorrhages in patients like this one? Consider that vessels reach the surface of the retina by making acute right and left turns, after exiting the optic nerve. This configuration creates significant shear forces. If blood flow increases by 60%, one of the smaller vessels, perhaps a lamina cribrosa capillary or peripapillary retinal arterial, could burst and bleed, causing disc hemorrhages.

How could such hemorrhages cause glaucoma? Remember that within the optic nerve head we can find Elschnig's scleral ring, where the optic nerve is at risk for compartment syndrome. This occurs in the optic disc drusen when calcium builds up inside proteins and the proteins press

on the optic nerve and cause nerve fiber layer tissue drop-out.

In these tight quarters, the disc hemorrhage could lead to a form of compartment syndrome and cause the nerve fiber layer to drop out. When the blood cleared, the patient could be left with a notch from blood that had pinched the nerve fiber layer tissue against the neural retinal rim. If you look closely at many patients who have disc hemorrhages, you will notice that the width of the hemorrhage corresponds exactly to the width of the focal nerve fiber layer defect.

This hypothesis is consistent with a finding that disc hemorrhages tend to precede localized nerve fiber layer visual field loss in spatially consistent locations in patients with open-angle glaucoma.[8] It is also possible that the blood is exerting a locally toxic effect on the nerve fiber layer tissue in this location. Again, though, these are just hypotheses.

Larger study of hyperperfusion

After detecting retinal autoregulation dysfunction in the 40-year-old woman in 1999, we included her in a larger study in which we looked at 18 glaucoma patients and eight age-matched controls. We identified retinal autoregulation dysfunction in seven of the patients, all of them women between 40 and 60 years of age and all them diagnosed with normal-tension glaucoma. We found normal retinal autoregulation in another seven patients. In four patients, we recognized a vasospastic phenomenon, in which blood flow to the retina surged when the patients reclined and then receded dramatically and significantly below baseline (seated) levels while the patients remained in a supine position. Interestingly, the patients with a vasospastic hemodynamic profile had the lowest ocular perfusion pressures at baseline (45 mm of mercury vs. 55 mm in the control patients vs. 51 mm in the hyperperfusion group). These findings are statistically significant, although more research is certainly needed.

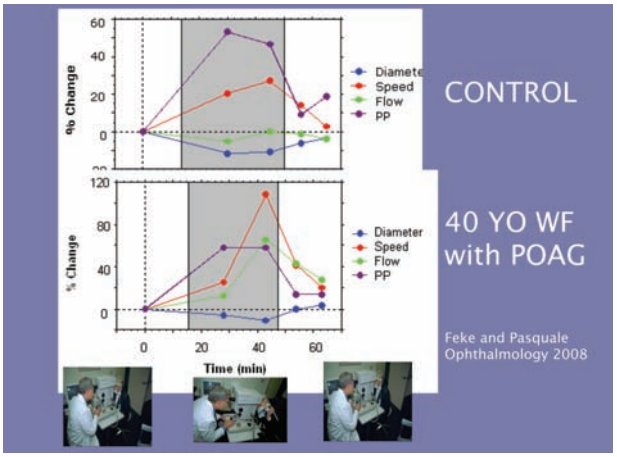
It is worth noting that the technology we used would not allow us to assess vessels with diameter lower than 90 microns. In the future, spectral domain technology might help us look at capillary flow in more detail. Perhaps compromised autoregulation can be explained by a dysfunctional autonomic nervous system, involving interaction between the glia and the vasculature. Other causes are also possible.

Exploring genetic causes

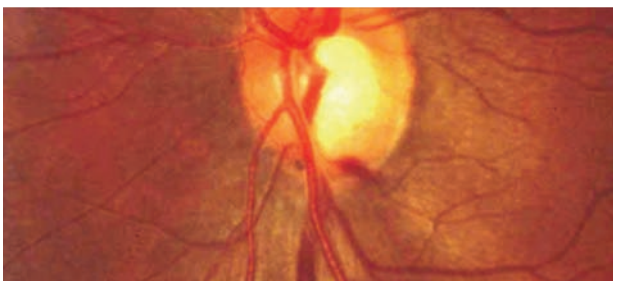
One other potential cause of disc hemorrhages in glaucoma is the activity of molecular mediators of retinal vascular response. My colleagues and I have focused on two mediators that may contribute to pathogenesis—endothelin and nitrous oxide. Vascular abnormalities that occur in patients with open-angle glaucoma may have a genetic cause, modified by environmental influences.

