

The ganglion cell complex and glaucoma

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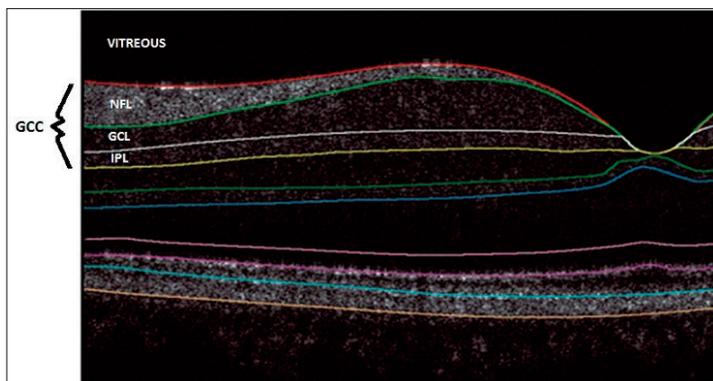
GLAUCOMA IN ALL its manifestations and clinical variants ultimately leads to destruction of retinal ganglion cells.

Different methods are available to detect ganglion cell damage, such as structural losses at the optic nerve head (for example, increased C/D ratio, neuroretinal rim thinning or notching) or changes in ganglion cell function such as threshold visual field defects. Existing clinical methods are limited in their ability to detect ganglion cell damage until there is significant loss. Approximately 40 per cent of ganglion cells need to be lost before an early glaucomatous threshold visual field defect is manifested,¹ and the typically slow progression in optic nerve head changes makes structural glaucoma detection difficult until significant rim tissue is lost.

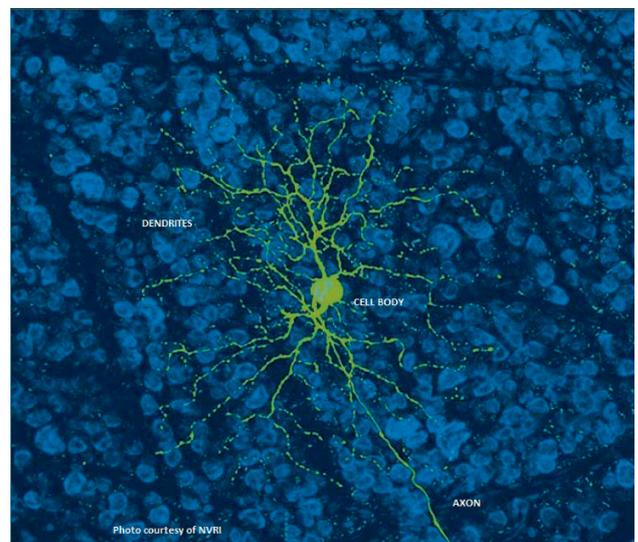
With the advent of scanning laser clinical instruments, newer methods have been developed to enhance earlier detection of glaucomatous damage. The Heidelberg Retinal Tomograph (HRT) tracks changes in optic nerve head physical characteristics such as rim area and cup volume. The GDx

(Carl Zeiss Meditec) looks for loss of ganglion cell birefringence in the circumpapillary retinal nerve fibre layer. Time domain (TD) and spectral domain (SD) OCTs measure the ganglion cell axons around the optic nerve head to determine nerve fibre layer thickness and the TSNIT curve. These existing clinical instruments concentrate on measuring the axons of the retinal ganglion cells adjacent to and on the optic nerve head.

However, retinal ganglion cells are large and complex cells extending from the inner retina all the way to the lateral geniculate nucleus (LGN) in the midbrain. Ganglion cells begin at the inner plexiform layer (IPL) where they synapse with the bipolar and amacrine cells of the middle retina. Their cell bodies (soma) make up the ganglion cell layer (GCL) of the inner retina, and the ganglion cell axons that emerge are the retinal nerve fibre layer (NFL). These axons traverse the retina, converging at the scleral foramen where they form the neuroretinal rim of the optic nerve head, before continuing on to the optic chiasm and LGN. On a cross-sectional OCT scan,



▲ Figure 1. The ganglion cell complex (GCC) consists of the three innermost layers of the retina: IPL (inner plexiform layer), GCL (ganglion cell layer), NFL (nerve fibre layer)



▲ Figure 2. An en face view of a retinal ganglion cell; the extensive dendritic arbour makes up the inner plexiform layer (IPL)

all three segments of the ganglion cells (IPL, GCL and NFL) are known as the ganglion cell complex (GCC) (Figure 1). In an en face view (Figure 2) we see that the ganglion cell synapses of the IPL are the largest area of the cell, forming an extensive branching dendritic arbour. The cell body (GCL) and axon (NFL) are relatively small compared to this dendritic tree.

