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Macular GCIPL loss precedes peripapillary RNFL loss in glaucoma with lower intraocular pressure

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**TITLE:** Macular GCIPL loss precedes peripapillary RNFL loss in glaucoma with lower intraocular pressure.

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**RUNNING HEAD**
Clinical covariates impacting detection of glaucoma progression using mGCIPL and pRNFL analysis.

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ABSTRACT

Purpose: To investigate which clinical measures influence whether an individual demonstrates earliest glaucomatous structural progression on peripapillary retinal nerve fibre layer (pRNFL) or macular ganglion cell-inner plexiform layer (mGCIPL).

Design: Prospective longitudinal cohort study.

Participants: 271 eyes from 207 individuals with statistically significant evidence of glaucomatous progression on optical coherence tomography (OCT)-Guided Progression Analysis (GPA) software were drawn from a total of 1271 eyes from 686 individuals categorized as glaucoma suspect or having early manifest glaucoma undergoing glaucoma surveillance.

Methods: Individuals demonstrating earliest evidence of longitudinal progression on mGCIPL GPA event analysis were compared to individuals demonstrating evidence of earliest longitudinal progression on pRNFL GPA event analysis.

Outcome Measures: Correlation of OCT event change analysis with intraocular pressure (IOP), clinical variables, and baseline thickness of the pRNFL and mGCIPL.

Results: IOP, baseline pRNFL thickness, baseline mGCIPL thickness and systemic hypertension were associated with location of first progression. Eyes demonstrating earliest longitudinal progression on mGCIPL had significantly lower maximum-recorded pre-treatment IOP (mean difference: 3.90mmHg, 95%CI: 2.37-5.43; p<0.001). The time interval between progression on pRNFL and progression on mGCIPL increased by 12.4 months for every 5mmHg increase in IOP (95%CI: 10.32 -15.72). Eyes demonstrating earliest longitudinal progression on mGCIPL had significantly lower baseline average pRNFL thickness than eyes progressing on pRNFL first (mean difference: 7.07μm; 95%CI: 4.38-9.77; p<0.001). Eyes progressing first on mGCIPL parameters were 3.03 times more likely to develop a new paracentral field defect than cases progressing first on pRNFL parameters (OR: 3.03; 95%CI: 1.26-7.28; p=0.01).

Conclusion: Clinical features, particularly pre-treatment IOP, influence whether structural glaucoma progression is detected earlier with mGCIPL or pRNFL imaging. These data support the utility of mGCIPL imaging in addition to pRNFL analysis for detection of glaucoma progression, particularly in patients with normal IOP.
ABBREVIATIONS:

- mGCIPL: macular ganglion cell-inner plexiform layer
- pRNFL: peripapillary retinal nerve fibre layer
- IOP: intraocular pressure
- NTG: normal tension glaucoma
- HTG: high tension glaucoma
- GPA: guided progression analysis
- RGC: retinal ganglion cell
- OCT: optical coherence tomography
- SITA: Swedish Interactive Threshold Algorithm
- HVF: Humphrey Visual Field
- EMG: Early Manifest Glaucoma
- GS: Glaucoma Suspect
INTRODUCTION

Spectral domain optical coherence tomography (SD-OCT) provides quantitative information about glaucomatous degeneration of retinal ganglion cell (RGC) components at the macula and peripapillary retinal nerve fibre layer (pRNFL). Assessment of pRNFL thickness has become routine in clinical glaucoma practice. Over the past decade, evidence has accrued to indicate that OCT changes at the macular ganglion cell-inner plexiform layer (mGCIPL) are also highly informative. Hou et al showed that in patients with confirmed glaucoma, mGCIPL monitoring is a more specific OCT metric for detecting further progression than pRNFL monitoring (95.5% vs 91.0%). The mGCIPL has a theoretical advantage over the pRNFL for detecting RGC degeneration, because 50% of RGCs are located at the macula, and RGC cell bodies are 10-20x thicker than their axons adjacent to the optic nerve head.

Several longitudinal studies have compared the ability of mGCIPL and pRNFL imaging to detect glaucoma progression, but the influence of clinical parameters, such as intraocular pressure (IOP), on the relative utility of these two testing strategies has received limited attention. The only clinical parameter to have been studied in detail is glaucoma severity. In moderate and advanced glaucoma, mGCIPL imaging is superior at detecting progression than pRNFL imaging, whereas in earlier stages of the disease the two testing strategies are approximately equivalent. It is unknown whether other ocular or systemic characteristics influence the timing and location of structural progression. The purpose of this study was to investigate covariates that could help to predict whether glaucoma progression on SD-OCT is likely to be detected earlier at the optic disc or macula.
METHODS

Participants
This investigation was a subanalysis of an ongoing, longitudinal, prospective, multi-centre observational cohort study of individuals initially classified as glaucoma suspect (GS) or having early manifest glaucoma (EMG) in South Australia. Inclusion criteria were: age between 18 and 85 years, and the ability to provide written consent, to perform reliable automated perimetry and to attend 6-monthly visits. Participants classified as GS and EMG were consecutively recruited at the Flinders Medical Centre and private ophthalmology practices in South Australia. EMG was defined as glaucomatous optic disc changes (disc grade ≥ 3 on the Disc Damage Likelihood Scale (DDLS) in the presence of early glaucomatous field changes (mean deviation better than -6dB), as per Hoddapp-Parrish-Anderson (HPA) criteria on at least 2 reliable Humphrey Visual Field (HVF) 24-2 SITA Standard Tests (Humphrey Field Analyzer; Carl Zeiss Meditec; Dublin, CA). The criteria for a reliable HVF included: fixation loss of 33% or less, false positive rates of 33% or less, and false-negative rates of 33% or less. Glaucoma suspects were defined as participants with an optic nerve head or neuroretinal rim appearance suspicious of glaucoma but without a glaucomatous visual field defect as per HPA criteria on a reliable HVF 24-2 SITA Standard test. A suspicious optic nerve head or neuroretinal rim was defined by a DDLS grade of 1 or 2 on stereo disc photography.

