NANOS/AGS Collaborative Session
Glaucoma: The Other Optic Neuropathy

NANOS 2015 Annual Meeting
February 26, 2015
Hotel del Coronado | San Diego, CA
This symposium is a combined program of AGS (American Glaucoma Society) and NANOS aiming to clarify the relationship between glaucoma and other optic neuropathies.

The structure of the symposium is divided into 3 sections: 1) Establishing whether there is a difference between high tension glaucoma and normal tension glaucoma. This section will explore the contribution of intraocular pressure to glaucoma as well other factors that might be contributory; 2) Differentiating glaucoma from other optic neuropathies and deciding whom to image with MRI; and 3) Looking at glaucoma as a neurodegenerative disorder and reviewing issues of neuroprotection and neuroregeneration.

Upon completion of this course, participants should be able to: 1) Identify important contributions to glaucomatous damage other than intraocular pressure; 2) Identify which glaucoma patients should be evaluated for other causes of optic neuropathy; and 3) Identify areas of research ripe for collaboration between glaucoma and neuro-ophthalmologic researchers.
9:05 a.m. – 9:13 a.m. Is Normal Tension Different than High Tension Glaucoma: Other Possible Factors

Martin B. Wax, MD

9:13 a.m. – 9:29 a.m. Pro and Con Debate: We Should Eliminate the Normal Tension in Glaucoma

Pro: Robert N. Weinreb, MD; Con: Robert Ritch, MD

9:29 a.m. – 9:44 a.m. Discussion

9:44 a.m. – 10:14 a.m. Break

10:14 a.m. – 11:15 a.m. Clinical Differences Between Glaucoma and Other Optic Neuropathies

10:14 a.m. – 10:22 a.m. The Morphological Difference Between Glaucoma and Other Optic Neuropathies

Claude Burgoyne, MD

10:22 a.m. – 11:15 a.m. Case Presentations

Helen V. Danesh-Meyer, MD, PhD, FRANZCO and Mark L. Moster, MD

Panel: Peter Savino, MD, Valérie Biousse, MD, Anne L. Coleman, MD, PhD, and Richard Lee, MD, PhD

11:15 a.m. – 12:32 p.m. The Neurology of Glaucoma

11:15 a.m. – 11:23 a.m. Glaucoma as a Neurological Disease

Helen V. Danesh-Meyer, MD, PhD, FRANZCO

11:23 a.m. – 11:31 a.m. The Brain in Glaucomatous Optic Neuropathy: Evidence for Trophic-Factor Mediated Self-Repair

David J. Calkins, PhD

11:31 a.m. – 11:39 a.m. What are the Common Neurodegenerative Pathways Relevant to Glaucoma?

Stuart J. McKinnon, MD, PhD

11:39 a.m. – 11:47 a.m. Neuro-Protection in Glaucoma: Where are We Going?

Leonard A. Levin, MD, PhD

11:47 a.m. – 11:55 a.m. Neuro-Regeneration for Glaucoma and other Optic Neuropathies

Jeffrey Goldberg, MD, PhD

11:55 a.m. – 12:03 p.m. Mitochondrial Disease and Glaucoma

Alfredo A. Sadun, MD, PhD

12:03 p.m. – 12:19 p.m. Debate: Non-IOP Lowering Therapies will be the Future of Glaucoma Management.

Pro: Harry Quigley, MD

Con: Christopher A. Girkin, MD, MSPH, FACS

12:19 p.m. – 12:32 p.m. Q & A

2:00 P.M. – 5:30 P.M. AGS/NANOS AFTERNOON SESSION

AGS will lead the afternoon collaboration with NANOS. The scientific session will feature the following topics: 1) The Pathogenesis of Optic Neuropathy: Glaucoma vs. The Rest and; 2) Optic Nerve Imaging: New Parameters and Techniques, a platform presentation session, poster viewing and an afternoon break.

**Please note that the CME credits for this session will be provided through AGS.**
LEARNING OBJECTIVES

1. Describe clinical aspects of glaucoma
2. List functional deficits associated with glaucoma
3. Define aspects of glaucoma pathology

CME QUESTIONS

1. By the time glaucomatous optic neuropathy can be diagnosed with currently available clinical technologies, approximately what percentage of retinal ganglion cells will have been lost?
   a. 5-10%
   b. 10-20%
   c. 25-35%
   d. >50%
   e. None of the above

2. How many people are affected by glaucoma worldwide?
   a. 1 million
   b. 10 million
   c. 50 million
   d. 70 million

3. The earliest clinically detectable sign of glaucomatous optic neuropathy is presently
   a. Thinning of the RNFL as measured by OCT
   b. An arcuate scotoma demonstrated by SAP
   c. Characteristic cupping of the ONH
   d. Characteristic alterations of the electroretinogram
   e. In a state of flux

KEYWORDS

1. Glaucoma
2. Optic Nerve
3. Retinal Ganglion Cells
4. Trabecular Meshwork
5. Retinal Nerve Fiber Layer

INTRODUCTION

The glaucomas are a collection of progressive optic neuropathies that can lead to irreversible damage to retinal ganglion cells (RGC) and their axons and eventual loss of vision if inadequately treated. In the early stages the disease is largely asymptomatic and it is estimated that only half of glaucoma patients are aware that they have the disease. Clinically, glaucoma patients show characteristic optic nerve head (ONH) and retinal nerve fiber layer (RNFL) changes. These include thinning of the neuroretinal rim, increased cup-to-disk ratio, peripapillary atrophy and attenuated RNFL (Figure 1). Visual field deficits, especially in the periphery, are also a hallmark of glaucoma presentation. The clinical utility of imaging devices is growing as the technology rapidly evolves. Instruments are faster, resolution is increasing, computational algorithms are more refined and normative databases are expanding to include a wider range of patients. A current trend in glaucoma clinical care is the development of metrics that combine information from multiple testing modalities.\textsuperscript{13, 14, 15} Pathologic changes noted in glaucoma include ONH, RNFL and macular (ganglion cell-inner plexiform layer) deficits as well as atrophy of the lateral geniculate nucleus (LGN) and visual cortex. A potential biomarker for glaucoma involves imaging apoptosis of RGCs. Glaucoma is a disease with multifactorial mechanisms. Advances in diagnostic instrumentation and out understanding of the pathology of the disease will benefit patients. An individualized treatment approach will result in improved patient outcomes.

Indeed, in aggregate the glaucomas are the most common optic neuropathy, affecting ~70 million people worldwide.\textsuperscript{1} The number of people (aged 40-80 years) with glaucoma globally is estimated to increase to 76 million by 2020 and 112 million by 2040.\textsuperscript{2} Three-quarters
of these have primary open-angle glaucoma (POAG) and women are affected more than men (55% of POAG, 70% of primary angle closure glaucoma (PACG), and 59% of all glaucoma).

The prevalence of POAG is highest in Africa, while the prevalence of PACG is highest in Asia. People of Japanese ancestry have a higher incidence of normal-tension glaucoma (NTG), in which damage occurs to the optic nerve without intraocular pressure (IOP) exceeding the statistically normal range. (generally between 10-20 mm Hg). Of concern is that while glaucoma prevalence is increasing globally, in resource poor areas access to ophthalmologists is severely limited.

It is estimated that in sub-Saharan Africa there are less than 3 ophthalmologists per million people while there are approximately 79 ophthalmologists per million in high-income countries. The major risk factors for POAG include older age, Black or Latino race, higher IOP and lesser central corneal thickness (CCT). In the early stages the disease is largely asymptomatic, so many people are unaware they have glaucoma until significant loss of vision occurs. It is estimated that only half of glaucoma patients are aware that they have the disease. Vision deficits in glaucoma patients are associated with worse on-road driving performance and an increased risk of falls.

Stereo fundus photos, long the gold standard for assessment of a patient’s ONH morphology, have been a key part of glaucoma diagnosis and evaluation of progression. These photos reveal much about the condition of the eye but grading is subjective and inter observer variability can be high. Techniques using computer assisted digital analysis of the ONH have been developed to improve intra and inter observer reproducibility.

There are several different visual field tests: standard automated perimetry (SAP), Swedish interactive threshold algorithm (SITA), frequency-doubling technology (FDT), matrix frequency-doubling technology, short-wavelength automated perimetry (SWAP), and high-pass resolution perimetry (HPRP). These tests provide valuable information about a patient’s visual function and are required in clinical trials for glaucoma therapeutics. The FDA requires functional testing in clinical trials in an effort to provide patients with drugs that offer meaningful changes in, or preservation of, visual function. Critics of visual field tests point to their subjectivity and the patient learning effects that make evaluation of disease progression difficult.

Imaging instruments, such as confocal scanning laser ophthalmoscopy (HRT), scanning laser polarimetry (GDx), and optical coherence tomography (OCT), can provide objective quantitative measures of the optic disc, retinal nerve fiber layer (RNFL) and macula. Confirming structural damage can help in the initial glaucoma diagnosis but a critical aspect of glaucoma management is follow-up. Imaging systems offer the prospect of detecting progression early so that changes to a patient’s treatment plan can be made to prevent visual impairment. Imaging tests also provide more objective measurements of glaucoma-relevant structures and can be compared from visit to visit but are not necessarily diagnostic themselves. They are intended to provide information that can be used in conjunction with clinical examinations and visual fields.

Positive correlations exist between functional data from visual fields tests and structural data from imaging devices such as optical coherence tomography (OCT). RNFL thinning measured by OCT is associated with visual field loss. Additionally, OCT studies demonstrate that thinning in the macular layers (ganglion cell and inner plexiform layers) also occurs in glaucoma, can occur early in the disease and

Fig 1: Normal optic nerve (A and C). Glaucomatous optic nerve (B and D) with characteristic changes including 1) Thinning of the neuroretinal rim, 2) Increased cup-to-disk ratio, 3) Vertical elongation of the cup, 4) Pitting or notching of the rim, 5) Quick angulations in the course of the exiting blood vessels, 6) Wedge-shaped dark areas - retinal nerve fiber layer damage, 7) Undermined disk margins, 8) Peripapillary atrophy, 9) Attenuated nerve fiber layer. Panels A and B with permission from Atlas of Ophthalmology; panels C and D courtesy of Dr Morton Smith, Washington University.
is also associated with visual field loss. A recent study indicates that a combined thickness value derived from the RNFL, ganglion cell layer (GCL), and inner plexiform layer (IPL) is the most useful parameter to evaluate structure and function within the central 10° in glaucoma. There is a school of thought that imaging provides better information early in the disease while visual fields are better later. Metrics that include data from both may be the most useful at most stages.

The effect of glaucoma on the inner and outer retina and macula has also been documented by studies using electrophysiology tests, notably the photopic negative response of the multifocal electroretinogram (mERG), the full-field photopic negative response (PhNR) and pattern ERGs (PERG). Electrophysiology testing has some advantage over visual field testing since it is less subjective and not as dependent upon patient “performance”. Newer iterations of electrophysiology instruments have improved discriminatory ability but are not as commonly available as perimetric instruments and are not as widely used. In experimental glaucoma models, electrophysiology deficits correlate with decreases in RNFL and both inner and outer retinal thickness. Highlighting the benefit of experimental models, the structural changes can be confirmed by histology. Of importance in evaluating structure/function data is that substantial structural loss occurs before functional loss is detected using existing visual field methods as has been reported by several groups. Comparing the number of RGCs topographically mapped to specific test points in the visual field in the same eyes among glaucoma patients, a 25% to 35% RGC loss was associated with statistical abnormalities in automated visual field testing. In an observational cohort study estimates of RGC numbers were made in eyes converting to glaucoma versus healthy eyes using a model that combines SAP sensitivity thresholds and retinal nerve fiber layer (RNFL) thickness measurements with spectral domain optical coherence tomography (SD-OCT). Compared with the average number of RGCs in the healthy group, glaucomatous eyes had an average RGC loss of 28.4%. In a prospective cross-sectional study using automated perimetry (Humphrey) field analyser and spectral domain OCT (Cirrus) HD-OCT, a structural loss of ~17% was necessary for functional loss to be detectable. Also, structure/function and structure/function relationships can change with age as well as glaucoma status.

Pathologic changes noted in glaucoma include the ONH, RNFL and macular (ganglion cell-inner plexiform layer) deficits described above as well as atrophy of the lateral geniculate nucleus (LGN) and visual cortex, which includes layer shrinkage and reduced neuron size and numbers. Experimental models of glaucoma have established the correlation between axon size, number of remaining axons and LGN atrophy. LGN atrophy may have potential as a biomarker of visual system injury or glaucoma progression in some patients, especially those with media clarity issues.

Another potential biomarker for glaucoma involves imaging apoptosis of RGCs. Several systems are in development, hoping to identify early cellular degeneration; perhaps even before permanent vision loss occurs. One system uses a wide-angle confocal laser scanning ophthalmoscope (cLSO) and fluorescently labeled annexin V to non-invasively visualize single retinal cells undergoing apoptosis in vivo. This has been given the acronym DARC (Detection of Apoptosing Retinal Cells). A series of in vivo studies using experimental models has been performed using DARC technology to evaluate RGC apoptosis. A second system in development for serial, non-invasive imaging of apoptosis is TcapQ488, which uses a cell-penetrating caspase-activatable peptide probe. Highly specific uptake by RGCs was noted following intravitreal injection of fluorophores conjugated to a modified cell-penetrating peptide sequence. Subsequent localization of apoptosing cells using retinal flat mounts from a rat model of NMDA-induced RGC degeneration helped validate the technology. The results from both imaging systems demonstrate the potential of this type of technique; clinically for direct assessment of retinal ganglion cell health in neurodegenerative diseases such as glaucoma and Alzheimers, but also in clinical trials to provide an assessment of the potential neuroprotective effects of novel drug candidates and their therapeutic efficacy. The development of a novel clinical endpoint would help fill an unmet need in glaucoma research and the development of therapeutics.

Advances in imaging instrumentation have facilitated the development of new models aimed at understanding the etiology of glaucomatous pathologic changes in the laminar cribrosa and clinical correlates of those changes. Trabecular meshwork (TM) changes in glaucoma are well documented histologically. Imaging technologies are being developed to quantify TM change serially and non-invasively, aiming to develop metrics for what constitutes meaningful change in glaucoma.

Glucoma is a disease with multifactorial mechanisms. Advances in diagnostic instrumentation and out understanding of the pathology of the disease will benefit patients. An individualized treatment approach will result in improved patient outcomes.

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C, consultant; R, honoraria, F, financial support; P, patent.

**CME ANSWERS**

1. c
2. d
3. a or e

**REFERENCES**


LEARNING OBJECTIVES

1. Describe the evolving role of IOP in relation to primary open-angle glaucoma

2. Measure the relative strength of IOP as a risk factor for glaucoma development and progression

CME QUESTIONS

1. The size of the general population with intraocular pressure of 21 mmHg or less is greater than the population with intraocular pressure 22 mmHg or higher by how much?
   a. 3 to 4 times
   b. 6 to 8 times
   c. 10 to 12 times
   d. 15 to 17

2. According to data from the Early Manifest Glaucoma Trial, every mmHg higher IOP corresponds to higher risk of glaucoma progression at what level:
   a. 0%
   b. 3%
   c. 11%
   d. 16%

3. True/False: The absolute risk reduction for developing glaucoma achieved with treatment of elevated IOP in ocular hypertension is of the same magnitude as the absolute risk reduction of a cardiovascular event achieved with treatment with statin therapy.

KEYWORDS

1. Intraocular Pressure
2. Primary Open-angle Glaucoma
3. Risk Factor
4. IOP Variability
5. Glaucoma Progression

INTRODUCTION

That IOP is intricately linked with primary open-angle glaucoma is well established. A historical perspective on the evolving role of IOP as synonymous with glaucoma to being a risk factor for glaucoma is explored. The predictive ability of IOP as a risk factor for POAG is calculated and compared with risk factors for cardiovascular disease, a disease for which there exists considerable data.

That intraocular pressure (IOP) and primary open-angle glaucoma (POAG) are intricately linked is well established. Yet, the IOP-POAG relationship has arguably been the subject of more controversy than any other topic in glaucoma, and our understanding of it continues to evolve.

Several decades ago, IOP, specifically 21 mmHg, defined glaucoma. Subsequent observations that some patients continue to lose vision despite well-controlled IOP has led to the assertion that IOP independent mechanisms may play a role in the glaucomatous process. Recent research on optic nerve related factors that are potentially involved in the glaucomatous process lends further credence to this view. As our knowledge of pathogenic mechanisms in glaucoma advances, IOP will undoubtedly be considered to play a less prominent role than in the past. Thus, in addition to IOP-lowering strategies, novel therapies to protect retinal and central visual system neurons are increasingly being investigated.

IOP had historically been considered the primary therapeutic endpoint. It has been argued that IOP is not in itself the primary variable of interest but should rather be considered as an intermediary factor that may affect the endpoint. Glaucoma comprises a continuum of disease states from undetectable disease, characterized by ganglion cell death and axonal loss at one end of the spectrum, to total loss of sight at the other end. Since the outcome of importance to the patient is symptomatic disease, particularly blindness, visual functioning is beginning to supplant IOP as the primary indicator of success or failure of treatment.

Despite shifting perspectives on IOP, what remains incontrovertible is that IOP is the most important risk factor for glaucoma development and progression. Population-based studies have repeatedly and consistently documented that elevated IOP is associated with higher prevalence and incidence of POAG that progressively higher IOP is
associated with progressively greater risk of POAG\textsuperscript{7}, and that elevated IOP is associated with greater progression of POAG\textsuperscript{8}.

The fact that a substantial proportion of subjects with POAG have initial IOPs below 21 mmHg does not diminish the strength of this IOP-POAG relationship. The size of the general population with IOPs less than or equal to 21 mmHg is ten to twelve times the size of the population with IOPs greater than 21 mmHg\textsuperscript{9}. Because of the sheer size of the at-risk population with IOPs in this lower range, this group can be expected to have a considerable number of subjects with glaucoma. However, according to data from the Baltimore Eye Survey, the relative risk of having glaucoma in eyes with higher IOPs (22 mmHg or higher) was 8.6 times that of eyes with lower IOPs (21 mmHg or lower)\textsuperscript{9}.

A causal relationship between IOP and glaucoma would be strengthened if incidence data were used rather than prevalence data. Unfortunately, only a few population-based incidence studies have been attempted due to the large cohorts and long follow-up periods necessary to obtain a sufficient number of newly diagnosed cases to ensure valid estimates. The Rotterdam Eye Study found the risk of developing POAG increased by 16\% per 1 mmHg in highest baseline IOP; the risk of developing glaucoma was three times higher if baseline IOP was greater than 21 mmHg\textsuperscript{10}. The Barbados Eye Study also found baseline IOP to be a significant risk factor for subsequent development of POAG\textsuperscript{11}. Causal inference is also strengthened considerably when a dose-response relationship can be demonstrated. The Collaborative Glaucoma Study demonstrated that among subjects with baseline IOP of less than 16, 0.8 percent subsequently developed glaucoma; the corresponding percentages for those with baseline IOPs of 16-19 mmHG, 20-23 mmHg, and 24 mmHg and higher were 1.4, 3.1, and 8.4\textsuperscript{12}.

Elevated IOP is not only a glaucoma risk factor but also a prognostic factor for glaucoma progression. In fact, this assumed relationship between elevated IOP and glaucoma progression has been the underpinning upon which glaucoma treatment has historically been based. A number of studies, most notably the Early Manifest Glaucoma Trial (EMGT)\textsuperscript{13}, have provided scientific validation. The EMGT followed a cohort of patients with early glaucoma in a clinical trial in which patients were randomized to IOP lowering treatment or no treatment. One finding from the study was that each higher millimeter of mercury of IOP on follow-up was associated with an approximate 10\% increased risk of glaucoma progression. That every millimeter of mercury of IOP lowering reduces the risk of glaucoma progression by some specific percentage amount that is quantifiable over the entire spectrum of IOPs has been called into question by Wilson and Singh\textsuperscript{14}. Nonetheless, that there is an association between elevated IOP and glaucoma progression is not contested and is confirmed by other studies.

Because IOP is the only known risk factor amenable to modification, it can be argued that it is the most important risk factor for POAG. One way to assess the strength of a risk factor is to calculate the treatment effect using measures such as Absolute Risk Reduction (ARR) and Relative Risk Reduction (RRR). Absolute risk reduction is the absolute difference in outcome between the control and treatment groups: untreated group disease risk minus the treatment group disease risk. Relative risk reduction measures how much the risk is reduced in the treatment group compared with the untreated group. It is measured as one minus the relative risk, in which relative risk equals the treated group disease risk (numerator) over the untreated group disease risk (denominator). Both absolute risk and relative risk measures have their advantages and disadvantages; in both, the higher the number the more effective the treatment.

Risk calculations have routinely been used with cardiac risk factors for predicting cardiac events. Although the epidemiology of POAG is not nearly as advanced as that of cardiovascular disease, some useful lessons can be learned from considering assessment and prevention strategies developed for coronary heart disease. For example, reduction of plasma low-density lipoprotein cholesterol levels has long been a therapeutic goal to prevent coronary mortality and morbidity, and a clinical trial evaluated the three year risk of a cardiovascular event with or without statin therapy\textsuperscript{15}. Using data from recently completed glaucoma clinical trials, the five year risk of glaucoma development (Ocular Hypertension Treatment Study)\textsuperscript{16} and the six year risk of glaucoma progression (Early Manifest Glaucoma Trial)\textsuperscript{13} with and without treatment can be calculated and compared with the statin therapy trial.

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<td>Statin trial (3 yr)</td>
<td>12.5%</td>
<td>49%</td>
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<td>OHTS (5 yr)</td>
<td>5.1%</td>
<td>54%</td>
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<td>EMGT (6 yr)</td>
<td>17%</td>
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Because of the different time lines, a direct comparison is difficult. However, it is clear that the greatest reduction in risk is with the statins in cardiovascular disease. Over a three year period, for every 100 subjects who received statin in the trial, 12.5% averted a bad cardiovascular event (ARR). Also, a cardiovascular event was reduced by 49% in the statin group compared to the control group (RRR). Nonetheless, the benefit from reduction of IOP is substantial for both the development of glaucoma and progression of glaucoma. Over a six year period, for every 100 subjects who received IOP lowering treatment, 17% had progression of glaucoma averted; over a five year period, the development of glaucoma was reduced by 54% in the IOP lowered treatment group versus non-treatment group.

A number of questions regarding the IOP-POAG relationship remain unanswered. Among these is the age-specific trend of intraocular pressure among the Japanese population, in which a very low prevalence of ocular hypertension and very high prevalence of POAG with IOPs in normal range are noted. Most American and European population-based studies that measured IOP have demonstrated an increasing IOP with increasing age. Though the magnitude of the IOP increase with age is small, this positive IOP-age correlation is at least consistent with the positive POAG-IOP correlation noted universally. However, several population-based studies have shown a negative correlation between IOP and age among the Japanese. Population differences in potentially confounding factors such as blood pressure and body mass index have been postulated, but reasons for this peculiarity are not well understood. This suggests that there are other factors at play in the glaucomatous process besides IOP and that these other factors may be relatively more important than IOP in causing glaucoma in elderly Japanese.

From initially defining glaucoma to defining glaucoma treatment success to being an intermediate factor for vision-related glaucoma endpoints, IOP’s role in glaucoma has become increasingly limited. The large numbers of patients with POAG and normal IOP, particularly among the Japanese, have even called into question the primacy of IOP in the pathogenesis of glaucoma. Focus on other factors such as decreased ocular perfusion pressure and ischemia, excitotoxicity, neurotrophic factor deprivation, oxidative stress, immune modulation, and exposures to potential biomarkers such as nitric oxide and endothelin have increased over recent years. Undoubtedly, the actual mechanism of glaucomatous damage is multifactorial and IOP is likely only one of many contributory factors.

Yet, IOP is unequivocally an important risk factor for the development of glaucoma and it is strongly prognostic of glaucoma progression. Lowering of IOP currently remains the only therapeutic option for mitigating the visual deficits associated with advancing disease, and the effect of treatment is substantial. Though diminished, IOP’s role in glaucoma is firmly established and the two will forever be inextricably linked.

CME ANSWERS
1. c. 0 to 12 times
2. c. 11%
3. b. False

REFERENCES


PHENOTYPIC DIFFERENCES IN NORMAL VS HIGH TENSION GLAUCOMA

Jonathan S. Myers, MD
Wills Eye Hospital
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LEARNING OBJECTIVES

1. Describe differences between normal tension glaucoma and primary open angle glaucoma in optic nerve and visual field findings
2. Cite studies reporting on optic nerve and visual field changes in normal tension and primary open angle glaucoma
3. Define the broad overlap in findings in most patients with normal tension glaucoma and primary open angle glaucoma

CME QUESTIONS

1. Focal Notching of the optic nerve is
   a. Always associated with normal tension glaucoma
   b. More often associated with primary open angle glaucoma
   c. Found in normal tension and primary open angle glaucoma
   d. Indicative of a non-glaucomatous optic neuropathy
   e. Rarely seen in glaucoma

2. Visual field defects in normal tension glaucoma
   a. May be more focal in some cases than in primary open angle glaucoma
   b. May be closer to fixation in some cases than in primary open angle glaucoma
   c. Are often identical to those seen in primary open angle glaucoma
   d. All of the above
   e. None of the above

3. Normal Tension Glaucoma and Primary Open Angle Glaucoma
   a. Are easily distinguished in individual cases based on optic nerve findings
   b. Are easily distinguished in individual cases based on visual field findings
   c. Are distinct entities with little overlap in research studies on optic nerve and visual field parameters
   d. Have not been adequately studied and compared in regards to their associated optic nerve and visual field findings
   e. Show broad overlap clinically and in studies, but have slight differences in the frequency of various findings

KEYWORDS

1. Normal Tension Glaucoma
2. Primary Open Angle Glaucoma
3. Optic Nerve
4. Visual Field

INTRODUCTION

Normal tension glaucoma (NTG) and high tension open angle glaucoma (HTG), are associated with characteristic patterns of optic nerve findings and associated patterns of visual field loss. In the past, most clinicians viewed NTG and HTG as distinct entities, with separate underlying pathophysiology and often different clinical presentations and courses. Optic nerve and visual field findings in NTG have been reported to be different than those in HTG. There has been increasing recognition of the shared features NTG and HTG. In more recent years, there has been a trend to consider these two entities as part of a spectrum of glaucoma with overlapping pathophysiology and clinical characteristics.