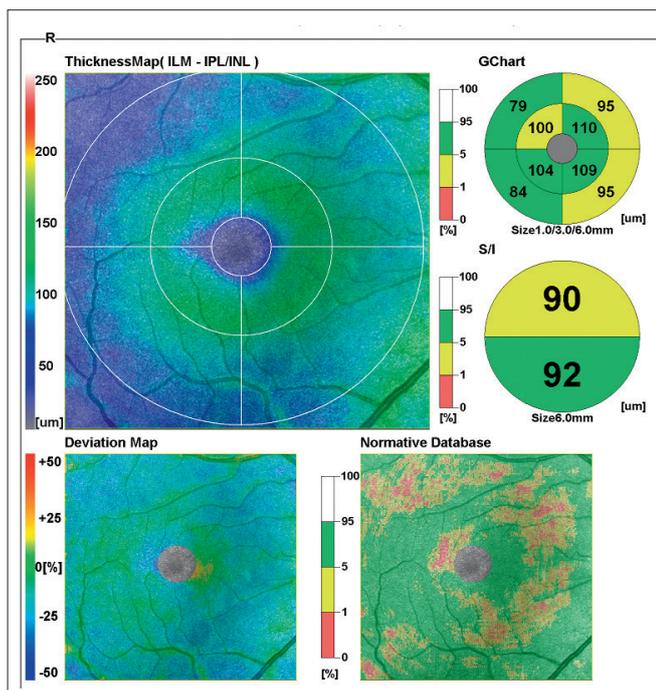
How retinal ganglion cells die

Research in animal models shows that when a retinal ganglion cells is damaged either by axotomy (severing the retrobulbar optic nerve) or raised intraocular pressure, the ganglion cells dies in a characteristic way. The earliest changes are seen as a loss of the dendritic arbour (IPL) due to mitochondrial fission (splitting).² Subsequently, the cell body (GCL) dies. Studies on axonal death show that first there is a loss of microtubules inside the axons (detected as a change in birefringence on GDx scans) prior to the eventual phagocytosis of the outer axon itself (detected as RNFL thinning on OCT scans) which in animal models may occur many months later.³

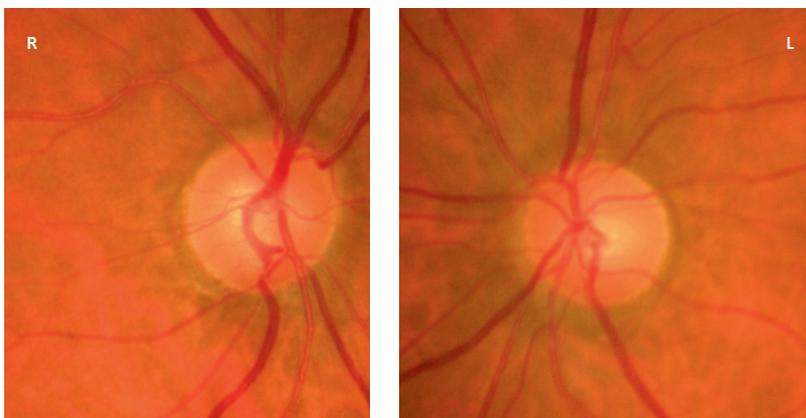
The advent of spectral domain (SD) OCT allows us greater optical resolution to accurately segment the inner layers of the retina into their constituent IPL, GCL and RNFL layers. Given that ganglion cells die initially from their synapses (IPL) and cell bodies (GCL), thinning of the ganglion cell complex (GCC) as detected by SD OCT may prove to be an earlier detector of glaucomatous damage than simple RNFL thickness around the optic nerve head.

