Purpose: The purpose of this study is to explore the attenuation coefficient (AC) of the retinal nerve fiber layer (RNFL) in spectral domain optical coherence tomography (OCT) images, in healthy eyes and eyes affected by glaucoma. To assess the relation between RNFL AC, disease severity, RNFL thickness, visual field sensitivity threshold, spatial location and age.

Patients and Methods: We analyzed peripapillary circle scans of a clinical OCT device (Spectralis OCT, Heidelberg Engineering, Heidelberg, Germany) in 102 glaucoma patients and 90 healthy controls. The images were fully automatically converted into depth-resolved AC images. Next, the median AC within the RNFL was calculated based on the Spectralis segmentation. We compared the RNFL AC between healthy, mild, moderate and advanced glaucomatous eyes and assessed the correlation with patient characteristics such as age and visual field sensitivity threshold (HFA, Carl Zeiss Meditec, Dublin, CA) in a generalized estimating equations (GEE) model. Finally, we explored the ability to discriminate between glaucomatous and healthy eyes by RNFL AC.

Results: Median RNFL AC decreased with increasing disease severity up to moderate glaucoma (P < 0.001) in all 4 sectors around the optic nerve head. The largest relative decrease occurred in the nasal sector. The RNFL AC (AUC, 0.834 ± 0.028) effectively discriminated healthy from glaucomatous eyes, although RNFL thickness (AUC, 0.975 ± 0.013) performed even better (P < 0.001). Prediction of visual field sensitivity improved significantly when RNFL thickness was augmented with RNFL AC as covariates (P < 0.001).

Conclusions: This study demonstrated that RNFL AC provides complementary information on the RNFL’s health compared with RNFL thickness measurements alone.

Key Words: glaucoma, retinal nerve fiber layer, spectral domain optical coherence tomography, attenuation coefficient

Glaucoma is a common optic neuropathy that can lead to irreversible vision loss and, when left untreated, even to blindness.1 Optical coherence tomography (OCT) may be used to measure retinal nerve fiber layer (RNFL) thickness at predefined locations in the retina, thereby assessing the local and diffuse RNFL thinning that occurs in glaucoma.2,3 RNFL thinning is the result of tissue apoptosis that occurs in the weeks after the retinal ganglion cells and their axons that form the RNFL are damaged.4 OCT can be used to distinguish glaucomatous eyes from healthy eyes based on the measured RNFL thickness.5,6,7

However, valuable structural information may be obtained from more than just thickness measurements, for example from the intensity of the OCT signal. Two types of data that, among others, can be derived from the OCT signal intensity are the RNFL reflectance and the RNFL attenuation coefficient (AC). RNFL reflectance can be used to discriminate between glaucomatous and healthy eyes.8,9,10 Also, a decrease in reflectance preceded the thinning of the RNFL in experimental glaucoma11 and turned out to be correlated to the rate of functional loss in glaucoma.12 These studies showed the potential of other OCT features than thickness alone to detect glaucomatous RNFL damage. Recently, we have introduced a method to estimate the AC of the RNFL from standard OCT measurements.13,14 The AC is an intrinsic optical tissue property that is related to the intensity of the OCT signal. It describes the amount of light scatter and absorption per unit of distance as light passes through a semitransparent medium. Several studies have shown that the structural integrity of a wide range of biological tissues can be assessed by their OCT-derived AC.15-20 In this study, we explore the RNFL AC in healthy eyes and eyes affected by glaucoma and how it relates to age, spatial location with respect to the optic nerve and disease severity.

In 2 preliminary studies on relatively few subjects, we demonstrated differences in the RNFL AC between healthy and glaucomatous eyes.13,15 These studies were limited by 3 major methodological constraints, though. Firstly, all the OCT images had to be segmented manually, which is time-consuming and therefore limits the feasible number of evaluated scans and subjects. Secondly, the RPE was used as a reference layer to calculate the RNFL AC,13 limiting its applicability to conditions that do not affect the RPE. Finally, the RNFL AC was only determined in 4 small 20-pixel wide sections of the OCT images instead of across the full peripapillary scans.