Nitric oxide could be the main culprit. The endothelial nitric oxide is made from an enzyme called nitric oxide synthase 3, which is on chromosome 7. In an article we recently authored in *Investigative Ophthalmology*, we assessed whether variants in this gene were associated with primary open-angle glaucoma.[9] When we looked at individual single nucleotide polymorphisms, we did not see a strong effect. We did see a modest association between a combined NOS3 haplotype and primary open-angle glaucoma.

We also found that NOS3 gene variants interacted with postmenopausal hormone use among women with high-tension glaucoma. These data suggest that NOS3 may be a vascular mediator in



Above is a comparison of the findings of retinal hemodynamic studies on two patients. The results on the bottom indicate the presence retinal vascular autoregulation dysfunction as a possible cause of disc hemorrhages in glaucoma.



The size and shape of disc hemorrhages often correspond to the width of nerve fiber layer defects, suggesting the possibility of these hemorrhages creating a compartment syndrome that contributes to nerve fiber layer fall-out.

the abnormal retinal vascular autoregulatory process seen in primary open-angle glaucoma.

Of course, other factors are involved. We are making a list of all the molecules that result in either vasodilation and vasoconstriction. Because of our knowledge of the architecture of the human genome gained from the HapMap project, we know exactly where the genes that code for these molecules are located. We also know the locations of the polymorphisms in those genes and which of the polymorphisms might be functional.

We plan to scan the whole human genome in 3,000 people with primary open-angle glaucoma and in 3,000 people without the disease. Once we have completed the scan (by June of 2010), we will assemble all of the markers that are associated with vascular tone in a panel. We will look at their relationships with primary open-angle glaucoma and with primary open-angle glaucoma subtypes, stratified by IOP and by gender. The top gene variants in that panel could be associated with the disease. Then we can assess if the markers interact with biomarkers of vascular endothelial dysfunction to try to identify new drug targets for primary open-angle glaucoma.

For the time being . . .

While we await developments, we still need to manage our patients – such as the one who comes in with primary open-angle glaucoma and disc hemorrhages despite IOP in the low normal range. Before deciding

to perform an outflow procedure with the goal of achieving an IOP in the single digits, I think we should establish a diurnal curve. Some of these patients have occult-elevated IOP at different times of the day and documentation of this tendency might justify surgical intervention. When we do not document an IOP spike, remember that sometimes a treatment can be worse than the disease.

There may be drugs that are well-suited for normal-tension glaucoma that is characterized by retinal autoregulation dysfunction. We took a 70-year-old white male with normal-tension glaucoma off of his medication and evaluated the hemodynamic status of his retina. His blood flow went from 5.5 µL (microliters) per minute while sitting up to about 11.5 µL per minute while lying down. We put him on brimonidine 0.2% (Alphagan), flattening his transient hyperperfusion while lowering his IOP. Even if we had not improved his IOP, we would likely have kept him on brimonidine 0.2% to improve his autoregulation.

We do not know if this treatment will produce a better outcome for such a patient, but we plan a randomized clinical trial to try to determine if brimonidine improves retinal vascular autoregulation in normal-tension glaucoma.

In another study, we will assess if fixed combination brimonidine 0.2%-timolol ophthalmic 0.5% (Combigan) or fixed combination dorzolamide hydrochloride 2%-timolol maleate 0.5% (Cosopt) improves retinal vascular autoregulatory abnormalities in patients with open-angle glaucoma. Basic science literature indicates that brimonidine 0.2% can be vasoactive at the level of the optic nerve head vasculature when applied topically.[10-12] We will continue to study this effect.

History of disc hemorrhages

Here is one more case: An 80-year-old man with a history of paracentral scotoma in his left eye, first noted in 1986, was referred to me in 2006 after developing increased peripheral loss in the left eye and a new paracentral scotoma in the fellow eye. I found a history of recurring hemorrhages by reviewing serial fundus photographs from 1986, 1991, and 1996.