NTG and HTG are both primary open angle glaucomas, without evidence of other associated pathologies. Thus the clinical findings, other than the elevated pressure in HTG, are confined to the optic nerve and visual field. Both NTG and HTG are characterized by optic nerve cupping, notching, larger disc size, RNFL loss, peripapillary pigment changes, lamina cribrosa thinning or defects, and disc hemorrhages. Visual field findings for both may include the full range from nasal step to arcuate defects, to central and temporal islands. However, some researchers have found significant differences in the associations of each of these with these two clinical entities, NTG and HTG.
Larger optic nerve size has been associated with a greater risk of the development of open angle glaucoma in some but not all studies. Disc size was not associated with the development of HTG in a subset of subjects in the Ocular Hypertension Treatment Study. In the Blue Mountains Eye Study, significant differences were not found between the disc size in NTG and that in HTG. However, in some studies in Asian populations larger discs were found in NTG vs HTG. In another study of Japanese patients this was not found. In all of these studies, the range of disc sizes of subjects with NTG significantly overlaps that of HTG; there is no single value that serves to separate these populations by disc size. Concentric cupping is another feature seen in glaucomatous eyes. Concentric cupping has been reported to be more prevalent in HTG than NTG. Focal thinning of the neuroretinal rim has been reported to be more common in HTG than NTG. Visual field defects are a hallmark of glaucoma, and, of course, follow a variety of patterns related to optic nerve damage. Focal defects have been reported to be more common in NTG than HTG. In the study by Hitchings et al., 30 eyes with NTG were compared with 30 eyes with HTG in terms of location and steepness of defect edge on Goldmann perimetry. More NTG eyes had steeper defects and defects closer to fixation. However, many eyes with HTG had steep sided defects close to fixation as well. In a similar but larger study (79 NTG eyes, 106 HTG eyes) Caprioli et al. also found NTG eyes to have more focal defects, closer to fixation on automated perimetry.

More recently, spectral domain optic coherence tomography (SD-OCT) has shown differences in NTG vs controls and HTG. Firat et al. found greater macular thickness and ganglion cell complex values in the NTG group, even in early field loss similar among the HTG and NTG groups. However, there was broad overlap in the range of values of all parameters. In a study by Kim et al., comparing SD-OCT of the nerve head and macula in about 50 normal, HTG, and NTG eyes, no differences were seen in optic nerve head measures. Differences were seen in the macular thickness and ganglion cell complex measures suggesting more focal loss in NTG and diffuse loss in HTG. These findings were of high statistical significance, but with broad overlap of the HTG and NTG groups.

Peripapillary pigment changes in the retina pigment epithelium have been correlated to progression in HTG but not NTG. In this large prospective study of 289 patients with HTG and 178 patients with NTG followed on average 4 years, a larger area of beta zone atrophy was associated with progression in the HTG group but not the NTG group. Disc hemorrhage was a factor associated with progression in the NTG group.

Several studies have looked at the lamina cribrosa with enhanced depth imaging in HTG and NTG. NTG eyes with disc hemorrhage have been reported to have thinner lamina cribrosa in the mid superior and mid inferior regions compared to normal and HTG eyes. In another study of 148 eyes, focal lamina cribrosa defects were associated with NTG and more frequent disc hemorrhages.

Visual field defects are a hallmark of glaucoma, and, of course, follow a variety of patterns related to optic nerve damage. Focal defects have been reported to be more common in NTG than HTG. In the study by Hitchings et al., 30 eyes with NTG were compared with 30 eyes with HTG in terms of location and steepness of defect edge on Goldmann perimetry. More NTG eyes had steeper defects and defects closer to fixation. However, many eyes with HTG had steep sided defects close to fixation as well. In a similar but larger study (79 NTG eyes, 106 HTG eyes) Caprioli et al. also found NTG eyes to have more focal defects, closer to fixation on automated perimetry. Chauhan et al. studied 40 pairs of eyes with NTG or HTG were compared, and the NTG eyes had more clusters of normal points, suggesting more focal defects on average.

In summary, NTG and HTG are both characterized by typical optic and visual field findings. There is ample evidence showing significant differences in the patterns of nerve and field damage, with the most common being more focal nerve and field damage in NTG compared to HTG. Statistically significant differences between the averages of these traits for the two populations have been reported, in some but not all studies. Additionally, the range of findings for each these entities is 80-90% shared with the other. Thus, there is great overlap in the features of these two conditions, such that most patients fall in a middle zone in which their findings could be associated with either condition. The appearance of the optic nerve or visual field in any one patient cannot be reliably distinguished as normal or high tension glaucoma in the vast majority of cases.

**CME ANSWERS**

1. c  
2. d  
3. e
REFERENCES


LEARNING OBJECTIVES
1. Define the nature of vascular dysfunction in primary open angle glaucoma across the spectrum of intraocular pressure
2. Describe what is meant by autonomic dysfunction as it relates to primary open angle glaucoma across the range of intraocular pressure

CME QUESTIONS
1. All of the following aspects of endothelial cell dysfunction may be important in primary open-angle glaucoma pathogenesis except:
   a. Increased endothelin-1 production
   b. Impaired nitric oxide signaling
   c. Sub-vascular endothelial plaque formation
   d. Reduction of circulating endothelial progenitor cells

2. Which of the following vascular beds exhibit abnormalities in primary open-angle glaucoma?
   a. Brachial artery
   b. Cerebral vasculature
   c. Retinal and choroidal vasculature
   d. Nail fold capillaries
   e. All of the above

3. Which of the following statements about autonomic dysfunction in primary open-angle glaucoma is true?
   a. Excessive sweating is a well-documented aspect of primary open-angle glaucoma
   b. Autonomic dysfunction is specific to normal-tension glaucoma and is not seen in high-tension variant of primary open-angle glaucoma
   c. Intraocular pressure is not under autonomic nervous system control
   d. An important feature of autonomic dysfunction in primary open-angle glaucoma is the reduced variability in heart rate.
   e. Normal tension glaucoma patients have a more profound dip in nocturnal blood pressure compared to age-matched controls

KEYWORDS
1. Primary Open-Angle Glaucoma
2. High-Tension Glaucoma
3. Normal–Tension Glaucoma
4. Vascular Dysfunction
5. Autonomic Dysfunction

INTRODUCTION
Primary open-angle glaucoma (POAG) is a progressive optic neuropathy that is often arbitrarily stratified by the intraocular pressure (IOP) level associated with initial damage into high-tension glaucoma (HTG) and normal–tension glaucoma (NTG) subtypes. Patients with both POAG subtypes exhibit a variety of ocular and non-ocular vascular abnormalities and there is no evidence these abnormalities predominate in one subtype or the other. Interestingly common genetic variation in NOS3 and the CAV1/CAV2 genomic regions, which code for proteins involved in setting vascular tone, are associated with POAG but these markers seem to stratify with POAG subtypes by sex or pattern of initial visual field loss. Overall it is clear that there is also cardiovascular autonomic dysfunction in HTG and NTG but it is unclear if this dysfunction is more common in NTG compared to HTG. It is largely unknown if other physiologic processes that are under autonomic control are abnormal in POAG. Overall POAG is likely a heterogeneous disease but stratifying cases by IOP level associated with initial optic nerve damage may be less useful than using other endophenotype approaches. Embracing the evidence suggesting systemic endothelial and autonomic dysfunction are operative in POAG will help move beyond an IOP-centric view of the disease and facilitate “tearing down the wall” that divides treating physicians and a better understanding POAG pathogenesis.

In primary open angle glaucoma (POAG), there are no obvious anterior segment abnormalities and the filtration angle is physically open but the optic head is excavated and the neuroretinal rim is eroded. There are no obvious clinical clues as to why the intraocular pressure (IOP) might be elevated in the subset referred to as high-tension...
glaucoma (HTG) cases. Furthermore, it is unclear why optic nerve pathology develops in patients whose IOP is in the statistical normal range (the so-called normal-tension glaucoma (NTG) cases). The ultimate goal in POAG is to define the disease in terms of biochemical pathways as opposed to describing it as an IOP-related optic neuropathy without obvious secondary cause. It is likely that the term POAG encompasses several disease mechanisms produced by distinct biochemical pathways. On an interim basis, it is reasonable to stratify POAG into HTG and NTG subtypes and ask if candidate disease mechanism are operative at lower or higher IOP at presentation. In fact, from an experimental perspective, it makes sense to first compare NTG patients to controls with comparable IOP in order to explore whether any putative mechanism makes the optic nerve vulnerable to degeneration in POAG. Here we will discuss whether two pathophysiologic processes—vascular dysfunction and autonomic dysfunction—are related to POAG stratified by the IOP level associated with optic nerve degeneration.

**VASCULAR DYSFUNCTION IN POAG**

Broadly speaking, vascular dysfunction refers to an inability of endothelial cells to transmit cellular and chemical signals from luminal surfaces to nearby tissues in a physiologic manner. Normally endothelial cells function to maintain normal vascular tone but they also play a role in immune processes, platelet adhesion and other functions. One hypothesis is that POAG is categorized by impaired endothelial signaling between both: a) the inner wall Schlemm’s canal endothelial cells as well as endothelial cells located in the ciliary body and the posterior longitudinal muscle that helps to set outflow resistance and b) the ocular vascular endothelial cell and underlying luminal smooth muscle for vessels that supply the RGCs (Figure 1, see below). This hypothesis could explain why POAG can occur across the spectrum of IOP but it does not consider the role of systemic endothelial cell dysfunction in the disease.

![The Ocular Endothelial Cell Dysfunction Hypothesis in Primary Open-Angle Glaucoma](Image)

**Figure 1:** The ocular endothelial cell dysfunction hypothesis in primary open-angle glaucoma (POAG). The outflow pathway contains classical vascular endothelial cells in the ciliary body that contributes to setting tone in the outflow pathway while the retina is rich in vessels that supply the optic nerve and retinal ganglion cells. When endothelial cell function is normal, as illustrated in the upper panel, vascular endothelium can transmit signals that cause posterior longitudinal smooth muscle relaxation and low resistance in the outflow pathway. Furthermore retinal vessels can dilate as needed to supply a healthy optic nerve. When endothelial cell function is impaired, as illustrated in the lower panel, there can be simultaneous constriction of posterior ciliary muscle and retinal/optic nerve vasospasm that contributes to glaucomatous optic neuropathy and visual field loss. This hypothesis allows for glaucomatous optic neuropathy to occur across a spectrum of IOP on the basis of vascular endothelial cell dysfunction.

What is the evidence for impaired endothelial cell dysfunction in POAG and what are biological mediators of this impairment? A pharmacologic intervention study in untreated NTG patients found that forearm blood vessels failed to dilate in the presence of exogenous acetylcholine. These findings suggest there is a generalized abnormality of vascular endothelium in POAG. Various researchers studied flow-mediated vasodilation in the brachial artery and found that both HTG and NTG patients had impaired responses compared to controls. One group provided evidence that impaired flow-mediated vasodilation was related to reduced circulating endothelial progenitor cells, which are a small group of cells partially derived from the bone marrow that serve to replace and repair the endothelial cell lining. Evans et al compared the changes in retrobulbar ocular blood flow in POAG patients with normal subjects during supine and upright posture. They concluded that posture change exposes a vascular autoregulatory abnormality in the vessels distal to the central retinal artery. Feke et al confirmed these results in HTG and NTG cases. In fact, in open-angle glaucoma (OAG), there is evidence for vascular dysregulation in the choroidal circulation, the optic nerve head circulation, the central retinal artery, and the perifoveal macular capillaries. In fact there is evidence that this vascular dysregulation extends to the cerebral vasculature.

Since compromised endothelial cell signaling plays an important role in POAG, an important question regards what are the biological mediators of this impairment? The answer to such question could translate into more rational treatments for POAG. There is compelling evidence that impaired nitric oxide (NO) signaling plays an important role in the endothelial cell dysfunction in POAG and this evidence comes from human genetics and laboratory studies. One study with no controls found no difference in two functional NOS3 (the gene coding for the enzyme responsible for NO generated by vascular endothelium) between HTG and NTG cases. On the other hand, several research groups that have evaluated genetic polymorphisms in NOS3 have implicated this system in POAG pathogenesis. In fact, the evidence is fairly strong.
that at least one NOS3 variant (−786 C/T) is associated with HTG and that the association is particularly strong in women. In addition, polymorphisms in the genomic region corresponding to the caveolin genes, which code for proteins that reciprocally control NOS3 activity in endothelial caveolar membranes, are also associated with POAG. The association between CAV variants and POAG was particularly strong for cases with early paracentral vision loss. While one study found that the maximum untreated IOP in early paracentral visual field loss cases (21.6 mm Hg) was lower than in early peripheral visual loss cases (28.3 mm Hg), it was still above the statistical norm. Another study did not find more paracentral visual loss in NTG versus HTG, suggesting there is a range of IOP at presentation for this pattern of glaucomatous damage.

When soluble guanylate cyclase (sGC is the intracellular receptor for NO) is knocked out in a murine model, IOP increases nominally (~1-2 mm Hg), there is abnormal retinal vascular reactivity to NO donors, and optic nerve degeneration ensues. Interestingly, a subset of female POAG patients with early paracentral visual loss tended to harbor a polymorphism (rs11722059) in the genomic region between GUY1A3 (codes for the sGC alpha 1 subunit of sGC) and GUY1B3 (codes for the sGC beta 1 subunit of sGC). This locus is in high linkage disequilibrium with another GUCY1A3 / GUYC1B3 variant (rs13139571) linked to blood pressure in a large European consortium. What emerges is that a biochemical pathway that starts with activation of acetylcholine receptors on endothelial cells and concludes with smooth muscle cell relaxation plays an important role in POAG pathogenesis (Figure 2, see below).

**Figure 2:** Simplified schematic of endothelial cell mediated smooth muscle cell relaxation mediated by nitric oxide. The vascular endothelial cell (blue) receives signals which activate NOS3 leading to formation of nitric oxide (NO). NO permeates into the smooth muscle cell (orange) and binds to sGC to mediate relaxation via biomarkers not shown. Endothelin-1 (ET-1), a 21 amino acid peptide made by vascular endothelium throughout the body including tissues relevant to glaucoma (non-pigmented ciliary epithelium, ciliary body muscle and iris), is a potent vasoconstrictor and serves to balance endothelial cell NO mediated vasodilation. Considerable interest in ET-1 emerged when perineural optic nerve delivery in rabbits produced optic nerve excavation and retinal ganglion cell loss without elevated IOP. However excitement that ET-1 might be a NTG biomarker was curbed when appropriately designed studies found that plasma ET-1 levels were not necessarily higher in NTG compared with HTG or CACG. In fact not every study shows that ET-1 levels are higher in glaucoma subtypes versus controls, but ET-1 is abnormally directed from the endothelial cell to the underlying smooth muscle and assessing serum levels may not be sufficiently sensitive to understand the role this peptide plays in glaucoma pathogenesis. Nonetheless interventional studies with NTG patients clearly implicate the endothelin processing system in POAG pathogenesis. For example the physiological increase in ET-1 plasma levels after shifting from supine to standing was absent in NTG patients. After cold-pressor challenge associated with donning a cooling head vestment for 30 minutes, POAG patients experienced a 34% increase in plasma ET-1 levels compared to only a 7% increase in controls. Patients with progressive POAG had higher ET-1 levels compared to those regarded as having stable disease. Interestingly, while untreated NTG patients had a normal systemic vasoconstriction when exposed to intra-arterial ET-1, intra-arterial injection of a selective endothelin A (ET_A) receptor antagonist (BQ123) produced less forearm vasodilation than in controls. This is a critical experiment because there are two endothelin receptors—ET_A and ET_B— and antagonism of ET_A unmasks ET_B related activation of the NO-sGC pathway that produces vasodilation. Therefore, antagonizing ET_A could expose an impaired NO-mediated response via ET_B activation, although other explanations must also be considered such as increased ET_B receptor mediated vascular tone or pre-existing poor ET_A-receptor mediated tone. The cardiology literature indicates that ET_A-receptor antagonist-mediated vasodilation is inhibited by blocking NO synthesis. Overall interaction with ET_A and ET_B receptors is intertwined with NO production, which is key for physiologically appropriate vascular regulation. It is clear that endothelin processing is abnormal in POAG, especially in NTG patients, but the problem is complex and may point more to NO than to endothelin itself. To complicate matters further the problem with NO may involve how NOS3 interacts with caveolin as retinal blood flow tends to respond passively to posture change in POAG. The possibility that genes involved in endothelin processing (see Figure 3 on next page for the ET-1 processing) are related to POAG has not been fully explored. The available studies show no relation between various endothelin processing variants and POAG after controlling for multiple comparisons but not every possible gene involved has been studied and small sample sizes could obscure real associations (see Figure 3 on next page for references regarding the relation between endothelin processing genes and POAG). This is in contrast
to the evidence of replication for associations between a functional variant in the NOS3 promoter region (-786C), which is adversely associated with HTG in women,16,22,23 and between CAV1/CAV2 variants and POAG.39,40

**Figure 3:** Endothelin-1 (ET-1) processing with chromosomal locations for processing enzymes. ET-1 is formed prepro ET-1 which is cleaved by a protease to big ET-1 which in turn is converted to the active 21 amino acid peptide that binds either ETA or ETB receptors. Receptor binding triggers vasoconstriction but ETB receptor activation also leads to compensatory endothelial cell relaxation. *Common gene variants in ET-1, ECE1, ETA, and ETB receptors have not been associated with POAG after accounting for multiple comparisons.*72-74

Abbreviations: ET-1; ECE= endothelial converting enzyme

How do we reconcile the impaired NO signaling paradigm in POAG with the observed increased disc hemorrhages which represents an important biomarker of glaucoma progression? First we must revisit optic nerve anatomic features that would make it vulnerable to damage in the face of vascular dysregulation. It is important to remember that central retinal artery occlusion produces selective retinal ganglion cell layer dropout, a feature shared with glaucoma. Furthermore a subset of POAG patients with paracentral loss will develop fairly profound loss of retinal sensitivity in a discrete visual field zone resembling vascular injury (see Figure 1). The optic nerve can be considered a neurovascular pedicle where vessels make acute turns as they emerge onto the retinal surface. This creates an opportunity for large shear forces to develop in the smaller vessels if significant alterations in blood flow develop. If shear forces exceed the loading capacity of the vessel wall, then the vessel will rupture and bleed. Second, the scleral ring of Elschnig serves to create a compartment syndrome in the pre-laminar portion of the optic nerve head. Thus optic nerve hemorrhages can act like space occupying lesions and compress RGC axons in the optic nerve head. Simple positional changes such laying down prompts physiologic increases in ocular perfusion pressure (blood pressure minus IOP) of ~ 30 mm Hg.6 Such hemodynamic changes must be accompanied by changes in vascular tone that keep ocular blood flow relatively constant. Studies of postural changes in ocular blood flow in POAG patients suggest that these vessels behave like passive sieves and that blood flow can paradoxically increase as much as 100% when patients recline for 30 minutes.41 It is suspected that some complex interaction between ET-1, NO and CAV1 is responsible for this aberrant retinal hemodynamic response to posture change. Such increases in retinal blood flow could translate into the kind of shear forces necessary to induced hemorrhaging in lamina cribrosa capillaries. In the Ocular Hypertension Treatment Study,42 the Normal Tension Glaucoma Study43 and the Early Manifest Glaucoma Study,44 disc hemorrhage was associated with disease progression in multivariable models that also account for IOP. Nonetheless disc hemorrhage does occur in chronic angle closure glaucoma, suggesting that it could also be a secondary event after IOP-induced optic nerve damage.45 The reason why disc hemorrhages might occur more commonly in NTG than HTG may relate to the fact that there is more of an opportunity to tamponade the micro-bleeding that occurs in HTG cases.

If disc hemorrhages in POAG reflect the workings of an impaired NO signaling system, then stopping disc hemorrhages should have therapeutic effect in this disease. Interestingly the Low Pressure Glaucoma Treatment Study46 found that brimonidine, an alpha 2 agonist that has vasomodulatory activity mediated through nitric oxide signaling,47 was effective in reducing visual field loss in NTG patients. Brimonidine use was also associated with less frequent occurrence of disc hemorrhages.48 The counter view that the study did not demonstrate the neuroprotective effect of brimonidine but rather the deleterious effect of timolol seems unlikely. First the disease progression in the timolol arm of the study (39% in 3 years) was comparable to the untreated arm of the CNTGS (35%).49 Second, in the EMTG, patients with OAG across the spectrum of IOP achieved neuroprotective benefited from treatment consisting of laser trabeculoplasty plus betaxolol versus observation.50

**AUTONOMIC DYSFUNCTION IN PRIMARY OPEN-ANGLE GLAUCOMA**

The autonomic nervous system (ANS) is housed in the medulla oblongata with the hypothalamus serving as an integrator. The ANS has parasympathetic, sympathetic and enteric arms that controls many bodily functions such as: body temperature, heart rate, breathing rate, perspiration, digestion, salivation, swallowing, coughing, sneezing, vomiting, sexual arousal and function, pupil diameter, and accommodation. The parasympathetic and sympathetic arms of the ANS have been the targets of therapeutic drugs used to treat glaucoma for over a century. The level of IOP may itself be partially controlled by the ANS. In fact, chemical stimulation of the dorsomedial and perifornical hypothalamus where central autonomic regulatory neurons are housed, causes marked rises in IOP.51 This is interesting
because diurnal variation in IOP is more variable in POAG patients compared to controls.52 This wider fluctuation in IOP coupled with instability of blood flow regulation probably contributes to POAG, regardless of IOP level. It should be pointed out that the ability to regulate retinal blood flow is not a pure autonomic function; rather, it is partially under local paracrine control (especially in the retina where vessels are not innervated9) with a neurovascular component in the retrobulbar optic nerve vessels.

Patients with Familial Dysautonomia (FD) who exhibit a wide array of autonomic function abnormalities uniformly exhibit an optic nerve phenotype. The optic nerve pathology seen in FD predominately involves the maculopapillary bundles, pathologic features shared by some POAG patients (as discussed above) and by patients with mitochondrial disease.54,55 Another disease with well known vasomotor and autonomic abnormalities is Nail Patella syndrome,56 a condition that represents a familial form of NTG57 caused by the LMX1B gene.58 Interestingly common variants in LMX1B are linked to both HTG and NTG.59 At this juncture the link between LMX1B and autonomic dysfunction is not entirely clear. What is quite remarkable is that three cardiovascular autonomic studies shows reduced low frequency heart rate variability in NTG patients versus controls and these changes are not accompanied by dramatically different blood pressure, even during the nocturnal period.60-63 However, other studies suggest features consistent with cardiac autonomic dysfunction are shared by HTG64,65 and exfoliation glaucoma patients.66 The problem of autonomic dysfunction seems inextricably linked to endothelial cell dysfunction in that affected NTG patients with low frequency heart rate variability tend to have paracentral visual defects and concomitant nail fold microvascular abnormalities.67 Furthermore these patients also tend to have higher plasma ET-1 than age matched controls.68 Aside from cardiovascular autonomic function, other bodily functions under the ANS are operative in NTG versus controls and these changes are not addressed if we are going to more favorably impact this disease. More study in the fields of genetic epidemiology, immunology and cardiovascular medicine are likely to contribute to an improved understanding of POAG. Hopefully we may someday drop the word primary from POAG and replace it with real descriptors that speak to the multiple etiologies that exist in this condition.

CONCLUSIONS

While one cannot claim that impaired NO and endothelin signaling represents a unifying hypothesis in our understanding of POAG, it does appear to play an important role in disease pathogenesis for both HTG and NTG cases. This discussion highlights the role of genetics in contributing to this process. It is important to point out that sub-endothelial plaque formation (atherosclerosis) is not an aspect of endothelial dysfunction in POAG.69 Furthermore, patients with POAG do not have increased risk of cardiovascular-related mortality.70 While studies focused on NTG patients have helped to highlight mechanisms involved in optic nerve degeneration in POAG, these mechanisms are operative in NTG and HTG. More studies with novel alternative stratification of POAG (such as stratifying disease on the basis of pattern of visual field loss) are needed.

The current body of knowledge reviewed here clearly implicates systemic processes in POAG and these processes need to be addressed if we are going to more favorably impact this disease. More study in the fields of genetic epidemiology, immunology and cardiovascular medicine are likely to contribute to an improved understanding of POAG. Hopefully we may someday drop the word primary from POAG and replace it with real descriptors that speak to the multiple etiologies that exist in this condition.

CME ANSWERS

1. c
2. e
3. d

REFERENCES

IS NORMAL TENSION DIFFERENT FROM HIGH TENSION: GENETIC/EPIDEMIOLOGIC FACTORS

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LEARNING OBJECTIVES

1. Identify differences in prevalence of normal-tension glaucoma among glaucoma patients world-wide
2. Recognize genes responsible for early-onset forms of normal-tension glaucoma and high-tension open angle glaucoma
3. Distinguish genes/genomic regions associated with adult-onset normal-tension glaucoma from those associated with adult-onset high-tension glaucoma

CME QUESTIONS

1. What population has the highest percentage of normal-tension glaucoma patients?
2. True/False: Mutations in genes causing familial forms of glaucoma are common.
3. True/False: Genes/genomic regions associated with intraocular pressure in population-based studies are also associated with normal-tension glaucoma

KEYWORDS

1. Normal-tension Glaucoma
2. Primary Open-angle Glaucoma
3. Intraocular Pressure
4. CDKN2BAS
5. Choroid Plexus

INTRODUCTION

Epidemiologic and genetic studies suggest that patients with normal tension glaucoma (NTG) have a different set of pre-disposing risk factors compared with patients with primary open angle glaucoma (POAG), high-tension open angle glaucoma (HTG) or ocular hypertension. In particular, Asians (especially Japanese) are at increased risk for NTG compared to other populations. Genetic studies of NTG families as well as NTG case/control samples have identified genes that could influence optic nerve susceptibility. A different set of genetic factors appear to influence risk of high-tension open angle glaucoma. These results suggest that the disease mechanisms underlying NTG may not be the same as those contributing to high-tension glaucoma.

NTG GLOBAL PREVALENCE

NTG prevalence varies widely among different ethnic populations ranging from 92% of the open angle glaucoma patients in the Japanese Tajimi study\(^1\) to 6% of open angle glaucoma patients in an Ethiopian clinic population\(^2\) (Figure 1, References 1-24). Interestingly, NTG prevalence is also higher in Japanese Americans compared to other ethnic groups suggesting that the NTG risk is due to genetic, rather than environmental, factors\(^25,26\). NTG prevalence is highest in Asians overall compared to Caucasians of European ancestry and African races\(^27\).
Early-onset open-angle glaucoma (familial NTG and juvenile open-angle glaucoma) is caused by rare mutations in TBK1 and OPTN (familial NTG) and MYOC (juvenile open-angle glaucoma). Although rare, these mutations have large biological effects and directly cause the disease. In contrast, late-onset open angle glaucoma (POAG and NTG) and ocular hypertension have been associated with common variants in a number of genes that individually have small biological effects. While these variants are statistically associated with disease risk they are not ‘causative’ (Figure 2).

Figure 1. Prevalence of NTG in populations world-wide. The percentage of open-angle glaucoma in each population is indicated on the Y-axis. Results from the Chinese (3 studies) and India (4 studies) were averaged. Bars are colored according to geographic distributions (Green, Asia; Blue, North American; Red, Africa; Purple, Europe; Orange, Australia).

GENETICS OF NTG, HTG, POAG AND IOP

Early-onset open-angle glaucoma (familial NTG and juvenile open-angle glaucoma) is caused by rare mutations in TBK1 and OPTN (familial NTG) and MYOC (juvenile open-angle glaucoma). Although rare, these mutations have large biological effects and directly cause the disease. In contrast, late-onset open angle glaucoma (POAG and NTG) and ocular hypertension have been associated with common variants in a number of genes that individually have small biological effects. While these variants are statistically associated with disease risk they are not ‘causative’ (Figure 2).