As our previous work described above, we have made several methodological improvements to enable the calculation of RNFL ACs in a robust and fully automated way. This allowed us, in the present study, to validate and extend earlier work on a considerably larger scale. The data was obtained with a regular clinical OCT system that also produced the automated RNFL segmentation that we used for further processing. In order to be independent of the RPE as a reference layer, we used a method that provides depth-resolved AC estimates without the need for a reference layer.14 We compared the RNFL AC between groups of
healthy and mild, moderate and advanced glaucomatous eyes. Furthermore, we assessed the correlation between various patient characteristics such as age and RNFL AC. We also wanted to explore the capability of RNFL AC to discriminate between glaucomatous and healthy eyes.

MATERIALS AND METHODS

Subjects and Data Selection

All subjects were selected from the Rotterdam Glaucoma Imaging Study. This is a longitudinal prospective study to explore the performance of both commercially available and newly developed, experimental imaging technologies for detecting and monitoring glaucomatous damage. The study population consists of approximately 120 glaucoma patients with mild to advanced glaucoma, that visit the Rotterdam Eye Hospital, Rotterdam, The Netherlands, every 6 or 12 months for glaucoma care and additional study related measurements. A group of 90 healthy control subjects with yearly scheduled visits were also included.

The healthy subjects were examined before the first study visit and were not included in the study if their intraocular pressure (IOP) was over 22 mm Hg or if their visual field showed glaucomatous abnormalities, including; a Glaucoma Hemifield Test Outside Normal Limits, a Mean Deviation and Pattern Standard Deviation below the fifth percentile and decreased sensitivity scores with <1% probability at 1 test location or clusters of decreased sensitivity scores with <5% probability at 2 test locations on the total deviation plot. All glaucoma patients received standard glaucoma care, including medications and glaucoma (implant) surgery if required. In addition, healthy subjects and glaucoma patients were excluded from participation if there was evidence for any concomitant systemic or ocular disease that could potentially lead to visual field defects, for example diabetes. Previous ocular surgery also led to exclusion, except for uncomplicated cataract surgery. To ensure complete recovery after cataract surgery, a 1-year study intermission was mandatory after surgery. The study protocol was approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam, The Netherlands. All participants provided written informed consent and the study adhered to the Declaration of Helsinki and GCP guidelines.

For each participant, the most recent data set in which all relevant measurements were successfully performed was selected for the current analysis. All eyes eligible for analysis were included. This means that we selected both eyes in patients that were bilaterally affected by glaucoma and both eyes in the control subjects. The data used in our analyses included age, sex, IOP, the OCT-system’s proprietary overall scan quality score, Standard Automated Perimetry (SAP) Mean Deviation and the SAP sensitivity thresholds.

Standard Automated Perimetry

SAP was performed with a Humphrey Field Analyzer (Carl Zeiss Meditec, Dublin, CA) and the 24-2 SITA Standard test program. Visual field measurements were excluded if they did not meet the standard HFA quality indices: fixation losses, false positives or false negatives over 20%. Each glaucomatous eye was assigned to one of 3 groups, based on the mean deviation (MD) of the SAP measurement: mild glaucoma (MD better than −6 dB), moderate glaucoma (MD between −6 and −12 dB) and advanced glaucoma (MD worse than −12 dB). Because we included age as a covariate in our analyses on spatial correlation with OCT data, we used the sensitivity threshold values at the individual locations of the HFA 24-2 grid rather than the age-adjusted total deviation values.

