Birefringent Properties of the Peripapillary Retinal Nerve Fiber Layer in Healthy and Glaucoma Subjects Analyzed by Polarization-Sensitive OCT

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PURPOSE. To study the circumpapillary retinal nerve fiber layer (RNFL) birefringence (BIR) of early glaucoma and age-matched healthy eyes using polarization-sensitive optical coherence tomography (PS-OCT).

METHODS. In this prospective cross-sectional study, we compared virtual circular PS-OCT B-scans with a diameter of 3.5 mm centered on the optic disc (OD) acquired with a PS-OCT prototype (860 nm center wavelength). Early glaucoma was defined by the glaucomatous appearance of the OD and a pathologic visual field test with a mean deviation (MD) better than −6 dB. The main outcome parameters were BIR, RNFL-thickness (RNFL-T), and phase retardation (RET). The BIR value at each virtual A-scan position was the quotient of the RET measured at the inner segment/outer segment junction divided by the RNFL-T.

RESULTS. The dataset comprised 49 early glaucoma patients (mean ± standard deviation [SD]: 64 ± 10 years) and 49 healthy control subjects (61 ± 9 years). Glaucomatous eyes showed a statistically significant lower BIR globally (mean ± SD: 0.108 ± 0.008°/μm vs. 0.112 ± 0.009°/μm, P = 0.033), superiorly (0.116 ± 0.017°/μm vs. 0.126 ± 0.013°/μm, P = 0.0001), and inferiorly (0.112 ± 0.011°/μm vs. 0.121 ± 0.011°/μm, P < 0.0001), and increased BIR in the temporal quadrant (0.088 ± 0.015°/μm vs. 0.078 ± 0.014°/μm, P = 0.0001) compared to healthy eyes.

CONCLUSIONS. We report a reduced BIR of the RNFL in early perimetric glaucoma, which can be interpreted as a sign of loss or change of intracellular microtubules and may contribute to a better understanding of early disease development. Prospective longitudinal studies are needed to determine whether BIR is altered in pre-perimetric human glaucoma before RNFL-T decline.

Keywords: birefringence, PS-OCT, Glaucoma, polarization-sensitive OCT

G laucoma is one of the leading causes of irreversible blindness worldwide.1 The disease progressively damages the optic nerve head (ONH) and causes a loss of retinal nerve fibers (RNFs). Patients themselves often recognize glaucoma only in advanced stages, because visual degradation is gradual and usually begins in the periphery of the visual field (VF). Therefore early glaucoma diagnosis is an important goal to provide early treatment and prevent progression to symptomatic stages. Nowadays, standard examinations for glaucoma assessment, apart from clinical examination, comprise optical coherence tomography (OCT) and VF examination. However, reproducibility of the subjective VF examinations is poor, and the peripapillary RNF layer thickness (RNFL-T) as measured by OCT is subject to great interindividual variability. As a result, early glaucoma diagnosis using these parameters is still challenging.2–10

The RNFL has birefringent properties that arise from parallel organized, intracellular microtubules within the RNFs.11–15 It is assumed that a loss or change of these microtubules occurs even before a loss of RNFL-T as an early sign of glaucoma development, as demonstrated in experimental glaucoma in nonhuman primates.16,17 In addition, according to Huang et al.,18 birefringence (BIR) alterations may first occur near the optic disc. Therefore circumpapillary BIR is an interesting biomarker that provides additional information about microstructural changes in the nerve fiber layer and could serve as a very early marker for glaucoma damage.

Because polarization-sensitive OCT (PS-OCT) can measure BIR in the human eye,13,15,19–22 this technique could contribute to the early diagnosis of glaucoma by detecting microstructural alterations within the RNFL. However, there are only limited published PS-OCT data...
from glaucoma patients demonstrating reduced RNFL BIR in human glaucoma.22–24

Furthermore, in previous studies23,25 A-scans with RNFL-T thinner than 100 μm were excluded from quantitative analyses, which obviates analyses of some healthy locations and even more importantly of locations with pathologically reduced RNFL-T in glaucomatous eyes.

The main objective of this study was to find evidence of a reduction of BIR in a larger cohort of early glaucoma compared to healthy eyes and to extend previous work studying BIR, RET, and RNFL-T using PS-OCT.

**MATERIALS AND METHODS**

Participants of the current study were part of the prospective cross-sectional “PS-OCT Fiber Tracing Study.” Subjects were recruited from October 8, 2018, to March 6, 2020, at the Center of Medical Physics and Biomedical Engineering and the Department of Ophthalmology and Optometry of the Medical University of Vienna. The study was approved by the local ethics committee and followed the tenets of the Declaration of Helsinki. Written informed consent was obtained from all subjects before participation.

Inclusion criteria for the healthy subjects were (1) men and women aged between 18 and 79 years; (2) refractive error (spherical equivalent) of −5.0 diopters (D) to 5.0 D; (3) normal findings in medical history unless the investigator considered an abnormality to be clinically irrelevant; (4) normal ophthalmic findings, especially normal appearance of the ONH and normal VF. Inclusion criteria and definition of early glaucoma were (1) same as above, but with the glaucomatous appearance of the ONH and repeatedly abnormal 24-2 VF; (2) mean deviation (MD) of VF better than −6.0 dB.