I was impressed that he did not show signs of progressive functional loss during the late 1980s and 1990s. Despite evidence of a very discreet, deep defect in the nerve fiber layer tissue, scanning laser polarimetry (GDx) showed considerable nerve fiber tissue reserve in both eyes after 2 decades of disease. His IOP had been kept under good control and was in the low teens. The tempo of his disease did not suggest that he needed aggressive treatment, such as a filter to reduce his pressure to below 8 mm Hg or 9 mm Hg. I projected that he would probably do fine as long as we could keep his pressures down in the low teens

Putting disc hemorrhages in perspective

So where does this information leave us? I believe I can comfortably say that disc hemorrhages, which can be elusive and easily missed, are red flags but, granted, they can also be red herrings if we do not keep our eyes on all aspects of our patient's care. I believe they are markers for a complex vasculopathy in glaucoma that has not been completely understood or addressed. We need to look carefully for disc hemorrhages, using ophthalmoscopy and frequent disc photos, and interpret them in the context of the patient's entire clinical picture.

Meanwhile, the molecular factors contributing to disc hemorrhages remain largely unknown, but we will continue to work to identify them. I believe we will succeed with further research.

1. Budenz DL, Anderson DR, Feuer WJ, Beiser JA, Schiffman J, Parrish RK 2nd, Piltz-Seymour JR, Gordon MO, Kass MA; Ocular Hypertension Treatment Study Group. Detection and prognostic significance of optic disc hemorrhages during the Ocular Hypertension Treatment Study. Ophthalmology. 2006 Dec;113(12):2137-43. Epub 2006 Sep 25.
2. Bengtsson B, Holmin C, Krakau CE. Disc haemorrhage and glaucoma. Acta Ophthalmol (Copenh). 1981 Feb;59(1):1-14.
3. Bengtsson B, Leske MC, Yang Z, Heijl A; EMGT Group. Disc hemorrhages and treatment in the early manifest glaucoma trial. Ophthalmology. 2008 Nov;115(11):2044-8. Epub 2008 Aug 9.
4. Yang CH, Hung PT, Lin LL, Hsieh JW, Wang TH, Wang IJ. Characteristics of optic disc changes in Taiwanese patients with primary angle-closure glaucoma. J Formos Med Assoc. 2003 Mar;102(3):183-8.
5. Gazzard G, Morgan W, Devereux J, Foster P, Oen F, Seah S, Khaw PT, Chew P. Optic disc hemorrhage in Asian glaucoma patients. J Glaucoma. 2003 Jun;12(3):226-31.
6. Hendrickx KH, van den Enden A, Rasker MT, Hoyng PF. Cumulative incidence of patients with disc hemorrhages in glaucoma and the effect of therapy. Ophthalmology. 1994 Jul;101(7):1165-72.
7. Miyake T, Sawada A, Yamamoto T, Miyake K, Sugiyama K, Kitazawa Y. Incidence of disc hemorrhages in open-angle glaucoma before and after trabeculectomy. J Glaucoma. 2006 Apr;15(2):164-71.
8. De Moraes CG, Prata TS, Liebmann CA, Tello C, Ritch R, Liebmann JM. Spatially consistent, localized visual field loss before and after disc hemorrhage. Invest Ophthalmol Vis Sci. 2009 Oct;50(10):4727-33. Epub 2009 May 20.
9. Kang JH, Wiggs JL, Rosner B, Hankinson S, Abdrabou W, Fan BJ, Haines J, Pasquale L. The Relation between Endothelial Nitric Oxide Synthase Gene Variants and Primary Open-Angle Glaucoma: Interactions with Gender and Postmenopausal Hormone Use. Invest Ophthalmol Vis Sci. 2009 Oct 8. [Epub ahead of print]
10. Rosa RH, Hein TW, Yuan Z, Xu W, Pechal MI, Geraets RL, Newman JM, Kuo L. Brimonidine evokes heterogeneous vasomotor responses of retinal arterioles: diminished nitric oxide-mediated vasodilation when size goes small. Am J Physiol Heart Circ Physiol 2006;29:H231-8.
11. Kent AR et al. Vitreous concentrations of topically applied brimondine tartrate 0.2%. Ophthalmology 2001;108:784-7
12. Kent AR, King L, Bartholomew LR. Vitreous concentration of topically applied brimonidine-purite 0.15%. J Ocul Pharmacol Ther. 2006 Aug;22(4):242-6.

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OGS Honoree Lecture

Clinical Testing: New Ideas and Implications Gonioscopy & Optic Disc Assessment

BY GEORGE SPAETH, MD

One of the most important words in medicine is "valid." Related to "value," valid means it conforms to the facts. Something valid is an accurate representation of reality. Obviously, we want our observations and diagnosis to be valid. However, that is not always the case.

