Diagnostic accuracy of ganglion cell complex substructures in different stages of primary open-angle glaucoma

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ABSTRACT • RÉSUMÉ

Objective: To evaluate diagnostic accuracy of substructure of ganglion cell complex versus peripapillary nerve fiber layer (NFL) thickness using spectral domain optical coherence tomography (SD-OCT) in different stages of glaucoma.

Methods: Thirty eyes were normal, 120 were glaucomatous. Glaucomatous eyes were classified into: early glaucoma (46), moderate glaucoma (48), and severe glaucoma (26). Perimetry and SD-OCT were done. Peripapillary NFL thickness, ganglion cell layer (GCL), macular NFL thickness, combined GCL and macular ganglion cell complex (GCC), were recorded. Area under receiver operating characteristic curves (AUCs) was used to verify performance of different OCT parameters.

Results: Peripapillary NFL, GCL, and GCC thickness values were significantly different in all stages of glaucoma. All comparisons were significantly different; normal versus early, early versus moderate and moderate versus severe. The best parameters that distinguished normal from early stage were: peripapillary NFL (AUC: 0.90), GCC (AUC: 0.75), early from moderate stage were: peripapillary NFL thickness (AUC: 0.85), GCL (0.81),GCC (0.81), moderate from severe stage were: GCC (AUC:0.95), macular NFL (AUC:0.91), GCL (AUC:0.89), and peripapillary NFL (AUC:0.88).

Conclusions: Peripapillary NFL and GCC thinning showed paradoxical course. The most diagnosed parameter in early glaucoma was peripapillary NFL and in severe glaucoma was GCC. In severe glaucoma, macular NFL showed higher diagnostic power than GCL and peripapillary NFL. Ganglion cell complex mapping may provide good alternative to optic disc imaging in advanced glaucoma with poor fixation.

Objet : Comparer – au moyen de la tomographie par cohérence optique en domaine spectral (SD-OCT) – la valeur de l’épaisseur des cellules ganglionnaires (GCC) à celle de l’épaisseur des couches de fibres nerveuses (NFL) péripapillaires en tant que marqueurs de différents stades du glaucome.

Méthodes : L’échantillon comptait 30 yeux normaux et 120 yeux glaucomateux (glaucome précoce, 46; modéré, 48; et grave, 26). On a procédé à la périmétrie et à la SD-OCT, puis consigné les paramètres suivants : épaisseur des NFL péripapillaires, épaisseur de la couche des cellules ganglionnaires (GCL), épaisseur de la NFL maculaire, et l’épaisseur combinée de la NFL maculaire + épaisseur de la GCL (GCC). La performance de divers éléments de l’OCT a été évaluée au moyen de la courbe (ASC) ROC.

Résultats : L’épaisseur des NFL péripapillaires, de la GCL et du GCC différaient significativement à tous les stades du glaucome. L’écart était significatif pour toutes les comparaisons – œil normal vs glaucome précoce; glaucome précoce vs modéré; et glaucome modéré vs grave. Les structures permettant de distinguer le plus nettement l’œil normal des yeux atteints de glaucome à divers stades ont été les suivantes : œil normal vs glaucome précoce : NFL péripapillaires (ASC : 0.90) et GCC (ASC : 0.75); glaucome précoce vs glaucome modéré : NFL péripapillaires (ASC : 0.85), GCL (0.81) et GCC (0.81); glaucome modéré vs glaucome grave : GCC (ASC : 0.95), épaisseur NFL maculaire (ASC : 0.91), GCL (ASC : 0.89) et NFL péripapillaires (ASC : 0.88).

Conclusions : L’amincissement des NFL péripapillaires et du GCC suivait un cours paradoxal. Le paramètre diagnostique le plus efficace du glaucome précoce était l’épaisseur des couches de fibres nerveuses; celui du glaucome modéré, l’épaisseur de laNFL péripapillaires. La performance de divers éléments de l’OCT a été évaluée au moyen de la courbe (ASC) ROC.