Figure 2. Genetics of Early-onset and Adult-onset primary open angle glaucoma. The relative biological impact (effect size) is shown on the Y-axis and the frequency of the mutation or variant is shown on the X-axis. OPTN and TBK1 mutations cause familial NTG, while MYOC mutations cause familial HTG. CDKN2BAS variants are associated with NTG and POAG; TMCO1, ABCA1 and FNDC3B variants are associated with POAG and IOP; CAV1 and AFAP1 variants are associated with POAG and GAS7 variants with IOP.
MUTATIONS IN MYOC CAUSE FAMILIAL HIGH-TENSION OPEN ANGLE GLAUCOMA
Missense mutations in MYOC (myocilin) cause early-onset (before age 50) autosomal dominant primary open angle glaucoma. MYOC missense mutations cause misfolding of the nascent polypeptide with subsequent endoplasmic reticulum stress. The small molecular chaperone, PBA (sodium 4-phenylbutyrate) can promote secretion of the misfolded protein, relieving ER stress and lowering IOP.

ADULT ONSET NTG GENES
The NEIGHBOR and GLAUGEN case/control genome-wide associations studies have revealed two genes/genomic regions associated with the common form of adult-onset NTG in Caucasians with European ancestry. Common variants in the genomic region coding for CDKN2BAS, a long non-coding antisense RNA (also known as ANRIL) are significantly associated with NTG, as well as POAG. This finding has been replicated in a number of populations, including the Japanese. CDKN2BAS regulates cell cycle division suggesting a role in retinal ganglion cell apoptosis. Common variants in a regulatory region on 8q22 are also associated with NTG. This regulatory region is most active in choroid plexus (producing cerebrospinal fluid) and ciliary body suggesting that it could have a role in the development of an adverse translaminar gradient that has been associated with NTG in some populations.

ADULT ONSET POAG (HTG) GENES
Genome-wide association studies have also identified several genes associated with high-tension open angle glaucoma.

CME ANSWERS
1. Japanese
2. False
3. False

REFERENCES


ABSTRACT
Our understanding of what role intracranial pressure (ICP) plays in the manifestation of the optic neuropathy called glaucoma is only beginning to develop. ICP can have a profound effect on the optic nerve. Edema of the optic nerve head is an accepted consequence of elevations in ICP. Venous pulsations are seen in some individuals with normal or low ICP. Therefore some local alterations in the structure, blood supply, or axonal transport as a consequence of changes in ICP, possibly in relation to intraocular pressure (IOP), is realistic. This talk will briefly summarize the relationship between ICP and the eye, hypothesized relationships with glaucoma and existing evidence.

CEREBROSPINAL FLUID PRODUCTION, FLOW AND RESORPTION
Cerebrospinal fluid (CSF) surrounds and fills cavities within the brain and spinal cord. Normal CSF volume is roughly 100 ml. The rate of CSF production is surprisingly high, with the entire volume being replaced several times each day. Most lies within the subarachnoid space and basal cisterns, with approximately 25% being contained within the ventricles. Normally, the osmolality of the CSF is equal to that of serum, with a similar electrolyte composition. There are small differences, for example, CSF sodium concentration is usually slightly less than that of serum. There is usually less glucose in CSF. However, the CSF ionic concentration can remain relatively stable despite serum fluctuations.

CSF is produced primarily by the choroid plexus, found in all four ventricles. The ventricular choroid plexus is similar to the ciliary body of the eye, being comprised of an epithelial lined highly vascular core. The epithelium pumps ions into the CSF space, creating an osmotic gradient and the influx of water. Similar to the ciliary body of the eye, CSF production is dependent on carbonic anhydrase. Thus carbonic anhydrase inhibitors are effective in lowering both ICP and IOP.

CSF passes from the lateral ventricles to the third ventricle and onto the fourth ventricle via the aqueduct of Sylvius. CSF exits the fourth ventricle to the subarachnoid space, through the middle foramen of Magendie and two lateral foramina of Luschka. CSF exits the subarachnoid space through arachnoid villi, located in the superior sagittal sinus. Obstruction of the CSF flow can result in elevations of ICP. If the obstruction is at the aqueduct of Sylvius, the lateral and third ventricles expand. This is called obstructive hydrocephalus. Should the obstruction be at the level of the arachnoid villi, the term non-communicating hydrocephalus may be applied.

Under normal physiologic conditions, CSF resorption equals production. The bulk of the CSF exits through the arachnoid villi of the superior sagittal sinus. Proposed alternative routes of CSF exodus include selective resorption through the choroid plexus, interstitial efflux to lymphatics of surrounding tissue, and by following olfactory nerves through the cribiform plate. The exact contribution of these alternate routes is debated; with most agreeing that the arachnoid villi provide the primary outflow pathway. Arachnoid villi are small out-pouchings of arachnoid through the overlying dura into a venous structure, such as the superior sagittal sinus. A granulation refers to a collection of villi. Arachnoid villi function as one-way valves that open under hydrostatic pressure. Thus with increased ICP (and therefore the gradient), flow increases.

NORMAL INTRACRANIAL PRESSURE
The craniospinal compartment is relatively closed. It is filled with neural tissue, blood and CSF. Should one component increase in volume, another must decrease in volume. Should this balance be disrupted, ICP may change. ICP is most commonly and easily measured via a lumbar puncture. You can find CSF pressure measured in units of mmHg and cm H₂O, which is derived from the actual measurement of mmCSF: 1.0 mmHg is roughly equal to 1.36 cm H₂O. Normal adult ICP is considered to be between 7 and 15 mmHg. The significance of ICP measurements between 15 and 18 mmHg is often of unclear. ICP greater than 18 mmHg is elevated. Normal ICP is lower in children and even less in infants.

ICP fluctuates slightly (i.e. CSF pulsation) with respiration and the cardiac cycle. It is also thought that arterial pressure contributes directly to ICP. The balance between mean arterial pressure (MAP) and ICP is important; the difference between MAP and ICP is called the cerebral perfusion pressure (CPP). Venous pressure is important as elevations can result in a reduction in the rate of CSF resorption, leading to increased ICP. This effect is more profound with acute changes in venous pressure, with compensatory mechanisms buffering ICP alterations with...
chronic venous pressure elevation. Body position affects ICP and its measurement. When assessing ICP, measurements in sitting and supine position will be affected by the resultant change in the height of the water column.

INTRACRANIAL PRESSURE AND THE EYE

INTRAOCULAR PRESSURE
It has been theorized that ICP has a direct effect on IOP, the most plausible proposed mechanisms being via effects on venous pressure within the cavernous sinus and in turn episcleral venous pressure. Intraocular pressure is determined by the balance between aqueous production and efllux through the trabecular meshwork, which is in part dependent on episcleral venous pressure. In animal models, a close correlation between ophthalmic arterial, venous pressure and a rise in ICP has been demonstrated. Also in support of this, a highly significant correlation between central retinal vein pressure and ICP estimated by ophthalmodynamometry has also been found. When an acute large increase in ICP has been induced in animals, small changes in IOP have been recorded. One of the most convincing observations was published in 2000 by Sheeran et al. They monitored ICP and IOP in intensive care unit patients. Although there was much variability, they found a significant correlation (p<0.0001) with a mean change in IOP of 1 mmHg per 11 mmHg change in ICP.

However, compensatory changes would be expected to buffer the effect of alterations in episcleral venous pressure. And when looking at chronic changes in ICP and IOP this is what is found. Studies in patients with chronically elevated ICP have not found such a close correlation. Lashutka et al described a loose correlation between ICP and IOP; however, due to the substantial variability, concluded that “changes in intraocular pressure are a poor predictor of changes in intracranial pressure.” Others have substantiated this finding.

PAPILLEDEMA
Alteration in ICP has many known effects on the eye. Papilledema refers to optic disk edema occurring secondary to elevated ICP. Pressure transmitted within the CSF along the subarachnoid space compresses the optic nerve, just posterior to the globe. This is thought to cause stasis of axoplasm and axonal swelling. It has also been suggested that vascular compression may contribute. Despite the presence of papilledema vision is relatively preserved. In the majority of cases, with early papilledema visual function (acuity and perimetry) is normal. Exceptions include marked edema with presumed vascular compression and infarction or adjacent retinal edema and hemorrhage. With chronic papilledema axonal loss is seen. Proposed mechanisms include a toxic effect of disrupted axonal transport and vascular compromise. As axonal swelling is not seen with glaucoma, the likelihood that pressure related axonal stasis contributes to glaucoma is negligible.

ABDUCENS NERVE
Increased intracranial pressure may also result in diplopia. This is one of the more common findings in patients with elevated ICP, and the result of unilateral or bilateral abducens nerve palsy. This is likely the result of pressure on the brainstem, with stretching the nerve(s) over the clivus.

BLOOD FLOW
Spontaneous venous pulsations (SVP) of the optic disk refer to visible pulsations of the retinal veins. Presumably SVP occurs when the pressure in the vein falls between IOP peak pressure during systole and trough pressure in diastole. The cessation of optic disk spontaneous venous pulsations (SVP) is often considered a sign of elevated intracranial pressure (ICP). This is thought to be due to ICP being transmitted to the venous outflow with elevation of central retinal vein pressure above intraocular systolic pulse pressure. Other factors may influence SVP presence. Optic disk edema occurring for reasons other than elevated ICP usually results in the loss of SVP. Presumably, disk edema causes a localized increase in venous pressure. This emphasizes the complexity in the relationship between ICP, optic disk structure and vascular pressure. Tortuosity of the retinal vasculature is also a common finding in patients with elevated ICP. Given that changes in blood flow at the optic nerve are to the degree that gross changes are visible with elevated ICP, it seems plausible that alteration in the ICP/ IOP gradient might have a contributory effect to blood flow and the development of glaucoma.

ORBIT
Dynamic changes in bone are well recognized. Osteoporosis is a very familiar disease. Neoplasm can alter bone via a number of mechanisms. In the orbit, bone invasion/erosion may be indicative of malignancy. The effects of intracranial hypotension on surrounding bone are less obvious and until recently largely unrecognized. In 1996, Meyer and colleagues described three patients with congenital hydrocephalus who developed bilateral enophthalmos following ventriculoperitoneal shunting (VPS). More recent a number of reports have described adult cases with acquired enophthalmos following VPS. This has been well demonstrated to be primarily due to bone remodeling secondary to intracranial hypotension. The change in the pressure gradient between the orbital soft tissue and CSF results in upward bowing of the orbit roof with expansion of the orbit volume. The term “sagging brain, sunken eyes syndrome” has been applied to the development of enophthalmos secondary to intracranial hypotension. This change in the bony structure of the orbit illustrates the power of chronic alterations in the pressure gradient between CSF and an adjacent cavity. If bone can be moved, an affect at the lamina cribosa of the optic nerve head might be a plausible consequence of an alteration in the ICP and IOP pressure gradient.
INTRACRANIAL AND INTRAOCULAR PRESSURE GRADIENT

Of the potential mechanisms that ICP might contribute to the development of glaucoma, a pressure gradient driven mechanical alteration of the structure of lamina cribosa seems most plausible. This has been the focus of a number of recent investigations.

A number of researchers have looked at ICP in cohorts of patients with glaucoma. For example Berdahl et al compared “ICP in subjects with primary open-angle glaucoma (POAG), normal-tension glaucoma (NTG), and ocular hypertension (OHT) with that in subjects with no glaucoma”. Relative to normals they found that lower ICP in both the POAG and NTG groups and elevated ICP in the OHT group. More recently Siaudvytyte et al. assessed translaminar pressure gradient (TPG) and neuroretinal rim area (NRA) in patient with POAG and NTG. They reported that translaminar pressure gradient was higher in glaucoma patients and that in the NTG group there was a reduction of NRA in patients with higher TPG. This study was arguably flawed in that ICP was not directly measured.

The lamina cribosa (LC) is the probable site of axonal injury in glaucoma and several studies have assessed the LC in glaucoma subjects. Spectral Domain Optical Coherence Tomography (SD-OCT) has been proposed to have resolution sufficient to detect small differences in optic nerve tissue dimensions, specifically LC thickness and position. In 2008, Schuman et al imaged the LC in humans using Spectral-domain Optical Coherence Tomography (SD-OCT). Images were suboptimal due to limited contrast, vascular shadowing, and signal fade at increasing optic nerve tissue depth. Subsequent studies sought to better optimize visualization. Inoue (2009) used 3D imaging software to reconstruct the LC from standard SD-OCT images. This attempt was limited by not being able to identify the LC reliably. Newer studies employ the use of Enhanced Depth Imaging (EDI) SD-OCT first used by Lee et al (2011) to image the LC in humans.

Several studies have looked at the LC in patients with glaucoma. Changes in LC position and prelaminar tissue thickness (PTT) after surgical IOP reduction have been assessed. Images were obtained before trabeculectomy or tube shunt placement. Significant anterior laminar displacement and increased PTT were described. Weinreb et al (2012) performed a similar study with patients undergoing trabeculectomy. They looked at changes in thickness of the LC and prelaminar tissue, and displacement of the LC. They also found a significant reduction in posterior displacement of the LC as well as an increase in LC thickness and pre-laminar tissue thickness after treatment.

Although these groups have employed SD-OCT to image the lamina cribosa in patients with glaucoma, few studies if any have used it to study the lamina cribosa in patients with intracranial hypertension or hypotension. In one recent case report it was demonstrated through enhanced-depth SD-OCT that the position of the LC changed after optic nerve fenestration. The prelaminar tissues (optic cup surface) were posteriorly displaced roughly 143 microns, while the anterior surface of the LC itself was posteriorly displaced by 137 microns. This was accompanied by a significant improvement in vision in both eyes.

SUMMARY

In summary, our understanding of the relationship between ICP and glaucoma is beginning to evolve. Alterations in ICP are known to influence optic nerve structure, blood supply and function. Whether ICP plays a significant role in the development of glaucoma in all or a subset of individuals remains to be determined. The possibility is arguably plausible and worthy of further consideration.

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IS NORMAL TENSION DIFFERENT THAN HIGH TENSION GLAUCOMA: OTHER POSSIBLE FACTORS

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I. DEFINITION

A. WHAT’S IN A NAME?
Open angle glaucoma (OAG) is the second leading cause of irreversible blindness in the United States, comprises 2 major syndromes: primary open angle glaucoma (POAG) and normal pressure glaucoma (NPG). POAG is a disease generally characterized by a clinical triad which consists of 1) elevated intraocular pressure (IOP); 2) the appearance of optic atrophy presumably resulting from elevated IOP; and 3) a progressive loss of peripheral visual sensitivity in the early stages of the disease, which may ultimately progress and impair central visual acuity. Primary open angle glaucoma affects approximately 0.5% of the American population and occurs in 1.3% of white and 4.7% of black Americans over the age of 40 (1.6 million persons). Studies have indicated, however, that a surprisingly high percentage of patients with open-angle glaucoma have findings identical to those of POAG but with a singular exception; namely, that the IOP has never been demonstrated to be elevated. This form of glaucoma is often called “low-tension glaucoma,” but also goes by the names “normal tension” and “normal pressure” glaucoma.

While numerous arguments and custom may be used to justify either of these terms, it is prudent to state the obvious: (1) there is nothing low about the range of IOPs in these patients, and (2) the use of the word “tension” is idiosyncratic to the field of ophthalmology since it is a term used to describe tonometric readings. We prefer the word “pressure” since it is more readily understood by our medical colleagues and patients alike. Furthermore, since there is no widely acknowledged effective therapy for this disorder, the use of the term “tension” is, unfortunately, most decriptive of the interactions that commonly occur between the physician and the patient with this disorder, and it is often anything but low! The remainder of this chapter will therefore refer to this syndrome as “normal pressure glaucoma.”

B. NATURAL HISTORY OF NORMAL PRESSURE GLAUCOMA
Several large population-based studies have documented the high prevalence of normal pressure glaucoma. Although a few of these studies suggest that as high 50% of open-angle glaucoma patients may indeed have normal pressure glaucoma, more conservative estimations prevail in numerous population studies. It is reasonable, therefore, to consider that approximately 25% of cases with open-angle glaucoma occur in the presence of normal IOP. Little is known about the natural history of normal pressure glaucoma. Most experts, however, believe that while the hallmark of this syndrome is progression of visual field loss (as it is for POAG), there are often long periods, often years, in which stability of the visual fields occur. Unfortunately, in some patients, episodes of rapid deterioration can and do occur within a relatively short time, typically over several months to 1 year. One long-term, population-based study found that 62% of patients with NPG showed progression of visual field loss over 5 years. This was significantly worse than the 42% of patients with POAG who were tested and followed similarly during the same period of time. NPG is therefore often a disease marked by continued visual field progression, but not all patients progress even during long periods of follow-up. In many patients, episodic deterioration is interrupted by long periods of stable visual function.

Several findings make normal pressure glaucoma particularly difficult for the ophthalmologist to treat. Most apparent, however, is that IOP, the target of conventional glaucoma therapy, is no longer an attractive parameter to lower, since it is already within the normal range in these patients. Several investigators have speculated, however, that further lowering of IOP may still be beneficial, presumably by facilitating vascular perfusion in the region of the laminar cribrosa, since blood flow will occur against less resistance in the presence of lowered IOP. No data, however, are yet available from a national prospective study which will address whether the rate of visual field loss in these patients is significantly altered in patients in whom the IOP is further lowered by 30% of baseline values. Ongoing studies in Britain, however, have suggested that visual field progression may indeed be minimized or limited in patients in whom IOP is lowered effectively by filtration surgery. Furthermore, in eyes of with asymmetric IOPs.
lower than 21 mm and visual field loss in one eye, the eye with the field loss almost invariably has the higher IOP\textsuperscript{11,12}. Such studies, coupled with our inability to offer better alternatives, serve as the basis for our rationale that effective IOP lowering, either by medication, laser or surgery, may be a useful therapeutic intervention for many of our patients.

II. MAKING THE DIAGNOSIS

A. A DIAGNOSIS OF EXCLUSION

The diagnosis of normal pressure glaucoma is a diagnosis of exclusion. Normal pressure glaucoma is defined by the clinical constellation of open iridocorneal angles, normal IOP, optic nerve damage, and visual field loss which is progressive in nature and may ultimately impair central vision. The first parameter that must be evaluated as an inclusion criterion is what precisely is meant by normal IOP? Although it is well known that IOPs obey a non-Gaussian distribution in humans\textsuperscript{13}, several studies specify a value which defines an acceptable entry criterion for patients with normal pressure glaucoma. These values generally range between 20 and 24 mm Hg, and although they may have some utility for clinical studies, they should be viewed with less importance in an office setting. They certainly are not a criteria for defining glaucoma. The key question the practitioner must ask himself is: do I think my patient has a pressure-related glaucomatous neuropathy or not? When IOPs are elevated to greater than 2 standard deviations of the population mean (e.g. > 30 mm Hg), it is often easier to guess the right answer. When pressures are in upper teens, however, the answer to this question is not nearly so easy to ascertain.

Another well accepted inclusion criterion for normal pressure glaucoma is progressive changes in either visual fields or optic nerve cupping. In many patients, changes in both visual fields and cupping will occur. However, deterioration of either constitutes legitimate glaucomatous progression.

Most importantly, the definition of normal pressure glaucoma implies the absence of alternative causes of optic neuropathy, such as meningeal disease, infections (e.g. syphilis), inflammation, ischemic disease, or compressive lesions which may account for anterior nerve fiber bundle loss. Additional alternative causes of optic neuropathy, of course, include those in which episodic or transient elevation of IOP has been documented previously such as occurs in steroid responsiveness, or as a sequelae of some forms of trauma or intraocular pathology.

Simple ophthalmoscopic examination is often helpful in ruling out ocular conditions that may mimic glaucomatous nerve fiber layer damage such as congenital anomalies of the optic disc, choroidopathies (i.e., toxoplasmosis lesions), or retinal lesions (e.g. retinoschisis). Most often, however, such conditions are easily seen. Furthermore, they generally constitute nonprogressive lesions and are therefore not easily confused with genuine glaucoma.

B. NORMAL PRESSURE GLAUCOMA IS A DISEASE WITH SEVERAL VARIANTS

Now that we have satisfactorily ruled out numerous possible causes of intrinsic glaucomatos neuropathy, as well as other conditions which may be resemble glaucomatous damage, we are left with a clinical entity whose name, normal pressure glaucoma, unfortunately tells us little about the pathophysiology that underlies this disorder. Since the hallmark of medical therapy is based on treatment directed at preventing or ameliorating specific pathological processes, it should be no surprise that we have little to offer for this illness. Fortunately, however, the clinician can often gain useful insight into the factors underlying glaucomatous damage in certain individual patients. It is becoming increasingly clear that normal pressure glaucoma is likely comprised of numerous clinical variants, or subsets, of patients in whom there appear distinct similarities and thus clues as to the etiology of neuronal cell death in many subsets of patients. The most common subsets of normal pressure glaucoma syndrome may be characterized by patients in whom:

- no known cause has been identified (idiopathic)
- there is a history of migraine headaches and/or peripheral vasospasm
- there is evidence of aberrant systemic, serum or retinal autoimmunity (i.e. specific anti-retinal antibodies)
- there is nocturnal systemic hypotension
- there has been hypovolemic shock (blood loss from transfusions, etc.)
- patients in whom there is hyposecretion in the presence of impaired outflow (so-called “burnt-out” glaucoma)
- falsely low IOP has been recorded due to excessive corneal thinness.

While distinctions in these heterogeneous patients with NPG may often be made clinically, the advent of modern genetic techniques makes it is likely that a hereditary factor will be identified for one or more of these subgroups. For example, the chromosomal loci of genes (2cen-q13) responsible for at least one form of NPG that typically manifests in the fifth decade has recently been identified\textsuperscript{14}.

The finding that there are identifiable ‘subsets’ of patients within the NPG syndrome, is not surprising in light of our contemporary views of glaucoma pathogenesis. In addition to high intraocular pressure, evidence is rapidly accumulating that prompts us to consider that damage to the optic nerve may be initiated or sustained by any number of the factors cited above, in addition to others such as excitotoxicity, neurotrophin insufficiency, peroxynitrite damage or others yet undefined. These different injurious influences then act through common final pathways that eventually disturb ion transport and...
activate the cellular proteases that accompany neuronal programmed cell death.

C. LABORATORY EVALUATION
Certainly, in order to rule out certain systemic considerations to help confirm the diagnosis of normal pressure glaucoma, it is reasonable to obtain several laboratory and/or radiologic tests. In general, there have always been two schools of thought which have tempered the clinical judgment of ophthalmologists regarding testing of these patients. There are those practitioners who will obtain almost no tests whatsoever. Conversely, there are those that will obtain every test imaginable. We would advocate that in general it is reasonable to perform limited testing to detect certain obvious disorders which are either treatable, or require further medical evaluation to assess potential treatment, and therefore should be performed on all patients with normal pressure glaucoma. The following tests should be viewed as the minimal essential testing to be performed, and their rationale are as follows:

(a) complete blood count with differential and platelets. There is no easier test to identify obvious blood dyscrasias, or common anemias, which may impair the delivery of oxygen to the high energy requirement tissues of the retina and optic nerve.

(b) antinuclear antibody panel (ANA). This test is a useful screen for collagen vascular disease, and other autoimmune abnormalities. A hospital generally offers ANA panels of varying complexity and we would advocate that the most complete panel offered, which typically tests for antibodies to extractable nuclear antigens such as Ro/SSA, La and Sm antibodies, are the most useful. Positive findings to the presence of these autoantibodies may signify the identification of the autoimmune subset of patients with normal pressure glaucoma.

(c) VDRL and FTA. One of the great masqueraders of glaucomatous optic neuropathy is indeed syphilitic disease. In our experience two out of every 100 patients with optic atrophy that have been referred to us for normal pressure glaucoma have tertiary syphilis which requires treatment.

(d) Serum immunofixation for paraproteins. In our experience in a tertiary care setting, approximately 10-15% of patients with normal pressure glaucoma have a monoclonal gammopathy (i.e. paraproteinemia), which is a clonal expansion of B cells which produces excessive serum immunoglobulin. While the majority of monoclonal gammopathies in an older adult population generally represents a benign condition (called “monoclonal gammopathy of undetermined significance”), approximately one-third of these gammopathies will turn out to be caused by lymphoproliferative disorders such as multiple myeloma or other neoplastic conditions. It is recommended, therefore, that the ophthalmologist test for this condition and if a paraproteinemia is found, refer the patient to a hematologist for further workup which may include a bone marrow aspirate. Although paraproteinemas can often be determined by obtaining full serum protein electrophoresis profiles, a much easier and cheaper test is available in most laboratories in which immunofixation testing is performed in order to detect a serum monoclonal protein.

Additional laboratory testing which may be useful in selected patients include the following:

(e) SMA12. It is not unreasonable to obtain electrolytes and studies of liver and renal function in patients in whom there is a high index of suspicion of such disease. We have found, however, that routine testing for these values has been rather unproductive in virtually all patients with normal pressure glaucoma.

(f) complement studies. Testing for C3 and C4 complement has been unproductive in our hands as a assessment of potential collagen vascular disease.

(g) B12 and folate. These two have likewise been unrevealing. Although they are often obtained when there is a high degree of suspicion of an intrinsic neuropathy affecting central vision, we have not found them to be of value in assessing patients with normal pressure glaucoma.

(h) cryoglobins. These may be useful in patients in whom there is Raynaud’s phenomenon or evidence of marked peripheral vasospasm, but is otherwise not very helpful.

D. RADIOLOGIC EVALUATION
There is considerable debate as to whether there is any utility in obtaining a CAT scan or MRI in patients with normal pressure glaucoma. Obviously, these tests are more useful in patients in whom there is a loss of central vision with preservation of peripheral vision, or in patients in whom chiasmal lesions are suspect. On the other hand, one might argue that it is not unreasonable to leave “no stone unturned,” especially in a patient in whom central vision is threatened (i.e. a progressive paracentral scotoma very near fixation). We have never found a positive MRI or CAT result which has uncovered a lesion that accounts for glaucomatous optic neuropathy although anecdotal reports exist of such findings. On the other hand, we would not consider it unwise to obtain radiologic brain or orbit studies to help eliminate any suspected source of optic nerve damage resembling glaucoma. This is perhaps especially true if a physician is practicing in a hostile medical legal
environment like the United States. One wonders: if Hippocrates were alive today would he amend the credo “Physician-do no harm” with “Physician- protect thyself?”

Finally, another useful clinical measurement that may be relevant in some cases is the assessment of corneal thickness. Certainly, patients who have had corneal refractive surgery, and high myopes, should have pachymetry performed since applanation tonometry underestimates IOP by 3 mm Hg for a 50 micron decrease in corneal thickness.