Optical Coherence Tomography

We used a commercially available SD-OCT system (Spectralis OCT, Heidelberg Engineering, Heidelberg, Germany) to image the peripapillary retina. Peripapillary circle scans were acquired by centering a circular B-scan (with a diameter of 3.4 mm) on the optic nerve head (ONH). The B-scans consisted of 768 A-scans and were based on 16 averaged scans. Any B-scan that did not meet the 20 dB quality cut-off was excluded. Conditions that cause low scan quality include media opacities such as floaters and cataract.

Data Processing

The raw SD-OCT signal contains information on the scattering and absorption but also depends on factors such as the strength of the incident light beam, media opacities, and the tissue anterior to a certain pixel. The latter effect is most clearly observed below blood vessels, which cast a shadow on deeper tissues. All SD-OCT scans and segmentations as produced by the built-in software (version 1.9.10.0) were exported as raw measurement data from the OCT instrument. The images were then automatically converted into RNFL AC images based on an earlier described method. In brief, the raw SD-OCT data was first compensated for the sensitivity decay of the OCT instrument. An earlier presented model of the sensitivity decay was fitted to sensitivity measurements, resulting in an estimate of the model parameter \( \omega \), denoting the ratio of the spectral resolution to the sampling interval, of 1.9. The corrected data was then used to estimate the attenuation coefficient \( \mu(i) \) for each pixel \( i \) in an OCT A-line:

\[
\mu(i) = \frac{I(i)}{2\Delta \sum_{j=1}^{N} I(j)},
\]

where \( N \) is the number of pixels in each A-line, \( \Delta \) is the axial sampling density and \( I(i) \) is the OCT signal after correcting for the sensitivity decay.

Some example SD-OCT images and the corresponding AC images have been presented in Figures 1A–D. The segmentation of the device was used to determine the RNFL thickness and the RNFL AC for the entire B-scan. These values were also calculated for 4 sectors: temporal (330 to 30 degrees), superior (30 to 120 degrees), nasal (120 to 240 degrees) and inferior (240 to 330 degrees). These 4 sectors were derived from the Garway-Heath mapping system with the superior 2 sectors (temporal superior and nasal superior) and the inferior 2 sectors (temporal inferior and nasal inferior) combined (see Figs. 1E–F). We constructed fitted curves by using loess smoothing (sampling proportion 0.1, first degree polynomial) to improve the comprehensibility of Figure 3.

Statistics

Any differences between the study groups were evaluated by Kruskal–Wallis tests which is a nonparametric equivalent of 1-way ANOVA. The RNFL thickness and
RNFL attenuation data was adjusted to reference levels of sex (male), age (60 y), and OCT scan quality (20 dB) to eliminate the between group differences. To evaluate the pairwise group differences and to study any trends in RNFL thickness and RNFL AC with disease severity we used 2 nonparametric tests; the Mann-Whitney U test and the Jonckheere-Terpstra test, respectively. The diagnostic accuracy of the RNFL AC and the RNFL thickness was evaluated by receiver operating characteristic (ROC) curves and the area under the curve (ROC AUC).

Generalized estimating equation (GEE) models were applied to estimate the RNFL AC, RNFL thickness and the SAP sensitivity thresholds while taking other covariates into account. This resulted in 3 different models with RNFL AC, RNFL thickness or SAP sensitivity as the dependent variables. Age, sex, OCT scan quality, and IOP were included as covariates. In contrast to conventional ANOVA models, GEE models do not assume that measurements are independent, which may not be the case when analyzing both eyes from participants. We considered P-values below 5% as statistically significant.

RESULTS

Subject Characteristics

A total of 185 eyes from 102 glaucoma subjects and 179 eyes from 90 healthy subjects were included. For the descriptive statistics and the trend and between group statistical tests we selected the right eye from each subject. If only the left eye of a subject was available, that eye was used. In the GEE analyses, both eyes were included. The population demographics and the group comparisons for all 4 groups have been presented in Table 1. SAP mean deviation and sensitivity thresholds were statistically significantly different between groups (P<0.001), reflecting the increasing glaucomatous damage in the groups. The subjects in the healthy group were approximately 10 years younger (P<0.001) and had a 0.7 to 1.8 dB higher OCT scan quality (P<0.018) compared with the glaucoma subjects. In the healthy group, approximately 60% of the subjects were women; compared with the glaucoma groups, where women represented, on average, 44% of the subjects (P<0.05). The IOP was not statistically significantly different between the groups. The RNFL thickness and RNFL AC data were corrected for differences in sex, age, and scan quality between the groups by adjusting the data to the reference of a healthy 65-year-old woman with an OCT scan quality of 28 dB.