I recently reviewed patients who we had diagnosed in our office as having angle closure. Over half of these patients had been examined by ophthalmologists during the previous visit, who had missed the fact that the patients had angles narrow enough to close. Either they had not gonioscoped the patients at all or gonioscoped them incompetently.

These patients, then, unnecessarily developed angle closure. **Our profession should be able to do better than that.**

I am also concerned by another aspect of glaucoma care that is often neglected – documentation of the optic disc. We know from Dr. Paul Lee at Duke University that half of all glaucoma patients do not have a note in their records on the condition of their optic discs.

What are we doing about these problems? We know that the most widely-used method to describe the anterior chamber angle is the Shaffer

grading system and the most widely-used system to describe the disc is the cup/disc ratio system. Although legitimate in certain situations, these approaches have significant limitations.

I want to discuss how we can improve in both of these areas. I will also discuss two interesting studies that show just far off we can get when we do not control our biases.

Barriers to overcome

Many doctors seem to think that gonioscopy is too difficult. Because their interpretations are often not valid, they do not trust the procedure. They do not find much clinical correlation between gonioscopy results and their own findings. However, we can correct this problem by using the right approach. Several years ago, we had a fellow who wanted to develop quantitative gonioscopy, using a reticule on a gonioprism. He trained a second-year medical student who did not know anything about ophthalmology, much less gonioscopy, and within 2 weeks the student was performing valid gonioscopy. The results were corroborated by ultrasound biomicroscopy (UBM). It showed that, with the right instruction, this technique can be mastered.

I believe this is a very important priority. Angle closure glaucoma is the leading cause of irreversible blindness in the Chinese and the Intuit and the second leading cause in Africans and Europeans. We need to be able to diagnose this accurately.

Some leading glaucoma specialists tell me they do not perform indentation gonioscopy because it is misleading. Yes, it can be misleading. If you press a little too hard, you artificially deepen the anterior chamber angle, risking a dangerous misinterpretation. However, if you want to distinguish between an anterior chamber angle without peripheral anterior synechiae and a chamber with anterior synechiae, you need to use indentation gonioscopy. To perform the procedure properly, you need to use the right lens—such as a Sussman, Posner, or Zeiss—to compress the cornea and displace the aqueous.

Accurate description of the angle configuration

It is essential to remember that there are three different aspects of the angle configuration: 1) the site of the iris insertion, 2) the angularity of the approach of the angle, and 3) the curvature of the peripheral iris.

Irs insertion. Few seem to realize that the iris does not always insert in the same place, even in normal individuals. These changes have many different causes, and are racially related. For example, the normal angle of a brown-eyed, hyperopic patient usually has a relatively anterior iris insertion, so that the iris attaches to the inner wall of the eye just posterior to the scleral spur. However, in a blue-eyed patient of European descent, the iris insertion is more posterior, so it is possible to see the ciliary body. In a normal myopic patient, on the other hand, you would see even more of the ciliary body.

The anterior chamber angle also varies, depending on the curvature of the peripheral iris. The iris is not always flat. Sometimes it bows anteriorly, sometimes posteriorly. Archimede Busacca reported this in 1945. Busacca, an Argentinean writing his *Atlas of Ophthalmology* in French, described a type of curvature very carefully.

The Shaffer system is based almost entirely on one characteristic, a line that is tangential to the iris and another tangential to the inner surface of the cornea. But, what if the iris bows? Where do you draw the line? The Shaffer system does not consider the position of the iris insertion. The iris may insert anteriorly to Schwalbe's line or behind Schwalbe's line or way back in the iris recess. That would still be the same angularity, but those are clinically very different anterior chamber angles. To describe these recesses, you have to account for these differences.

Part of the problem in considering the anterior chamber "angle" is the word "angle" itself. In fact, we are considering the anterior chamber "recess," not the angle.

Evaluating the anterior chamber recess

I use the A-B-C-D-E system to document these different positions of the iris insertion. Specifically, an A insertion means that the iris is inserted Anterior to Schwalbe's line. In a B insertion the iris inserts Behind Schwalbe's line. With a C recess you can just see the scleral spur but not see the Ciliary body. A D insertion stands for a Deep angle in which it is possible to see the ciliary body. An E recess stands for an Extremely deep recess in which one can see around 1 mm of the ciliary body.

A recesses are always pathologic. They can occur in shallow or deep anterior chambers. An A insertion in a deep chamber is a classic sign of neovascular glaucoma. It is important to know that a C recess would be unusual in a myopic patient, but a D recess would not be unusual in a myopic patient.