III. SIGNS AND SYMPTOMS OF NORMAL PRESSURE GLAUCOMA

A. OCULAR CLUES

1. Intraocular pressure

   It is well known that IOP fluctuates throughout the day. Most humans have their highest IOPs in the mid morning. Patients who are evaluated at a single visit, however, may have IOPs within the normal range during their visit and do not necessarily manifest elevated IOP at that time. For this reason, we often prefer to obtain IOP measurements not only during multiple office visits, but at various times of the day, in order to get a truer sense of an individual's IOP range. Furthermore, we find that serial recording of diurnal pressure measurements is often useful to obtain an accurate representation of the average IOP readings in the eye. Although it sounds quite simple, in practice, it is not easy to obtain detailed recordings over a 24 hour period. Most practitioners have the capability of recording pressure measurements throughout the day when their office is open, say from 8 a.m. to 5 p.m. In general, when we receive diurnal measurements for this time period, we are usually satisfied that we have obtained a sampling that will detect an episodic increase during the day. Our own diurnal measurements, however, are made throughout the night as well, with the absence of the midnight to 6 a.m. time period. Of course, patients who have an aberrant light/dark cycle, such as those individuals who work in the evening, should have their IOPs preferably tested during the 12-16 hour period following their arousal from sleep.

2. Optic nerve atrophy

   Documenting progressive optic nerve atrophy is generally straightforward. Sure, there are lots of sophisticated instruments that have the ability to define numerous optic nerve parameters in a moderately objective way. Some of the nerve fiber layer or optic nerve analyzers that are on the market are able to give fairly reproducible and reliable estimates of specific measurements such as cup to disk diameter, neuroretinal rim area, nerve fiber layer height, etc. It is important to keep in mind, however, that no computer has yet been able to answer the obvious questions we would like to ask about the key parameters of interest; namely, what, exactly, constitutes a significant change of any given parameter measured at various time points. More importantly, if we don’t have access to one of these wonderful research-grade instruments, can we make such assessments at all? Fortunately, there is evidence that a good set of stereo disk photographs interpreted by an astute observer, is perhaps the most reliable way to assess meaningful changes in the optic nerve head. By far the most important change that should be assessed is a loss of neural tissue which is assessed by the presence of cupping which of course represents atrophy of both neural as well as glial tissue. We would advocate that three dimensional viewing is extremely advantageous in order to perform this assessment. If this is not possible, certain clues such as the displacement of vessels, or the increased appearance of laminar pores, may assist the viewer who only has access to two dimensional analysis. In short, one does not need high technology in order to assess optic nerve progression; rather, all that is needed is a good brain.

   It should be noted that patients with normal pressure glaucoma often present at a later stage of their disease than their counterparts with POAG. Therefore, it is not unusual that ophthalmologists will be faced with patients in whom cupping is quite advanced and in many cases almost complete. In these patients, the progression of optic nerve cupping will not be nearly as productive as the clinical assessment of progressive visual field loss in order to characterize the clinical course of their glaucoma.

   Another parameter which has received increased attention recently is the appearance of parapapillary (also called “peripapillary”)atrophy. This atrophy of the retinal pigment epithelium adjacent to the optic nerve head has been found to be active and dynamic, and accompanies the glaucomatous process. Alone, an increase in peripapillary atrophy is not considered a diagnostic indicator of optic nerve progression. However, the clinicians’ index of suspicion that progressive nerve damage has occurred should certainly be elevated if progressive changes in peripapillary atrophy occur with time.

3. Optic disc hemorrhages

   The presence or recurrence of one or more splinter hemorrhages on the optic disc surface has long been considered an important prognostic sign indicating the development and/or progression of glaucomatous damage. The greater incidence of these hemorrhages in patients with NPG as opposed to POAG is particularly troubling. It suggests that such hemorrhages are not merely the result of structural changes that accompany glaucomatous optic neuropathy, but in the case of NPG, an ominous sign of neurovascular infarction and hence, eventual progressive optic nerve deterioration. Some observers believe that the appearance of such hemorrhages are not always followed by further field loss. In fact, such hemorrhages have been observed in nonglaucomatous populations. However, many studies report further field loss does indeed occur in the nerve fiber bundles in which such disc hemorrhages were present. Such hemorrhages usually occur at the upper and lower poles of the optic nerve.
head, especially inferotemporally. They are often found in association with disc “notching” of the neuroretinal rim, and this too is strong evidence that their presence is of pathogenic importance. We are inclined to view their appearance most pessimistically. When such hemorrhages occur, they often prompt our serious reconsideration of current therapy (or lack thereof). Succinctly put, the appearance of hemorrhages in NPG patients tells us whatever we’re doing to minimize future loss isn’t working.

4. Visual field loss
No assessment of glaucoma can be made without confirmation that there has been a progressive decrease in light sensitivity of the peripheral and/or paracentral visual field. Since visual field analysis is performed by several instruments among practitioners, no generalized statement can be made regarding the precise definition of what constitutes visual field progression. Utilizing the automated perimetry (Humphrey) field analyzer automated perimetry (Humphrey) Instruments, San Leandro, CA), our criteria for visual field abnormalities on the computerized parametric test include a corrected pattern standard deviation with a $p$ value < 0.05 or a glaucoma hemifield test outside normal limits obtained with at least two reliable and reproducible visual field examinations. Visual field damage is considered to have progressed if there was a change in 5 or more points with at least 3 being contiguous compared with their baseline values based on the glaucoma change probability analysis (STATPAC). Utilizing manual perimetry (Goldmann), kinetic visual field defects typically include reproducible nasal steps of at least 10 degrees in width or paracentral scotomas over 5 degrees in width, in addition to classical Seidel and Bjerrum scotomas. The criteria for progression commonly consists of a reproducible and meaningful increase in either the depth or the extent of the scotoma.

Although it has been the subject of considerable controversy, most observers would agree that characteristic changes of the visual field often seen in patients with normal pressure glaucoma include visual field defects that are steeper, deeper, and closer to fixation than those seen in patients with POAG. If paracentral defects are present, it is often helpful to utilize the 10-2 program of the Humphrey instrument, and if visual acuity is poor, the use of a size V stimulus as opposed to a III stimulus is often preferred. There are many patients on whom we alternate 30-2 and the 10-2 programs on sequential visits.

B. SYSTEMIC CLUES

1. Vasospasm
Patients with vasospasm constitute one of the earliest and best-characterized subsets of patients with NPG. The literature is replete with studies which measure the vascular diameter of fingernail bed capillaries in response to temperature changes in order to assess peripheral vasospasm in these patients. Should the clinician feel inadequate if they do not have access to such nifty technology? The answer of course is a resounding no! The eminent Dr. Stephen Drance has taught us that the easiest way to assess the presence of vasospasm is simply to shake your patients’ hands. Naturally, some patients may have cold hands if they are nervous, as may merely by a visit to the doctor, but your perception of cold hands should prompt directed questions to ascertain whether or not the patient is vasospastic. Are their feet cold? Do they sleep with socks on, particularly in the summertime? There is a high yield of positive responses to these questions in patients who have cold hands detected with a simple hand shake.

An additional history that is often given by vasospastic individuals with NPG is that of migraine headaches. In attempting to elicit this history, it is important to keep in mind that the presentation of migraine headaches spans the gamut from classical migraine to acephalgic migraine, in which the headaches are not nearly as prominent as visual disturbances associated with spasm of either retinal or cerebral vessels.

Not only is one of the largest subset of normal pressure glaucoma patients those that are vasospastic, but it is a group for which some rational, however unproven therapy, may be considered. Recently, the use of calcium channel antagonists has been popularized for the treatment of normal pressure glaucoma. We consider the evidence that this family of compounds is efficacious in this disorder to be anecdotal and not utterly convincing. In addition, there are many studies which have shown that these compounds in fact do not favorably alter visual field progression in either normal pressure or high pressure glaucoma. Complicating this issue even further have been a number of interesting reports which suggest that patients who use these drugs chronically may experience an increased risk of cancer. Before offering patients calcium channel blockers, consider this: If the practitioner does not experience enough “tension” already in dealing with patients with this disease, one can only imagine what happens when informing patients of these studies? We nevertheless do advocate the use of these drugs in select individuals, particularly the ones with frank and overt manifestations of peripheral vasospasm and/or migraine headaches, in which visual field worsening has occurred despite other efforts such as maximum reduction of IOP.

Calcium channel antagonists vary in their selectivity of cardiac as opposed to vascular receptors. Although ideally one would expect that the most vasoselective calcium channel antagonist is the one which should be used, the reality is that initiating therapy with these compounds will often result in untoward side effects such as headache, and lethargy. Therefore we generally initiate therapy with nonselective agents such as verapamil (Calan SR) prior to escalating our therapy with more selective compounds such as nifedipine (Procardia). (Nimodopine, the most vaso-selective compound, is prohibitively expensive and therefore not a practical choice.) The use of calcium...
channel antagonists is a two-edged sword in more than one way. Effective concentrations may ameliorate vasospasm to a significant degree. However, excessive doses of this compound may cause decreased cardiac ionotropy and chronotropy, which would decrease cardiac output, and thus result in decreased ocular perfusion and be counterproductive. Patients should be explicitly told to monitor their pulse rate and blood pressure when using this family of compounds.

2. Systemic hypotension
Here lies one of the most treatable of conditions that affect a subset of patients with normal pressure glaucoma. Many factors affect blood flow to the optic nerve, including systemic blood pressure, vascular resistance, and intraocular pressure. A meaningful way to integrate the relationship between IOP and blood pressure is to refer to “perfusion pressure”, which is the systemic diastolic blood pressure minus IOP. Studies suggest that the risk of glaucomatous damage increases markedly when diastolic perfusion pressure decreases below 50 mm Hg. Several studies have been performed which demonstrate that patients with both the high and low pressure form of glaucoma (in fact all individuals), may experience nocturnal hypotension in which their blood pressure drops precipitously during the evening hours of sleep. This of course, decreases perfusion pressure and likely increases the risk of optic neuropathy. In our experience this syndrome occurs most typically in thin, older individuals, particularly women.

We have been impressed that self monitoring of blood pressure with automated sphygmomanometry devices readily available at the local pharmacy have detected profound decreases in diastolic pressures which routinely fall to lower than 60 mm Hg. In these individuals we take a common sense approach and prescribe the use of a salt tablet after dinner as a dietary supplement. Salt tablets are generally available in any health food store and usually consist of 450 mg of sodium chloride and 50 mg of potassium chloride. In these patients, we are often successful in preventing nocturnal hypotension by increasing salt and fluid intake prior to retiring. Of course, the long-term effects on their optic neuropathy is unknown. However, we believe it is reasonable to advocate such an approach to facilitate ocular circulation and minimize the potential harm of lowered systemic blood pressure.

3. Is there a role for aberrant autoimmunity?
Many patients in whom we suspect that there is an autoimmune component to their optic neuropathy can be identified easily by history taking. Questions relating to the presence of musculoskeletal and joint involvement, skin rashes, and sicca complex are all useful in order to identify these potential patients. However, we should recognize the fact that the presence of such symptoms are generally nonspecific. In fact, in the case of sicca complex and/or dry eyes we all know that this condition is quite common in elderly individuals, not just those with glaucoma. The most useful hallmark, therefore, of potential autoimmune involvement in glaucomatos optic neuropathy is eliciting a history in patients in whom there is another autoimmune disease. We have identified several patients who belong to this subset of normal pressure glaucoma based on the clues that they have other autoimmune diseases such as Paget’s disease, Addison’s disease, multiple myeloma, chronic inflammatory peripheral neuropathy, hypothyroidism, etc. In fact, the epidemiologic association of patients with autoimmune disorders obtained from their history was the first clue which identified the subset of patients in whom autoimmunity may mediate the optic neuropathy that occurs in this disease. Of course, it is beyond the scope of the ophthalmologist to treat these other disorders. Also discouraging is that the treatment of autoimmune diseases is in its infancy. The use of global suppressants such as steroids are often successful in ameliorating certain symptoms of several diseases, but the price is often high, and furthermore there is no evidence to suggest that the relief of symptoms due the use of steroids or other immunosuppressants will have any favorable bearing on the glaucomatous process, if indeed a similar autoimmune mechanism underlies both diseases. Nevertheless, it is the responsibility of the ophthalmologist to be alerted by the presence of accompanying autoimmune diseases, and to work closely with a patient’s internist throughout the course of the patient’s care. Certainly, any use of steroids in these patients must be monitored closely in order to detect steroid responsiveness, which, if it occurs, can certainly compromise an already glaucomatous nerve.

IV. TREATMENT
A. WHEN AND WHY TO TREAT CONSERVATELY
Much as it may seem like an anathema to the practicing glaucoma specialist, we would nevertheless state that not all patients with progressive visual field loss require treatment. Let us take for example a 90 year old woman with documented normal pressure glaucoma for 30 years. During that period of time she has had excellent visual acuity in both eyes, but with perhaps a paracentral scotoma in one eye and an arcuate defect in the other eye which has been noted to undergo episodic worsening over a 30 year period. We do not believe it would be unreasonable to consider that minimal, if any, impairment in functional vision would occur in this patient’s expected lifespan if left untreated. Thus, the ophthalmologist’s decision to treat should be tempered by several considerations such as the age of the patient, the level of visual function, and the level of visual needs. On the other hand, there are certainly patients in whom we would advocate aggressive treatment is worth pursuing, although again it must be emphasized that the efficacy of any treatment is unclear. Certainly, the
younger the patient the more inclined we are to consider treatment since there is an increased span of years during which further deterioration may occur.

The decision to treat based on the usual hallmarks of progression, namely, changes in the visual field or the optic nerve, garner the most attention. However, we believe that in many patients, the occurrence of repetitive optic nerve hemorrhages are the harbinger of future field loss, and their appearance often prompts us to treat aggressively. This is certainly true in younger patients.

Assuming one has made the decision that treatment should be initiated to minimize future damage, the basic tenets for treatment vary little from those of other glaucomas. The risks and benefits of any individual therapy, whether it be medical, laser or surgical, must be ascertained on an individualized basis. Patients with multiple allergies to the use of topical drops would certainly make the further use of that modality unattractive. Similarly, patients who are tremulous or uncooperative may not be able to successfully undergo argon laser trabeculoplasty. Finally, older patients who have had multiple ocular surgeries or who have undergone multiple filtering procedures that have been unsuccessful may not be the best candidates for further filtering surgery due to the risk of intraoperative complications such as exsudative hemorrhage.

In general, we do not advocate surgery for patients in whom the IOP is controlled in the low teens. We are much more likely to recommend pressure lowering by whatever means necessary for patients whose IOP > 15 mm Hg. The reason for this is twofold and is rather obvious. First, it is difficult to guarantee successful filtering surgery that yields pressure in single digits or the low teens. Obviously, the desired potential benefit following filtering is limited in an eye whose initial pressures are not that different that obtained post-operatively. Secondly, although it appears that there is a continuum of visual field loss with IOP in glaucoma (with low pressures generally having the least loss), it is again by no means clear that a pressure of 9 mm Hg is necessarily better than a pressure of 12 mm Hg in a disease in which the contributing role of any level of IOP is poorly understood. We would also add that we would have no quarrel for those that feel differently and for whom the reduction of IOP in the single digits is a sought after goal in all patients.

Regarding filtering surgery, we generally prefer the use of a guarded trabeculectomy procedure employing the use of releasable sutures to obtain low pressures early in the postoperative course while avoiding the frequent complications of full-thickness procedures such as choroidal effusions and flat chambers. We routinely employ antimetabolites in all filtering surgery although the dose and duration are varied based on individual patient characteristics. We use low doses of mitomycin C (0.2 mg/ml for 2 minutes) in individuals with thin sclera such as myopes, particularly in young myopic males since this group is one in whom hypotonous maculopathy antimetabolite therapy has been reported.

B. FUTURE OUTLOOK

The best opportunities for treatment will obviously be based on evidence that a specific pathologic mechanism mediates neuronal cell death in any or all of the variants of normal pressure glaucoma. Unfortunately, at the present time there is little evidence that any presumed mechanism of glaucomatous neuropathy is specific to normal pressure glaucoma as opposed to POAG. Although it is likely that many normal pressure and high pressure glaucoma patients may share a common mechanism for their neuropathy, the identification of these mechanisms need to occur prior to rationalizing optimum strategies for treatment. Unfortunately, it is entirely too premature to expect that neuroprotectants, immunosuppressive agents or gene therapy will be available to us in the immediate future. At present, common sense which tempers sound medical judgment offers the best opportunity for decreased morbidity resulting from the diminished optic nerve function that may accompany this most perplexing optic neuropathy.

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DEBATE: THAT WE SHOULD ELIMINATE THE TERM ‘NORMAL TENSION GLAUCOMA’
POSITION: CON

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HISTORICAL
- First mentioned by von Graefe in 1857 – rejected out of hand
- Before 1950, glaucoma divided into congestive and non-congestive, acute or chronic
- 1950s – population studies to define mean IOP in population
- 2 standard deviations from mean, ≤21 mmHg, accepted as normal IOP

DEVELOPING CONCEPTS OF GLAUCOMA
- IOP ≥22 mmHg became equated with glaucoma in the late 1950s and early 1960s
- Patients were treated irrespective of optic nerve head or visual field damage on the basis of IOP alone
- Other factors, such as central corneal thickness, were unknown
- 1960s – concepts of cup/disc ratio, ocular hypertension
- Early reports of non-IOP risk factors largely ignored – nocturnal hypotension, central corneal thickness, low cerebrospinal fluid pressure – rediscovered after 20-30 years

NORMAL-TENSION GLAUCOMA
- Until the last 2 decades, thought to be rare.
- Despite publications, many ophthalmologists, including academicians, found any excuse to rationalize why a patient could not have glaucoma at normal IOPs
- Diagnosis was often markedly delayed, patients being referred with severe visual field loss because discs and fields were often not examined in patients with IOPs in the mid-teens
- It was not incongruous for a patient with IOP 21 mmHg to be diagnosed differently from a patient with IOP 22 mmHg

- Collaborative Normal-Tension Glaucoma Study showed lowering IOP could be beneficial
- Also marked the beginning of differentiation into subgroups according to progression rate – vasospasm, disc hemorrhage, migraine, cardiovascular disease
- People with elevated IOP can also have other risk factors for damage

TERMINOLOGY
Until this point, I have avoided the question as to whether the term “normal-tension glaucoma” should be considered valid or retained in usage.

Part of this is because I don’t think I could fill 7 minutes simply presenting an argument to retain it. You have heard my colleague, Bob Weinreb, argue why we should eliminate the term. I will present reasons for continuing to use it, with the caveat that we are all on the same page when we do so.

POAG WAS LONG DIVIDED INTO OCULAR HYPERTENSION, GLAUCOMA (HTG, NTG)
It took years of arguing before getting people to drop glaucoma from every other entity. People never said “neovascular ocular hypertension” or “chronic angle-closure ocular hypertension”.

We know very well now that IOP exists on a continuum, and there is no dividing line between one mmHg and another. We define glaucoma without using the term IOP. We know that IOP is not the disease itself, as once thought, but a risk factor, albeit the most important known risk factor and to date, the only one inarguably proven to modify outcomes when treated.

What term could we use to replace NTG? When we speak of other risk factors, we speak not of IOP-independent risk factors, but risk factors other than IOP that contribute to a propensity for glaucomatous damage.

But we don’t say this when speaking – it takes too long to say. Nor when we speak of other risk factors, do we say “glaucoma associated with low nocturnal blood pressure”
or “obstructive sleep-apnea associated glaucoma”. The terms are unwieldy and perhaps overly inclusive or insufficiently precise. Thus, knowing what we are speaking about when we use the term NTG, we can retain using it, at least for the time being.

It is also useful, but not necessary terminologically, for separating groups in clinical trials. If all the patients in one arm of a trial have IOP consistently <21 mmHg and those in the other arm have IOP consistently ≥24 mmHg, we can use NTG and HTG to describe these groups in looking for differences between them, whatever those may be.

Since the literature is replete with this terminology, changing it at this point without a specific and widely understood replacement would lead more to confusion than to consolidation.
LEARNING OBJECTIVES

1. Recognize that “cupping” is a clinical term that is non-specific
2. Recognize the clinical features of a “glaucomatous” form of “deep” “cupping”
3. Describe how intraocular pressure, cerebrospinal fluid pressure lamina cribrosa and peripapillary scleral connective tissue material properties and ocular perfusion pressure influence optic nerve head biomechanics at all levels of intraocular pressure and determine the form of cupping present in all forms of optic neuropathy
4. Recognize that optic nerve head connective tissue deformation and remodeling are the defining causes of what clinicians comfortably call “deep” or “glaucomatous” cupping. While we necessarily focus on how retinal ganglion cell axons are insulted within the optic nerve head in glaucoma — optic nerve head biomechanics likely determines the level of pressure and the location at which that damage will occur in a given eye
5. Describe why we should expect to find IOP-related optic neuropathies at all levels of IOP that demonstrate shallow forms of “cupping”
6. Recognize that the continuum between age related axon loss—glaucoma at “normal levels of IOP”—and glaucoma at elevated levels of IOP—is governed by optic nerve head susceptibility

KEYWORDS

1. Glaucoma
2. Optic Nerve Head
3. Biomechanics
4. Cupping
5. Optic
6. Neuropathy
7. Aging

The defining features of a “glaucomatous” optic neuropathy include “glaucomatous” “cupping” which is difficult to describe and therefore phenotype except when “deep” cupping is present and is accompanied by some degree of excavation of the rim tissues beneath Bruch’s Membrane Opening (BMO) and/or the anterior scleral canal opening. Discussion of what constitutes “glaucomatous” cupping are made difficult by the fact that “cupping” is a clinical term which is used to describe optic nerve head (ONH) structural change in all forms of optic neuropathy, however “cupping” is also used as a synonym for the pathophysiology of glaucomatous damage to the ONH. Because the clinical and pathophysiologic contexts for “cupping” are seldom clarified there is a confusing literature regarding the presence, importance and meaning of “cupping” in a variety of disorders. We have previously proposed that only “laminar” or “deep” forms of “cupping” (those that include a connective tissue component) are pathognomonic for glaucoma. We have further clarified that even a glaucomatous form of “cupping” is only one manifestation of the underlying pathophysiologic processes which drive the optic neuropathy of glaucoma. Cupping is therefore a manifestation of the neuropathy of glaucoma, not the optic neuropathy itself. Damage to the retinal ganglion cell axon within the ONH is a second component of the optic neuropathy of glaucoma, but it also is not the optic neuropathy itself. The clinical phenomenon of cupping has two principal pathophysiologic components in all optic neuropathies: prelaminar thinning and laminar deformation (Figure 2). We define prelaminar thinning to be the portion of cup enlargement that results from thinning of the prelaminar tissues due to physical compression and/or loss of RGC axons. We define laminar deformation or laminar cupping to be the portion of cup enlargement that results from permanent, IOP-induced deformation of the lamina cribrosa and peripapillary scleral connective tissues following damage and/or remodeling. We propose that the defining phenomenon of glaucomatous cupping is deformation and/or remodeling of the neural and connective tissues of the ONH, which is governed by the distribution of IOP-related connective tissue stress and strain, regardless of the mechanism of insult or the level of IOP at which that deformation and/or remodeling occurs. Said in another way, “glaucomatous
“cupping” is the term clinicians use to describe the clinical appearance and behavior the ONH assumes as its neural and connective tissues are deformed and/or remodeled: 1) in a pattern and 2) by the several pathophysiologic processes governed by IOP-related connective tissue stress and strain. ONH Biomechanics can help us understand why a given optic nerve head will demonstrate a certain form of “cupping” and at what level of IOP that might happen. Animal models are allowing us to tease apart the important components of cupping in IOP-related and non-IOP related forms of optic neuropathy. A paradigm change in SDOCT ONH, RNFL and Macular imaging should improve our ability to phenotype all forms of damage to the visual system including glaucoma.

THE OPTIC NERVE HEAD (ONH) IN GLAUCOMA

While glaucomatous damage to the visual system likely includes important pathophysiologies within the retinal ganglion cell (RGC) body, photoreceptors, peripheral RGC axon and its synapse, lateral geniculate body and visual cortex, strong evidence suggests that damage to the RGC axons within the lamina cribrosa of the ONH is a central pathophysiology. Recent studies in the monkey, rat, and mouse support the importance of the ONH, by describing profound alterations and axonal transport disruption within the prelaminar, laminar and retrolaminar tissues of the ONH at the earliest detectable stage of experimental glaucoma (EG).

The ONH tissues make up a dynamic environment wherein 1.2 to 2.0 million RGC axons converge, turn, and exit the eye through the inner (Bruch’s Membrane opening) and outer (scleral) portions of the neural canal (Figure 1, see next page). Within the scleral portion of the canal, the bundled axons pass, through a 3-dimensional (3D) meshwork of astrocyte-covered, capillary containing, connective tissue beams known as the lamina cribrosa (Figure 1, see next page). Within the lamina, axonal nutrition is thought to depend upon the movement of oxygen and nutrients from the laminar capillaries, through the laminar beam extracellular matrix, across the astrocyte basement membrane into the astrocyte, finally reaching the peripheral and central axons of each bundle, via cell processes.