Enrolled participants underwent six-monthly ophthalmic evaluation. IOP measurements were undertaken using Goldmann applanation tonometry. Longitudinal IOP data was reviewed to determine maximum, minimum, mean and maximum pre-treatment IOP. In cases where patients were already on treatment at baseline, patient records were reviewed to determine maximum IOP prior to treatment commencement. Optic disc and structural assessment were undertaken using slit lamp examination and stereo-photography of the optic disc, and SD-OCT analysis of the pRNFL and mGCIPL. Longitudinal visual field data was obtained using achromatic HVF 24-2 SITA Standard perimetry. Reliable HVFs were assessed for visual field progression using the HPA criteria. Spatial assessment of visual field defects was undertaken as per Kang et al whereby the paracentral visual field was deemed to be the central 16 points in the 24-2 pattern. The peripheral visual field was deemed to be all other areas of the visual field (i.e. nasal steps, bjerrum or temporal wedge). New visual field defects were subsequently characterised as either: paracentral only, peripheral only, or both paracentral and peripheral. The study protocol allowed participants to have additional reviews during the six-month interval, with additional HVF and SD-OCT testing performed, if the treating clinician felt that their clinical situation warranted more frequent follow-up. Participants’ past medical and medication history was obtained at study enrolment and reviewed during monitoring.
using a general health screening. This health screening consisted of a questionnaire and a baseline assessment of systolic and diastolic blood pressure.

Clinical management was at the discretion of the treating ophthalmologist. The study design allows individualized treatment of participants and therefore provides maximal generalizability to the ophthalmic community, particularly in regions with a predominantly Anglo-European population. Patient treatment data was also obtained to assess whether different classes or IOP lowering medications would influence the site of structural progression. This study followed the tenets of the Declaration of Helsinki, and ethical approval for this study was granted by the Southern Adelaide and Flinders University Clinical Research Ethics Committee, South Australia.

Optical Coherence Tomography imaging

SD-OCT imaging was performed using the CIRRUS HD-OCT (Carl Zeiss, Meditec; Dublin, CA). pRNFL and mGCIPL imaging was performed with optic disc and macular cube scans, respectively. Upon enrolment, each participant in this study underwent 2 baseline optic disc and macular cube scans, and repeat optic disc and macular cube scans performed at every subsequent review (6 monthly). SD-OCT scans were captured by an experienced operator using CIRRUS FastTrac eye-tracking technology. Scans with a signal strength of <7, motion artefact, poor centration, or segmentation errors were discarded by the operator and rescanning was performed at the same visit. Scans were re-checked for artefact by an investigator (HM) before being included in GPA calculations.

Guided Progression Analysis

GPA data from CIRRUS HD-OCT Software version 9.5 was reviewed. GPA quantitates progressive thinning of the pRNFL and mGCIPL using both event analysis and trend analysis. Event analysis evaluates change in pRNFL and mGCIPL thickness in individual superpixels (1 superpixel = 4 x 4 pixels) between the follow-up scan and the two baseline scans. Three summary parameter graphs summarise overall change in the mGCIPL and pRNFL thickness by plotting the mean superior thickness, mean inferior thickness, and mean total thickness of the mGCIPL and pRNFL. Using event analysis, a data point is colored yellow (“possible loss”) on the summary parameter graph when the value falls outside the range of test-retest variability, and is colored red (“likely loss”) if the change is confirmed on a subsequent follow-up scan. Trend analysis assesses for a statistically significant rate of thinning over time by performing linear regression of the data points in each of the 3 summary parameters of mGCIPL and pRNFL (average, superior, and inferior thickness).
This study was a review of mGCIPL and pRNFL GPA event analysis using the average, superior, and inferior thickness summary parameters. We classified individuals as having robust evidence of SD-OCT progression if they showed “likely loss” (red data points) on two consecutive visits in any mGCIPL or pRNFL summary parameter (Figure 1). Individuals therefore required at least 5 SD-OCT scans (2 baseline scans at their enrolment visit and 3 follow-up scans) to reach this study’s endpoint for inclusion in the analysis. This highly stringent definition of progression was chosen to offset any possibility of stable individuals being erroneously classified as demonstrating progression due to test-retest variability of individual scans (a false positive result). If an individual showed “likely loss” on two consecutive visits in a mGCIPL summary parameter before any pRNFL summary parameter, then they were classified as ‘mGCIPL-first’. Conversely, if an individual showed “likely loss” on two consecutive visits in a pRNFL summary parameter before any mGCIPL summary parameter then they were classified as ‘pRNFL-first’. Individuals demonstrating earliest longitudinal structural progression on mGCIPL (classified as ‘mGCIPL-first’) were compared to individuals demonstrating earliest longitudinal structural progression on pRNFL (classified as ‘pRNFL-first’). Individuals demonstrating earliest longitudinal progression on pRNFL and mGCIPL at the same time point were excluded from the comparative analysis.