Lower RNFL AC and RNFL Thickness Corresponds to Higher Glaucoma Severity

A statistically significantly lower RNFL AC and thinner RNFL was found for glaucomatous eyes compared with healthy eyes (P<0.001). The second column of Table 2 (“All locations”) lists the medians of the RNFL AC, the RNFL thickness, the SAP sensitivity threshold values and their 95% confidence intervals. Each of the 3 parameters decreased with increasing glaucoma severity (Jonckheere-Terpstra test; P<0.001). A graphical representation of the RNFL AC and RNFL thickness is given in Figure 2, which suggests a strong decrease of the RNFL AC up to moderate glaucoma, but not beyond that stage. The Mann-Whitney U test confirmed the statistically significant difference (P<0.05) between all disease groups for RNFL AC, except between moderate and advanced glaucoma (P=0.99). For RNFL thickness, we found a similar trend, with a statistically highly significant change up to moderate glaucoma and no change between moderate and advanced glaucoma (P=0.32). The notably larger confidence intervals for moderate glaucoma were because of the relatively small number of eyes included in the moderate glaucoma group.

To evaluate how the RNFL AC differs between the 4 sectors, each sector has been plotted in Figure 2A. We found that the RNFL AC in the nasal sector was low compared with the other 3 sectors, which all showed more similar values. All sectors showed a decrease up to moderate glaucoma. In contrast, the RNFL was relatively thin in both the nasal and the temporal sector (see Fig. 2B) and showed a large relative decrease in the superior and inferior sectors compared with the nasal and temporal sectors. Appendix 1
lists the statistical significance of the between group differences for each of the sectors.

Glucomatous RNFL Changes Visualized With TSNIT Plots

We used TSNIT plots to visualize the local RNFL AC and RNFL thickness differences between control eyes and the 3 glaucoma severity groups. Figure 3A shows the smoothed median RNFL AC values for each individual A-line, stratified by glaucoma severity. The RNFL AC curve for healthy eyes showed a double hump shape with the highest values in the superior and inferior regions. Figure 3A shows that the RNFL AC decreased with increasing glaucoma severity in all regions. This is in agreement with the trend of a decreasing RNFL AC and RNFL thickness with increasing glaucoma severity shown in Figure 2. An RNFL AC floor level of around 1.5 mm$^{-1}$ remained in all regions except for the nasal region where a further reduction to approximately 1.0 mm$^{-1}$ was found in moderate and advanced glaucoma. Nasally, the relative loss of RNFL AC was the largest for mild glaucoma compared with healthy eyes; in all other regions, the RNFL AC continued to decrease in the mild and moderate stages. Compared with RNFL thickness in Figure 3B we found a higher variability in RNFL AC. Noteworthy differences were also found in the temporal and nasal regions upon visual comparison. Although the temporal RNFL thickness decreased only very little with disease severity, this figure suggests that the RNFL AC in that sector decreased markedly with increasing glaucoma severity up to moderate glaucoma. In the nasal region, the depression that was observed in RNFL thickness was not as evident as in the nasal region of the RNFL AC plots. The statistical analysis of the correlation between RNFL AC and RNFL thickness with visual field data will be presented below.