WHAT, REALLY, IS GLAUCOMATOUS “CUPPING”? “Cupping” is a clinical term which is used to describe ONH structural change in all forms of optic neuropathy. However, “cupping” is also used as a synonym for the pathophysiology of glaucomatous damage to the ONH. Because the clinical and pathophysiologic contexts for “cupping” are seldom clarified there is a confusing literature regarding the presence, importance and meaning of “cupping” in a variety of disorders. Cupping in glaucoma is highly variable. We have previously proposed that only “laminar” or “deep” forms of “cupping” (those that include a connective tissue component) are pathognomonic for glaucoma.
Figure 1. Glaucoma, cupping and axonal insult within the optic nerve head (ONH). The ONH is made up of prelaminar, laminar and retrolaminar regions (A). Within the clinically visible surface of the Normal ONH (referred to as the optic disc) (B), central retinal vessels enter the eye and RGC axons appear pink due to their capillaries (which are principally supplied by branches from the posterior ciliary arteries (PCA) in (C). The primary site of RGC axon insult in glaucoma is within the lamina cribrosa (schematically depicted with axon bundles in (D), isolated by trypsin digest in a scanning electron micrograph in (E) and drawn with stippled extracellular matrix (ECM), central capillary (red) and surrounding astrocytes (yellow with basement membranes in black) (F). Blood flow within the ONH, while controlled by autoregulation, can be affected by non IOP-related effects such as systemic blood pressure fluctuation and vasospasm within the retrobulbar portion of the PCAs. Additional IOP-induced effects may include compression of PCA branches within the peripapillary sclera (due to scleral stress and strain) and compression of laminar beam capillaries reducing laminar capillary volume flow (C and F). There is no direct blood supply to the axons within the laminar region. Axonal nutrition within the lamina (F) requires diffusion of nutrients from the laminar capillaries, across the endothelial and pericyte basement membranes, through the ECM of the laminar beam, across the basement membranes of the astrocytes, into the astrocytes, and across their processes to the adjacent axons (vertical lines). Chronic age-related changes in the endothelial cell and astrocyte basement membranes, as well as IOP-induced changes in the laminar ECM and astrocyte basement membranes may diminish nutrient diffusion to the axons in the presence of a stable level of laminar capillary volume flow. The clinical manifestation of IOP-induced ONH structural change is most commonly “deep cupping” (G) but in some eyes cupping can be shallower accompanied by pallor (H). Z-H = circle of Zinn-Haller; PCA = posterior ciliary arteries; NFL = nerve fiber layer; PLC = prelaminar region; LC = lamina cribrosa; RLC = retrolaminar region; ON = optic nerve; CRA = central retinal artery. (A) Reprinted with permission from Arch Ophthalmol; (C) reprinted with permission from The Glaucomas. St. Louis: Mosby; 1996:177–97; (D) reprinted with permission from Optic Nerve in Glaucoma. Amsterdam: Kugler Publications; 1995:15–36; (E) reprinted with permission from Arch Ophthalmol; (F) reprinted with permission from Arch Ophthalmol.
Figure 2. All Clinical Cupping, Regardless of Etiology, is a Manifestation of Underlying “Prelaminar” and “Laminar” Pathophysiologic Components. A. Normal ONH. To understand the two pathophysiologic components of clinical cupping, start with (B) a representative digital central horizontal section image from a post-mortem 3D reconstruction of this same eye (white section line in (A)) - vitreous top, orbital optic nerve bottom, lamina cribrosa between the sclera and internal limiting membrane (ILM) delineated with green dots. (C) The same section is delineated into principle surfaces and volumes (Black – ILM; purple - prelaminar neural and vascular tissue; cyan blue line – Bruch’s Membrane Opening (BMO)-zero reference plane cut in section; green outline – Post-BMO Total Prelaminar area or a measure of the space below BMO and the anterior laminar surface). (D) Regardless of the etiology, clinical cupping can be “shallow” (E) or “deep” (F) (these clinical photos are representative and are not of the eye in (A)). A prelaminar or “shallow” form of cupping (G, black arrows) is primarily due to loss (thinning) of prelaminar neural tissues without important laminar or ONH connective tissue involvement. Laminar or “deep” cupping (H, small white arrows depict expansion of the green shaded space) follows ONH connective tissue damage and deformation that manifests as expansion of the total area beneath BMO, but above the lamina. Notice in (H) that while a laminar component of cupping predominates (white arrows) there is a prelaminar component as well (black arrows). While prelaminar thinning is a manifestation of neural tissue damage alone, we propose that laminar deformation can only occur in the setting of ONH connective tissue deformation and remodeling. **Reprinted with permission**26
We now further clarify that even a glaucomatous form of “cupping” is only one manifestation of the underlying pathophysiologic processes which drive the optic neuropathy of glaucoma. Cupping is therefore a manifestation of the neuropathy of glaucoma, not the optic neuropathy itself.

Damage to the retinal ganglion cell axon within the ONH is a second component of the optic neuropathy of glaucoma, but it also is not the optic neuropathy itself. While the pathophysiology of RGC axon damage is of fundamental importance in preserving vision, it may be only one component of, (or secondary to) the larger pathophysiologic events that drive the neuropathy.

The clinical phenomenon of cupping has two principal pathophysiologic components in all optic neuropathies: prelaminar thinning and laminar deformation (Figure 2, see left). We define prelaminar thinning to be the portion of cup enlargement that results from thinning of the prelaminar tissues due to physical compression and/or loss of RGC axons. We define laminar deformation or laminar cupping to be the portion of cup enlargement that results from permanent, IOP-induced deformation26 of the lamina cribrosa and peripapillary scleral connective tissues following damage and/or remodeling.

The clinical hallmarks of a glaucomatous optic neuropathy are “glaucomatous cupping” of the tissues of the optic nerve head (ONH) (Figure 3, see below) — a progressive posterior displacement of the surface of the ONH and progressive excavation of the prelaminar tissues beneath the anterior-most aspect of the scleral canal, the anterior scleral ring (Figure 1, see p. 529) — and glaucomatous visual field loss, which most commonly starts as a nasal step and progresses through an arcuate scotoma to full hemifield loss. These clinical hallmarks distinguish a glaucomatous optic neuropathy from the many other optic neuropathies in which damage to the RGC axons, either at the nerve head or within the orbital optic nerve and chiasm, leads to RGC death. Although exceptions exist, ischemic, inflammatory, and compressive damage to the nerve head, orbital optic nerve, or optic chiasm usually results in pallor and atrophy of the nerve head but little or no excavation of the remaining rim tissue (Figure 1, right). In addition, in these entities the pattern of axon damage within the optic nerve, and as detected by visual field testing, is usually different from that of glaucomatous optic neuropathy.

Thus, while the ONH is the primary site of damage for a group of optic neuropathies, only a subset of these disorders assume the clinical appearance and behavior commonly associated with the term glaucomatous. What then constitutes a “glaucomatous” cupping? We propose that the defining phenomenon that underlies the glaucomatous optic neuropathies is deformation and/or remodeling of the neural and connective tissues of the ONH, which is governed by the distribution of IOP-related connective tissue stress and strain, regardless of the mechanism of insult or the level of IOP at which that insult occurs. Said in another way, “glaucomatous cupping” is the term clinicians use to describe the clinical appearance and behavior the ONH assumes as its neural and connective tissues are deformed and/or remodeled: 1) in a pattern and 2) by the several pathophysiologic processes governed by IOP-related connective tissue stress and strain.

**OPTIC NERVE HEAD BIOMECHANICS**

The biomechanical paradigm of glaucomatous ONH damage does not argue that the ONH is the earliest or only site of damage. ONH biomechanics provides a framework for explaining how IOP-related stress (force/cross-sectional area of the tissue experiencing that force) and strain (a measure of local deformation of a tissue induced by applied stress) within the load-bearing tissues of the ONH influence the physiology and pathophysiology of all three ONH tissue types at all levels of IOP. These include: 1) the connective tissues (load-bearing connective tissues of the peripapillary sclera, scleral canal wall, and lamina cribrosa), 2) the neural tissues (RGC axons), and 3) the cells which exist alone or in contact with both 1 and 2 (astrocytes, glial cells, endothelial cells, and pericytes and their basement membranes).20-44,47-51

![Figure 3](image_url)
ONH biomechanics provides a logic by which non IOP-related risk factors such as ischemia, inflammation, auto-immunity, astrocyte and glial molecular biology are influenced by or interact with the effects of IOP.\textsuperscript{43, 44} ONH biomechanics attempts to combine these "non-IOP-related" factors with laminar and peripapillary scleral connective tissue geometry and material properties (strength, stiffness, structural rigidity, compliance and nutrient diffusion properties) to explain the physiology of normal ONH aging, ONH susceptibility to IOP, and the clinical manifestation of all forms of optic neuropathy (Figure 4, see below).

To understand when, why and how the lamina deforms at a given level of IOP in a given eye, and perhaps also to understand some portion of the contributing mechanisms of axonal insult in glaucoma, the biomechanical determinants of the translaminar pressure gradient or the transition from intraocular pressure to retrolaminar tissue pressure experienced by the ONH tissues are illustrated and explained in Figure 5, see right. The importance of this gradient to axonal physiology is separately discussed below. The key messages of this and the following sections are five-fold. First, energy is required for axon transport and the translaminar pressure gradient may increase the energy requirements of the RGC axons within the lamina cribrosa. Second, IOP-related stresses and strains within the ONH connective tissues are complicated and do not necessarily lead to deformation of the lamina out of the plane of the sclera. Third, scleral canal expansion that tightens the lamina within the canal and lessens posterior laminar deformation, still increases strain within the lamina. Fourth, posterior deformation of the lamina is likely not required for axon transport compromise. Fifth, IOP-related stress and strain within the ONH connective tissues may independently affect the delivery of nutrients to the RGC axons (and therefore affect axon transport) in the presence or absence of frank laminar deformation.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4.png}
\caption{Whether, Over the Course of a Lifetime, an Eye Demonstrates the “Neuropathy of Aging” or the Neuropathy of Glaucoma Lies in ONH Susceptibility. For a given ONH, IOP generates low or high levels of stress depending upon the 3D architecture (geometry) of the ONH connective tissues (size and shape of the canal, thickness of the lamina and sclera) - (Susceptibility 1). Some ONHs will have relatively low stress at high IOP (d). Others will have high stress at low IOP (e). Whether a given level of IOP-related stress is physiologic or patho-physiologic depends upon the ONH's microenvironment (Susceptibility 2). Strong connective tissues, a robust blood supply and stable astrocytes and glia increase the chance of Normal ONH Aging (right – bottom). While the existence of a neuropathy of aging is controversial, the difference between “normal” age-related axon loss (if it is shown to exist) and the development of glaucomatous damage is a matter of ONH susceptibility. \textit{Reprinted with permission\textsuperscript{42}}}
\end{figure}
Figure 5. Principle distribution of forces, pressures and the translaminar pressure gradient within the optic nerve head (ONH), A. Cut-away diagram of IOP-induced mechanical stress in an idealized spherical scleral shell with a circular scleral canal spanned by a more compliant lamina cribrosa. In this case, the majority of the stress generated by IOP/orbital pressure difference (red arrows on the inner surface of the sclera) is transferred into a hoop stress borne within the thickness of the sclera and lamina (blue arrows) that is concentrated circumferentially around the scleral canal (green arrows). B. Note that the pressure behind the lamina is not simply cerebral spinal fluid (CSF) pressure but is retrolaminar tissue pressure (RLTP) which has been demonstrated to be approximately 0.82 x CSF + 2.9 mm Hg by Morgan, et al in dogs. C. The difference between IOP and the retrolaminar tissue pressure is the translaminar pressure difference which generates both a net posterior (outward) force on the surface of the lamina (the red arrows over the lamina) and a hydrostatic pressure gradient (the translaminar pressure gradient - schematically shown in green) within the neural and connective tissues of the pre-laminar and laminar regions. Note that the in-plane hoop stress transferred to the lamina from the sclera is much larger than the stresses induced by the translaminar pressure difference. CSF directly influences laminar position through its effect on the translaminar pressure difference. CSF may also effect scleral flange position within the region it projects to the sclera (Figure 2), but in most eyes, because the projection of the CSF space is minimal this is not likely important (the CSF space within Panels B and C in this figure is greatly expanded due to perfusion fixation). IOP has a similar direct effect on laminar position, but has an additional (and potentially more important) effect on laminar position through the peripapillary sclera. However, while the magnitude of the translaminar pressure difference may be small relative to the stresses within the sclera and lamina, the axons experience it as the translaminar pressure gradient the steepness of which is influenced by the thickness of the tissues over which it is experienced. The translaminar pressure gradient, as such, may serve as a primary barrier to axon transport and flow within this region and an important physiologic determinant for the ONH axons and cells. \textit{Reprinted with permission.}
The difference between intraocular and orbital pressure establishes “engineering” or “mechanical” stresses (force/cross-sectional area of the tissue bearing the load) within the ONH neural and connective tissues, the magnitude of which are determined by the level of IOP and the 3D geometry or architecture of the tissues that carry them.\textsuperscript{53, 54, 55} These mechanical stresses are separate from physiologic stress which we define as physical and metabolic changes within a cell in response to alterations in its environment. The direct (outward) effect of intraocular pressure on the internal limiting membrane of the ONH prelaminar tissues is resisted by the pressure within the retrolaminar optic nerve tissues (retrolaminar tissue pressure) and the outward expansion of the scleral canal which pulls the lamina cribrosa “tight” within the canal, effectively increasing its resistance to outward deformation.\textsuperscript{53, 54}

The important concept for this discussion is that engineering models suggest that the stresses generated by the IOP/orbital pressure difference within the scleral connective tissues are far higher than the direct (outward) stresses on the neural and connective tissues of the lamina (Figure 2, see page 530).\textsuperscript{51, 53, 55} How the ONH connective tissues respond to a given distribution of mechanical stress is determined by their material properties. A growing body of experimental\textsuperscript{44} and theoretical work\textsuperscript{49-51, 55} supports the concept of a laminar-scleral dynamic in which the net compliance or rigidity of the sclera exerts a large influence over the magnitude of lamina cribrosa deformation at all levels of IOP. While a previous study of ONH surface deformation in dogs suggested substantial ONH surface movement with acute IOP elevation,\textsuperscript{56} recent studies in which laminar deformation was measured directly, suggest variable magnitudes of posterior laminar deformation follows acute IOP elevation in normal monkey\textsuperscript{17, 57, 58} and human eyes.\textsuperscript{59}

How the axons respond to laminar deformation, (when present) cannot be separated from other axonal effects of the constituent neural and connective tissue stresses and strains. Said in another way, the components of IOP-related stress and strain that drive ONH connective tissue deformation may not be the components that influence axon transport. Glaucomatous damage to the RGC axons within the ONH may not simply occur at locations with the highest levels of IOP-related connective tissue strain or stress. Rather as neural and connective tissue stress and strain increase, axon physiology may become compromised at those locations where the translaminar tissue pressure gradient is steepest (Figure 5, see previous page) and/or where the axon’s energy supply is or otherwise becomes most vulnerable. In these regards, it is important to clarify the separate concepts of the translaminar pressure difference (the difference between IOP and the retrolaminar tissue pressure) - which generates a net posterior (outward) force on the surface of the lamina and the translaminar pressure gradient (schematically shown in green in Figure 2) which is the hydrostatic pressure gradient within the neural and connective tissues of the pre-laminar and laminar regions created by the translaminar pressure difference. Note that the in-plane hoop stress transferred to the lamina from the sclera is much larger than the stresses induced by the translaminar pressure difference (Figure 5, see previous page). CSF directly influences laminar position through its effect on the translaminar pressure difference. CSF may also effect scleral flange position within the region it projects to the sclera (Figure 2, see page 530), but in most eyes, because the projection of the CSF space is minimal this is not likely important (Figure 2, see page 530).

IOP has a similar direct effect on laminar position, but has an additional (and potentially more important) effect through the peripapillary sclera (Figure 2, see page 530). However, while the magnitude of the translaminar pressure difference may be small relative to the stresses within the connective tissues of the sclera and lamina, the axons separately experience it as the translaminar pressure gradient, the steepness of which is influenced by the thickness of the tissues over which it is experienced. In eyes with thin laminas or in which the lamina becomes thin through the course of the neuropathy,\textsuperscript{52, 60-63} the translaminar pressure gradient may serve as a primary barrier to axon transport and flow within this region and an important physiologic determinant for the ONH axons and cells.

To understand if and how the lamina cribrosa will deform and remodel at a given level of CSF- and IOP-induced load requires engineering finite element models that take into account the geometry of the ONH connective tissues and their constituent material properties. Taken together these components determine the net “structural stiffness” of the lamina cribrosa and peripapillary sclera. It is this net structural stiffness combined with the inherent propensity of the astrocytes to remodel the laminar extracellular matrix of the lamina at a given level of strain, that will determine whether a given eye demonstrates a shallow or deep form of cupping at a given translaminar pressure difference (Figure 6, see next page).
We have characterized glaucomatous damage to the ONH tissues within the monkey model of chronic unilateral experimental IOP elevation using 3D histomorphometric and more recently SDOCT imaging techniques. Our studies together suggest that early “cupping” (0-30% optic nerve axon loss) in the monkey ONH includes profound deformation and remodeling of lamina cribrosa that includes “posterior” (outward migration) of both the anterior and posterior laminar insertions, laminar thickening, that may involve recruitment of the retrolaminar orbital septa into more transversely oriented laminar beams. These laminar alterations occur in the setting of variable scleral canal expansion and myelin remodeling within the immediate retrolaminar orbital optic nerve (Figures 7, 8 and 9).

As the neuropathy progresses, there is eventual thinning of the lamina, further expansion of the scleral canal and splitting of the peripapillary sclera above the anterior most projection of the sub-arachnoid space (Figure 10). While our findings in advanced glaucoma, support classic descriptions of advanced monkey and human disease, our findings of profound laminar remodeling early in the neuropathy are important because they depict “deep” glaucomatous cupping to be an active process of deformation and cellular remodeling – rather than the passive response of deformation alone. Our findings in monkeys need to be confirmed in human disease. New SDOCT-based approaches to deep optic nerve head imaging, (Figures 11 and 12 and covered briefly in the final section) should not only make this possible (targeting human ocular hypertensive patients) but will eventually allow for the characterization of the laminar component of cupping in all forms of clinical optic neuropathy.

Figure 6. Differences in ONH connective tissue structural stiffness and/or remodeling may underlie “shallow” and “deep” forms of glaucomatous cupping in monkeys and humans. Deep (far left) and shallow (far right) forms of human glaucomatous cupping occur at all ages and IOP levels but are classically seen in youthful and elderly eyes, respectively. We have proposed that the ONH connective tissues “harden” with age and that on average aged eyes should demonstrate a shallower form of cupping (i.e. a shallower “phenotype”) as a result. Spectral domain optical coherence tomography (SDOCT) ONH B-scans (green, lower left) from the EG eye of a young (left) and old (right) monkey, when the eye was normal (upper) and at the second confirmation of confocal scanning laser tomography (CSLT) detection of ONH surface change in the young eye (lower left) and at the (later) pre-sacrifice data set in the old eye (lower right). All images were obtained after 30 minutes of manometer controlled IOP (10 mm Hg). In both eyes, while prelaminar neural tissue thickness alterations are present, laminar deformation is also apparent as an increase in the magnitude of space between the Bruch’s membrane opening reference plane (red line) and the anterior lamina cribrosa surface (gold dots). Laminar deformation in the old eye is far less than in the young eye and this profound difference in laminar deformation occurred in the setting of a cumulative IOP insult that was approximately 5 times greater in the old eye. Reprinted with permission.

“CUPPING” IN FOUR MONKEY OPTIC NEUROPATHY MODELS

We have characterized glaucomatous damage to the ONH tissues within the monkey model of chronic unilateral experimental IOP elevation using 3D histomorphometric and more recently SDOCT imaging techniques. Our studies together suggest that early “cupping” (0-30% optic nerve axon loss) in the monkey ONH includes profound deformation and remodeling of lamina cribrosa that includes “posterior” (outward migration) of both the anterior and posterior laminar insertions, laminar thickening, that may involve recruitment of the retrolaminar orbital septa into more transversely oriented laminar beams. These laminar alterations occur in the setting of variable scleral canal expansion and myelin remodeling within the immediate retrolaminar orbital optic nerve (Figures 7, 8 and 9).
Our Central Hypothesis Regarding ONH Connective Tissue Damage In “Laminar” Cupping.

“Deep”, “laminar” or “glaucomatous” cupping is a manifestation of ONH connective tissue deformation, remodeling and/or damage which can be caused by either IOP-related or non-IOP-related insults. (See Figure 5). However, regardless of the primary insult to the ONH connective tissues, their deformation (if present) is driven by IOP-related connective tissue stress and strain. Thus the presence of ONH connective tissue deformation in any optic neuropathy is evidence that the level of IOP at which it occurred, (whether normal or elevated) is too high for the connective tissues in their present condition.

(A) Schematic of normal laminar thickness (x) within the scleral canal with scleral tensile forces acting on the scleral canal wall. (B) Early IOP-related damage in the monkey eye (Figure 6) includes posterior bowing of the lamina and peripapillary sclera accompanied by neural canal expansion (mostly within the posterior (outer) scleral portion) and thickening (not thinning) of the lamina (y). In our studies to date this appears to represent mechanical yield (permanent stretching) and or remodeling of the lamina rather than mechanical failure (physical disruption) of the laminar beams. (C) Progression to end-stage damage includes profound scleral canal wall expansion (clinical excavation) and posterior deformation and thinning of the lamina (z) by mechanisms that are as yet uncharacterized. If all other aspects of the neuropathy are identical, the stiffer the lamina, the more resistant it will be to deformation. Whether this is better or worse for the adjacent axons is a separate question that remains to be determined. Reprinted with Permission

Figure 7. Our Central Hypothesis Regarding ONH Connective Tissue Damage In “Laminar” Cupping.

Figure 8. Profound Subsurface Structural Change Accompanies the Onset of CSLT-Detected Clinical Cupping in the Young Adult Monkey Eye but this May be Different in the Old Monkey Eye. Upper: Normal lamina cribrosa (unhatched), scleral flange (hatched), prelaminar tissue (beneath the internal limiting membrane - brown line), Bruch’s membrane (solid orange line), Bruch’s Membrane Opening (BMO) zero reference plane (dotted orange line), Border tissue of Elschnig (purple line), choroid (black circles) are schematically represented in the upper illustration. Lower: Overall changes in the ONH surface and subsurface architecture at the onset of CSLT-detected ONH surface change in experimental ocular hypertension in young adult monkey eyes are depicted below. Posterior bowing of the lamina and peripapillary scleral flange, thickening of the lamina and thickening (arrows) not thinning of the prelaminar neural tissues (brown shading) underlie posterior deformation of the ONH and peripapillary retinal surface (dotted brown to solid brown ILM). Thus, while expansion of the clinical cup and deformation of the surface are clinically detectable at this early stage of the neuropathy, because they occur in the setting of prelaminar tissue thickening, (not thinning), clinical cupping in experimental ocular hypertension in these young adult eyes is “laminar” in origin, without a significant “prelaminar” component (Figure 3). Because aged eyes will have (on average) stiffer connective tissues, we predict they will demonstrate less laminar and more prelaminar cupping at the onset of clinically detectable ONH surface change – a prediction that has been confirmed in a recent study (Figure 6, above). Adapted from Yang, et al
Figure 9. The pathophysiology of early experimental glaucomatous damage to the monkey ONH includes not only “thickening” but regional “migration” of the laminar insertion away from the sclera to the point that “complete pialization” of the laminar insertion is achieved in a subset of eyes. Neural canal landmarks (Red – Neural Canal Opening (end of Bruch’s Membrane); Blue – Anterior Scleral Canal Opening; Yellow – Anterior Laminar Insertion; Green – Posterior Laminar Insertion; Purple – Posterior Scleral Canal Opening) and segmented connective tissue (dark grey - lamina cribrosa; purple - peripapillary sclera; light green - pial sheath) within digital section images from the inferior region of the normal (top) and the contralateral early experimental glaucoma (bottom) ONH of a representative monkey. Note that in most normal monkey eyes, the lamina inserts into the sclera as is demonstrated in this monkey’s normal eye (top). However at an identical location in the early experimental glaucoma eye of this animal (bottom) in addition to the lamina being thickened and posteriorly deformed, the laminar insertion has migrated outward such that both the anterior and posterior lamina effectively insert into the pial sheath. While regions of laminar insertion into the pia have been reported in normal human eyes, these findings are the first to suggest that active remodeling of the laminar insertion from the sclera into the pia is part of the pathophysiology of “glaucomatous” ONH damage. This phenomenon when present has important implications for the mechanism of axonal insult within these regions. Adapted from Burgoyne, et al.
Characteristics of three monkey experimental models of non-glaucomatous optic neuropathy have recently been reported.70-73 Not surprisingly, optic nerve head features of pallor without excavation were described in unilateral experimental anterior ischemic optic neuropathy (AION),71, 72 and pallor accompanied by diffuse retinal nerve fiber layer thickness (RNFLT) loss without evidence of laminar deformation was demonstrated by SDOCT in a subset of animals that underwent chronic experimental CSF lowering.73 In a paper during the AGS portion of this joint meeting70, our group will additionally describe the lack of SDOCT-detected lamina cribrosa deformation in the setting of profound ONH rim and RNFLT loss in 5 animals followed longitudinally after unilateral surgical optic nerve transection. These findings are in prominent contrast to SDOCT detection of profound laminar deformation in the setting early chronic IOP elevation.66

The lack of laminar deformation in the AION, CSF-lowering and ONT models, while not surprising, is important because it distinguishes them from chronic unilateral IOP elevation. The findings of no laminar deformation in the setting of RNFL loss in a subset of eyes following chronic CSF lowering are important for two reasons. First it suggests that the magnitude of translaminar pressure increase induced by CSF lowering from 12 to 4 mmHg is not enough to overcome the scleral tensile forces (at normal levels of IOP) holding the lamina taut within the canal. Second, it suggests that while the translaminar pressure increase that resulted from primary CSF lowering did not induce laminar deformation, the translaminar pressure gradient increase likely contributed to axonal compromise at normal levels of intraocular pressure in a subset of eyes. This finding suggests the translaminar pressure gradient may be a risk factor for RGC axon loss at all levels of IOP. Finally, the presence of laminar deformation/remodeling in chronic IOP elevation is important because it confirms those phenomena to be defining features of the optic neuropathy of chronic IOP elevation that are detectible at its earliest clinically detectable stage.

The search for a model of low tension glaucoma is important to identify non-IOP-related insults that are capable of not just damaging axons in a glaucomatous pattern but weakening the ONH connective tissues such that they deform and remodel at previously tolerated levels of “normal” IOP. We do not have an experimental model of glaucoma that does not require IOP elevation. Using SDOCT to detect early deformation of the lamina should be considered a target until proven otherwise. New SDOCT imaging paradigms for phenotyping the ONH/RNFL/Macular tissues should enhance this kind of characterization (Figures 11 and 12).
OCT PARADIGM CHANGE IN PHENOTYPING THE ONH/RNFL/Macula OF MONKEYS AND HUMANS

Whether the desire is to detect laminar deformation in a human patient in which the role of IOP and its lowering is uncertain, or to detect laminar deformation in experimental models of normal pressure glaucoma, a new paradigm for SDOCT image acquisition and regionalization relative to the anatomic axis between the SDOCT detects fovea and the centroid of Bruch’s Membrane opening should enhance our ability to phenotype all forms of damage to the visual system in diseases that are dear to both of our subspecialties. A growing literature is using SDOCT and adaptive optics imaging to characterize the ONH cupping in all stages and manifestations of monkey and human glaucoma.

Figure 11. SDOCT ONH, RNFL and Macular data sets acquired relative to the SDOCT determined Foveal to BMO (FoBMO) axis. FoBMO ONH 768 x 256 grid, 48 radial, RNFL Circle and macula Grid scans identically acquired using eye tracking each time the eye is imaged after BMO, its center and the fovea are anatomically identified during the first imaging session.

Figure 12. FoBMO ONH phenotyping will include quantification of ONH tilt, torsion and the neural canal minimum. We have published extensively on the ONH parameters ALCS-D-BMO, ALCS-D-BM, BMOD-BM, PLTT, MRW, MRA and Rim Volume ((A) and (B)). The SDOCT definitions of ONH torsion and tilt are evolving. ONH torsion will be defined as the angle of the long axis of the disc margin ellipse relative to the vertical FoBMO axis (C - lower center). BMOD torsion will be defined as the angle of the long axis of the BMO ellipse relative to the FoBMO vertical axis (shown as zero in C - upper middle) because BMO is a circle. ONH tilt will be defined as the angle between a line connecting the nasal BMO point and the temporal SDOCT projection of the Disc Margin within the FoBMO B-scan (C – lower right). The neural canal minimum defines the smallest cross-sectional area through which the RGC axons pass using all BMO (red) and anterior scleral canal opening (blue) points (lower right).
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INTRODUCTION

The retinal ganglion cells (RGC) and their axons that form the optic nerve are anatomically and developmentally an extension of the central nervous system (CNS). In fact, the optic nerve should not be considered a “nerve” but rather a CNS white matter “tract” containing glia of the white matter of the brain and spinal cord namely oligodendrocytes, astrocytes, and microglia, rather than Schwann cells. The optic nerve is also surrounded by meninges like other white matter tracts. It is not surprising that pathological processes that affect the optic nerve, therefore, demonstrate changes in the brain. However, many ophthalmologists and members of the lay public have focused on glaucoma as exclusively a disease of the eye and have been surprised when pathological abnormalities have been identified in the part of the afferent visual pathway that extends into the brain. This view has been pervasive because the recognized risk factors associated with glaucoma have been ocular: intraocular pressure, corneal thickness, abnormalities of the trabecular meshwork and the angle. Many investigators have highlighted that this perception has resulted in lost opportunities.