Primary open-angle glaucoma (POAG) is defined as chronic progressive optic neuropathy accompanied by characteristic cupping and atrophy of the optic disc, visual field loss, open angle, and no obvious systemic or ocular cause.1 Standard perimetry cannot detect visual field defects until 20%–40% of ganglion cells have been lost.2 Staging glaucomatous damage into broad categories such as mild, moderate, and severe enhances management and promotes careful assessment of clinical damage, thereby facilitating monitoring for stability versus progression.3 Measuring nerve fibre layer (NFL) thickness by optical coherence tomography (OCT) enables an objective and quantitative assessment of glaucomatous structural loss.1 Peripapillary NFL thickness evaluation and optic nerve head (ONH) analysis had been considered for discriminating between different glaucoma stages with variable results.4,5 Ganglion cell complex (GCC) parameter was found to have significantly higher diagnostic power than total macular thickness parameters. It has been proven to be a useful method for glaucoma diagnosis and has potential for tracking glaucoma progression.6,7

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Different studies showed the beneficial role of GCC analysis in glaucoma-suspect, ocular hypertensive, and POAG patients. In addition, the clinical validity of GCC in advanced stages of glaucoma had been addressed; however, little concern had been paid to the role of GCC individual substructure (nerve fibre layer, ganglion cell layer, and inner plexiform layer) in different stages of POAG. The aim of this study was to evaluate the diagnostic accuracy of GCC substructure versus peripapillary NFL thickness in different stages of POAG.

**Patients and Methods**

This was a nonrandomized, cross-sectional study. Eyes were considered glaucomatous if they had 2 consecutive abnormal visual field test results associated with ONH damage characteristic of glaucoma with or without elevated intraocular pressure (≥21 mm Hg). Eyes were considered normal if they had intraocular pressure <21 mm Hg, normal optic disc appearance, and normal visual field. Normal visual field was defined as a mean defect and pattern standard deviation within 95% normal limits and a glaucoma hemifield test result within normal limits.

Patients who met the following criteria were excluded from the study: poor fixation, history of intraocular surgery, secondary glaucoma, systemic or ocular diseases, history of stroke, diseases that affect the macular area, and high myopia greater than –6 diopter.

All patients underwent measurement of best-corrected visual acuity, slit-lamp biomicroscopy, gonioscopy, Goldmann applanation tonometry, stereoscopic ophthalmoscopy of the optic disc, standard automated perimetry, and SD-OCT.

**Visual Field Testing**

Visual field testing was done with Humphrey field analyzer (Carl Zeiss Meditec, Inc, Dublin, California, USA) using Swedish Interactive Thresholding Algorithm standard strategy, program 24-2. A visual field was considered abnormal if it showed the following criteria: 3 nonedge contiguous points demonstrating a threshold sensitivity loss (p < 5%) with at least one of the points depressed at p < 1%, or a >10-dB difference across nasal horizontal midline at 2 or more adjacent locations. In addition, abnormal glaucoma hemifield test was required.

The severity of glaucomatous damage was graded into early, moderate, and severe according to Hodapp, Parrish, and Anderson classification.

**Imaging with OCT**

Optical coherence tomographic scanning was performed using Topcon 3D OCT-1000 mark II (Topcon, Tokyo, Japan). Data were obtained with the raster scanning technique (128 horizontal scans) centred on ONH covering a square area (6 mm × 6 mm × 1.7 mm). Total acquisition time was about 3.7 seconds.

Peripapillary NFL thickness maps and 3.4-mm-diameter circumpapillary OCT image can be generated from the 3D OCT data and can be manually or automatically repositioned to provide accurate centration around the ONH. Mean thickness of peripapillary NFL measurements outside 95% normal limits that were confirmed on 3 repeat scans were considered to be abnormal thinning.

ONH parameters included disc diameter, cup area and cup volume, cup-to-disc ratio horizontal and vertical, rim area, and rim volume.

The ganglion cell complex was measured using the scan protocol GCC. All patients had vertical cube scans of the macula (7 mm × 7 mm, 128 horizontal B-scans with 512 A-scans each) centred 1 mm temporal to the fovea. The area within 0.75 mm of the foveal centre was excluded because the GCC was too thin to be measured in that region.