FIGURE 2. A, The median RNFL ACs and their 95% confidence intervals for each of the 4 groups, stratified by sector and all sectors combined. The connecting dotted lines visualize the statistically significant trend per sector and all sectors combined (Jonckheere-Terpstra, $P < 0.001$). Between groups comparison showed statistically significant differences between all groups ($P < 0.05$) except between healthy and mild glaucoma in the temporal sector, between mild and moderate glaucoma in the nasal sector and between moderate and advanced glaucoma in both the nasal sector and the average of all sectors. B, The median RNFL thicknesses and their 95% confidence intervals for each of the 4 groups, stratified by sector and all sectors combined. The connecting dotted lines visualize the statistically significant trend per sector and all sectors combined (Jonckheere-Terpstra, $P < 0.001$). Between groups comparison showed statistically significant differences between all groups ($P < 0.05$), except between mild and moderate glaucoma in the nasal sector and between moderate and advanced glaucoma. Figure 2 can be viewed in color online at www.glaucomajournal.com.

FIGURE 3. A, The median RNFL AC (thin gray lines) for the healthy subjects and the mild, moderate and, advanced glaucoma patients plotted for each of the 768 A-scans of the peripapillary B-scan. Please note the clear distinction between healthy subjects, mild glaucoma and moderate glaucoma in all regions. The nasal RNFL AC shows a relatively strong decrease up to mild glaucoma whereas in the other regions this decrease continues up to moderate glaucoma. B, The characteristic double hump shape in RNFL thickness data (thin gray lines) with low variability compared with RNFL AC. The bold lines represent fitted curves based on loess smoothing (sampling proportion 0.3, first degree polynomial). Figure 3 can be viewed in color online at www.glaucomajournal.com.
Diagnostic Power of RNFL AC and RNFL Thickness

To evaluate the diagnostic power of both RNFL AC and RNFL thickness, we used a GEE model. In our model, RNFL AC showed significant predictive ability to discriminate healthy from glaucomatous eyes (AUC = 0.834 ± 0.028), although RNFL thickness (AUC, 0.975 ± 0.013) performed significantly better ($P < 0.0001$).

Table 2 shows the performance of the individual sectors in discriminating healthy from glaucomatous eyes. It demonstrates that the RNFL AC diagnostic performance was similar for all 4 quadrants (AUC, 0.76 to 0.82), whereas the RNFL thickness scored best in the superior and inferior sectors (AUC, 0.96). For an extensive list of all AUCs and their sensitivities at 90% and 95% specificity please refer to Appendix 1.

Correlations Between RNFL AC and RNFL Thickness and Visual Field Data

Both eyes of subjects were included in a GEE model, as GEE models still produce valid parameter estimates when the data of both eyes is correlated. We assessed the contribution of RNFL AC to the prediction of the SAP sensitivity threshold after correcting for several factors, including RNFL thickness. This data has been presented in Table 3. In our model, the SAP sensitivity threshold increased by 1.15 dB ($P < 0.001$) with every 1 mm$^{-1}$ higher RNFL AC. This means that after we corrected for the RNFL thickness in the sensitivity model, the RNFL AC resulted in a highly statistically significant improvement of the SAP sensitivity threshold prediction. In other words, both the RNFL AC and RNFL thickness instead of only RNFL thickness significantly improved the structure and function correlation in our data. A 1 mm thicker RNFL corresponded to a mean increase of 0.23 dB ($P < 0.001$) in SAP sensitivity threshold. Furthermore, a 1 mm Hg increase of IOP corresponded to a mean increase of 0.16 dB ($P < 0.05$) in SAP sensitivity threshold. The remaining factors that we explored (sex, age, and OCT scan quality) showed no statistically significantly association with SAP sensitivity thresholds.

To further explore the differences between factors that contributed to the RNFL AC and the RNFL thickness, we evaluated 2 additional GEE models: one that modeled the RNFL AC and one with the RNFL thickness as the response. All factors and model estimates have been listed in Table 3. Both RNFL AC and thickness were significant factors for explaining the other variable. SAP sensitivity threshold was the only other covariate that was significant for both models, indicating that sensitivity threshold was positively correlated with both RNFL AC and thickness. Age, IOP, and OCT scan quality affected only the RNFL AC, whereas sex was only statistically significantly correlated with RNFL thickness.