Angularity. The second aspect of angle configuration that needs to be considered is its angularity, which can be estimated in approximately five degree increments. It has been confirmed with UBM that this is possible. Although this is not a precise measurement, it does not need to be more precise than that. The angularity decreases gradually in patients older than about two years of age. When it decreases to less than ten degrees, the incidence of angle closure rises.

The Shaffer system relates primarily to the angularity of the angle recess. However, even this is difficult to evaluate validly and reproducibly with the Shaffer system, because the iris is not always flat. When the iris bows, where does one choose to draw the line tangential to the iris?

The third aspect of the angle that must be evaluated, then, is the curvature of the peripheral iris. There are four possibilities in this regard: 1) the iris may be flat without any curvature, 2) it may bow anteriorly, 3) it may bow posteriorly, or 4) it may have a sudden bend in it. Each of these needs to be described separately. When the iris has a configuration in which there is no apparent bowing, it is designated as an f, that is, flat. A b signifies that the iris bows anteriorly, indicating that there is either some component of pupillary block, or that the iris is being pushed forward by something like the anterior surface of the crystalline lens. A c configuration is one in which there is a posterior concavity. Such a configuration is typically seen in myopes. A posterior curvature of the iris has been said to be characteristic of the pigment dispersion syndrome, but this entity can occur in the absence of posterior bowing of the iris. The symbol p is used to designate a plateau iris configuration. The plateau iris configuration is the situation in which the iris closely parallels the curvature of the peripheral cornea for less than a millimeter of its most peripheral portion, and then bends sharply to become flat, and no longer following the curvature of the cornea; thus, the peripheral-most portion of the recess is narrow (or even closed) but the anterior chamber depth is not shallow. The recess,

then, is narrow, but the angularity is not narrow. It is for this reason that a plateau iris cannot be diagnosed without gonioscopy. The anterior chamber in such cases typically looks to be of normal depth, misleading the observer into thinking that the recess is normal. Again, this plateau configuration is designated with a p.

By combining these three different, independent descriptors of the recess configuration, it is possible to characterize the recess fully and quantitatively. It is not possible to do that by using just one descriptor, as in the Shaffer or the Sheie systems. It is for this reason that those systems are not satisfactory.

The description of the anterior chamber angle configuration, then, involves putting together the three different aspects, so that the description includes the site of the iris insertion, the angular approach to the recess, and the configuration. In some cases it is not possible to see the site of the insertion because the iris bows forward anteriorly. Where that is the case then one indicates that the apparent site of the insertion is different from the real position. This is shown by putting the apparent site in parenthesis and the actual insertion not in parenthesis. Thus, in a patient in whom the angle was narrow, say ten degrees, and bowed anteriorly, it would be quite likely that it would not be possible to see past the bowing of the iris to discern the actual position of the iris insertion. If the most posterior portion of the angle that could be seen was the posterior trabecular meshwork, then that would appear to be a "B" angle. But with indentation the aqueous would be displaced into the periphery, pushing the iris posteriorly, and revealing the actual site of insertion which could be a "C," a "D," or an "E." But for this purpose we will call at the position of the anterior ciliary body a D. Thus, this angle will be called a (B)D.

Using this system of notation makes it possible to describe accurately and comprehensively the entire recess in, literally, seconds.

Gonioscopy with a gonioscopic mirror or lens also permits establishing qualitative aspects of the angle, such as the presence of a foreign body, a recession or a hyphema. It allows noticing complications such as vitreous or iris into the sclerectomy or a retained piece of lens nucleus in the inferior recess. Furthermore, it allows quantitation of the amount and type of pigmentation, characteristics which are frequently the key to an accurate diagnosis in conditions such as the pigment dispersion syndrome, siderosis, melanoma, and the exfoliation syndrome.

"Machine" gonioscopy

None of the instruments presently available for performing evaluation of the anterior chamber angle allow accurate recognition of certain characteristics of the recess which are essential to a correct diagnosis, such as the qualitative aspects just mentioned. Furthermore, recognition of the posterior trabecular meshwork's exact position is often difficult or impossible, making clinical correlation difficult or impossible. For these reasons, gonioscopy performed by a trained observer using a gonio lens or a gonio mirror will remain the standard of care for the foreseeable future.