Three thickness maps were generated by the machine for the macular scan:

1. Macular NFL: thickness measured from the internal limiting membrane (ILM) to posterior boundary of macular NFL.
2. Ganglion cell layer: GCC thickness measured from the posterior boundary of NFL to the posterior boundary of inner plexiform layer (IPL). This map represents both ganglion cell layer (GCL) and IPL because delineation of GCL alone is difficult.
3. GCC: thickness measured from ILM to outer IPL boundary. Therefore, this map represents macular NFL plus GCL and IPL (NFL + GCL + IPL)

Three corresponding probability (significance) maps were generated for the 3 thickness maps denoting areas of focal or generalized deviation from an age-, sex-, and race-matched database. In addition, average thickness of...
AUC of 0.5 indicates a completely worthless test.

Table 1 summarizes the baseline characteristics of the glaucoma (26). There were 76 males and 24 females.

Glaucomatous eyes were further classified into early glaucoma (46), moderate glaucoma (48), and severe glaucoma (26). There were 76 males and 24 females. Table 1 summarizes the baseline characteristics of the study population. No difference was detected between normal and glaucomatous eyes with regard to age and refractive error. A statistically significant difference of intraocular pressure and mean deviation was detected between the normal and glaucomatous eyes.

OCT measurements could discriminate all stages of glaucomatous damage. Peripapillary NFL measurement values were significantly different in all stages of glaucoma \( p < 0.001 \). Similarly, GCC values were significantly different in all stages of glaucoma \( p < 0.001 \). Regarding GCC substructures, all GCL comparisons were significantly different \( p < 0.001 \): normal versus early, early versus moderate, and moderate versus severe (Table 2). However, macular NFL values were significant when comparison was made for early versus moderate \( p < 0.004 \) and moderate versus severe only \( p < 0.001 \). Macular NFL failed to differentiate normal from early stages of glaucoma \( p = 0.29 \). In severe stages, mean peripapillary RNFL thickness was 71 \( \mu \)m and the range of thickness was 42–97 \( \mu \)m, while mean GCC thickness was 77 \( \mu \)m and the range of GCC was 58–87 \( \mu \)m.

**STATISTICAL ANALYSIS**

Analysis was done using SPSS version 16. Mean values of peripapillary RNFL, GCL, macular NFL, and GCC thickness were compared between the normal and whole glaucoma group using the \( t \) test. Mean thickness in different stages was compared using 1-way analysis of variance followed by the Sidak post hoc test for multiple comparisons. Receiver operating characteristic (ROC) curves described the ability of each parameter to differentiate between different glaucoma stages by using area under the curve (AUC). An AUC of 1 (100% sensitivity and 100% specificity) represents a perfect test, whereas an AUC of 0.5 indicates a completely worthless test.

**RESULTS**

A total of 150 eyes of 100 patients were enrolled in this study. Thirty eyes were normal; 120 were glaucomatous. Glaucomatous eyes were further classified into early glaucoma (46), moderate glaucoma (48), and severe glaucoma (26). There were 76 males and 24 females. Table 1 summarizes the baseline characteristics of the study population. No difference was detected between normal and glaucomatous eyes with regard to age and refractive error. A statistically significant difference of

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Area</th>
<th>Cutoff point</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
<th>( p )</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripapillary NFL</td>
<td>0.901</td>
<td>100.5</td>
<td>96.7</td>
<td>86.1</td>
<td>&lt;0.001</td>
<td>0.828–0.974</td>
</tr>
<tr>
<td>GCL</td>
<td>0.733</td>
<td>71.5</td>
<td>53.3</td>
<td>82.6</td>
<td>0.001</td>
<td>0.621–0.844</td>
</tr>
<tr>
<td>Macular NFL</td>
<td>0.651</td>
<td>33.5</td>
<td>100</td>
<td>37</td>
<td>0.026</td>
<td>0.529–0.774</td>
</tr>
<tr>
<td>GCC</td>
<td>0.756</td>
<td>101.5</td>
<td>93.3</td>
<td>50</td>
<td>&lt;0.001</td>
<td>0.649–0.863</td>
</tr>
<tr>
<td>Rim area</td>
<td>0.731</td>
<td>1.21</td>
<td>90</td>
<td>65.2</td>
<td>0.001</td>
<td>0.618–0.845</td>
</tr>
</tbody>
</table>

GCL, ganglion cell layer; GCC, ganglion cell complex; NFL, nerve fibre layer.