**DISCUSSION**

This study showed that both RNFL AC and RNFL thickness were statistically significantly lower for glaucomatous eyes compared with healthy eyes. We also found a
decrease of RNFL AC and RNFL thickness with increasing disease severity. This applied to all sectors around the ONH, which confirmed earlier, preliminary findings in a considerably smaller sample size. Inclusion of a significantly larger sample was possible because of the improved automated RNFL AC quantification methods that were used in the present study.

Pairwise comparisons between the subject groups showed that the RNFL AC decreased with increasing glaucoma severity up to moderate glaucoma. Despite the RNFL AC and RNFL thickness measuring fundamentally different properties of the RNFL, they behave similarly in glaucoma progression. However, several important differences were found as well. The RNFL AC decrease occurred in all 4 sectors around the ONH but most prominently in the nasal sector. Interestingly, in mild glaucoma the nasal RNFL AC was affected more than in the other stages of the disease. This suggests that the RNFL AC is affected nasally at an earlier stage of the disease than in the other sectors. Such a phenomenon was not observed for RNFL thickness, where little thinning between the disease stages was found in the nasal and temporal sectors. Taken together, our results showed that changes in RNFL AC and RNFL thickness behave differently in glaucomatous progression and that they may complement each other in our understanding of the disease.

We found that the AUC of the RNFL AC was similar across all sectors. By contrast, the RNFL thickness performed best in the superior and inferior sectors, which is in agreement with earlier reports. The sectorial differences in diagnostic power again indicate that the RNFL thickness and RNFL AC may behave differently throughout the 4 sectors. For simple discrimination between healthy and glaucomatous eyes, RNFL thickness turned out to be a stronger parameter than RNFL AC, although the AUC for RNFL thickness that we found was at the high end of what has been reported in the literature.

**RNFL AC Data is Complementary to RNFL Thickness Data**

Because both RNFL AC and thickness decreased with an increase in glaucoma severity, the RNFL AC was shown to be a significant factor in explaining RNFL thickness and

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**TABLE 3.** The Mean Increase and 95% Confidence Interval for SAP Sensitivity Threshold, RNFL AC and RNFL Thickness for Each Unit Increase of the Factors

<table>
<thead>
<tr>
<th>Factors</th>
<th>Unit</th>
<th>Mean SAP Sensitivity Threshold (dB) Increase Per Unit</th>
<th>Mean AC (mm⁻¹) Increase Per Unit</th>
<th>Mean Thickness (μm) Increase Per Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNFL AC</td>
<td>mm⁻¹</td>
<td>1.15 (0.71-2.33)</td>
<td>n/a</td>
<td>3.09 (1.01-5.07)</td>
</tr>
<tr>
<td>RNFL thickness</td>
<td>μm</td>
<td>0.23 (0.20-0.26)</td>
<td>0.011 (0.006-0.016)</td>
<td>NA</td>
</tr>
<tr>
<td>SAP sensitivity threshold</td>
<td>dB</td>
<td>0.20 (0.08-0.33)</td>
<td>0.17 (1.43-1.97)</td>
<td>7.65 (3.66-11.64)</td>
</tr>
<tr>
<td>Age</td>
<td>y</td>
<td>NS</td>
<td>-0.011 (-0.017 to -0.006)</td>
<td>NS</td>
</tr>
<tr>
<td>Sex</td>
<td>M/F</td>
<td>NS</td>
<td>NA</td>
<td>7.65 (3.66-11.64)</td>
</tr>
<tr>
<td>IOP</td>
<td>mm Hg</td>
<td>0.16 (0.04-0.28)</td>
<td>-0.015 (-0.027 to -0.004)</td>
<td>NS</td>
</tr>
<tr>
<td>OCT scan quality</td>
<td>dB</td>
<td>0.043 (0.024-0.062)</td>
<td>0.043 (0.024-0.062)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data derived from Generalized Estimation Equations model.
NA indicates not applicable; NS, not statistically significant.
vice versa. The RNFL AC was also a highly significant predictor of visual field sensitivity in a GEE model after correcting for all covariates, including RNFL thickness. This showed that RNFL AC and RNFL thickness provided complementary information for predicting the visual field based on the RNFL AC and thickness. This is in line with results presented by Gardiner et al who evaluated if changes in RNFL thickness and RNFL reflectivity information predict the rate of visual field loss. They concluded that the combination of these 2 parameters may improve the structure-function correlation.12