GCC scans

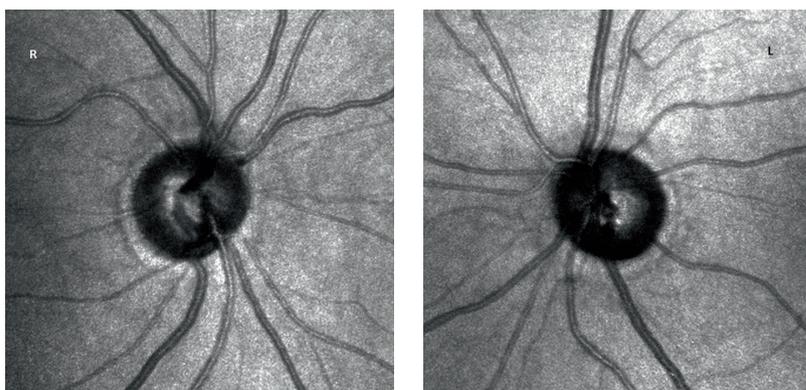
Approximately 50 per cent of the total retinal ganglion cells synapse in the central 5 mm of the macula.⁴ All commercially available OCTs perform GCC scans that are centred on the fovea to a diameter of between 6 mm and 9 mm, depending on the instrument and software settings. The results can be displayed as an absolute GCC thickness, as well as a probability map compared to the OCT's normative database (Figure 3). Depending on the OCT used, average GCC thickness is approximately 95 to 100 microns. Progression analysis software allows comparison of baseline and follow-up scans to determine if there has been any progressive thinning of the GCC.



▲ Figure 3. R eye GCC chart (Nidek RS3000). In this instrument the macular region is divided into eight sectors around the fovea which respect the horizontal raphe. There is never a thickness reading centrally as the fovea does not contain any ganglion cells.



▲ Figure 4. Disc photos of patient MP; note the subtle loss of the ISNT rule inferiorly in the R eye



▲ Figure 5. Scanning laser ophthalmoscope disc images (Nidek RS 3000)

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CASE REPORT

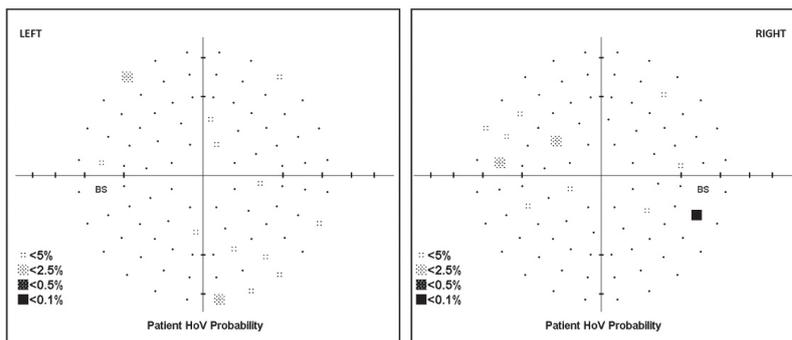
MP is a 53-year-old white female followed as a glaucoma suspect since 2004 due to ocular hypertension. Her pertinent clinical findings were:

IOP: R 23-26, L 22-26 mmHg.
 Pachymetry: R 561, L 555 microns.
 Gonioscopy: open to posterior trabecular meshwork OU.
 Slitlamp: no pigment dispersion or pseudoexfoliation. General health: no medication, no drug allergies. Family history: no glaucoma.

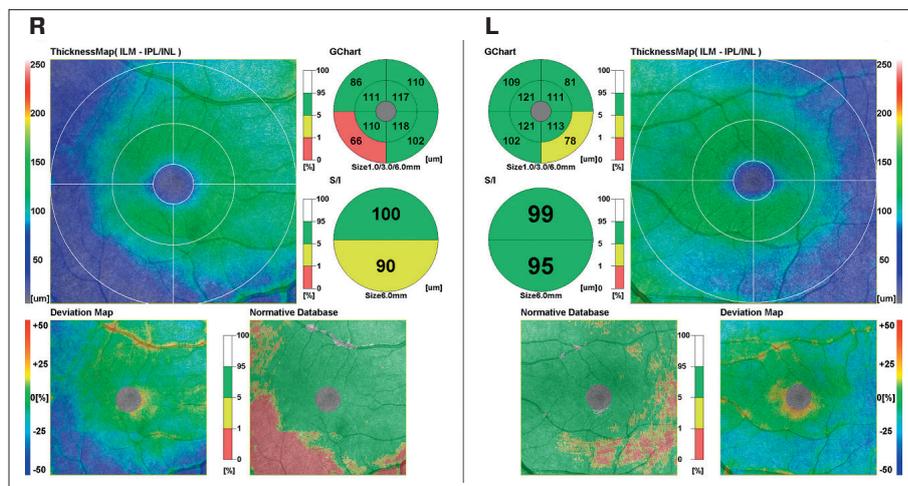
Optic nerve head assessment (Figure 4) showed a subtle loss of the ISNT rule inferiorly in the R eye. This was more evident in the OCT confocal scanning laser ophthalmoscope image (Figure 5) due to the difference in laser light absorption by the neuroretinal rim tissue compared to the more reflective lamina cribrosa. Threshold visual fields were reliable and did not show any clusters of loss or glaucomatous defects in either eye (Figure 6).