**Figure 1. A representative individual progressing on mGCIPL-first.**

Individuals were classified using the Guided Progression Analysis (GPA) software of the CIRRUS HD-OCT (mGCIPL is displayed in rows a and b, and pRNFL in rows c, d). Rows ‘a’ and ‘c’ are thickness maps, rows ‘b’ and ‘d’ are thickness change maps (from baseline) and rows ‘e-g’ are graphs of thickness summary parameters of mGCIPL and pRNFL. This individual had 2 baseline scans performed upon enrolment at age 74 years. The interval between baseline scans was so small that the 2 baseline data points appear as 1 on the summary parameter graphs. The mGCIPL GPA event analysis shows “possible loss” at Exam 5 in both the average (row e) and inferior (row g) thickness summary parameters, indicated by orange shading of the data point (orange arrows). These parameters are shaded red (“likely loss”) in Exams 6-8 (red arrows). The mGCIPL thickness change map indicates the area where structural change is occurring. In contrast, the pRNFL GPA event analysis has not shown “possible” (orange) or “likely loss” (red), even by Exam 8. This eye was therefore classified as ‘mGCIPL-first’, with this study’s end-point being reached at Exam 7 (“likely loss” on 2 consecutive scans).
Statistical Analysis:

Baseline data for mGCIPL-first cases were compared to pRNFL-first cases across a range of demographic and clinical characteristics, including age, sex, eye enrolled, duration of follow-up, IOP, central corneal thickness, refraction, optic disc area, vertical cup-disc ratio, glaucoma treatment, baseline pRNFL and mGCIPL thickness, and comorbid medical conditions.

Differences in independent variables between the dichotomous categorical outcome (mGCIPL-first or pRNFL first) were tested statistically by fitting a univariate generalised linear model (GLM) with mixed-effects to account for inter-eye correlation. GLMs were fitted with the glmer() function in the lme4 package (v1.1-18-1) in R (v3.4.1). A multivariate generalised linear model with mixed effects was then fitted using careful consideration of those variable likely to explain or confound the outcome, as well as a combination of stepwise variable selection for variables with a p-value <0.1 on univariate analysis. Random effects on intercept were fitted at the single level of individual patient ID to account for inter-eye correlation.

Receiver operating characteristic (ROC) curves were constructed and Youden’s index was used to determine the IOP values and baseline retinal thickness values that optimally distinguished mGCIPL-first cases from pRNFL-first cases. The predictive performance of threshold values was evaluated using Fisher’s exact test and positive predictive values.

All of the continuous variables studied followed a normal distribution based on the Shapiro-Wilk test and graphical Q-Q plots. Statistical analyses were performed using freely available R software (v3.4.1) and commercially available software SPSS for Windows version 23 (SPSS Inc., Chicago, IL, USA). Reported p values are 2 sided and the probability level for statistical significance was initially set at 0.05. As this study evaluated 36 independent systemic and ocular variables, this threshold value was subsequently adjusted to 0.001 after Bonferroni correction.
RESULTS

Figure 2 shows a flow chart depicting how eyes were selected for this study. CIRRUS HD-OCT GPA data were reviewed for 1271 eyes of 686 participants. A total of 389 eyes were excluded due to insufficient SD-OCT scans and 74 eyes were excluded for inadequate scan quality, leaving 808 eyes from 452 participants with sufficient duration of follow-up and scan quality to reach the study endpoint. There were 271 eyes of 207 individuals that reached this study’s endpoint of robust SD-OCT progression. 26 eyes (4%) demonstrated structural progression on pRNFL and mGCIPL parameters at the same time point and were excluded from analysis, leaving 245 eyes from 188 participants. 111 eyes (45.3%) from 80 participants demonstrated structural progression on pRNFL-first, of which 77 demonstrated structural progression on the average summary parameter, 59 demonstrated progression on the superior quadrant summary parameter and 71 cases demonstrated progression first on the inferior quadrant parameter. 71 of the 77 cases (92%) progressing on the average pRNFL parameter also demonstrated focal progression on either the superior or inferior summary parameters. 134 eyes (54.7%) from 108 participants demonstrated structural progression on mGCIPL-first. 117 mGCIPL-first eyes progressed on the average summary parameter, 69 mGCIPL-first eyes progressed on the superior summary parameter and 87 mGCIPL-first eyes progressed on the inferior summary parameter. 104 of the 117 cases (88.9%) that progressed on the average mGCIPL parameter also demonstrated focal progression on either the superior or inferior summary parameter. In total, 6282 pairs of optic disc and macular cube scans (12,564 scans) were included for progression analysis.

Figure 2: Selection and classification of eyes using CIRRUS SD-OCT Software

GS = glaucoma suspect; EMG = early manifest glaucoma

Analysis of participant demographics and medical history

The mean age for the entire cohort was 66.72 ± 9.15 years, 44.5% were male and the mean duration of follow-up was 32.43 ± 14.36 months. Univariate analyses into patient demographics and medical history revealed that although individuals demonstrating earliest longitudinal structural progression on mGCIPL (mGCIPL-first) had a higher rate of systemic hypertension than individuals progressing on pRNFL-first, there were no significant differences between mGCIPL-first individuals and pRNFL-first individuals in terms of age, duration of follow-up, sex, eye reaching endpoint, or other systemic medical conditions (Table 1).
Analysis of ocular structural characteristics

Eyes progressing on mGCIPL-first had a significantly thinner pRNFL and mGCIPL at baseline than eyes progressing on pRNFL-first (Table 2). Baseline average pRNFL thickness had the strongest association with progression on pRNFL-first or mGCIPL-first (p<0.001). Hedges’ g test for comparing baseline average pRNFL thickness between mGCIPL-first cases and pRNFL-first cases was 0.64, indicating large effect size. There were no significant differences between mGCIPL-first eyes and pRNFL-first eyes in terms of central corneal thickness, optic disc area, vertical cup-disc ratio, or refractive error.

Analysis of ocular clinical characteristics

Eyes progressing on mGCIPL-first had significantly lower maximum recorded pre-treatment IOP (p<0.001) and maximum recorded IOP (p=0.001), as well as lower mean and minimum IOP values (p=0.006, p= 0.035 respectively) (Table 3). The IOP measurement that was most strongly associated with progression on pRNFL-first or mGCIPL-first was maximum recorded pre-treatment IOP. There was no statistically significant difference in the prevalence of glaucoma treatment prior to structural progression between the two groups. There also was no statistically significant difference in the prevalence of cases treated with Prostaglandin Agonists, Beta-blockers, Alpha-2-agonists or Carbonic Anhydrase Inhibitors (Table 3).