In both groups, the following exclusion criteria were applied: (1) history of ocular trauma; (2) ocular surgery within the last three months before study inclusion; (3) evidence of any eye disease except refractive error unless the investigator considered an abnormality to be clinically irrelevant; (4) astigmatism of 2.0 D or worse; (5) history of hyperdispersion glaucoma. Baseline characteristics are shown in Table 1. Peripapillary RNFL-T, RET, and BIR were studied in 49 healthy and 49 eyes with early glaucoma, of which 38 had primary open-angle, three pseudoexfoliative, three juvenile glaucoma, three chronic angle closure, and two pigment dispersion glaucoma. Baseline characteristics are shown in Table 1.

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In the present study, we collected data from 179 normal and 66 early glaucoma participants (one eye each) out of which we had to exclude 69 eyes for quality issues (image quality, corneal compensation, stitching), leaving datasets of 51 glaucoma patients and 125 healthy subjects. Because patients with diabetes mellitus showed alterations in RET of 51 glaucoma patients and 125 healthy subjects. Because our prototype PS-OCT machine had no standardized quality measure, all recorded images were checked for the correctness of corneal BIR compensation.28 Images where the stitching algorithm failed or that showed poor-quality corneal compensation were excluded from the analysis. The segmentation of the virtual circular scans was checked for correctness and manually corrected if necessary.

The Humphrey Field Analyzer II with the STA-Standard algorithm and stimulus size III was used for the 24-2 VF tests. Outcome parameters were VF MD and pattern standard deviation (PSD). Similar to what was described before, we determined the affected VF hemispheres.29 The following criteria had to be met in the 24-2 VF tests to determine whether a VF hemisphere was considered affected: at least three contiguous points on the pattern deviation probability plot with sensitivity reduced to $P < 0.05$, including at least one point at $P < 0.01$ on two consecutive VF tests no more than two weeks apart.

Statistical analyses were performed using SPSS Version 21. Global and sector-wise means including 95% confidence intervals (95% CI) of the virtual circular B-scan parameters, as well as age, eye length, intraocular pressure, cup-to-disc ratio, VF 24-2 MD, and VF 24-2 PSD were compared between healthy and glaucoma subjects using the t-test for independent samples. A χ² test was used to compare frequencies of sex between healthy and glaucoma. Receiver operating characteristics were calculated to compare the diagnostic accuracy of our main outcome variables. Repeatability was tested by calculating intraclass correlation coefficients and pooled standard deviation of RNFL-T, RET, and BIR. $P$ values $< 0.05$ were considered statistically significant.

**RESULTS**

Peripapillary RNFL-T, RET, and BIR were studied in 49 healthy and 49 eyes with early glaucoma, of which 38 had primary open-angle, three pseudoexfoliative, three juvenile glaucoma, three chronic angle closure, and two pigment dispersion glaucoma. Baseline characteristics are shown in Table 1.
in Table 1. No statistically significant difference between normal and glaucoma eyes was observed for gender, age, intraocular pressure, and eye length. As anticipated, cup-to-disc ratio, VF MD, and PSD were significantly different in the glaucoma group.

A comparison of averaged RNFL-T, RET, and BIR globally over the circular B-scan and in the temporal, superior, nasal, and inferior sectors of both study groups is shown in Table 2. Boxplots for raw data visualization are shown in Figure 1. RNFL-T and RET were statistically significantly lower in glaucomatous eyes compared to healthy eyes globally and in all quadrants.

Compared to the healthy controls, glaucomatous eyes showed a statistically significant lower BIR globally (0.108° ± 0.008°/μm vs. 0.112° ± 0.009°/μm, \( P = 0.033 \)), as well as in the superior (0.116° ± 0.017°/μm vs. 0.126° ± 0.013°/μm, \( P = 0.0001 \)) and inferior quadrant (0.112° ± 0.011°/μm vs. 0.121° ± 0.011°/μm, \( P < 0.0001 \)). In the temporal quadrant, we observed a larger BIR in glaucoma (0.088° ± 0.015°/μm vs. 0.078° ± 0.014°/μm, \( P = 0.0001 \)) compared to healthy eyes.

Figure 2 shows wide-field en-face PS-OCT maps of a healthy subject (left column: A, C, E) and a glaucoma patient (right column: B, D, F) for RNFL-T (A, B), RET (C, D), and BIR (E, F). As can be seen in the healthy eye, the highest values of RNFL-T, RET, and BIR were observed near the large vessels on the temporal superior and inferior regions of the circular B-scan. Contrary to that, the glaucomatous eye (B, D, F) shows reduced RNFL-T, RET, and BIR, particularly in the inferior hemisphere that exhibits glaucomatous damage. It is noticeable that the BIR (E, F) appears to change only slightly along the nerve fiber bundles, whereas RNFL-T and RET drop rapidly along this path. The BIR map of the healthy eye (C) yields two more-or-less uniform areas, where bundles that serve the macula have low BIR and bundles that go elsewhere have much higher BIR. In the circular B-scans (G, H), we also recognized a clear RNFL defect in the temporal-inferior region in the glaucomatous eye (H), in which the RNFL-T and the RET are reduced. The circular scan of the healthy eye (G) shows the typical double-hump pattern with RNFL-T and RET peaks at the temporal superior and inferior positions.