*Significant \( p < 0.05 \).
The best parameters that distinguished moderate from severe stage were GCC (AUC: 0.95), rim area (AUC: 0.95), and macular NFL (AUC: 0.91), followed by GCL (AUC: 0.89) and average peripapillary NFL (AUC: 0.88). Cutoff values of GCC, rim area, macular NFL, GCL, and average peripapillary NFL were 88.0, 0.67, 30.5, 58.5, and 0.785, respectively (Table 5, Fig. 3).

DISCUSSION

Several studies have documented that the diagnostic power of GCC is similar to peripapillary NFL thickness in glaucoma diagnosis. However, these results cannot be directly compared to the current study because of lack of glaucoma staging. The present study found that the paradoxical pattern of GCC substructure loss versus peripapillary NFL loss in different stages of glaucoma is peculiar and may indicate how glaucomatous damage develops and progresses. Peripapillary NFL showed high diagnostic power in the early stages of glaucoma. This is in agreement with many previous studies and could be explained by the fact that peripapillary scans sample all axons of NFL, whereas GCC scans sample only macular axons. The diagnostic power of peripapillary NFL decreased progressively through the moderate stage and became the lowest in severe stages. A similar finding was detected by Mwanza et al., who emphasized that AUCs of average and inferior peripapillary NFL decreased gradually from discriminating between normal and all glaucomatous eyes (discrimination 1; AUC: 0.95) to discriminating between normal from eyes with mild glaucoma (discrimination 2; AUC: 0.89–0.91) and moderate from severe glaucoma (discrimination 3; AUC: 0.66–0.78).

Ganglion cell loss is the primary event in glaucoma pathogenesis. Tan et al. investigated the diagnostic accuracy and reproducibility of GCC as measured by stratus OCT compared with the circumperipapillary NFL thickness measurements. They employed existing data from patients enrolled in the Advanced Imaging for Glaucoma Study. The AUCs for the GCC parameters were 0.80 and 0.90 for preperimetric and perimetric glaucoma, respectively. In the current study, the diagnostic accuracy of GCC complex and/or its substructure,

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<th>p</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripapillary NFL</td>
<td>0.853</td>
<td>95.5</td>
<td>60.9</td>
<td>98</td>
<td>&lt;0.001</td>
<td>0.774–0.931</td>
</tr>
<tr>
<td>GCL</td>
<td>0.817</td>
<td>65.5</td>
<td>63</td>
<td>89.6</td>
<td>&lt;0.001</td>
<td>0.730–0.904</td>
</tr>
<tr>
<td>Macular NFL</td>
<td>0.652</td>
<td>32.5</td>
<td>71.7</td>
<td>60.4</td>
<td>0.011</td>
<td>0.539–0.765</td>
</tr>
<tr>
<td>GCC</td>
<td>0.813</td>
<td>97.5</td>
<td>73.9</td>
<td>79.2</td>
<td>&lt;0.001</td>
<td>0.726–0.900</td>
</tr>
<tr>
<td>Rim area</td>
<td>0.642</td>
<td>0.92</td>
<td>80.4</td>
<td>54.2</td>
<td>0.018</td>
<td>0.530–0.735</td>
</tr>
</tbody>
</table>

GCL, ganglion cell layer; GCC, ganglion cell complex; NFL, nerve fibre layer.
*Significant p < 0.05.
including GCL and macular NFL, showed weak diagnostic power in early stages. This may be explained by the fact that quantitative parameters used for evaluation of GCC in the present study reflected global rather than focal GCC loss. Focal losses were analyzed by probability symbols that indicate degree of deviation from normal but could not be translated into meaningful statistical data. Therefore, analysis techniques may miss the focal damage that is usually prevalent in early stages.

Different studies\textsuperscript{17,18} reported that, in advanced glaucoma, the measurement of GCC thickness has diagnostic power similar to the measurement of peripapillary NFL thickness. In the current study, the diagnostic power of GCC increased progressively through moderate stage until it became maximal in the severe stage, where it supersedes peripapillary NFL. It was noticeable that AUC for severe glaucoma (where global loss is prevalent) was higher than that from other studies (0.95). Better performance of GCC in severe stages may be due to better fixation in the central macular field (away from the area of nasal field loss), easier detection of inner plexiform boundaries than posterior border of attenuated NFL, and absence of the peripapillary degeneration that affects outcome measurements at the disc region. The recordable performance of GCC in advanced glaucoma may have implications in severe stages in which GCC mapping may represent a good alternative to optic disc imaging, which usually fails because of encroachment of nasal field loss on patient fixation target.