Logistic Regression analysis

A multivariate logistic regression was performed to evaluate how systemic and ocular covariates influenced the probability that a patient would progress on pRNFL-first. We evaluated covariates with a p-value <0.1 on univariate analysis, and to prevent co-linearity, only the most significant variable within each subgroup of IOP, baseline pRNFL and baseline mGCIPL variables was selected. The following variables were assessed: maximum-recorded pre-treatment IOP, baseline average pRNFL thickness, baseline inferior mGCIPL thickness, and systemic hypertension. Initial analysis demonstrated that baseline inferior mGCIPL thickness did not statistically significantly contribute to the model (P=0.419). These were subsequently excluded. The final model was expressed with the formula:

$$mGCIPL/pRNFL \sim \text{max pre-treatment IOP} + \text{baseline pRNFL thickness} + \text{hypertension} + (1|\text{ID})$$

where; (1|ID) represents the random effect on slope for each participant.

In the final model, maximum pre-treatment IOP had the largest effect on OCT progression pattern (OR 1.19, 95% CI: 1.077-1.316; P<0.001). That is, for every 5 mmHg increase in maximum-recorded
pre-treatment IOP, the odds of progressing first on pRNFL increased 2.5 times. Baseline pRNFL thickness had a more modest effect on the OCT progression pattern (OR 1.10, 95% CI: 1.03-1.17; P=0.002). For every 10µm increase in baseline pRNFL thickness, the odds of progressing first on pRNFL increased 2.5 times. Finally, a concurrent diagnosis of hypertension showed a trend towards affecting OCT progression pattern (OR 0.33, 95% CI: 0.11-1.02; P=0.055).

Sub-analysis of treatment naive individuals

To investigate the possible confounding effects of IOP treatment, a sub-analysis was performed of individuals who were treatment naive during the period of SD-OCT progression (n=131 eyes (48%), mGCIPL-first = 78 eyes, pRNFL-first = 53 eyes). Treatment naive eyes progressing on pRNFL-first had significantly higher maximum-recorded IOP (OR 1.4, 95% CI: 1.03-1.96; P=0.030), with an effect size greater than was observed in the general cohort. Treatment naive eyes progressing on pRNFL first also had a greater baseline pRNFL thickness (OR 1.15, 95% CI:1.01-1.32; P=0.031) than those patients progressing on mGCIPL-first.

Sub-analysis of Early Manifest Glaucoma and Glaucoma Suspects

The baseline visual field data for all patients was reviewed and 8 eyes were excluded because of missing baseline visual fields. Sub-analyses were performed for individuals classified as Early Manifest Glaucoma (EMG) at baseline (n=89 eyes, 37%) and individuals classified as Glaucoma Suspect (GS) at baseline (n=146 eyes, 63%).

A multivariate generalised linear model with mixed effects was fitted to baseline EMG/GS classification for the variables of baseline IOP and RNFL thickness at baseline. Firstly, patients with thinner baseline average pRNFL were more likely to be EMG cases than GS cases (OR 0.93, 95% CI:0.89-0.97; P<0.001). EMG and GS case had similar maximum recorded pre-treatment IOP (OR 0.94, 95% CI:0.87-1.00; P=0.062; Supplementary Material, Table 1).

Considering EMG cases alone, the relationship between pattern of OCT progression and the explanatory variables of maximum IOP and baseline RNFL thickness was sustained. Eyes progressing on mGCIPL-first had a significantly lower maximum-recorded pre-treatment IOP than eyes progressing on pRNFL-first (OR 0.81, 95% CI:0.66-0.98; p=0.032). Eyes progressing on mGCIPL-first also had lower baseline average pRNFL thickness (OR 0.91, 95% CI:0.84-0.99; p=0.031).
For GS cases, eyes progressing on mGCIPL-first showed a trend towards a lower maximum-recorded pre-treatment IOP (OR 0.79, 95% CI:0.60-1.02; P=0.073), and a similar baseline average pRNFL thickness (OR 0.98, 95% CI:0.83-1.14; P=0.75).

Correlation of Structural and Functional progression

This study also correlated the structural progression observed on mGCIPL and pRNFL monitoring to visual field progression on HVF assessments. 82 cases (n = 47 GS cases and n = 35 EMG cases) demonstrated a new or worsening visual field defect during monitoring, as per the HPA criteria. Of the 82 cases, 40 cases were mGCIPL-first progressing cases and 42 cases were pRNFL first progressing cases. 20 eyes demonstrated only paracentral defects, 34 eyes demonstrated only peripheral defects and 31 eyes demonstrated both paracentral and peripheral defects. Eyes progressing first on mGCIPL parameters were 3.03 times more likely to develop a new or worsening paracentral field defect than cases progressing first on pRNFL parameters (OR: 3.03; 95%CI: 1.26-7.28; P=0.01). Eyes progressing first on pRNFL parameters were 1.79 times more likely to develop a new or worsening peripheral field defect than cases progressing first on mGCIPL (OR: 1.79; 95%CI: 1.12-2.86; P=0.01). Cases that demonstrated solely a new or worsening peripheral visual field defect had a statistically significantly higher maximum recorded pre-treatment IOP than eyes that demonstrated a new or worsening paracentral visual field defect (mean difference: 2.96mmHg; 95%CI: 0.48-5.44; P=0.02).