Both studies showed that the structure-function correlation improved by taking both RNFL thickness information and additional OCT signal intensity information into account. In glaucoma, the thickness of the existing RNFL may be seen as an indirect measure of the tissue that no longer exists; a correlation between RNFL thickness and function is therefore intuitive. A fundamental question is whether the remaining RNFL is normal or affected. As the RNFL AC provides information about the optical properties of the tissue that composes the RNFL, our data suggest that the remaining RNFL is already affected by the disease processes.

We found that the RNFL AC went down with age, which has not been described in the literature before. Adjusting RNFL AC for age would be of particular importance when comparing ACs between groups with different age profiles or in case the RNFL AC will be used for longitudinal assessment of glaucoma patients. Possible explanations for the RNFL AC decrease with increasing age could be the loss of nerve fibers or other age related changes within the RNFL. In addition, a lower signal to noise ratio caused by media opacities could result in a small bias in the estimation of the RNFL AC. Our data showed that the thickness of the RNFL did not significantly decrease with age, whereas other investigators showed that the RNFL thickness decreases with an increase in age.27–29 and that media opacities may lead to an underestimation of the RNFL thickness.30

OCT scan quality was only correlated to RNFL AC values. We would expect an intensity based feature such as the RNFL AC to be more sensitive to noise than a morphologic feature such as thickness. Further improvements to the AC estimation algorithms would be needed to reduce this effect of noise on the estimated AC. The negative association between IOP and RNFL AC that we found indicates that a lower RNFL AC is observed for a higher IOP. Because a higher IOP is related to more severe glaucoma,2 this association shows that RNFL AC could be a measure of the structural integrity of the RNFL. Sex only contributed to the RNFL thickness model, where we measured a thicker RNFL in women. The thickness difference between sexes that we found is in contrast to the literature where no statistically significant RNFL thickness differences have been reported between women and men.31–33

Structural RNFL Properties and Anatomic Differences Between ONH Sectors May Explain the Observed Sectoral RNFL AC Differences

RNFL bundle size, path and distribution vary between the sectors around the ONH.3,4,34 These anatomic differences may explain the temporal-nasal RNFL AC difference that we observed. The RNFL is the thickest at the superior and inferior bundles and the typical double hump shape in RNFL thickness plots is correlated with the trajectory of the main retinal blood vessels and the relatively high concentration of nerve fiber bundles around these vessels.35–38 The local concentration of RNFL tissue in the superior and inferior bundles may also translate into a higher volumetric concentration of bundles.39 This would explain the higher AC values in these sectors.

The RNFL AC is a tissue property and is not expected to be affected directly by loss of tissue per se, but rather by structural changes within the tissue. The RNFL AC reflected these tissue changes in all 4 sectors around the ONH and most prominently in the nasal and temporal sectors. In near infrared imaging, such as spectral domain OCT, most of the light scattering in the RNFL is thought to be because of the larger cylindrical structures such as axon bundles, whereas the contribution of other cylindrical structures such as microtubules is considered to be relatively small.40,41 A difference in the density of axon bundles, as a result of glaucomatous damage or physiological anatomic differences, could explain the difference in RNFL AC values between sectors. Studies that compare RNFL axonal counts in sectors surrounding the ONH have so far not been performed in humans.42