Machines have definite advantages. In the first place they can be operated by a technician. Furthermore, they can obtain data using bright or dim or virtually no visible illumination. This is an important consideration. The size of the pupil and the state of accommodation affect the recess configuration. It is for this reason that, when performing gonioscopy at the slit lamp in patients with narrow recesses, it is essential to use a small beam directed into the recess, so it does not shine through the pupil. In certain situations it may even be important to have the room darkened.

The advantage of anterior segment optical coherence tomographic evaluation of the angle is the lack of need for any topical anesthetic, and the non-invasive nature of the procedure. However, it is not always easy to visualize the entire anterior chamber angle, especially superiorly, as the lid may make the visualization of this area difficult or impossible. It should be remembered that the position of the patient during gonioscopy will affect the configuration of the recess. Thus, recesses examined with Koeppe lenses will frequently appear significantly deeper than those evaluated at the slit lamp with indirect gonioscopy, because the former is performed with the patient supine and the latter with the patient sitting. So it is with ultrasound biomicroscopy, which is performed with the patient in a supine position, in contrast to anterior segment OCT. UBM also has the disadvantage that it requires placing a weight on the cornea which has risks in certain situations, and is likely to distort angle configuration from its unindented state.

For the reasons just mentioned, gonioscopy with a machine is appropriately considered an ancillary technique, and is not a standard of care.

In summary, regarding gonioscopy, gonioscopy is an essential part of a comprehensive evaluation of a patient's ocular health. Performed properly, the technique using a gonioscopic lens or mirror provides valid, clinically-useful information that can be obtained quickly and safely. There are excellent references to consult, including the online material prepared by Alward of the University of Iowa, www.gonioscopy.org.

Evaluating the optic disc

Most ophthalmologists today use the cup/disc ratio system as the method of estimating whether a disc is healthy or abnormal. However, the cup/disc ratio system has such serious flaws that it should no longer be used for this purpose. The two major flaws are that the cup/disc ratio does not consider the position of the cup or the size of the disc.

It is the neuroretinal rim that correlates with the health of the optic nerve. The size of the cup is a function of the size of the disc and the width of the rim. Whether cups are big or little is of little direct importance. On the other hand, the width of the rim is a measure of the amount of neuroretinal rim tissue and is enormously important and closely related to the presence or absence of visual field defects. Thus, it is the rim that needs to be considered. Rather than estimating the cup/disc ratio, it is appropriate to estimate the rim/disc ratio.

Consider two patients, both of whom have cup/disc ratios of 0.5. In one patient the cup is totally concentric with the disc, so that the rim/disc ratio is 0.25 in all areas. In the other eye, the cup is eccentrically placed so that in one area, the rim/disc ratio is 0.5 and in another area the rim/disc ratio is 0. The first disc will definitely not have a visual field defect. The second disc, with a rim/disc ratio of 0, will always have a visual field defect. This illustrates that even though discs can have the same cup/disc ratio, one can be completely healthy and the other absolutely pathologic. Cup/disc ratios do not take into account the position of the cup, and therefore are frequently invalid representations of whether the disc is healthy or sick.

The second problem relates to the size of the disc. The number of neurons passing through the disc is relatively standard. The cross-

sectional area of the rim is fairly well associated with the number of neurons. In a small disc, less than 1.5 millimeters in diameter, the neurons typically fill up the entire small space through which they are exiting. However, in a large disc with a diameter of greater than 2 millimeters, the cross-section is exponentially larger than in the small disc, so they do not fill up the space. Thus, small discs tend to have small cups normally, and big discs tend to have big cups. In order to assess the healthiness of a cup or a rim, then, requires knowing the size of the optic disc.

Recognizing that the width of the rim corrected for disc size correlated with the number of nerve fibers running through the disc, a system was devised to stage optic discs, based on the width of the rim and the size of the disc. This is called the Disc Damage Likelihood Scale (DDLS). The system is easily learned and is valid, that is the stage of the disc, from 1 to 10, correlates well with the amount of visual field loss. The figure demonstrates this system, indicating how the rim/disc ratio is used to stage the health of the nerve.