Subanalysis of individuals progressing on both pRNFL and mGCIPL

In total, 93 eyes reached this study’s endpoint for structural progression on both mGCIPL and pRNFL but at different time points. A sub-analysis was performed to evaluate how the maximum recorded pre-treatment IOP impacted the time interval between progression being demonstrated on mGCIPL and pRNFL. For every 5mmHg increase in maximum-recorded pre-treatment IOP, the interval between progression on pRNFL and mGCIPL extended by 12.4 months (95%CI: 10.32 -15.72; R-squared=0.857). In eyes with a maximum recorded pre-treatment IOP <14mmHg, progression on mGCIPL preceded progression on pRNFL by approximately 14.02±8.40 months, whereas in eyes with a maximum recorded pre-treatment IOP >30mmHg, progression on mGCIPL lagged behind pRNFL progression by approximately 31.29±2.41 months (Figure 3).

Figure 3: Impact of maximum recorded pre-treatment IOP on the time interval between progression being demonstrated on pRNFL and mGCIPL
Legend: For each value of maximum recorded pre-treatment IOP, the mean time interval between progression being demonstrated on mGCIPL and pRNFL was plotted. A positive value indicates that progression on pRNFL occurred prior to progression on mGCIPL.

A multiple linear regression analysis was conducted to evaluate how maximum recorded pre-treatment IOP and baseline average pRNFL thickness affected the time interval between progression on pRNFL and progression on mGCIPL. For every increase of 5mmHg in maximum pre-treatment IOP, the timing of progression on pRNFL relative to mGCIPL increased by 0.59 years (95%CI: 0.28-0.90, p<0.001) and for every 5µm increase in baseline average pRNFL, the time interval increases by 0.17 years (95%CI: 0.015-0.32, p=0.03).
Figure 4 shows the ROC curve for maximum recorded pre-treatment IOP and baseline average pRNFL thickness. Combining these variables resulted in higher predictive performance than either variable in isolation (AUC for combination ROC curve = 0.771; 95%CI: 0.684-0.8570). When these two variables operate in tandem, the optimal threshold values are 22.0mmHg for maximum recorded pre-treatment IOP and 80.5µm for average baseline pRNFL thickness.

Figure 4: ROC curves for maximum recorded pre-treatment IOP and baseline average pRNFL thickness

Figure 5 illustrates the distribution of pRNFL-first and mGCIPL-first progressing cases, according to whether the maximum recorded pre-treatment IOP and baseline average pRNFL is greater or less than the optimal threshold values. Individuals with a maximum recorded pre-treatment IOP < 22mmHg and baseline average pRNFL thickness < 80.5µm were 2.91 times more likely to progress on mGCIPL-first than pRNFL-first (positive predictive value 74.6%; 95%CI: 73.3-89.1%). In contrast, individuals with a maximum-recorded pre-treatment IOP ≥ 22mmHg and baseline thickness ≥ 80.5µm were 3.6 times more likely to progress on pRNFL-first than mGCIPL-first (positive predictive value 69.7%; 95%CI: 53.8-82.0%).

Figure 5: Distribution of pRNFL-first and mGCIPL-first progression according to IOP and baseline pRNFL thickness

Legend: Each quadrant shows the distribution and the proportion of eyes demonstrating progression on pRNFL-first or mGCIPL-first. Quadrants are defined according to the optimal threshold values for maximum recorded pre-treatment IOP (22mmHg, horizontal line) and baseline average pRNFL thickness (80.5µm, vertical line).
**DISCUSSION**

Although both optic disc and macula parameters have excellent specificity for glaucoma progression, several investigators have used Venn diagrams to elegantly illustrate that in the majority of progressing individuals, structural progression is detected on only one of the two tests. Hou et al recently demonstrated that although pRNFL progression and mGCIPL progression are mutually predictive, neither test can substitute for the other and both parameters may be used in combination to optimise early detection of disease progression in glaucoma patients. This is the first study to specifically investigate covariates impacting on the relative utility of optic disc and macula parameters to detect longitudinal structural change. We found that IOP and baseline pRNFL thickness are predictive of whether structural progression will be detected first at the optic disc or macula, which in turns predicts whether a patient is more likely to first manifest a paracentral or peripheral visual field defect.

Our results support a novel association between lower IOP and glaucomatous structural change manifesting at the mGCIPL prior to the pRNFL. Of all ocular and extra-ocular covariates studied, maximum pre-treatment IOP had the strongest association with the site that first demonstrated statistically significant structural progression after study enrolment. The pathophysiological explanation for this observation remains unclear. However, one biologically plausible explanation is that those individuals with lower IOPs form an endophenotype of glaucoma which manifests loss of thickness in the mGCIPL earlier than at the peripapillary axons. Whether this difference is related to macular ganglion cell somata, ganglion cell dendrites, or other retinal cell subtypes remains to be determined. The CIRRUS SD-OCT, like most OCT platforms, does not segment the Retinal Ganglion Cell layer (GCL) from the Inner Plexiform Layer (IPL) as the border between these structures is not readily distinguishable, therefore we do not yet know whether earliest structural change is being driven by the GCL, IPL, or both. Conceivably, structural change within the ganglion cell and inner plexiform layers is more widespread throughout the retina, but is detected at the macula because that is where the SD-OCT is targeted. Using a cross-sectional design, Kim et al reported that glaucomatous RNFL defects tended to occur closer to the macula in individuals with lower IOP than those with higher IOP, but no other studies have investigated this association.