Consideration of glaucoma in the context of the CNS reveals that it has significant similarities with other neurodegenerative disorders. This is an exciting paradigm shift in thinking and has played a critical role in the recent advances in understanding glaucoma pathophysiology. This paper will review the changes in the brain that occur in glaucoma as well as focus on the similarities glaucoma displays with other neurodegenerative disorders.

CHANGES IN THE BRAIN THAT OCCUR IN GLAUCOMA

In glaucoma the RGC is the principal cell type injured. The components that are located within the eye are the RGC dendrites, cell body and unmyelinated axons. However, the majority of the RGC lies within the brain forming the intraorbital, intracanalicular, and intracranial components of the optic nerve, optic chiasm, and optic tract. The axons relay information to several nuclei, but the majority of the axons synapse in the lateral geniculate nucleus (LGN). Other areas of the brain that receive input from axons are the pretectal nucleus, superior colliculus, and suprachiasmatic nucleus.

Axonal degeneration after optic nerve injury, including glaucoma, occurs both in retrograde direction (towards the proximal cell body) and towards the distal axon terminal (orthograde or Wallerian degeneration) at the LGN. In addition, transynaptic degeneration, the process whereby damage is transmitted through synaptic connections along anatomic and functional neuronal pathways, occurs in glaucoma as well as other CNS diseases. Therefore, secondary changes in the brain are well-recognized to occur following various types of optic nerve injury including glaucoma. Research suggests that in glaucoma the entire visual pathway is involved extending from the retina to the visual cortex. Studies on glaucoma-associated changes in the human brain have included postmortem investigations, assessed biochemical changes (e.g., cytochrome oxidase) in primates or more recently, neuroimaging. These changes include atrophy of the LGN laminae corresponding to the injured optic nerve and a reduction in visual cortex thickness corresponding to the termination of the LGN relay neurons.

Neuroimaging studies have expanded our understanding of brain involvement in glaucoma providing evidence that these changes in the brain correlate with the clinical severity of glaucoma. Diffusion tensor imaging reveals that the white matter of the visual pathway (optic tract and radiations) shows proportional damage to that seen in the structural and functional changes in the optic nerve while 3T MRI also correlate LGN atrophy with stage of glaucoma. An intriguing finding revealed by 3T MRI studies is that there are widespread abnormalities in the brain beyond the afferent visual pathway including the reduction in bilateral gray-matter volume in the lingual gyrus, calcarine gyrus, postcentral gyrus as well as in the right cuneus, right inferior occipital gyrus, left paracentral lobule, and right supramarginal gyrus.

One emerging concept in neurodegeneration that requires further exploration in glaucoma but potentially could explain these widespread findings is that of spread of disease through pathological proteins. Other lines of investigation have also suggested that the changes in the brain in glaucoma extend beyond the afferent visual pathway. Blood flow abnormalities
have been shown to occur in the brain of patients with both primary open angle glaucoma and ‘normal tension glaucoma’. These include abnormal regional cerebral blood flow patterns, vasoreactivity to hyperoxia, diffuse white matter lesions, and lacunar infarcts.12–15 These findings suggested widespread change in cerebral perfusion and a partial cerebrovascular insufficiency in glaucoma. Recently, numerous studies have further delineated these vascular abnormalities using functional MRI.16–19

The changes that have been described in the afferent visual pathway are consistent with our understanding of the processes of transsynaptic degeneration. However, the widespread changes in the brain identified in glaucoma patients is intriguing and requires further investigation.

FEATURES OF GLAUCOMA THAT ARE CONSISTENT WITH NEURODEGENERATIVE DISORDERS

Although the etiologies of neurodegenerative disorders are diverse, they share many common elements. Glaucoma is increasingly recognized to share these features to varying extent with neurodegenerative processes such as Alzheimers Disease (AD), Parkinsons Disease (PD), Huntingtons Disease (HD), and amyotrophic lateral sclerosis (ALS) amongst others.

1. Age-related
2. Genetic predisposition
3. Predilection for sub-population of neurons
4. Mechanism of Cell Injury
5. Early functional deficits that precede loss of neuronal substrates

FEATURES COMMON WITH OTHER CNS DISORDERS

AGE

Age is the greatest recognized risk factor for glaucoma with the likelihood of developing glaucoma increasing nearly 7-fold after 55 years. Although neurodegenerative disorders can occur at any age, they tend to become more common with aging with a geometrically increasing prevalence. Although the exact reasons for this are unclear, several possible explanations have been postulated. These include mitochondrial changes with age, change in susceptibility of the affected tissues to injury, and deposition or increased levels of one or more substances that increases toxicity or loss of protective substances. Investigators have suggested that this may suggest shared pathophysiological mechanisms between glaucomatous optic neuropathy and other neurodegenerative disorders.20, 21

GENETIC PREDILECTION

Most neurodegenerative disorders have a less common variant that occurs at a younger age that has a strong genetic origin, and a sporadic form that manifests later in life which has not been associated with a specific gene mutations although still may be associated with a genetic predisposition. For example, early onset forms PD, AD and HD comprise less than 10% of cases. The more prevalent form of neurodegenerative disorders are late-onset and are thought to be a combination of genetic susceptibility with other risk factors although the pathophysiological mechanisms overlap the early onset forms. Similarly, primary open angle glaucoma in children and young adults often follows simple Mendelian genetics whereas primary adult-onset open angle glaucoma rarely does.22, 23

PREDILECTION FOR CERTAIN POPULATION OF CELLS

A common feature of neurodegenerative disorders is a predilection for specific cell populations although there is extension to involvement of other cell types especially in advanced stages. For glaucoma the RGC are selectively involved, AD tends to have a predilection for hippocampal cells, PD for the nigrostriatal dopaminergic neurons, and ALS for upper and lower motor neurons.24 However, there is a strong body of evidence that suggests that photoreceptors and other retinal neurons are involved in glaucoma.25

MECHANISM OF CELL INJURY

Neurodegenerative diseases share common pathways in the process of cell death which ultimately occurs largely by apoptosis. The pathophysiology is a complex process with many of the areas still to be elucidated. However, there are some important emerging concepts.

MISFOLDING PROTEINS

The vast majority of neurodegenerative disorders have been shown to involve aberrant processing, misfolding and aggregation of proteins. Several types of abnormal aggregated proteins have been identified. In AD, extracellular β-amyloid (Aβ ) senile plaques and intracellular neurofibrillary tangles composed of abnormally phosphorylated tau protein have been identified. The fibrillation of tau proteins occurs in frontotemporal dementia (FTD). Alpha-synuclein in the Lewy bodies of Parkinson disease (PD) and in Lewy body disease are also recognized features.26

In glaucoma misfolding of proteins has become increasingly studied. Tau proteins have been detected in horizontal cells in human glaucoma retina.27 However, in mouse models of ocular hypertension there is a loss of tau proteins in the retina.28 This absence of tau is thought to be secondary to proteolysis and has been suggested to contribute to the pathogenesis of RGC death. The mechanism is thought to be an increase in calcium, which in turn activates calpain, a calcium-dependent protease. One possible explanation for the failure to detect phosphorylated tau is that tau protein is cleaved by calpain before detection is possible.29 However, investigators have shown that amyloid
The precursors of the amyloid precursor protein (APP) are abnormally processed and neurotoxic amyloid species are upregulated in retina of rodents exposed to chronically elevated eye pressure.

**ACTIVATION OF GLIA**

Activation of glia occurs in all neurodegenerative disorders and is thought to be closely linked with the pathogenesis of these conditions. Astrocytes maintain neuronal homeostasis by mediating extracellular ion and neurotransmitter balance, regulating vascular flow and blood-brain barrier integrity, and secreting a host of growth factors and neurotrophic factors. In neurodegenerative disorders, astrocytes become reactive, hypertrophic and migratory. In glaucoma, activated astrocytes at the optic nerve head have been proposed to secrete matrix metalloproteases and signal a variety of cytokines and growth factors that may result in optic nerve head excavation. Activation of Muller glia has also been described following chronic ocular hypertension. Furthermore, retinal astrocytes and connex 43 protein, the main astrocytic gap junction protein, have been shown to be upregulated in human post-mortem glaucomatous eyes. Once activated, glia may provide some supportive function such as degradation and elimination of amyloid precursor protein peptides, but can also release damaging cytokines and chemokines that contribute to the pathogenesis.

**NEUROINFLAMMATION**

Neuroinflammation has become recognized to be a contributor to a wide range of chronic neurodegenerative disorders such as AD and PD. Recently, there has been a growing body of research that has revealed that neuroinflammation also has a role in glaucoma. Complement proteins, tumor necrosis factor (TNF-alpha), and serum amyloid A (an acute marker for inflammation and infection) have been shown to be upregulated in human post-mortem glaucomatous eyes. Once activated, glia may provide some supportive function such as degradation and elimination of amyloid precursor protein peptides, but can also release damaging cytokines and chemokines that contribute to the pathogenesis.

**CONCLUSIONS**

The focus on the brain changes in glaucoma provides opportunities as well as challenges. On the one hand, it should not detract from exciting developments that are emerging from the study of ocular risk factors such as scleral changes and advances in trabecular meshwork pathology. Therefore, the findings that there are brain changes in the CNS should not be viewed as mutually exclusive or independent from the ocular processes, but rather intricately connected. Furthermore, the relative sequence of brain and RGC damage needs to be clarified. However, further understanding of the CNS involvement in glaucoma should provide intriguing insights into the progression of the disease as well as opportunities for new therapies.

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LEARNING OBJECTIVES
1. Describe that pathogenesis in glaucoma involves the entire optic projection to the brain, especially retinal recipient areas in important hypothalamic, thalamic, and midbrain nuclei.
2. Describe that axonal dysfunction including deficits in active transport is an early event in most age-related neurodegenerative disorders including glaucoma. When axon dysfunction is prevented, outright degeneration of the optic nerve is also abated.
3. Describe that in models of glaucoma, central brain targets respond to early loss of transport from retinal ganglion cell axons with increased brain-derived neurotrophic factor. This increase is concurrent with an interval of persistence of synapses and post-synaptic neurons.

CME QUESTIONS
1. Explain how glaucoma involves early events in the brain but should not be considered a “brain disease” per se.
2. Explain how preservation of anterograde axonal transport from the retina to the brain is a useful outcome measure for testing experimental neuroprotective therapies.
3. Describe evidence that central brain structures in the optic projection do not quickly degenerate even after depletion of axonal transport from the retina and that mechanisms of self-repair could be relevant.

KEYWORDS
1. Neurodegeneration
2. Axon Transport
3. Superior Colliculus
4. Brain-derived Neurotrophic Factor
5. Astrocyte

ABSTRACT
As in other age-related neurodegenerative diseases, progression of neurodegeneration in glaucoma involves early axonopathy. In glaucoma, this is marked by degradation of active transport along retinal ganglion cell (RGC) axons stretching from the retina to the brain. In experimental systems, transport degradation fails first in the most distal site in the optic projection, the superior colliculus of the midbrain. Even as degradation progresses from one retinotopic sector to the next, important structures in the affected sectors persist, including RGC synapses to SC neurons. This structural persistence defines a therapeutic window of opportunity and is accompanied by focally increased brain-derived neurotrophic factor (BDNF) in hypertrophic SC astrocyte glia. Thus, central brain structures in glaucoma may respond to disease-relevant stress by induction of mechanisms useful for maintaining retinal signals.

A NEUROBIOLOGICAL PERSPECTIVE OF GLAUCOMA
Increasingly the focus in glaucoma research from a neuroscience approach is shifting away from the elimination of retinal ganglion cell (RGC) bodies, which is late in progression, and towards degeneration of the optic projection to the brain, which includes many early events in pathogenesis (Calkins, 2012). As this shift occurs, the involvement of a broader neuroscience community brings with it the realization that experimental studies of vision loss and its mechanisms in glaucoma are useful tools for understanding neurodegeneration more broadly and potential therapeutic targets in other diseases of the central nervous system (CNS). This is especially so for age-related diseases (Trovato Salinaro et al., 2104; Namekata et al., 2014).

Linking glaucoma to CNS disease, however accurate, bears with it the danger of a prominent misunderstanding – that glaucoma is at its etiological roots a brain disease; something that begins in the brain and affects the eye. It is not, nor should it be construed as one, even though certain early events in pathogenesis are observed in the brain before the retina or optic nerve (Crish et al., 2010). In its most general terms, and with some noteworthy exceptions (Wax et al., 2008), glaucoma is a family of diseases in which sensitivity to intraocular pressure (IOP) causes degeneration of the optic projection through stress most likely conveyed at the nerve head through complex interactions of astrocytes and microglia.
interactions with the RGC axon. Many of these interactions involve biomechanical stressors that affect axon function (Burgoyne, 2011; Chidlow et al., 2011). That this stress can be read early in the brain, where RGC axon terminals form connections with post-synaptic neurons, is not tantamount to the disease originating in the brain. To state otherwise, as popular media have done in recent years, is a misinterpretation of empirical data that tends towards sensationalism.

This caveat does not in any way diminish the importance of considering glaucoma from a neuroscience viewpoint that focuses on the brain. We are beginning to understand that the optic projection and visual brain are not passive during progression. Quite the contrary; the retina (Ward et al., 2014), optic nerve head (Fu and Stretevan, 2012), and higher visual structures (Sponser et al., 2014) all demonstrate compensatory mechanisms to counter loss of function. Mechanisms of plasticity, remodeling and adaptability ultimately are just as relevant for glaucoma – and by extension other CNS diseases – as they are for complex synaptic functions in the healthy brain. Thus, translational research targeting new therapies must evolve from an exclusive focus on how glaucoma progresses from IOP-related stress at the nerve head to a new spotlight on intrinsic mechanisms that counter loss of function.

**EARLY PATHOGENESIS INVOLVES THE OPTIC PROJECTION**

The optic projection is defined by the path of RGC axons out of the retina and through the optic nerve to their termination targets in the brain (Figure 1). RGC axons from each eye cross at the optic chiasm to form the ipsilateral and contralateral projection to the brain. From there, axonal terminals provide synapses to neurons in several important nuclei. In primates, the primary target for RGC axons is the lateral geniculate nucleus (LGN) of the thalamus, which relays visual information directly to the primary visual cortex. In rodents, nearly every RGC projects primarily to the superior colliculus (SC) of the midbrain, with axon collaterals extending to other nuclei including the LGN. In all mammals, the SC is the most distal pre-cortical target that this stress interacts with the RGC axon itself. The relative sustainability of retrograde transport is likely to have implications for therapeutic interactions between RGC axon terminals and the post-synaptic brain structures with which they interact.

Several important points arise from rodent studies utilizing degradation of active anterograde transport in the SC as a functional outcome measure. Age is the critical determinant of transport failure, with elevated IOP serving as an additional stressor that increases the likelihood of failure (Crish et al., 2010; Calkins, 2012; Calkins and Horner, 2012; Calkins, 2013). In multiple experimental models, both chronic and inducible, deficits in anterograde transport are detected earlier than a variety of other pathogenic outcomes – including axon degeneration in the optic nerve and RGC body loss in the retina. This chronology renders transport read-out in the SC a convenient outcome measure for experimental interventions (Lambert et al., 2011; Bosco et al., 2012; Dapper et al., 2013; Ward et al., 2014). Finally, degradation of axon transport in the SC is spatially progressive, filling in from one retinotopic sector to the next. In early progression, a given SC is very likely to contain both affected and unaffected regions. This provides a convenient internal control for investigations directed at how post-synaptic structures in the optic projection respond to glaucomatous challenges (Crish et al., 2013), a topic we take up below.

**A THERAPEUTIC WINDOW IN PROGRESSION**

Across different experiment models, both chronic (e.g., DBA2J mouse) and inducible, degradation of anterograde axonal transport to the brain marks the beginning of an important window of opportunity for intervention. This window is defined by the interval during progression between the onset of deficits in axon function and actual degeneration of RGC axons in the optic projection, which occurs later. These functional deficits can be detected quite easily, either through axonal or retinal physiology (Quigley and Addicks, 1980; Baltan et al., 2010; Saleh et al., 2007). A similar interval likely exists in human glaucoma, with reversal of physiological deficits with timely IOP-lowering interventions (Sehi et al., 2010). Experimental interventions that target this period of functional quiescence in the projection and are successful in restoring axon transport also abate subsequent steps in pathogenesis. For example, daily topical application of a potent and highly selective inhibitor of retinal p38 MAPK activity was effective at stopping progression entirely in the microbead-occlusion...
Figure 1. Retinal Projection in the Rodent Brain. Schematic diagram illustrates the dominant contralateral projection of the retina in the rodent visual system. RGC axons exiting the retina through the optic nerve head cross at the optic chiasm to join either the ipsilateral or contralateral optic tract in the brain. Central targets for RGC axons are highly conserved across mammals and include the suprachiasmatic nucleus (SCN) of the hypothalamus (HT) and the olivary pretectal nucleus (OPT), nucleus of the optic tract (NOT), and posterior pretectal (PPT) nucleus of the pre-tectum in the subcortical midbrain. In primates, the lateral geniculate nucleus (LGN) of the thalamus is the primary RGC recipient. Across mammals, the superior colliculus of the midbrain is the most distal direct target for ascending RGC axons. In rodents, all or nearly all RGCs project to the colliculus, while extending axon collaterals to nuclei lying more proximal to the retina (e.g., anterior to the colliculus; Linden and Perry, 1983). There are numerous inter-species differences in the strength of specific RGC projections.

Figure 2. Deficits in Axon Transport Progress Distal to Proximal. (A) Cross-section (coronal plane) through the superior colliculus (SC) from an 8 month DBA2J mouse following intravitreal injection of cholera toxin β (CTB). Dashed line demarcates fully intact anterograde transport of CTB in retinal recipient region in superficial SC (sSC), just dorsal to deep (dSC). (B) In same brain, anterograde transport of CTB is also intact in structures more proximal to the retina, including the olivary pretectal nucleus (OPT) and dorsal and ventral lateral geniculate nucleus (dLGN and vLGN), as well as the suprachiasmatic nucleus (SCN), as shown in (C). The contralateral SC from the same brain (D) demonstrates a complete depletion of CTB transport from the retina. In distal to proximal progression, transport has also failed in the OPT, but persists at a residual level in the LGN (E). More proximally, in the SCN (F), axonal transport remains intact. Scale = 500 µm (A,B, D,E) or 100 µm (C,F).
inducible rat model (Dapper et al., 2013), as was systemic delivery of the alpha-2 adrenergic receptor agonist brimonidine in another inducible model (Lambert et al., 2011). In these cases, for control/vehicle cohorts, deficits in anterograde transport exceeded axon degeneration in the optic nerve which, in turn, exceeded RGC body loss in the retina (see Figure 4 of Calkins, 2012). Correspondingly, with treatment, rescue of transport was a surrogate marker for survival of RGC axons and bodies.

A key avenue of investigation addresses how RGC post-synaptic targets in the brain respond to glaucomatous stressors and whether this response includes mechanisms that promote RGC axon survival. At one end of the spectrum, post-mortem samples of LGN from human patients with significant field loss show significant loss of tissue (Gupta et al., 2006). With prolonged exposure to elevated IOP, non-human primate LGN demonstrates significant depletion of neurons post-synaptic to RGC axon terminals (Harwerth et al., 2002; Weber et al., 2000; Yucel et al., 2003). Even so, loss of LGN neurons generally lags by 20-30% RGC axon degeneration in the optic nerve (Yucel et al., 2003). Our studies show similar persistence of post-synaptic neurons and of RGC synaptic terminals in the SC well after axonal transport from the retina is depleted completely (Crish et al., 2010). This is so for both the DBA2J mouse model of hereditary pigmentary glaucoma as well as the microbead model. Thus, just as RGC axons in the optic nerve persist for a period of time following loss of anterograde transport, so too do their axon terminals and synapses with relay neurons in the brain.

POSSIBLE MECHANISMS OF SELF-REPAIR IN GLAUCOMA

Structural persistence in the optic projection is testimony to the resilience of the CNS and offers clues to possible intrinsic pro-survival mechanisms in glaucoma. Post-synaptic structures in the brain respond to disease-relevant stressors including degraded axon transport in ways thought to promote recovery of axon activity. This response may include a certain degree of synaptic remodeling to compensate for loss (Kimura et al., 2006; Hennigan et al., 2007 and Song et al., 2008). In retinotopic sectors of depleted transport in the SC, astrocyte glia become hypertrophic compared to SC regions with intact transport (Figure 3). These same astrocytes demonstrate increased levels of brain-derived neurotrophic factor (BDNF) that is likely sequestered after release from SC neurons (Crish et al., 2013). These changes occur prior to elimination of important structures in the SC, including synapses from RGC axons and dendrites of SC post-synaptic neurons, visualized with antibodies against MAP2 (Figure 4). Increases in BDNF occur with other injury models, including NMDA-induced excitotoxicity and acute elevations in IOP (Tanaka et al., 2009; Sasaoka et al., 2008; Zhang et al., 2009).

Why would retinorecipient targets in the brain respond this way to disease-relevant stressors? One possibility lies in the fact mentioned earlier that retrograde axonal transport in the optic projection persists in glaucoma as long as RGC axons themselves. BDNF is implicated in axonal guidance and RGC dendritic arborization during development (He et al., 2003). Whereas retinal-derived BDNF inhibits dendritic arborization, BDNF shuttled in retrograde fashion along RGC axons promotes outgrowth (Cohen-Cory and Lom, 2004). In the adult visual system, when IOP is elevated acutely, retrograde transport of exogenously applied BDNF from the SC to the retina is greatly diminished (Pease et al., 2000). Thus, SC-derived BDNF might be uploaded to RGC neurons in retinotopic sectors challenged by degradation of anterograde axon transport for the purpose of protecting RGC dendritic arbors in the retina. Supporting this hypothesis, combined application of exogenous BDNF to the eye and brain is far more effective in protecting RGCs than application to the eye alone (Chen and Weber, 2004; Weber et al., 2010).

In our studies, however, the greatest fraction of BDNF was found to be in stored membrane vesicles and not observed directly in RGC axon terminals (Crish et al., 2013). We argued that perhaps vesicle-stored BDNF in SC neurons is released and sequestered by astrocytes in response to diminished RGC axonal transport to promote synaptic activity and survival. In the CNS, BDNF contributes broadly to maintenance of synaptic function and plasticity of neural circuits (Huang and Reichert, 2001; Lessmann et al., 2003). Astrocytes are likely to play an important role. In the hippocampus, astrocytes expressing the TrkB.t1 receptor isoform bind extracellular BDNF for storage prior to re-release into the extracellular space (Alderson et al., 2000). This pathway could explain the high levels of BDNF in both SC neurons and astrocytes as a mechanism to conserve local excitatory interactions from RGC axon terminals (Crish et al., 2013). In support of this, in experimental Huntington’s disease, over-expression of BDNF in astrocytes conserves striatal synapses (Giralt et al., 2011). Such a mechanism could also contribute to the highly plastic coordination of residual retinal input to the brain to optimize binocular visual field coverage observed recently in human patients (Sponsel et al., 2014).

CME ANSWERS

1. Functional outcomes involving the retinal ganglion cell axon reflect the physiology and state of the entire ganglion cell. Pathogenic outcomes in the brain, such as degradation of anterograde transport, likely reflect stress originating within the optic nerve head or retina. To call glaucoma a “brain disease” implies there is strong evidence for causal or etiologically events originating in the brain in most types of glaucoma.
2. Deficits in axon transport from retina to brain occur early in relevant animal models, with outright degeneration of the optic nerve and retina later. Experimental interventions that are successful in preserving axon transport also preserve optic nerve and retinal structure.

3. Careful studies of progression indicate that axon degeneration in the optic nerve and loss of neurons in the SC in particular lag considerably behind depletion of axon transport. As well, RGC synapses to SC neurons remain, as do SC dendritic processes. One possible mechanism in the SC is the focal elevation in BDNF in retinotopic regions of transport deficit – which could work to preserve synapses and associated structures.

Figure 3. Deficits in Axon Transport Increase BDNF Focally. (A) Coronal section through the SC of an 10 month DBA2J mouse following bilateral intravitreal injection CTB shows fully intact anterograde transport in sSC from one eye (dashed line) with degradation of signal in the opposing SC (arrows). Staining for glial acidic fibrillary protein (GFAP) shows increased astrocyte hypertrophy in same SC, while brain-derived neurotrophic factor (BDNF) also increases. (B) SC from a 10 month DBA2J mouse shows bilateral deficit in anterograde transport of CTB, corresponding to a more uniform distribution of hypertrophic GFAP-labeled astrocytes and BDNF. Higher magnification images of the midline (boxed region) between the two SC (C) shows depleted CTB signal corresponding to increased BDNF in hypertrophic astrocytes. Scale = 200 µm (A,B) or 20 µm (C).
Figure 4. Persistence of Neuronal Structure. (A) SC from an 3 month DBA2J mouse shows a focal deficit in CTB transport from the retina (arrow) near the midline to the fellow SC, which has intact transport (dashed line). (B) Label for microtubule-associated protein 2 (MAP2) in the dendritic arbors of SC neurons remains unchanged despite the transport deficit. (C) Higher magnification images of the midline between the two SC (boxed region in A,B) shows increased BDNF where CTB transport is depleted and consistent MAP2 staining. Scale = 20 µm for C.

REFERENCES


Glaucoma is an age-related, chronic neurodegeneration of the optic nerve. The molecular and cellular pathologies that characterize glaucoma are shared by other chronic neurodegenerations such as Alzheimer’s disease, Parkinson’s disease, and amyotrophic lateral sclerosis. Therapies directed at treating chronic neurodegenerations could potentially be used to treat glaucoma, and conversely therapies that are successful in treating glaucoma could be used to treat other chronic neurodegenerations.