The necessary steps are to determine the size of the optic disc by any standard method, to measure the rim/disc ratio, that is the width of the rim in comparison to the diameter of the disc, or in most cases where rim is absent, the circumferential extent of rim absence. The figure below shows examples of small discs, average-sized discs and large discs, to highlight the importance of considering disc size. Where the rim/disc ratio is approximately 0.3, the patient has a stage 2 DDLS if the disc is of average size. However, a 0.3 rim/disc ratio in a large disc would be a stage 1, and in a small disc a stage 3. Thus, the DDLS is calculated for the average-sized disc and then one stage subtracted for discs which are larger than 2.0 millimeters in diameter, and one stage is added for discs which are smaller than 1.5 millimeters in diameter. As long as there is any rim present, an average-sized disc has a DDLS of 5 or smaller. Visual field defects are not present when a DDLS is a 4 or less. In most patients in whom the DDLS is a 5, that is where the rim/disc ratio is less than 0.1 in an average-sized disc, a field defect will be present. When there is no rim in an average-sized disc, a visual field defect is always present. If the circumferential extent of rim absence is less than 45 degrees in an average-sized disc, that is a stage 6. In a large disc that would be a stage 5; and in a small disc that would be a stage 7.

One further example in this regard to stress the importance of disc size – if a patient had a rim/disc ratio between 0.1 and 0.2, in an average-sized disc that would be graded as a stage 4. There would be no visual field loss present. However, if a small disc had a rim/disc ratio between 0.1 and 0.2 that would not be a stage 4 but rather would be a stage 5. In such cases the visual field defect would almost always be present. In contrast, in a large disc with a rim/disc ratio between .1 and .2, the disc would be appropriately staged as a grade 3 DDLS, not a grade 4. As such, there would never be a visual field defect present. This points out that with the same rim/disc ratio in some cases there will almost always be a visual field defect present, and others with exactly the same rim/disc ratio there will never be a visual field defect present. Cup/disc ratios will not provide that type of information. In the examples given, assuming that the cup is concentric, then all three discs of the cup/disc ratio will be 0.7. However, in the small disc with a concentric cup which is 0.7, a visual field defect will always be present, and in a large disc with a cup/disc ratio of 0.7, a visual field defect will never be present. Until this is well understood, it is not possible to evaluate discs validly.

Many reports have confirmed the validity of the DDLS system and its superiority to cup/disc ratios. Myers, at Wills Eye Institute, compared the DDLS to OCT III and Heidelberg Retinal Tomography III in a prospective study of 76 patients with open-angle glaucoma compared to 26 normal controls. He found that the DDLS was as accurate or more accurate than the OCT and HRT in distinguishing between patients who had glaucoma and those who did not, based on the sensitivity and specificity and the development of a receiver operating curve. Others have also reported similar findings.

THE DISC DAMAGE LIKELIHOOD SCALE

	Narrowest width of rim (rim/disc ratio)				Examples		
DDLS Stage	For Small Disc <1.50 mm	For Average Size Disc 1.50-2.00 mm	For Large Disc >2.00 mm	DDLS Stage	1.25 mm optic nerve	1.75 mm optic nerve	2.25 mm optic nerve
1	.5 or more	.4 or more	.3 or more	0a			
2	.4 to .49	.3 to .39	.2 to .29	0b			
3	.3 to .39	.2 to .29	.1 to .19	1			
4	.2 to .29	.1 to .19	less than .1	2			
5	.1 to .19	less than .1	0 for less than 45°	3			
6	less than .1	0 for less than 45°	0 for 46° to 90°	4			
7	0 for less than 45°	0 for 46° to 90°	0 for 91° to 180°	5			
8	0 for 46° to 90°	0 for 91° to 180°	0 for 181° to 270°	6			
9	0 for 91° to 180°	0 for 181° to 270°	0 for more than 270°	7a			
10	0 for more than 180°	0 for more than 270°		7b			

The Disc Damage Likelihood Scale (DDLS) can be used to stage the optic disc based on three ranges of disc size and three ranges of optic nerve size.

The importance of minimizing bias

Finally I want to stress the importance of acknowledging the impact of bias on our clinical decisions. Many studies will indicate how bias affects the decisions we make. In reality, however, if we are in any way thoughtful, we do not need such studies to convince us of the fact that bias distorts our opinions.

In one study three readers were given 100 disc photographs and asked to determine, based on the appearance of the disc photograph alone, whether the patient had glaucoma or not. Of those 100 photographs, 50 photographs showed the optic discs of patients who had mild or moderate glaucoma and the other 50 the discs of patients who as best as could be determined did not have glaucoma. When the readers were shown the disc photographs, with each photograph there was an accompanying visual field, but the readers were told not to use the visual field as the basis for deciding whether or not the patients had glaucoma, and to make such a decision based solely upon their interpretation of the optic disc. What the readers did not know is that the visual fields that accompanied the disc photographs were not always the visual fields from the patient whose disc photograph was being shown. In some cases with glaucoma the accompanying field was normal, and in some patients who had no glaucoma the accompanying visual field was abnormal. The readers called discs glaucomatous more than twice as often when the disc photograph was accompanied by an abnormal field, and less than half as often when the photograph was accompanied by a normal visual field.