It is interesting that the optimal threshold level of maximum pre-treatment IOP as determined by our analysis was in the range of 21-22mmHg which is comparable to historical cut-offs between Normal Tension Glaucoma (NTG) and High Tension Glaucoma (HTG) in caucasian populations. We recognise that this is an arbitrary value which relates specifically to the entry criteria and cases.
included in our study. It may be useful to highlight the distribution of pRNFL-first and mGCIPL-first cases by IOP, but our results are not necessarily generalisable to other populations or patient subgroups. This study did not set out to categorise participants into HTG and NTG subtypes, preferring to consider all participants as being part of a continuous spectrum. Nonetheless, our finding that individuals with lower pre-treatment IOP tended to demonstrate earliest longitudinal progression on mGCIPL is consistent with what has historically been represented in the literature in regards to the NTG phenotype. This is further supported by our finding that patients demonstrating structural progression first on mGCIPL are more likely to develop a paracentral field defect. NTG is reportedly associated with focal glaucomatous damage at the macula and visual field defects that are central or paracentral.\textsuperscript{12,15,16} Macular parameters on SD-OCT are therefore well-suited to detect glaucomatous damage in such patients, in contrast to pRNFL parameters which have been reported to be less sensitive at detecting glaucomatous macula defects.\textsuperscript{17,18} In this study, eyes with early macular progression were therefore associated with several features consistent with historic descriptions of NTG.\textsuperscript{19}

The current understanding of the cytoskeletal changes during glaucoma might explain the association between IOP and the site of initial detectable structural defects. The pRNFL is comprised mostly of retinal ganglion cell axons, whereas the mGCIPL contains the RGC cell bodies, RGC dendrites and RGC axons.\textsuperscript{2} Several studies have suggested that elevated IOP compresses axons at the level of the lamina cribrosa to induce retrograde axonal deterioration.\textsuperscript{20–22} It is biologically plausible that individuals with elevated IOP could manifest structural progression on pRNFL-first, as we found in this study. In individuals with normal IOP, the same pathological process of retrograde axonal deterioration at the optic nerve head may be less relevant. It is therefore possible that glaucoma participants with lower IOP lose retinal ganglion cells via an alternative pathogenic pathway.\textsuperscript{16} This would be in agreement with our findings and those of Jung et al and Baniasadi et al. These two studies showed that NTG causes focal thinning of the macula, whereas HTG causes more diffuse thinning.\textsuperscript{23,24} The integration of these findings with our work suggests that the transition along the POAG spectrum from NTG to HTG is associated with an endophenotype transition from focal, deep macula-first structural defects to diffuse, shallow optic-disc first structural defects. Although more research is required to investigate this hypothesis and to characterise the structural changes observed, our results illustrate an interesting association between IOP and the initial site of detectable structural progression.
Three previous studies have used trend analysis of OCT data to evaluate rates of mGCIPL and pRNFL thinning in individuals with progressive mild and advanced POAG.\textsuperscript{4,5} In these studies, the rate of pRNFL thinning slowed dramatically in advanced glaucoma, whereas the rate of mGCIPL thinning remained relatively steady in advanced disease and still maintained good sensitivity for detecting progression.\textsuperscript{4,6,25} This may help explain why patients with thinner pRNFL at baseline were more likely to progress first on mGCIPL monitoring. In contrast, mGCIPL and pRNFL have been found to have similar efficacy for detecting progression in mild glaucoma.\textsuperscript{4,5} Our study excluded individuals with mean deviation worse than -6dB, and yet we still found that even in this cohort of early manifest glaucoma and glaucoma suspects, baseline pRNFL and mGCIPL thickness influenced the detection of glaucoma progression. In addition, the inclusion of both so-called “pre-perimetric” glaucomas and glaucoma suspects (as defined by disc appearance) provided a basis for capturing very early disease.

Some of these subjects showed no progression on either structural parameters and do not have, and may never develop glaucoma. We do not see this as a limitation, but as providing an important internal control. The subanalyses by disease and treatment status clearly showed the findings to be independent of both disease stage and treatment status. The present study indicates new insights into the initial manifestations of glaucoma by using longitudinal analysis with event based endpoints early in the disease process.

Although this study revealed useful new insights into characteristics that influence SD-OCT GPA event analysis progression, we acknowledge potential limitations. The use of the CIRRUS SD-OCT event analysis algorithm to detect structural progression does present some limitations. Event analysis algorithms detect structural change by comparing the change in thickness from baseline for a given parameter to the reproducibility coefficient of a normative database.\textsuperscript{26} Without knowledge of this figure, due to proprietary reasons, it may be difficult to ascertain the false positive rate of progression. We attempted offset this uncertainty by only classifying eyes as showing SD-OCT progression when “likely loss” was confirmed twice. This stringent definition of progression combined with the high precision of SD-OCT imaging makes it extremely likely that the structural change detected on SD-OCT represents definite and progressive thinning of the mGCIPL or pRNFL. The fact that our findings were also replicated on visual field analysis further suggests that the change is real. Above all though, we elected for this methodology because it is an algorithm that is standardised across all CIRRUS pRNFL and mGCIPL monitoring and therefore well suited to real-world glaucoma monitoring. Criticism may also arise because RNFL event analysis has been suggested to be less sensitive than RNFL trend analysis.\textsuperscript{26} There currently however exists a paucity of data comparing the sensitivities of mGCIPL event analysis and trend analysis algorithms to make a
similar claim. Hou et al have recently reported that mGCIPL GPA event analysis has a specificity >95% and that pRNFL GPA event analysis has a specificity >90% for progressive glaucoma, so it would appear that event analysis is well suited for detecting glaucomatous change. Another debate regarding event analysis could arise as to whether the nasal pRNFL regions influence the specificity of the glaucomatous structural progression analysis observed using the average pRNFL parameter. We have performed a further analysis (Supplementary table 2) which confirmed the major findings when pRNFL progression was confined to the inferior and superior regions more traditionally involved in glaucoma.