OUTLINE

1. Classification of chronic neurodegenerations
   a. Early-onset forms (autosomal dominant, “familial”)
      i. Alzheimer’s disease
      ii. Huntington’s disease
      iii. Parkinson’s disease
   b. Late-onset forms (multi-factorial)
      i. Alzheimer’s disease
      ii. Huntington’s disease
      iii. Parkinson’s disease
      iv. Amyotrophic Lateral Sclerosis

2. Affected pathways
   a. Cellular aging (senescence):
      i. Protein folding/chaperones
      ii. Ubiquitination/proteasome function
      iii. Autophagy/lysosomes
      iv. Programmed cell death (apoptosis)
   b. Cellular components:
      i. Axonal transport/integrity
      ii. Synaptic function
      iii. Mitochondrial function
   c. Gene regulation
      i. Excitotoxicity/oxidative stress
      ii. DNA damage/repair
      iii. micro RNA
   d. Neuroinflammation
      i. Complement activation
      ii. Tumor necrosis factor
      iii. Astrocyte activation

INTRODUCTION

Recent work in our laboratory has shown that glaucoma and Alzheimer’s disease (AD) share similar molecular and cellular pathways that may contribute to neuronal loss in glaucoma. Investigating these shared pathways will yield valuable clues to aid in rational drug design for the treatment of glaucoma.

CLASSIFICATION OF CHRONIC NEURODEGENERATIONS

Most neurodegenerations can be classified into one of two categories: Specific genetic mutations cause autosomal dominant (“familial”), early-onset forms of AD, Huntington’s disease (HD), and Parkinson’s disease (PD). Fortunately, the incidence of these familial types of neurodegeneration is low, comprising less than 10% of total cases of AD, HD, and PD. These mutations have been exploited to create transgenic mouse models that have greatly aided in the understanding of the pathobiology of these diseases. The more prevalent category of neurodegenerations includes AD, PD, and amyotrophic lateral sclerosis (ALS, or Lou Gehrig’s disease). These sporadic diseases manifest in the later decades of life and are not associated with specific gene mutations, as are the familial forms of the diseases—facts that closely parallel those in open-angle glaucoma. Mounting evidence shows that late-onset neurodegenerations are characterized by a combination of genetic susceptibility and environmental exposure with mechanisms that overlap those in the familial or early-onset forms of the disease.

ALZHEIMER’S DISEASE

AD is a progressive, debilitating neurodegeneration and is the most common form of dementia. It causes loss of neurons in the hippocampus and cerebral cortex, leading to short-term memory loss.
It is characterized by the formation of aggregated proteins composed of amyloid-β known as amyloid plaques, and neurofibrillary tangles, composed of hyperphosphorylated tau protein. Amyloid-β is cleaved from the membrane-bound protein amyloid precursor protein (APP) by enzymes termed secretases. The pathologic forms of amyloid-β are cleaved by β and γ-secretases; these are currently target components of drugs under development for the treatment of AD. A third secretase, the α subtype, cleaves APP to form a soluble form that is important in neuronal survival and synaptic maintenance.

APP is the most abundant protein in the optic nerve. It is rapidly transported in the optic nerve in small vesicles and is transferred to the axon plasma membrane and synapses. The incidence of glaucoma is significantly higher in AD patients than in age-matched controls: 26% versus 5% in a German population and 24% versus 9% in a Japanese population. Furthermore, progression of visual field defects is accelerated in patients with open-angle glaucoma and AD versus patients with open-angle glaucoma without AD. We have shown that APP is abnormally processed, and neurotoxic amyloid-β species are upregulated in the retina of rats and mice exposed to chronically elevated eye pressure. Hyperphosphorylated tau protein has also been detected in the retinas of glaucoma patients. Glaucoma and AD are characterized by synaptic degeneration in the brain, which implies that it is not just a disease of the eye, but of the brain as well.

Memantine, a treatment approved by the U.S. Food and Drug Administration for AD, has been used in a clinical study for the treatment of human glaucoma. Unfortunately, the clinical endpoints (preservation of visual fields) for the study were not reached, probably due to an ineffective mechanism of action. However, directly targeting the formation of amyloid-β has shown promise in preserving retinal ganglion cells (RGCs) in a rat glaucoma model.

PARKINSON’S DISEASE

PD is the most common neurodegenerative movement disorder, caused by loss of dopaminergic neurons in the substantia nigra of the brain. It is characterized by slowness of movement, difficulty in walking, rigidity, and shaking. As with AD, cognitive and behavioral problems and dementia occur in advanced stages of PD. Hereditary forms of PD show mutations in α-synuclein and phosphatase and tensin homolog (PTEN), and pathology shows eosinophilic cytoplasmic inclusions of fibrillar, misfolded proteins termed Lewy bodies.

The synucleins are a family of proteins with unknown function, but a link between γ-synuclein and PD and other synucleinopathies has been established. γ-Synuclein has been noted to be deposited in a specific area of the optic nerve head where myelin begins to be expressed. A recent study has shown that optic nerve astrocytes digest and process RGC axonal processes, suggesting that optic nerve head astrocytes are important for normal maintenance of RGCs. Axonal material found inside these astrocytes contained a protease-resistant form of γ-synuclein, possibly contributing to the loss of RGCs in glaucoma.

PTEN is a negative regulator of the mammalian target of rapamycin (mTOR) pathway and in wild-type adult mice, mTOR activity is suppressed and protein synthesis is impaired in axotomized RGCs. Of note, deletion of PTEN promotes axon regeneration after optic nerve crush in mice, and the manipulation of PTEN and mTOR pathways is an exciting new therapeutic approach to promoting axon regeneration after central nervous system injury, both in the eye and in the brain and spinal cord.

AMYOTROPHIC LATERAL SCLEROSIS

ALS is a progressive, fatal motor neuron disease caused by the degeneration of neurons located in the ventral horn of the spinal cord and the corticospinal neurons that provide their afferent input. It is characterized by rapidly progressive weakness, muscle atrophy, fasciculations, spasticity, dysarthria, dysphagia, and respiratory compromise. Mutations in Cu/Zn superoxide dismutase (SOD), a potent antioxidant enzyme, cause 2% to 3% of ALS cases. Oxidative stress and the expression of reactive oxygen species have been linked to the pathogenesis of glaucoma, and the use of antioxidants such as SOD analogues represents a promising therapeutic approach to the treatment of glaucoma.

NEUROINFLAMMATION

Neuroinflammation is rapidly emerging as a major contributor to the development of chronic neurodegenerations such as AD and PD, as well as glaucoma. Complement proteins are part of the immune system that aid antibodies and phagocytic cells in clearing pathogens. C1q is the first element in the classic complement activation pathway, and it activates several proteases (C1r, C1s, C2, C3, and C4) that initiate opsonization and anaphylactic reactions that attract phagocytic cells. Increased neuronal
C1q expression occurs in AD, and C1q has been shown to be upregulated in mouse and monkey glaucoma models. In a recent study involving a model of inherited mouse glaucoma, the normal developmental mechanism of complement-mediated synapse elimination was aberrantly reactivated in retinal astrocytes. The authors conjectured that C1q tags retinal synapses for early elimination and drives dendritic atrophy and axon degeneration that occur in glaucoma.

Tumor necrosis factor (TNF)-α is an inflammatory cytokine, and its receptors TNFR1a and TNFR1b have been noted to be upregulated in the retinas of glaucoma patients. In a study of a mouse glaucoma model of elevated IOP, the absence (knockout) of the TNFR1b gene afforded robust neuroprotection of RGCs and their axons. Serum amyloid A is an acute-phase marker of inflammation and infection, and gene-profiling studies of glaucoma have shown upregulation of serum amyloid A in the trabecular meshwork and retina of glaucoma patients.

MICRO-RNA REGULATION OF GENE EXPRESSION IN CHRONIC NEURODEGENERATIONS

Because glaucoma and other chronic neurodegenerations share common genetic mechanisms, it is critical to understand how the expression of genes are regulated in the retina and optic nerve in glaucoma, as this knowledge may enable rational drug design for therapeutic intervention. Recent investigations into the regulation of gene expression have focused on micro (mi)RNAs, which are short, endogenously expressed, noncoding RNAs that bind to the 3' untranslated region of messenger RNA, targeting it for downregulation or degradation.

Several laboratories have shown significant changes in expression of some miRNAs in the brains of AD patients. Down regulation of these miRNAs is believed to contribute to increased production and accumulation of amyloid-β in these brains. Other miRNAs dysregulated in AD, such as miR-27b, -34a, and -146a, have been hypothesized to contribute to AD pathogenesis by increasing oxidative stress and inducing inflammation. In a recent report, human astrocytes from normal individuals were cultured in vitro and treated with interleukin-6 to induce astrogliosis, a detrimental cellular process that occurs in AD brains. Levels of miRNAs were assayed, and miRNA-125b was noted to be upregulated. When miRNA-125b activity was repressed with antisense miRNA-125b, glial cell proliferation and increased expression of CDKN2A (cyclin dependent kinase inhibitor 2A) were found. CDKN2A is a miRNA-125b target and negative regulator of cell growth. CDKN2A downregulation has been noted in advanced AD and Down’s syndrome brains, disorders associated with astrogliosis. The authors reasoned that miRNA-125b upregulation contributes to cell cycle defects and the astrogliosis that is characteristic of neurodegeneration. This finding may be of major importance, given recent reports of a significant association between polymorphisms in CDKN2BAS and open-angle glaucoma.

Given the relationship between AD and glaucoma, we hypothesize that in glaucoma, the retina and optic nerve experience changes in miRNA expression similar to those reported in the brains of AD patients. The observation of changes in expression of specific miRNAs associated with glaucoma should be useful in elucidating the pathogenic mechanisms involved in the loss of RGCs and could identify novel therapeutic targets.

SUMMARY

Glaucoma is an age-related, chronic neurodegeneration of the optic nerve. The molecular and cellular pathologies that characterize the disease are shared by other chronic neurodegenerations such as AD, PD, and ALS. Therapies directed at treating chronic neurodegenerations have potential for use in treating glaucoma; conversely, therapies that are successful in treating glaucoma could be used in treating other chronic neurodegenerations. The following are targets for therapeutic intervention in chronic neurodegenerations and glaucoma:

- Axonal transport and integrity
- Autophagy and lysosomes DNA damage and repair
- Excitotoxicity and oxidative stress
- Gene regulation and miRNA
- Mitochondrial function
- Neuroinflammation
- Programmed cell death (apoptosis)
- Protein folding and chaperones
- Synaptic function
- Ubiquitination and proteasome function

Finally, many of the molecular and cellular pathologies that characterize chronic neurodegenerations could be detected first in the eye, leading to earlier diagnosis and more effective treatments.
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LEARNING OBJECTIVES

1. Describe new developments in designing clinical trials for neuroprotection
2. Recognize the importance of power calculations in the development of neuroprotective therapies
3. Describe how drug delivery impacts neuroprotective efficacy in the clinical setting

CME QUESTIONS

1. Which of the following is not relevant to calculating the n needed in a clinical trial of neuroprotection?
   a. Significance level sought
   b. False negative rate
   c. Critical value for difference sought between groups
   d. Standard deviation of the outcome measure
   e. None of the above (all are relevant)

2. Proof-of-concept studies are valuable because:
   a. They make Phase 3 studies unnecessary
   b. They are the main source of toxicity/safety data
   c. They are typically powered to have an 80% or 90% chance of detecting an effect
   d. They use a mixture of non-human primates and humans

3. Drug delivery for neuroprotection should meet the following criteria:
   a. Allow sufficient drug to reach the target
   b. Lead to preservation of the RGC axon
   c. Lead to preservation of the RGC soma
   d. Lead to preservation of the RGC dendrites
   e. All of the above

KEYWORDS

1. Neuroprotection
2. Power Calculations
3. Clinical Trial Design
4. Proof-of-concept Studies
5. Drug Delivery

INTRODUCTION

As any user of Google Maps or Mapquest knows, to get to where one is going, it helps to know where is the starting point. For neuroprotection, there have been numerous excellent reviews on the topic of the current status of neuroprotection in glaucoma. These include cell culture studies, animal models testing therapies, clinical trials, the two largest of which are still unpublished, and even systematic reviews. Yet all of this work can be summarized in a very simple “FROM” box: there is no convincing clinical evidence that neuroprotection works in glaucoma. The subject of this syllabus is a discussion of some future possibilities for a Rd. map that takes us “TO” evidence for neuroprotection in glaucoma.

TRANSITIONAL BREAKDOWN AND ANIMAL MODELS

There are many neuroprotective drugs and other strategies that have been developed and work in laboratory animals, but this has not translated into clinically available compounds in glaucoma and other optic neuropathies. The reasons for this are manifold, and reflect mostly a general problem of translational research that is common to many other areas of medicine. One of the issues is that the preclinical models used for testing neuroprotective drugs and other interventions may not reflect the human disease as accurately as desired. For example, there are animal models that have significant intraocular inflammation or that require reactive tissue processes for the model to take place. Inflammatory or other injury processes may confound the study of neuroprotective drugs because inflammation or injury itself can have pro- or anti-neuroprotective effects. Another reason for translational breakdown is when models that are based on acute injuries or rapid progression are used to model a chronic disease such as human glaucoma, which takes place over decades. Finally, there is increasing realization that biomechanical processes at and around the optic nerve head may help explain features of glaucomatous progression. Rodent optic nerve heads, which are frequently used in glaucoma models, differ substantially from primate optic heads. It therefore is not be surprising if translational issues arise from using mouse and rat models to assess neuroprotection in glaucoma.

We can expect that better models will arise over the next decade. Nonhuman primates have ocular and optic nerve architecture very similar to humans, but their routine use in neuroprotection studies is difficult because of the
complexity and expense of carrying out studies in large numbers of animals. In the past, a study of a small number of rhesus or cynomologous macaque monkeys has been considered less helpful in preclinical research because of the high likelihood that there will be a false negative result because of the small n associated with the study. The marmoset is a much smaller nonhuman primate that has a fovea, retina, and optic nerve architecture very similar to other primates. Marmosets may thus be good candidates for the final stage of preclinical research before initiating clinical trials because of the ability to perform nonhuman primate studies in larger numbers. This would increase the likelihood of translation to the human.

**BETTER CLINICAL TRIALS**

Based on the failure of two large clinical neuroprotection trials to meet their primary endpoints, based on press releases, there has been great interest in better ways to perform clinical trials. Some issues are discussed in a recent review. One of the suggestions has been to use proof-of-concept trials as a first step in the neuroprotection development process, with continuation to pivotal Phase 3 studies if the treatment shows an obviously good result. If proceeding would be futile, development stops, i.e. a *futility trial*.

The issues of decision-making with respect to proof-of-concept trials are complex, and are highly dependent on power calculations. In general, the number of patients required to do a clinical trial in neuroprotection (or any other test) depends on four factors. The first is the alpha level for significance, which is typically less than 0.05. This means that a positive outcome is expected to be seen by chance less than one out of twenty times. The second factor is the power, i.e. the likelihood that if there really is an effect of the intervention, the trial will be able to detect it. This is usually chosen as 80% or 90%, implying that 20% or 10% (respectively) of the time, even an inherently effective intervention will show up as being ineffective in the trial, i.e. a false negative result.

The third factor is the *difference* that is being sought between the two groups being studied, e.g. placebo and active drug. For neuroprotection, this could be something like how much the mean deviation progression rate should decrease for an intervention to be neuroprotective, e.g. a decrease of 50% or more. The fourth factor is the variability of the population values for the endpoint, e.g. the standard deviation of the mean deviation progression rate in the population that is likely to be studied.

The classic methods for powering clinical trials may not be applicable to the development process for risk-associated development pathways such as neuroprotection. For example, most clinical trials that are submitted to the FDA have historically used power values of 80% or 90%, as mentioned above.

A new approach that our group has been studying is to reconsider how the power calculations interact with the financial analysis, in the case where a pharmaceutical company is deciding whether or not to perform a clinical trial and how it will be performed. This is based on the financial aspects of drug development. In general, companies develop drugs to make profits. There is a high risk associated with the development of any new drug, and this has historically been true for the development of neuroprotection strategies, for which there are almost no examples besides memantine in Alzheimer disease, tirilazad mesylate in subarachnoid hemorrhage in Australia, and riluzole in amyotrophic lateral sclerosis.

The basis of the mixed-model decision analysis is that the expected value in present-day dollars (or euros or pounds) represents the long-term revenue stream corrected for the development or other costs associated with the drug approval. For the sake of argument, this can be assumed to be in the billions of dollars.

This means that if a Phase 2 clinical trial is used as a proof of concept for drug development in neuroprotection, and the trial is powered at 80%, then 20% of the time false negative results will be seen. This means that the possibility of a multibillion dollar revenue stream has the potential of being abandoned, while if the trial had been powered at a more stringent level, then this false-negative rate would be lower. In other words, there is an opportunity cost by under-powering a study. Although historically, powers of 80% and 90% have been used, this analysis would suggest that much higher powers should be used, given the balance between the cost of actually performing the trial and the expected value in the long term. There is also value to performing more than one Phase 2 studies, with different approaches, and letting a positive value in any trial advance further development.

Similar analyses can be done for Phase 3 clinical trials, with the understanding that they are usually two independent trials, one of which may yield a significant result and one which may not. There are situations where the power should be even higher than would otherwise be for a single trial.

There are several strategies that are being studied to decrease the number of patients required and/or the length of time that they need to be followed for a trial of neuroprotection to take place. One approach is to take patients with high progression rates, so that the effect size will be greater compared to the variability in the measurements of the progression rate. A closely related approach is to improve the homogeneity of the groups, so that the variability is less and the signal-to-noise ratio is greater.

Calculations based on modeling and on real data suggest that in many cases, a trend-based analysis of visual field data may have more sensitivity and specificity for assessing the effects of an intervention such as neuroprotection than event-based analyses. The literature on this has been developed over the last few years, and reflects the fact...
that several visual fields can be used to calculate the slope with fairly good confidence intervals, and therefore an intervention which changes the slope over time of visual field progression can be detected with a higher ratio of effect size divided by standard deviation than within event-based analysis. The caveat is that the number of fields and the stage of the disease matters, meaning that the design of the clinical trial is critical to any perceived advantage. An excellent discussion relating to issues in comparing methods for field progression analysis has been recently performed9. Although it is impossible to predict, in the future, this and related information might be used to design optimal neuroprotection studies that would require fewer patients than with past trial designs.

**DRUG DELIVERY OF NEUROPROTECTIVE AGENTS**

Glaucoma and almost all other optic neuropathies begin with damage to the axon of the retinal ganglion cell. The study of neuroprotective drugs (or non-drug therapies) requires that the intervention address the injured part of the retinal ganglion cell, either directly or indirectly. For example, if a drug were to protect only the cell body (soma) of the retinal ganglion cell without protecting the axon, then an injury to the axon at the optic nerve head from glaucoma may result in a “zombie” RGC that cannot transmit information to the rest of the brain because its axon was lost or nonfunctional. This concept of the importance of the site of injury for neuroprotection10 is critical for development of future therapies. Recent research has focused not only on protecting the soma but also the axon. The study of the WldS mouse and rat, where their axons within the optic nerve head from glaucoma may result in a “zombie” RGC that cannot transmit information to the rest of the brain because its axon was lost or nonfunctional. This concept of the importance of the site of injury for neuroprotection10 is critical for development of future therapies. Recent research has focused not only on protecting the soma but also the axon. The study of the WldS mouse and rat, where the axons do not undergo Wallerian degeneration after injury, has been helpful in understanding the importance of preserving the axon11,12. More recently, the importance of the involvement of the dendritic tree as a response to injury has come about, as well as the response of target neurons of retinal ganglion cells within the lateral geniculate nucleus and elsewhere13,14.

Along these lines, it will be important to deliver drug or other interventions to the locations involved in the injury. Under the best of circumstances, a topically delivered drug could reach the retinal ganglion cell bodies, their dendrites, their axons within the retinal nerve fiber layer, and even their axons within the optic disk. However, the drug would not reach the retrobulbar optic nerve, and therefore for a disease such as indirect traumatic optic neuropathy, where the damage occurs at the optic nerve canal, a topical drug would not be helpful. On the other hand, such an approach may be feasible in glaucoma if there is enough penetration in and around the disk and laminar tissues, where the damage likely occurs. A variety of drug delivery methods have been developed and it is expected that these will continue to improve over subsequent years. Both sustained-release formulations within the eye and in other depot spaces will continue to be optimized. Systemic delivery is always an option, but may be less helpful when there are adverse effects associated with this approach.

There is a recognition that transcleral penetration of drugs after topical delivery may be more important than transcorneal penetration when the goal is to deliver drug to the retina and anterior optic nerve head. Overall, advances in drug delivery are likely to be part of the critical path that will eventually enable clinical neuroprotection in glaucoma.

**CME ANSWERS**

1. e
2. c
3. e

**REFERENCES:**

LEARNING OBJECTIVES

1. Describe that failure of retinal ganglion cell survival and axon regeneration underlies the permanent loss of vision in glaucoma and other optic neuropathies.

2. Describe that loss of survival and growth signals, including neurotrophic factors and electrical activity, contribute to retinal ganglion cell dysfunction in these diseases.

3. Describe the potential efficacy measures that could be used to study neuro-regenerative therapies in optic neuropathies.

CME QUESTIONS

1. Electrical activity is __________ for retinal ganglion cells after injury.
   a. Good
   b. Bad

2. Regenerative failure is attributable to problems in __________.
   a. Retinal ganglion cells
   b. The optic nerve environment
   c. Both

3. In a chronic disease such as glaucoma, is it easier to measure __________.
   a. Neuroprotection
   b. Axon Regeneration
   c. Neuroenhancement

KEYWORDS

1. Neurotrophic Factors
2. Electrical Activity
3. Axon Regeneration
4. Neuroprotection
5. Neuroenhancement

INTRODUCTION

Retinal ganglion cells degenerate in glaucoma and other optic neuropathies, and regenerative failure leads to permanent loss of vision in these diseases. RGC axons injured in the optic nerve fail to regrow back to their targets in the brain, and the cell bodies die a short time afterwards. Here we will discuss recent data revealing new signalling pathways regulating RGC survival and regeneration, and approaches to reversing regenerative failure in the visual pathway.

The search for treatments that promote the survival and regeneration of retinal ganglion cells (RGCs) in optic neuropathies including glaucoma and ischemic optic neuropathy remains a major goal for basic and clinical research. Neurotrophic factors impact the survival, proliferation, differentiation and function of neuronal cells, and one such neurotrophic factor, ciliary neurotrophic factor (CNTF), has begun to bridge from laboratory to human studies.

CNTF is a well-studied neurotrophic factor shown to act as an injury-activated signal to protect neural tissues, including the retina. CNTF is expressed in the retina under stressful conditions, such as experimental ocular hypertension and optic nerve trauma, where it directly stimulates intracellular signaling called the Jak-STAT cascade in retinal Muller glial cells, retinal ganglion cells (RGCs) and astrocytes. It is thought that the activation of the STAT3 signaling pathway directly mediates the neuroprotective effect of CNTF on neuronal cells. Also, glial cells activated by STAT signaling are associated with protection of neurons from neuronal degeneration.

Animal models of retinal degeneration have shown good evidence to suggest that CNTF has a neuroprotective effect on photoreceptors. CNTF protein injection into the vitreous cavity and intraocular adenovirus-mediated gene transfer of CNTF prevent the photoreceptor cell death in rodent retinal degeneration models.

Similarly, in pre-clinical models CNTF provides a neuroprotective effect in RGCs against severe stress such as optic nerve trauma. Long-term delivery using adeno-associated virus serotype 2 (AAV2) vectors that express a secretable form of CNTF make long term delivery to RGCs possible. AAV2-CNTF intravitreal injection 1 week before optic nerve trauma enhanced RGC survival almost fourfold compared with control retinas at 7 weeks.
AAV2-CNTF intravitreal administration in laser-induced glaucoma can exert a significantly protective effect against axon loss in the optic nerve.\textsuperscript{10}

Interestingly, CNTF has an additional positive effect, promoting axon regeneration after optic nerve damage in pre-clinical models. Purified RGCs extensively elongate their neurites in the presence of CNTF in culture.\textsuperscript{11, 12} In vivo, CNTF enhances RGC axon regeneration in the optic nerve. Intravitreal application of CNTF\textsuperscript{13} and AAV2-CNTF injection\textsuperscript{4} substantially enhanced the regeneration of damaged axons into a sciatic nerve graft after optic nerve axon transection.

Thus, numerous preclinical studies support the idea that CNTF may be both neuroprotective against RGC loss, and also promote regeneration in the optic nerve. The key question is, can CNTF slow the progression of visual field loss, or even bring vision back, in human patients with glaucoma or other optic neuropathies? Recently, another method of long-term CNTF delivery to the retina, using an intravitreal implant, has been developed (Neurotech, RI). A small capsule of human cells engineered to secrete CNTF are enclosed in a semi-permeable membrane that allows CNTF secretion into the vitreous. Human testing in pre-clinical models. Purified RGCs extensively elongate their neurites in the presence of CNTF in culture.\textsuperscript{11, 12} In vivo, CNTF enhances RGC axon regeneration in the optic nerve. Intravitreal application of CNTF\textsuperscript{13} and AAV2-CNTF injection\textsuperscript{4} substantially enhanced the regeneration of damaged axons into a sciatic nerve graft after optic nerve axon transection.

The data also showed that CNTF secretion was maintained at similar levels even after 2 years,\textsuperscript{14} suggesting that long-term drug delivery is feasible in the human eye.

Would CNTF work in humans to support RGC survival or encourage optic nerve regeneration? A pair of phase I trials in glaucoma and in non-arteritic ischemic optic neuropathy recently completed 18 months of follow-up. Although phase I trials are designed to evaluate safety in the patient population being studied, suggestion of biological activity will be expected to drive further investigation in later-phase trials.

Although much work will have to be done to cycle back and forth between human testing and pre-clinical development, the premise of moving promising candidate therapies from the laboratory to the clinic raises the hope of identifying new treatments for patients.

(Adapted from ref 15)

CME ANSWERS

1. (a) Although electrical activity in excess is thought to lead to “excitotoxicity” and cell death of retinal ganglion cells, after optic nerve injury providing extra electrical activity appears to promote retinal ganglion cell survival and growth, both in animal models and in early data from human testing.

2. (c) Deficits both in the glial environment of the optic nerve, and in the retinal ganglion cells themselves, contribute to regenerative failure in optic neuropathies. Newer data increasingly support the premise that manipulating retinal ganglion cells directly can promote survival and regeneration.

3. (c) We have no current methods to measure axon regeneration in humans, and in chronic diseases like glaucoma it may take longer to measure neuroprotection than to measure acute increases in visual function, termed neuroenhancement.

REFERENCES:


MITOCHONDRIAL DISEASE AND GLAUCOMA

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LEARNING OBJECTIVES

1. Describe the clinical characteristics of mitochondrial optic neuropathies (MON)
2. Explain the risk factors of the optic disc size and shape for MON
3. Describe reasons why some glaucomas have mitochondrial impairments as a pathophysiological mechanism

CME QUESTIONS

1. What are the two hereditary MONs?
2. Which hereditary MON may masquerade as glaucoma?
3. What protein helps regulate optic nerve size and is the pathophysiological basis of DOA?