That is, the readers were four times as likely to call a disc glaucomatous when they thought the field was abnormal as when they thought the field was normal. Remember – they were making this decision allegedly solely based on the appearance of the optic disc.

In the second study three readers were given 250 pairs of disc photographs taken from 250 different patients. They were asked to compare photograph A with photograph B of these 250 patients, and to conclude whether photograph B was better, the same, or worse than photograph A. The same readers were then given the same photographs, though in different order, and asked to go through the same procedure. However, at the first reading the readers had been told that the B photographs had been taken after the A photographs had been taken, chronologically. At the second reading the readers were told that the order of the photographs was random, and that in some cases the A photographs may have been taken first, and in some cases the A photographs may have been taken second chronologically. Remember, however, that this was not in actuality the case. In all cases, both of the first reading and the second reading, the order of the photographs was the same. Nevertheless, the readers were 1.5 times more likely to believe a disc had gotten worse when they thought that the second photograph of a pair was obtained later in the course of the disease, and they were half as likely to think the discs had gotten worse when they did not know the order. Thus, the observers were three times as likely to interpret a disc as having worsened, merely based on knowing the order of the slides they were reviewing.

The results of these studies are deeply disturbing. They show that our standard way of evaluating clinical information is seriously flawed. We are all told to make sure that we consider all the ancillary information before we come to a conclusion. But when we do so, we inevitably bias ourselves and make it literally impossible to be sure that the conclusions we are making are valid. It is well established that when clinical research trials are performed that observations are obtained in a way which limits bias as much as possible. Nevertheless, we also know that exactly the opposite characterizes the way we care for patients. In fact, many of those who are asked to interpret data, such as CT scan, or a disc photograph, will refuse to do so unless there is accompanying clinical material. We force ourselves into being biased the way we are presently practicing.

Conclusions

The validity of the data that we use and the conclusions we reach strongly determines the quality of the care that we provide. At present we are doing a bad job. Regarding gonioscopy, patients are either not examined, or if they are examined they are usually examined by a system which is not valid. Regarding disc evaluation, patients are frequently not examined, and when they are the health of their disc is evaluated using a system which is not valid. When we consider data we do not mask ourselves and we do not limit our biases, therefore frequently coming to incorrect conclusions. If we have a serious interest in providing good care, we need to change our practices. We need to gonioscope patients and use a valid gonioscopic technique. We need to examine the disc and use a valid method of establishing the disc nature, such as the Disc Damage Likelihood Scale. We need to do everything we can to limit our biases.

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About the Optometric Glaucoma Society

The Optometric Glaucoma Society (OGS) was formed nine years ago to promote excellence in the care of patients with glaucoma through professional education and scientific investigation. The major objectives are to:

- Promote education of the membership and other health-care providers related to all forms of glaucoma
- Promote the acquisition of new knowledge about glaucoma, in part through the development of clinical research within optometry that is related to glaucoma
- Facilitate the dissemination of information about glaucoma to health-care providers and the public
- Establish collaborative relationships with other related organizations.

The group photograph from the 2009 Optometric Glaucoma Society Annual Meeting, Orlando, Fl.

The OGS has 89 members, coming from several countries around the world. The organization is equally divided between clinicians and scientists, with ODs, PhDs, and MDs making up our membership. The OGS is a member of the World Glaucoma Association (WGA). Six members are on the faculty of the 2009 World Glaucoma Congress.

Additional information, including membership information and the application for membership, may be found on the OGS Web site at www.optometric-glaucomasociety.org. The OGS is involved in several programs related to improving optometric glaucoma education. We publish a quarterly electronic journal that is free of charge and available to anyone who wishes to subscribe. Individuals may sign up at the OGS Web site. We also publish an annual glaucoma handbook that is a review of glaucoma diagnosis and management. This is also available for free and distributed to 35,000 optometrists. Also, the OGS holds a glaucoma residents program each fall. One resident from each school participates. And, finally, the OGS in collaboration with the American Optometric Foundation funds an Ezell Fellowship in Glaucoma, which is intended to enhance the opportunities for post-graduate optometric glaucoma research.

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