We recognise that a potential limitation was that by permitting clinicians to treat participants at their discretion, patient treatment was therefore heterogeneous. We believe however that this model does possess some merits. This model provides maximum generalisability to the ophthalmic community treating Anglo-European individuals. It additionally enabled us to assess whether different glaucoma treatments affect the site of structural progression. We further assessed this limitation by including the maximum-recorded pre-treatment IOP and conducted a sub-analysis of treatment naive patient. The fact that this parameter was the most significant parameter and that the treatment naive subanalysis demonstrated higher levels of effect size indicates that differential treatment does not underlie the main outcomes of this study. We also recognise a potential limitation that our population occupies a spectrum of both rate and stage of disease. We believe however that this provides an important application to the real-world glaucoma clinic, whereby patients do present at different stages of disease. For instance, the superiority of mGCIPL monitoring to detect progression in patients with thinner baseline pRNFL highlights the utility of mGCIPL in these patients.

In conclusion, using a robust prospective study design with a large cohort and employing stringent definitions of SD-OCT progression, we found a significant association between maximum-recorded pre-treatment IOP, baseline pRNFL thickness, and earliest structural progression on mGCIPL or pRNFL. Specifically, longitudinal structural change in participants with lower pre-treatment IOP and thinner baseline pRNFL thickness tends to be detected on mGCIPL before pRNFL. These results support the clinical utility of using mGCIPL in addition to pRNFL imaging to monitor progression in glaucoma suspects and individuals with early manifest glaucoma, particularly in those with normal IOP and thinner pRNFL.
REFERENCES:


of the pathogenesis. Eye 2018;32:924–930.


Table 1: Analysis of patient demographics and medical history for mGCIPL-first and pRNFL-first progressing individuals

<table>
<thead>
<tr>
<th>Patient demographics</th>
<th>mGCIPL-first (n=134)</th>
<th>pRNFL-first (n=111)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>67.4 ± 9.2</td>
<td>64.8 ± 9.1</td>
<td>0.129</td>
</tr>
<tr>
<td>Sex: male (%)</td>
<td>51.12</td>
<td>33.01</td>
<td>0.923</td>
</tr>
<tr>
<td>Eye reaching endpoint: Right (%)</td>
<td>50.44</td>
<td>43.63</td>
<td>0.467</td>
</tr>
<tr>
<td>Follow-up (months)</td>
<td>33.15 ± 13.21</td>
<td>30.12 ± 12.81</td>
<td>0.483</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Medical History</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Migraine (%)</td>
<td>58.84</td>
<td>41.22</td>
<td>0.254</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>43.07</td>
<td>24.29</td>
<td>0.025</td>
</tr>
<tr>
<td>Antihypertensives (%)</td>
<td>38.05</td>
<td>19.81</td>
<td>0.017</td>
</tr>
<tr>
<td>Baseline Systolic BP (mmHg)</td>
<td>132.61±20.34</td>
<td>130.30±18.54</td>
<td>0.296</td>
</tr>
<tr>
<td>Baseline Diastolic BP (mmHg)</td>
<td>73.11±14.77</td>
<td>71.68±15.82</td>
<td>0.531</td>
</tr>
<tr>
<td>Raynauds Phenomenon (%)</td>
<td>7.58</td>
<td>1.83</td>
<td>0.106</td>
</tr>
<tr>
<td>Diabetes Mellitus (%)</td>
<td>11.63</td>
<td>14.32</td>
<td>0.566</td>
</tr>
<tr>
<td>Depression (%)</td>
<td>19.52</td>
<td>17.97</td>
<td>0.233</td>
</tr>
<tr>
<td>Myocardial Infarction (%)</td>
<td>8.31</td>
<td>8.92</td>
<td>0.964</td>
</tr>
<tr>
<td>Stroke/TIA (%)</td>
<td>4.53</td>
<td>8.03</td>
<td>0.396</td>
</tr>
<tr>
<td>Disc Haemorrhage (%)</td>
<td>14.55</td>
<td>8.23</td>
<td>0.113</td>
</tr>
</tbody>
</table>

* p-value significant after Bonferroni correction (threshold = 0.05/36 = 0.001)

p-values derived from univariate generalised linear models with mixed effects
<table>
<thead>
<tr>
<th>Structural Parameter</th>
<th>mGCIPL-first (n=134) μ±SD</th>
<th>pRNFL-first (n=111) μ±SD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline average pRNFL thickness (μm)</td>
<td>79.51 ± 10.93</td>
<td>86.58 ± 10.65</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Baseline pRNFL thickness in superior quadrant (μm)</td>
<td>94.67 ± 16.58</td>
<td>102.61 ± 19.13</td>
<td>0.002</td>
</tr>
<tr>
<td>Baseline pRNFL thickness in inferior quadrant (μm)</td>
<td>97.94 ± 19.02</td>
<td>106.61 ± 19.13</td>
<td>0.006</td>
</tr>
<tr>
<td>Baseline average mGCIPL thickness (μm)</td>
<td>73.66 ± 10.98</td>
<td>76.16 ± 7.98</td>
<td>0.104</td>
</tr>
<tr>
<td>Baseline mGCIPL thickness in superior sectors (μm)</td>
<td>74.02 ± 6.94</td>
<td>76.05 ± 9.78</td>
<td>0.056</td>
</tr>
<tr>
<td>Baseline mGCIPL thickness in inferior sectors (μm)</td>
<td>71.63 ± 8.39</td>
<td>75.83 ± 9.44</td>
<td>0.0114</td>
</tr>
<tr>
<td>Central Corneal Thickness (μm)</td>
<td>550.02 ± 40.52</td>
<td>546.58 ± 37.98</td>
<td>0.536</td>
</tr>
<tr>
<td>Optic disc area on Cirrus SD-OCT (mm²)</td>
<td>1.95 ± 0.44</td>
<td>1.99 ± 0.508</td>
<td>0.773</td>
</tr>
<tr>
<td>Vertical Cup-Disc Ratio</td>
<td>0.68 ± 0.12</td>
<td>0.67 ± 0.11</td>
<td>0.144</td>
</tr>
<tr>
<td>Spherical equivalent (dioptres)</td>
<td>-0.07 ± 2.37</td>
<td>+0.19 ± 1.9</td>
<td>0.258</td>
</tr>
</tbody>
</table>