GCC scans performed in February 2011 showed a significant reduction in ganglion cell thickness in the inferotemporal sector of the R eye ($p < 0.01$) and borderline thinning in the same sector of the L ($p < 0.05$) (Figure 7). RNFL scans were normal in both eyes. A decision was made to repeat the GCC scans in six months to check for any progressive thinning.

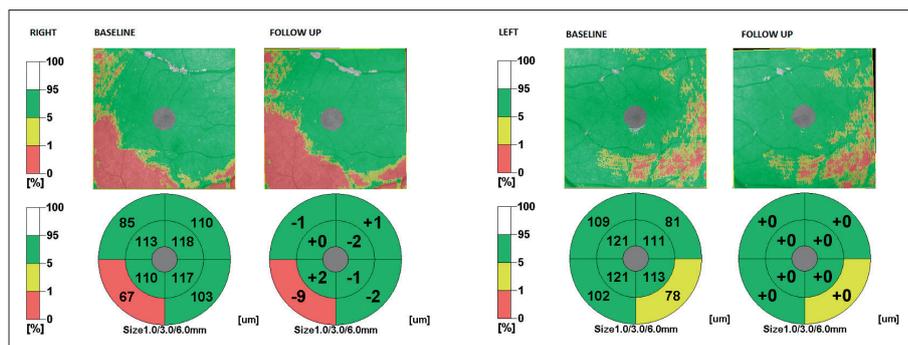
On review in October 2011, follow-up GCC scans were performed and compared to baseline. The defective R inferotemporal sector thinned by a further nine microns, while the equivalent suspicious sector in the L eye remained unchanged (Figure 8). A change of five or more microns is considered significant as it is greater than the test-retest variability of the OCT instrument.⁵



▲ Figure 6. Threshold visual fields (Medmont M700)



▲ Figure 7. Baseline GCC scan showing R > L thinning of the inferotemporal sectors

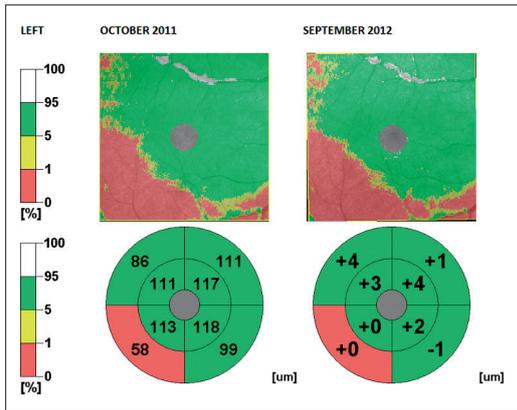


▲ Figure 8. GCC progression analysis shows a loss of nine microns in ganglion cell thickness in the R inferotemporal sector between February 2011 and October 2011 but no changes in the L eye