Because the RNFL AC is related to an intrinsic tissue property, it may be sensitive to the first changes of the cellular structure of the fiber bundles instead of the following onset of the decreasing RNFL thickness. The literature shows that the AC of in vitro cultured fibroblasts can be used to differentiate between viable, apoptotic and necrotic cells based on their AC in OCT data, showing a normal, increase and decrease of the AC, respectively.20

In 2 more recent studies, Fortune et al43,44 found that the RNFL retardation in scanning laser polarimetry (SLP) images could detect changes in the optical properties of the RNFL before the onset of RNFL thinning in OCT images. Local RNFL retardation can also be quantified with polarization-sensitive optical coherence tomography (PS-OCT) that measures the polarization state differences of the target tissue. RNFL retardation levels measured with PS-OCT have been shown to correlate with the integrity and orientation of the axonal substructure of the RNFL,39,45,46 and have been shown to decrease in glaucomatous eyes.10,39 The RNFL retardation decrease in glaucomatous eyes shows similarities with the RNFL AC decrease that we found in this study. Both RNFL AC and RNFL retardation could prove to be complementary to the standard thickness OCT images and they support each other as promising new methods for the detection of changes in the RNFL structure. A comparison between PS-OCT and RNFL AC in the same subjects would be interesting as it may help understand what these structural changes encompass. Our current study was cross-sectional in nature. It would be of interest to assess RNFL AC over time in individual eyes and to explore how each of them corresponds to glaucoma progression.

CONCLUSIONS

This study demonstrated that RNFL AC provides complementary information on the RNFL’s health compared with RNFL thickness measurements alone. Our results suggest that the RNFL that remains after glaucomatous thinning is also affected by glaucoma. The RNFL AC provides fundamentally different information than RNFL thickness and it should not be considered as a substitute parameter. Instead, thickness and AC may be jointly assessed for a more comprehensive evaluation of glaucoma.
### APPENDIX

#### APPENDIX 1

|          | Healthy vs. Mild | Mild vs. Moderate | Moderate vs. Advanced | Healthy vs. Moderate | Healthy vs. Advanced | Mild vs. Advanced | AUC (SE) | Sensitivity at 95% | Specificity (%) | Sensitivity at 90% | Specificity (%) |
|----------|------------------|-------------------|----------------------|---------------------|---------------------|-------------------|---------|-------------------|----------------|------------------|----------------|}
| Thickness B-scan | <0.001 0.001 0.316 | <0.001 0.001 | <0.001 | <0.001 0.001 0.97 (0.01) | 93 | 97 |
| Temporal | 0.001 0.040 0.914 | <0.001 0.001 | 0.002 0.83 (0.03) | 59 | 67 |
| Superior | <0.001 0.005 0.358 | <0.001 0.001 | 0.001 0.96 (0.01) | 85 | 93 |
| Nasal | <0.001 0.008 0.143 | <0.001 0.001 | 0.002 0.90 (0.02) | 51 | 65 |
| Inferior | <0.001 <0.001 0.394 | <0.001 <0.001 0.96 (0.01) | 84 | 92 |
| RNFL AC B-scan | 0.010 0.004 0.993 | <0.001 | <0.001 0.83 (0.03) | 48 | 60 |
| Temporal | 0.201 0.003 0.461 | <0.001 | <0.001 0.76 (0.03) | 34 | 46 |
| Superior | 0.047 0.001 0.223 | <0.001 | <0.001 0.82 (0.03) | 47 | 55 |
| Nasal | 0.009 0.482 0.403 | 0.007 | <0.001 0.063 0.78 (0.03) | 29 | 44 |
| Inferior | 0.044 0.016 0.349 | <0.001 | <0.001 0.80 (0.03) | 33 | 47 |

Right panel: area under the receiver operating characteristics with sensitivity at 95% and 90% specificity.

### REFERENCES