Leung et al.\textsuperscript{19} found that measurement of macular NFL thickness offered no advantage over measurement of total macular thickness for glaucoma detection, whereas circum-papillary NFL thickness outperformed macular NFL and total macular thickness in the ability to detect glaucoma. Lack of glaucoma staging and the use of time-domain OCT might have affected their outcome results. In the present study, macular NFL attained lowest diagnostic power of the GCC substructure in early stages of glaucoma, but in severe stages it supersedes both GCL and peripapillary NFL thickness measurement.

As expected, GCC showed higher diagnostic power than either of its individual component (macular NFL and GCL). GCL had higher diagnostic power than macular NFL in early and moderate stages, whereas macular NFL supersedes GCL only in severe stages of the disease. This finding may highlight pathogenesis of early stages of glaucoma and confirms that retinal ganglion cell (GCL) loss precedes axonal loss (NFL loss).

Knight et al.\textsuperscript{20} reported that there is essentially a “floor” effect associated with advanced RNFL loss, in which the OCT can no longer differentiate thickness change from glaucoma from noise in the measurements. They expected that RNFL thins to the point that there is almost no residual GCL, where it becomes very difficult to detect significant change with OCT.\textsuperscript{20} In the present study, mean RNFL thickness in the severe stage (71 μm) was comparable to mean GCC thickness (77 μm). However, the minimum value of GCC thickness (58 μm) was much higher than that of RNFL (42 μm). Thus, GCC measurement may reflect less floor effect. This finding may be related to the degree of severity of mean deviation in our study, where the average value (−15 dB) was less than that from other studies.\textsuperscript{10,21} In addition, this may be attributed to less density of supporting glial tissue and vascular network at the macular region compared to the disc.

![ROC Curve](image)

**Fig. 3** — The receiver operating characteristic curve of the best parameters for discriminating between moderate and severe stage of glaucoma. "AVERAGE" refers to average peripapillary nerve fibre layer thickness. "AverageG3" refers to average ganglion cell complex (GCC) thickness. "AVG2" refers to average ganglion cell layer (GCL) thickness. "MNFLAVG" refers to average macular nerve fibre layer thickness.

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<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripapillary NFL</td>
<td>0.888</td>
<td>78.5</td>
<td>89.6</td>
<td>80</td>
<td>&lt;0.001</td>
<td>0.790–0.985</td>
</tr>
<tr>
<td>GCL</td>
<td>0.898</td>
<td>58.5</td>
<td>77.1</td>
<td>100</td>
<td>&lt;0.001</td>
<td>0.826–0.969</td>
</tr>
<tr>
<td>Macular NFL</td>
<td>0.911</td>
<td>30.5</td>
<td>66.7</td>
<td>100</td>
<td>&lt;0.001</td>
<td>0.847–0.976</td>
</tr>
<tr>
<td>GCC</td>
<td>0.950</td>
<td>88</td>
<td>85.4</td>
<td>100</td>
<td>&lt;0.001</td>
<td>0.900–1.000</td>
</tr>
<tr>
<td>Rim area</td>
<td>0.956</td>
<td>0.67</td>
<td>83.3</td>
<td>100</td>
<td>&lt;0.001</td>
<td>0.911–1.000</td>
</tr>
</tbody>
</table>

GCL, ganglion cell layer; GCC, ganglion cell complex; NFL, nerve fibre layer.

\*Significant \( p < 0.05 \).
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region. Further studies on a larger subset of population targeting progression of the GCC substructure over long period of time can better delineate this effect.

A limitation of this study is that its findings cannot be generalized because results address only the Topcon OCT. Other OCT systems have different technical features, databases, and segmentation algorithms.

In conclusion, peripapillary NFL and GCC thinning showed a paradoxical course. The most sensitive parameter in early glaucoma was peripapillary NFL and in severe glaucoma was GCC. Ganglion cell complex substructure showed another paradox. GCL was the most sensitive parameter in early stages, and macular NFL was the most sensitive in the severe stage. GCC mapping may provide good alternative to optic disc imaging in advanced glaucoma with poor fixation.

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Footnotes and Disclosure:

The authors have no proprietary or commercial interest in any materials discussed in this article.

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