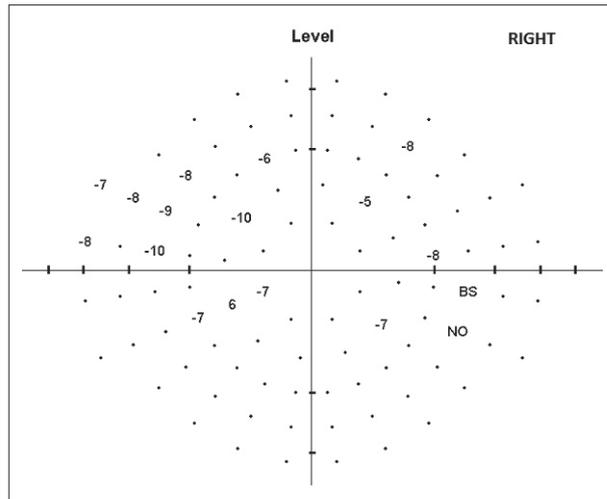
Due to the progressive nature of the GCC loss in the R eye, the patient was diagnosed with R preperimetric open angle glaucoma and commenced on gt latanoprost nocte in both eyes with a target IOP of 18 mmHg. The patient was compliant and responded to therapy, and target pressure was readily achieved. A post-treatment follow-up GCC scan was taken in September 2012 and compared to the pretreatment

scan of October 2011. This now showed stability in the damaged R eye inferotemporal sector and no further GCC thinning (Figure 9).

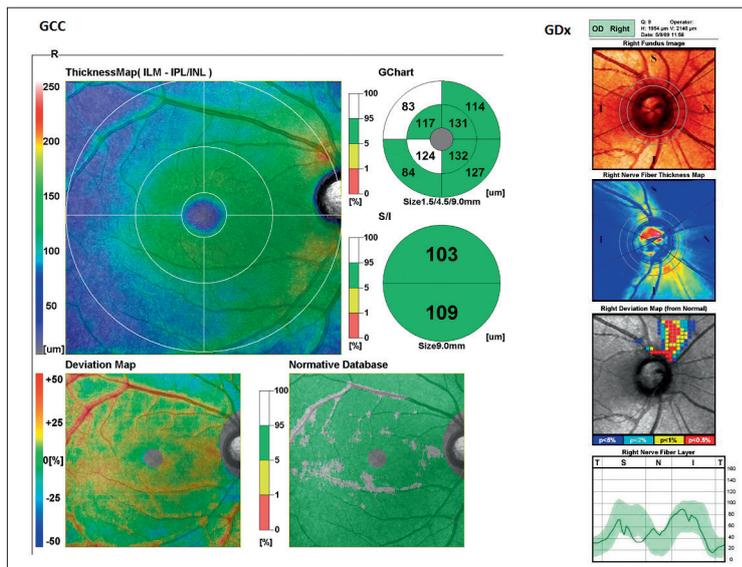
An inferotemporal loss of GCC would eventually be expected to result in a superior nasal visual field defect, that is, a superior nasal step. Further examination of the patient's initial visual field map did confirm a cluster



▲ Figure 9. GCC progression analysis after commencement of glaucoma treatment in the R eye. The damaged inferotemporal sector had stabilised due to the lower IOP and there was no further ganglion cell loss.



▲ Figure 10. A cluster of decreased sensitivity (-7 to -10 dB) in the R superior nasal step area of the visual field corresponding to the damaged R inferotemporal sector on GCC analysis



▲ Figure 11. A superonasal defect mapped by GDx scanning laser polarimetry remained undetected on the GCC scan. GCC analysis only scans the macular region temporal to the optic nerve head.

of mildly reduced sensitivity in the superior nasal step area of the R eye, albeit not to a level reaching statistical significance (Figure 10). In this instance, the GCC scan was able to detect glaucomatous damage prior to obvious changes in disc cupping and visual fields that may have been overlooked clinically; left untreated, further erosion of the inferior disc rim tissue and development of a deep

superior nasal step visual field defect would have been the likely outcome.

Limitations of the GCC

All ophthalmic instruments have limitation and GCC is no exception. Commercially available SD OCTs that measure GCC can only scan and statistically analyse the macular region; therefore, no information is provided regarding damage to areas nasal to the

disc. Figure 11 shows a patient with a superior nasal RNFL defect detected on GDx analysis but not seen on GCC because damage fell outside of the scanned area. Macular pathology that causes abnormal thickening of the inner retina such as macular oedema or retinal fibrosis will interfere with GCC measurements. This prevents accurate comparison with the normative database, as well as interfering with detection of change over time. Figure 12 shows an epiretinal membrane causing above-average thickening of the GCC.