* p-value significant after Bonferroni correction (threshold = 0.05/36 = 0.001)

p-values derived from univariate generalised linear models with mixed effects
Table 3: Ocular Clinical characteristics (IOP and treatment history) of eyes progressing on mGCIPL-first and pRNFL-first

<table>
<thead>
<tr>
<th>Ocular Clinical Characteristic</th>
<th>mGCIPL-first (n=134)</th>
<th>pRNFL-first (n=111)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intra-Ocular Pressure</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum recorded pre-treatment IOP (mmHg)</td>
<td>18.53 ± 6.02</td>
<td>22.43 ± 6.12</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Maximum recorded IOP (mmHg)</td>
<td>20.44 ± 5.54</td>
<td>23.2 ± 6.0</td>
<td>0.001*</td>
</tr>
<tr>
<td>Mean IOP during SD-OCT surveillance (mmHg)</td>
<td>15.49 ± 2.65</td>
<td>18.87 ± 5.69</td>
<td>0.006</td>
</tr>
<tr>
<td>Minimum recorded IOP (mmHg)</td>
<td>13.01 ± 2.56</td>
<td>14.46 ± 3.73</td>
<td>0.035</td>
</tr>
<tr>
<td><strong>Treatment History</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Participants treated medically prior to OCT progression (%)</td>
<td>41.79</td>
<td>52.15</td>
<td>0.174</td>
</tr>
<tr>
<td>SLT (%)</td>
<td>17.31</td>
<td>29.22</td>
<td>0.999</td>
</tr>
<tr>
<td>Trabeculectomy (%)</td>
<td>0</td>
<td>0.91</td>
<td>0.934</td>
</tr>
<tr>
<td>Patients with &gt;1 medication (%)</td>
<td>15.67</td>
<td>17.11</td>
<td>0.553</td>
</tr>
<tr>
<td>Cases treated with a Prostaglandin Agonist (%)</td>
<td>34.32</td>
<td>39.64</td>
<td>0.596</td>
</tr>
<tr>
<td>Cases treated with a Beta-blocker (%)</td>
<td>18.73</td>
<td>25.21</td>
<td>0.189</td>
</tr>
<tr>
<td>Cases treated with an Alpha-2 agonist (%)</td>
<td>4.54</td>
<td>9.92</td>
<td>0.155</td>
</tr>
<tr>
<td>Cases treated with a Carbonic Anhydrase inhibitor (%)</td>
<td>8.21</td>
<td>6.31</td>
<td>0.506</td>
</tr>
</tbody>
</table>

IOP = intraocular pressure.
SLT = selective laser trabeculoplasty
* p-value significant after Bonferroni correction (threshold = 0.05/36=0.001)
Macular GCIPL loss precedes peripapillary RNFL loss in glaucoma with lower intraocular pressure.

INVESTIGATOR DETAILS

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Prof Jamie E Craig, FRANZCO 1 (corresponding author)
### Supplementary table 1: Summary statistics for Glaucoma Suspect and Early Manifest Glaucoma cases.

<table>
<thead>
<tr>
<th></th>
<th>Glaucoma Suspect (n = 146)</th>
<th>Early Manifest (n = 89)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pRNFL first progressing cases (%)</td>
<td>41%</td>
<td>45%</td>
<td>0.020</td>
</tr>
<tr>
<td>Maximum recorded pre-treatment IOP (mmHg)</td>
<td>20.46± 6.15</td>
<td>19.33±5.27</td>
<td>0.062</td>
</tr>
<tr>
<td>Baseline Average RNFL thickness (μm)</td>
<td>84.32±10.72</td>
<td>78.71±11.23</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age (years)</td>
<td>65.06±9.18</td>
<td>68.61±9.28</td>
<td>0.003</td>
</tr>
<tr>
<td>Sex: male (%)</td>
<td>44.46</td>
<td>42.8</td>
<td>0.866</td>
</tr>
<tr>
<td>Eyes reaching endpoint: Right (%)</td>
<td>55.91</td>
<td>38.38</td>
<td>0.007</td>
</tr>
</tbody>
</table>

### Supplementary table 2: Replication of Results with exclusion of pRNFL average parameter

<table>
<thead>
<tr>
<th></th>
<th>pRNFL-first (n = 95)</th>
<th>mGCIPL-first (n = 134)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum Pre-treatment IOP (mmHg)</td>
<td>21.98±6.02</td>
<td>18.53±6.02</td>
<td>0.002</td>
</tr>
<tr>
<td>Baseline average pRNFL thickness (μm)</td>
<td>85.56±10.74</td>
<td>79.51±10.93</td>
<td>0.002</td>
</tr>
</tbody>
</table>

As a review of our methodology, we performed an alternative analysis where the average pRNFL summary parameter was excluded from the definition of structural progression. Eyes were subsequently reclassified as pRNFL-first (based on either superior or inferior regions) or mGCIPL-first. 16 cases no longer exhibited pRNFL first progression and were excluded. To replicate the most significant findings, a generalised linear model with mixed effects was fitted to the maximum pre-treatment IOP and baseline average pRNFL thickness. Cases that demonstrated structural progression on pRNFL parameters first had
a significantly higher maximum pre-treatment IOP before treatment (P=0.002) and thicker baseline average pRNFL thickness (P=0.002).
PRÉCIS

Using guided progression analysis of optical coherence tomography, longitudinal glaucomatous structural change in individuals with lower IOP is detected sooner with macular ganglion cell-inner plexiform layer thickness than with peripapillary retinal nerve fiber layer thickness.