KEYWORDS

1. Mitochondrial Optic Neuropathies
2. Glaucoma
3. Dominant Optic Atrophy

There are several mitochondrial optic neuropathies (MON). There are probably many glaucomas. So perhaps it is not surprising that there exist overlaps in these two conditions. More importantly, however, the pathogenic mechanisms recently elucidated in MON may provide insights and opportunities in the management of at least some glaucomas. Furthermore, there is a growing body of evidence that mitochondrial disease may affect tissues of the eye other than retinal ganglion cells (eg. trabecular meshwork and the optic nerve) and may even directly alter some of the dynamics that determine intraocular pressure. The purpose of this talk and manuscript is to delve into the relationship between mitochondria and glaucoma. In particular, we will address these considerations:

1. There are many similarities between some glaucomas and mitochondrial optic neuropathies (MON).
2. These similarities are greatest for low tension glaucoma that often present with paracentral scotomas that remind us of MON.
3. Dominant Optic Atrophy (DOA) is a hereditary MON with particular connection to glaucoma including the optic disc appearance.
4. Leber’s Hereditary Optic Neuropathy (LHON), another MON, has disc size as a risk factor for visual loss and the severity of this loss (large discs do better).
5. We are exploring the role of OPA-1 on development of the optic nerve as well as the degeneration of DOA.
6. Mitochondrial metabolism and dynamics may play a role in the pathology of glaucoma and provide clues to new therapeutic approaches.

MON represent a group of optic neuropathies that can be genetic, nutritional or toxic in basis. For example, hereditary mitochondrial optic neuropathy may be in the autosomal form as DOA or maternally inherited through mtDNA mutations as LHON. In the former case, over 212 mutations have been described. There are 3 major mtDNA mutations that produce LHON.

The clinical presentation of MON is characterized by bilateral loss of central vision, dyschromatopsia, central or cecocentral scotomas. Ophthalmoscopic features during the acute/subacute stage often reveal a hyperemic optic disc and peripapillary retinal nerve fiber layer swelling. With time, temporal pallor of the optic disc develops. There is no relative afferent pupillary defect due in part to symmetric optic nerve involvement. The fibers of the papillo-macular bundle (PMB) are most susceptible due to their long unmyelinated segment in the retina and their small caliber. Preferential involvement of the PMB is a feature common to a wide range of acquired and genetic mitochondrial optic neuropathies.

Nutritional optic neuropathies are often (but not always) mitochondrial as well. Deficiencies, especially of B-12 and folic acid can impair mitochondrial metabolic pathways and produce diseases that mimic LHON or DOA. An ever larger body of toxins, especially antibiotics, have been shown to impair mitochondrial function and also mimic or produce a similar clinical profile. Drugs proven to cause MON by blocking oxidative phosphorylation include ethambutol, chloramphenicol, linezolid, erythromycin, streptomycin,
and antiretroviral drugs\(^1\). Once again, the small fibers of the PMB are the main site of injury.

Leber’s hereditary optic neuropathy is characterized by severe visual loss, which may manifest acutely or subacutely in young adulthood\(^2\). In 1988, the genetic basis was determined to be due to a mitochondrial DNA (mtDNA) point mutation\(^3\). This Wallace mutation, at nucleotide 11778/ND4, was discovered first and later 14484/ND6 and 3460/ND1 were established as other common mtDNA mutations\(^4\). These three mutations affected components of respiratory complex I, and account for about 95% of LHON cases.

The typical story would be that of a young adult male who first notices abrupt and profound loss of vision in one eye, and then, weeks to months later, suffers a similar loss of vision in the other eye. Less commonly, LHON occurs later in life and may occur in women at menopause. There is evidence that estrogen, by controlling mtDNA copy number, is protective, explaining the menopausal association as well as the gender bias for conversion\(^5\). Environmental factors such as smoke and excessive alcohol, may act as triggers for visual loss in LHON\(^2, 3, 11-15\).

The size of the optic disc can play a role in the pathogenesis of LHON\(^16\). The optic disc was found to be larger in LHON carriers than in LHON-affected, suggesting that a small optic disc may be a risk factor for LHON carriers to convert to affected. In fact, amongst LHON-affected, larger optic discs were also associated with a better visual outcome and the propensity for some recovery of vision\(^16\). It is intriguing to consider that mechanical factors, such as those that may play a role in the pathogenesis of glaucoma or anterior ischemic optic neuropathy, may also influence the outcome in LHON.

Dominant optic atrophy is autosomal in genetics and in most cases due to a mutation in the OPA1 gene\(^17, 18\). It affects both genders equally and usually presents as a slow and insidious progressive visual loss starting in prepubescence. The central scotoma is smaller and grows more slowly than in LHON and the optic disc atrophy usually confined to the temporal side\(^19\). The temporal disc may also become excavated or cupped in appearance\(^19\). It is not surprising, then, that the main differential diagnosis in DOA is for low tension glaucoma. Furthermore, the optic disc in DOA is smaller than in controls, suggesting a role for OPA1 in regulating apoptosis and thereby controlling the size and shape of the optic nerve head\(^20\).

The optic disc area may also correlate with the rapidity and severity of visual field progression in patients with low tension glaucoma\(^21\). And, indeed, OPA1 polymorphisms have been found to correlate with both normal tension and primary angle glaucoma\(^22\). Hence, DOA has certain clinical similarities with glaucoma, and especially, in the absence of high intraocular tension, with low tension glaucoma. More intriguing, OPA1 probably plays an important role in both diseases.

On the flip side, there is substantial evidence that patients with primary open angle glaucoma may have mitochondrial impairment. In particular, Lee and colleagues found that lymphoblasts from glaucoma patients had complex-I impairments leading to decreased ATP production in many ways analogous to that seen in LHON that leads to RGC death\(^23\). Furthermore, mitochondrial DNA polymorphisms are not uncommon in low tension glaucoma\(^24\). Hence, it is likely that at least some glaucomas have mtDNA mutations or polymorphisms as a risk factor. Mitochondrial dysfunction probably predisposes RGCs to glaucoma damage\(^25\). Furthermore, elevations of intraocular pressure may damage mitochondria through oxidative stress\(^26\).

This overlap in glaucoma and mitochondrial impairment that leads to retinal ganglion cell (RGC) death should not be surprising. We are reminded that RGCs are probably exquisitely sensitive to mitochondrial dysfunction due to their having a very long unmyelinated segment and that the PMB fibers, by virtue of their small caliber, have a particularly poor “mitochondrial stress index” imposed because of the exposed membrane-to-mitochondrial volume ratio\(^3, 8\). Superimposed, of course, may be stressors at the lamina cribrosa that relate to pressure gradients.

The role of mitochondria in apoptosis makes it a major “final common pathway”. Glaucoma is now sometimes viewed as a neurodegenerative disease of the optic nerve. The accelerated death of RGCs and their axons may be due to primary mitochondrial impairment and the role of mitochondria in apoptosis. Neuro-ophthalmologists and glaucoma specialists interested in the optic nerve head, have much to talk about.

CME ANSWERS

1. LHON and DOA
2. DOA
3. OPA-1

REFERENCES


INTRODUCTION
While IOP-lowering eyedrop treatment is effective at decreasing glaucoma’s visual function loss, its disadvantages require renewed effort to improve the future of glaucoma treatment. This will involve a change to sustained delivery of medications directly to the eye, and, agents whose mechanism of action is to block retinal ganglion cell death by pathways other than and in addition to pressure lowering.

The treatment of the glaucomas evolved relatively slowly over the last 50 years, as the use of gonioscopy separated open angle from angle closure and secondary glaucomas. The role of the level of intraocular pressure (IOP) in all forms of glaucoma was established by scientifically valid methods of tonometry (Goldmann) applied in population-based studies. Open angle glaucoma (OAG) is now understood to be an optic neuropathy in which IOP is an important risk factor, regardless of whether its level in the individual patient is above or within the range found in the population (so-called normal). In every population around the world yet studied, one-half or more of those with OAG undergo retinal ganglion cell (RGC) death at “normal” levels of IOP (Tielsch). For those with angle closure and secondary glaucoma, it is predominately higher than normal IOP levels that produce RGC loss.

Lowering of IOP has been shown to reduce the incidence and progression of glaucoma in large controlled clinical trials, including those evaluating pre-injury OAG suspects (OHTS), those with early OAG (EMGT), and those with OAG whose damaging IOP level was in the normal range (CNTGS). We do not yet have definitive information on what the most desirable level to which IOP should be lowered in the typical person with OAG. Target IOP in various controlled trials varied from 20% to 40% below baseline. While some have advocated dramatic lowering of IOP in all glaucoma eyes, suggesting that 12 mm Hg is a form of magic potion (Palmberg), the risk/benefit ratio for treatment to achieve such levels is not acceptable.

One recent evaluation of a cohort of treated persons from Canada showed that standard treatment achieved very slow worsening of measured function in the vast majority of eyes (Chauhan).

In fact, the proportion of those with OAG who will become blind or seriously impaired represents a minority of those with the disease, even if one includes those who remain undiagnosed. This is not to ignore the fact that the glaucomas are the second-leading cause of world blindness (Quigley). But, the large number of blind glaucoma persons derives from the substantial prevalence of disease, not from a high rate of morbidity. Yet, the 10-15% of OAG glaucoma patients who progressively worsen at a substantially greater rate than the majority (Broman) represent a challenge for improvement. There could be several approaches to further decrease vision loss from glaucoma.

Some would argue that we simply need to do a better job with IOP-lowering. IOP therapy has several drawbacks that are only partially amenable to change. Eyedrop treatment has at least 3 major problems. First, patients adhere to drop treatment poorly in many cases. Even under ideal monitored conditions, patients take only 70% of doses of drugs that are well-tolerated and provided free in studies (Friedman). Interventions to increase adherence are moderately successful when they use reminder systems (Boland). Yet, some groups are not amenable to any presently tested intervention, and no study has generated close to ideal adherence in any population. Second, the side-effects of all drops on the eye are well-known, including pain, redness, overt allergic reaction, and chronic scarring/shrinkage of the subconjunctival connective tissues (Schwab). Each class of IOP-lowering drugs has, in addition, its own panoply of undesirable effects on the eye and systemically. Third, some persons are refractory to successful IOP-lowering, producing no effect despite actually documented delivery to the eye.

Laser and surgical treatment to lower IOP have further problems. Despite development and aggressive marketing of SLT laser angle treatment in the last decade, no improvement in efficacy or safety has been demonstrated over the ALT laser method used since 1978. Nearly one-third of laser-treated eyes have no IOP lowering, while among the remainder, it is the exception, not the rule, to achieve a target range of IOP without additional eyedrops (GLT). As to trabeculectomy surgery, it has been shown under controlled trial conditions to equal the benefit of eyedrops in vision preservation (CIGTS study). But, its use is...
limited by the rate at which it is associated with more rapid cataract development, hypotony, and late infection (Solus).

IOP lowering therapy could be improved by any one of several approaches, though none of these appears ready to be applied to large groups in replacement of eyedrops. First, sustained delivery of drugs (Hanes) could improve adherence and reduce side effects of frequent chronic eyedrops use, especially if delivered subconjunctivally (Wong). Second, a variety of “new” glaucoma surgical procedures, often involving innovative micro-devices are being tested. While their promise is to decrease detrimental effects while achieving safe IOP levels, none of these has been tested in a controlled clinical trial against standard IOP lowering medical or surgical treatment. Thus far, the IOP lowering by new surgeries has been less impressive than even prostaglandin eyedrop treatment alone (Samuelson).

For the short-term, then, clinicians will continue to use the present quiver of IOP-lowering approaches, since they generally are effective enough. But, this discussion was intended to point out what the future will bring to glaucoma therapy. Our vision should be multiple approaches that go beyond the blinders imposed by IOP-lowering alone. Death of RGC is the nature of glaucoma’s detrimental effects and, thus, RGC preservation by any means possible should be our goal—an approach generally referred to as neuroprotection.

The fundamental feature of glaucomatous optic neuropathy is disruption of normal structure and function of the RGC axon within the optic nerve head, leading to dual parallel processes of RGC cell body and RGC axon death (Levkovitch-Verbin). In human glaucoma, including in eyes with normal IOP, the differentiation of glaucoma from other optic neuropathies that share the same injury site quite clear. Despite past anecdotes of “cupping” in non-glaucoma eyes that putatively blurred the differences among these disorders, the phenotype of OAG is different from conditions such as ischemic optic neuropathy, optic neuritis, Leber’s optic neuropathy, and optic disc drusen. In general, the major difference is that glaucoma is associated with connective tissue stretching and rearrangement along with RGC loss, while the others involve RGC loss alone. The disorder closest to glaucoma in its pattern of RGC loss/field loss, ischemic neuropathy, has now been repeatedly shown not to develop deepening and widening of the nerve head (Danesh-Meyer).

There are several insults that may begin the glaucoma process at the nerve head and each of these may be amenable to therapeutic intervention, including:

- altering ganglion cell body injury response
- altering ganglion cell axon injury response
- scleral and lamina connective tissue treatment

• improved autoregulation of nutritional blood flow
• mitigation of glial cell cytokine release

The RGC death process uses similar pathways to that of programmed cell death for poorly targeted RGCs in embryological life, known as apoptosis. Interventions that directly block this process have been shown to delay RGC death in animal models (McKinnon).

To show in more detail some of these potential mechanisms, we can begin with approaches that alter the response of the RGC body to injury. Among these are neurotropin overexpression (Pease), calcineurin inhibition (Grosskreutz), β secretase inhibition (Cordeiro), blockade of glutamate excitotoxicity (Sharma), and inhibition of tumor necrosis factor α (Nakazawa), among others. Each of these has proven to extend RGC life in rodent models of elevated IOP. These approaches would be most effective if they aim at restoring equilibrium to the RGC soma soon after the first injury signal arrives from the axon at the nerve head (“upstream” therapy). Recent research by Welsbie and colleagues at Wilmer illustrates such an approach, which was identified in a large library screening of potential drugs to extend the life of RGC. Activation of c-Jun N-terminal kinase and subsequent activation of phosphorylated c-Jun has been shown to be a “death pathway” for RGC, including in models of glaucoma (Levkovitch-Verbin). Welsbie identified preceding activation in dual leucine zipper kinase (DLK) as a key event in this pathway and found what is now a series of inhibitors, which were delivered in a sustained delivery formulation to the vitreous cavity of rats with experimental glaucoma, protecting RGCs from death). Inhibition of apoptosis by genetic knockout of the bax gene in mice produced animals with remaining RGC long after loss of their axons (John). While this would not lead to any functional vision, it is proof that the adult RGC soma can remain alive, despite loss the axon—a potentially useful state if regrowth of the axon can be encouraged (Goldberg).

The inhibition of RGC axon loss has been suggested by specific mechanisms. Rats with the slow Wallerian degeneration (Wld^+) mutation can undergo RGC somal loss with retention of apparently intact axons to the brain (Martin), showing that the axonal death process can also be mitigated, and is separable from somal loss. It may be that combination therapy that protects both the RGC soma and axon will be more effective than either alone, though this has not been tested experimentally.

Epidemiological evidence for glaucoma risk factors suggests approaches to altering RGC injury and death by one of several means. The strength and response to IOP-generated stress is transmitted to RGC axons by the sclera and lamina cribrosa. Myopes, whose sclera and optic nerve heads are known to differ in thickness and configuration, are more susceptible to OAG (Boland). The protection of RGC by alteration of the scleral response and that of the lamina...
Cribrosa connective tissue is suggested by several lines of evidence, notably the demonstration that the Aca23 mutant mouse is significantly resistant to experimental glaucoma damage. This mouse has a mutant in collagen 8α and at baseline has larger eyes than the corresponding wild type C57Bl/6 mouse. Detailed study of the resistance conferred by this mutation is underway. Another initial experiment tested the hypothesis that increased cross-linking of scleral connective tissue would alter susceptibility to glaucoma injury. Indeed, mice with glyceraldehyde-induced stiffer sclera were more susceptible to losing RGC than controls. These experiments suggest that beneficial alteration of the sclera will more likely involve increasing its elasticity, rather than stiffness.

There is considerable evidence that poor nutritional blood flow to the nerve head, exemplified most consistently by low perfusion pressure, increases both incidence (Tielsch) and progression of OAG (Leske EMGT). While much has been written on the role of poor blood supply for glaucoma, there is minimal evidence for an approach that would beneficially increase nutrition. Acute experiments show it is the perfusion pressure that determines acute autoregulatory failure in animals, but chronic experiments have not been published that show benefit from vascularly active agents. Small clinical trials with calcium channel inhibitors have suggested some improvement in the rate of decline, though confirmation of these findings has not been provided (Araie). This may be a particularly difficult area to duplicate in animals, as the age-related changes in vessels that may be present in the (subset) of those with glaucoma and poor nutritional flow may not be present, even in aged animals. Furthermore, the susceptibility to experimental RGC glaucoma damage is different among strains of mice (Steinhart). The application of a systemic drug for neuroprotection, as in the calcium channel inhibitors, has the disadvantage of serious side effects that limit continuation, as well as detrimental off-target effects. Glaucoma patients have, most often, no symptoms of their disease. Any new medication that has serious or modestly frequent side effects is a non-starter for the glaucoma market. For agents that might have difficulty with oral delivery side effects, or, that would have blood—retinal barrier issues, the delivery through the sclera by depot injection would be a better approach.

While not detectable from clinical features, the contribution of astrocytes of the nerve head was potentially suspected and confirmed by studies showing abnormal cytokine release (TNFα). There is substantial information that TNFα is released in the glaucomatous optic nerve head and that its inhibition or elimination of its receptors is beneficial to RGC survival (Nakazawa). The presumed source of the cytokine is/are glia, including astrocytes of the optic nerve. Several approaches to TNFα inhibition are presently being implemented in treatment of systemic diseases. However, these are delivered either orally or intravenously, and have considerable side effects (Ma). For glaucoma, a more acceptable delivery system and side effect profile would be needed.

It is worth mentioning that glutamate toxicity was considered one of the potential mechanisms by which RGCs die in glaucoma, based on work that was done by a now-discredited investigator. Perhaps stimulated by this body of work, a controlled clinical trial of oral memantine was conducted, but announced some years later as having failed to achieve its end point—without providing any data on what really happened. This study has acted to “poison the well” for any subsequent research into glaucoma neuroprotection—the attitude being that it is too difficult and too expensive to develop a new glaucoma drug. On the contrary, the deficiencies in study design and implementation, as well as the poor choice of the drug, were known prior to its apparent failure in the trial. An objective analysis shows that neuroprotection trials, carried out in an efficient manner, using visual fields as the end point, can be conducted effectively in time frames typical for the testing of drugs in other chronic diseases (Quigley).

The future of glaucoma therapy will be to terminate the use of daily eyedrops and to move to methods of sustained delivery of drugs to the eye by systems that are now available for packaging agents efficiently. It is likely that these methods will involve the use of both IOP-lowering and non-IOP drugs to maximize the chance that visual impairment can be kept as low as possible. In some patients, the IOP-dependent part of their glaucomatous pathology may be so low or so difficult to achieve that only neuroprotective therapy will be used. In others, the ease and safety of IOP-lowering may have been demonstrated to have produced stability in function for previous long periods, and, in these eyes, neuroprotection would not be appropriate.
LEARNING OBJECTIVES

1. Describe the argument that the mechanisms of glaucomatous injury and how all of these mechanisms (vascular, mechanical, tissue remodeling, and neurodegenetation) are intimately related to the loading force within the eye (IOP)

2. Describe the relationship and differences between stress (the loading force; IOP) that is delivered to the posterior scleral and optic nerve and resultant tissue strain (the response of the tissue due to stress)

3. Describe that even at normal levels of IOP, significant tissue strain and deformation can occur that could result in compromise of the load bearing connective tissues of the optic nerve and generate significant axonal injury

CME QUESTIONS

1. The following are constant risk factors for the development of glaucoma
   a. Thin cornea
   b. Diabetes
   c. Female gender
   d. Myopia

2. True/False: Elevated stress (IOP) is required for potentially damaging tissue strain (deformation).

3. Focal acquired optic nerve pits are related to
   a. Lower rates of glaucoma progression
   b. Thick corneas
   c. Optic disc heme
   d. Alpha zone atrophy

KEYWORDS

1. Glaucomatous Optic Neuropathy
2. Intraocular Pressure
3. Lamina Cribsola
4. Ocular Biomechanics

INTRODUCTION

Point-Counterpoint: IOP lowering therapies will always play a role in the future of glaucoma management.

Lowering of intraocular pressure (IOP) either with medications or surgical interventions has been the mainstay of glaucoma therapy for well over 100 years and remains the only proven method to retard or prevent the development or progression of the disease. However, despite the fact that IOP lowering therapy is available, patients still may progress despite lowering of IOP. In addition, patients can develop glaucoma even at a low IOP. These findings have stimulated the development of alternate hypotheses regarding the pathogenesis of glaucomatous through non-IOP related mechanisms. There is clear evidence that vascular changes that alter perfusion to the optic nerve, changes in the supportive tissues of the lamina cribrosa, immunologic and excitotoxic/neurodegenerative changes all play an important role in the development of glaucoma. However, I will argue in this debate that despite these insults, that IOP lowering therapy will always remain a valid and important treatment for glaucoma. This is because IOP load is integrally related to changes in the vasculature and the supportive cellular and connective tissue components of the lamina cribrosa and thus play a key role in the axogenic injury to the optic nerve that is critical to the development of glaucoma.

The lamina cribrosa provides structural and functional support to the retinal ganglion cell (RGC) axons as they pass from the relatively high-pressure environment within the eye to a lower pressure region in the retrobulbar cerebrospinal space. To protect the RGCs in this unique anatomic region, the lamina has developed into a complex structure composed of a three-dimensional network of flexible beams of connective tissue, nourished by a capillary bed primarily arising from the short posterior ciliary arteries penetrating the immediate peripapillary sclera. This intra-scleral and intra-laminar vasculature is unique in that it is encased in load-bearing connective tissue, either within the scleral wall adjacent to the lamina cribrosa, or within architecture of the laminar beams themselves. Thus, this “end-organ” capillary bed is completely encased in load bearing connective tissues that is under constant strain (tissue deformation) that varies across every second, every hour and every day throughout life creating a unique environment.
situation in which mechanical effects are inseparable from vascular and other non-IOP mechanisms of injury.

The anatomy of the lamina cribrosa and peripapillary sclera merits several considerations regarding the etiology of glaucomatous cupping and implies that the classic “mechanical” and “vascular” mechanisms of glaucomatous injury are inseparably intertwined. For example, prior to structural damage, purely IOP-related stress could detrimentally affect the blood supply to the laminar segments of the axons by direct deformation of the capillary-containing connective tissue structures or due to changes in the extracellular matrix that may limit the diffusion of nutrients to RGC axons in the laminar region. Reciprocally, primary insufficiency in vascular supply to the laminar region could induce connective tissue changes that would serve to weaken the laminar beams, making them more prone to failure under similar levels of IOP-related mechanical stress.

To incorporate these concepts within a global hypothesis, a biomechanical model of glaucomatous optic neuropathy has been proposed. This model proposes that IOP-related stress (force/cross sectional area) and strain (local deformation of the tissues) play an essential and causative role in the pathophysiology of the changes seen in all of the tissue types within the optic nerve head and in its blood supply. These not only include the lamina cribrosa, the scleral canal wall, and the peripapillary sclera, but also the cellular components of these tissues, including astrocytes, glial cells, endothelial cells, and pericytes, along with their basement membranes and the RGC axons. Regardless of the primary insult in glaucomatous injury, IOP-related stress and strain in the laminar connective tissues are key elements in this model and are dependent on the optic nerve head architecture and material properties of these connective tissues.

Applying this model to the theoretical question as to the future role of IOP-lowering therapy has important implications in that IOP will always be one of the primary drivers of injury even at low levels of IOP since significant tissue strain is still realized at the optic nerve head. For example, the classic observations that disc hemorrhages occur, does not necessarily imply a primarily vascular etiology. Disc hemorrhages could easily be explained due to remodeling and resultant rupture the laminar beams with the blood dissecting onto the disc surface within an axonal bundle. This could occur based on an entirely mechanical insult. That disc hemorrhages have been strongly associated with signs of significant laminar remodeling as in the case of acquired optic nerve pits lends some credence to this hypothesis. I am not arguing that other mechanisms are not playing an important role, simply that IOP related mechanism are still important to injury at all IOP levels.

Further evidence for the important role of the biomechanical model in the development of glaucoma can be seen in the many large multicenter prospective studies focused on identifying the risk factors associated with the development or progression of glaucoma. The results of the several randomized prospective trials have identified the risk factors associated with the development or progression of glaucoma (Table 1). Factors that appear most consistent across several studies include the level of IOP, age, central corneal thickness, increased optic disc cupping, and African ancestry, which are all independently associated with glaucomatous progression. It is important to note that all of these risk factors have a biologically plausible association that can be directly explained within a biomechanical model. These factors are all associated with either the level of IOP, the severity of disease (visual field severity), or factors that may relate to the biomechanical properties of the optic nerve head (age, African ancestry, corneal thickness, increased cupping).

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<thead>
<tr>
<th>Risk Factor</th>
<th>Prospective Study</th>
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<tbody>
<tr>
<td>Increasing Age</td>
<td>AGIS, CIGTS, EMGT, OHTS, EGPS</td>
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<tr>
<td>African Ancestry</td>
<td>AGIS, CIGTS, CNHTS OHTS (Univariate)</td>
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<tr>
<td>Visual Field Severity</td>
<td>EMGT, AGIS, OHTS, EGPS</td>
</tr>
<tr>
<td>Follow-up IOP</td>
<td>EMGT, CNHTS</td>
</tr>
<tr>
<td>Cup-Disc Ratio</td>
<td>OHTS, EGPS</td>
</tr>
<tr>
<td>Corneal Thickness</td>
<td>OHTS, EGPS</td>
</tr>
<tr>
<td>Pseudoxefallation</td>
<td>EMGT</td>
</tr>
<tr>
<td>Initial IOP</td>
<td>EMGT</td>
</tr>
<tr>
<td>Female Gender</td>
<td>CNHTS</td>
</tr>
<tr>
<td>Male Gender</td>
<td>AGIS</td>
</tr>
<tr>
<td>AGIS, Advanced Glaucoma Intervention Study, EMGT, Early Manifest Glaucoma Treatment Study (1), CIGTS, Collaborative Initial Glaucoma Treatment Study (1), CNHTS, Collaborative Normal Tension Glaucoma Study (1), OHTS, Ocular Hypertension Treatment Study (1), EGPS, European Glaucoma Prevention Study (1)</td>
<td></td>
</tr>
</tbody>
</table>

Other factors that have been associated include diabetes, which can effect the material properties of connective tissues due to advance glycosylation end-products and disc hemorrhages, which as stated above could be secondary to structural remodeling of the lamina cribrosa in response to IOP-related or vascular injury. In short, all relevant risk factors for glaucoma can be explained within the context of a biomechanical model. Since IOP is a critical, but not exclusive, exposure input to this mode, lowering of IOP should remain a critical piece in the management of glaucoma.

CME ANSWERS
1. a
2. false
3. c
REFERENCES:


7. Lesk MR, Hafez AS, Descovich D. Relationship between central corneal thickness and changes of optic nerve head topography and blood flow after intraocular pressure reduction in open-angle glaucoma and ocular hypertension. *Arch Ophthalmol* 2006;124:1568-1572.