At present there are about 10 different manufacturers of SD OCTs, and each instrument segments the retinal layers and measures the ganglion cells with differing accuracy and repeatability.⁶ The GCC scan area varies from a 6 mm (six segment) circle centred on the fovea (Cirrus) to a much larger (in area) 9 mm square (eight segment) that reaches to the disc (Nidek). The definition of the GCC also varies between manufacturers: most consider the GCC to be made up of the IPL + GCL + NFL (for example, Optovue; Nidek) while Carl Zeiss Meditec uses only IPL + GCL and ignores the NFL, and Topcon gives three different IPL/GCL/NFL.

Due to the newness of the technique and lack of standardisation between OCT units, it is difficult to compare GCC instruments to each other, or

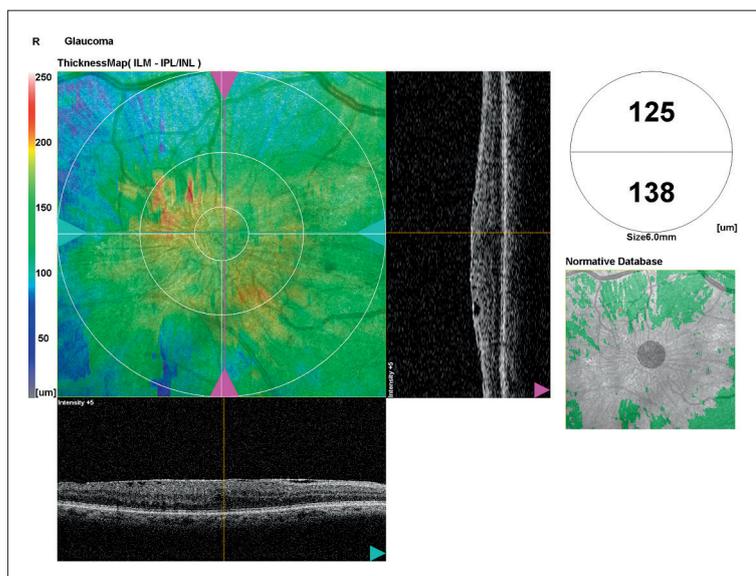
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to existing glaucoma instruments in terms of sensitivity and specificity for glaucoma detection and diagnosis.⁷ GCC scans are more repeatable and have less inter-scan variability than RNFL scans, but for the Cirrus OCT do not appear to detect glaucoma any earlier than RNFL scans.⁸ Future research will help elucidate the particular combination of instrument/scan layer/scan area that is most sensitive and specific for glaucoma diagnosis.

Conclusion

There is no single test or instrument or clinical finding that can make a definitive diagnosis of glaucoma. GCC analysis is an exciting new technique that provides an extra piece of data in the glaucoma puzzle. It appears to detect ganglion cell death via loss of synapses and loss of cell bodies at an earlier stage than existing methods that only look at nerve fibre (axonal) loss. Progressive loss of GCC has been helpful in making the decision to treat ocular hypertensives and patients with preperimetric glaucoma prior to the development of frank visual field defects.



▲ Figure 12. An epiretinal membrane causing abnormal thickening of the GCC. This thickening will mask any glaucomatous ganglion cell loss.

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Mutant myocilin possible root cause of increased eye pressure

RESEARCHERS at the Georgia Institute of Technology in Atlanta, USA have identified molecules with the potential to block the accumulation of a toxic eye protein that can lead to early onset of glaucoma. In their study, the researchers implicated a mutant form of a protein called myocilin as a possible root cause of increased eye pressure. Mutant myocilin is toxic to the cells in the part of the eye that regulates pressure. These genetically-inherited mutants of myocilin clump together in the front of the eye, preventing fluid flow out of the eye, which then raises eye pressure—a cascade of events that can lead to early onset-glaucoma. The researchers are now focusing on mapping the structure of myocilin to learn more about what myocilin does.

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Association of risk factors and glaucomatous damage

Both IOP and perfusion parameters appear to contribute independently—at least in part—to morphological and functional glaucomatous damage, according to a study published in the *Journal of Glaucoma*. Investigators analysed the association between the extent of glaucomatous damage and presumed risk factors for the disease in 50 patients with untreated primary open-angle glaucoma. The investigators found mean retinal nerve fibre layer thickness to be significantly associated ($P < .05$) with IOP, retinal arterial diameter, and choroidal blood flow. They observed that the visual field mean defect was associated with ocular perfusion pressure, laser Doppler flowmetry volume, and IOP variability.

J Glaucoma, August 2013