Figure 3 shows the mean and standard deviation of the RNFL-T (A), RET (B), and BIR (C), of the circular B-scans comparing healthy versus glaucoma eyes. The mean RNFL-T and RET showed the typical double-hump pattern in both healthy and glaucoma eyes, whereas the BIR appeared to show a single depression in the temporal region and remained fairly constant in the remaining temporal-superior, nasal, and temporal-inferior regions. Comparing the temporal

### Table 1. Subjects’ Characteristics

|                | Healthy \( n = 49 \) | Glaucoma \( n = 49 \) | \( P \) Value |
|----------------|---------------------|---------------------|--------------|
| Gender         |                     |                     | 0.681        |
| Female         | 28                  | 30                  |              |
| Male           | 21                  | 19                  |              |
| Age (y)        | 61 ± 0              | 64 ± 10             | 0.227        |
| Eye length (mm)| 23.8 ± 1.0          | 24.1 ± 1.0          | 0.128        |
| Intraocular Pressure (mm Hg)| 14.8 ± 2.5 | 15.8 ± 4.0 | 0.128 |
| C/D Ratio      | 0.34 ± 0.14         | 0.74 ± 0.14         | <0.001       |
| VF Mean deviation (dB) | −0.39 ± 1.09 | −3.03 ± 1.33 | <0.001 |
| VF Pattern standard deviation (dB) | 1.69 ± 0.39 | 4.63 ± 2.28 | <0.001 |

\( C/D \) Ratio, cup-to-disc ratio.

### Table 2. Comparison of RNFL-Thickness, Retardation, and Birefringence in Healthy Versus Glaucoma in the Given Quadrants

|                | Healthy \( n = 49 \) | Glaucoma \( n = 49 \) | \( P \) Value |
|----------------|---------------------|---------------------|--------------|
| **RNFL-Thickness (μm)** |                     |                     |              |
| Global         | 99 ± 12             | 69 ± 13             | <0.001       |
| Temporal       | 72 ± 12             | 59 ± 13             | <0.001       |
| Superior       | 122 ± 16            | 84 ± 24             | <0.001       |
| Nasal          | 81 ± 15             | 58 ± 13             | <0.001       |
| Inferior       | 130 ± 21            | 77 ± 21             | <0.001       |
| **Retardation (°)** |                     |                     |              |
| Global         | 11.5 ± 1.9          | 7.5 ± 1.6           | <0.001       |
| Temporal       | 5.6 ± 1.5           | 5.0 ± 1.2           | 0.04         |
| Superior       | 15.7 ± 2.7          | 9.9 ± 3.6           | <0.001       |
| Nasal          | 10.4 ± 2.3          | 7.0 ± 1.9           | <0.001       |
| Inferior       | 15.8 ± 3.2          | 8.8 ± 3.0           | <0.001       |
| **Birefringence (°/μm)** |                     |                     |              |
| Global         | 0.112 ± 0.009       | 0.108 ± 0.008       | 0.03         |
| Temporal       | 0.078 ± 0.014       | 0.088 ± 0.015       | 0.00         |
| Superior       | 0.126 ± 0.013       | 0.116 ± 0.017       | 0.00         |
| Nasal          | 0.128 ± 0.013       | 0.119 ± 0.014       | 0.00         |
| Inferior       | 0.121 ± 0.011       | 0.112 ± 0.011       | <0.001       |

SD, standard deviation.
with the nasal quadrant, a statistically significant higher RET and BIR was found nasally in healthy and glaucoma subjects. A difference between nasal and temporal RNFL-T was only found in healthy individuals.

The following analysis hypothesized that the unaffected VF hemifield acts as a surrogate for pre-perimetric glaucoma and may indicate BIR decrease before structural loss. Table 3 shows a subgroup breakdown to analyze RNFL-T, RET, and BIR of the superior and inferior quadrant with respect to the affected glaucomatous VF hemisphere. While the subgroups were collected according to the patients’ affected VF hemisphere (superior vs. inferior; see Methods section for definition), for the ease of understanding in the following we nevertheless denominated the subgroups as if they were selected based on the associated (opposite) RNFL hemisphere. For example, we denominated the group of patients with affected superior VF hemisphere (n = 27) as inferior RNFL affected and vice versa. Because we excluded patients in whom both VF hemispheres were affected (n = 3), we could designate for the purpose of this comparison the opposite hemisphere of the affected RNFL as unaffected in the sense that the related VF hemisphere was normal.

As expected, the affected RNFL quadrants show significantly larger reductions of RNFL-T and RET compared to healthy eyes, especially when looking at patients where the inferior RNFL was affected (Table 3). This difference relative to healthy eyes was larger when looking at patients with affected inferior RNFL (RNFL-T −48% affected vs. −23% unaffected; RET −52% vs. −27%), compared to patients with affected superior RNFL (RNFL-T −42% affected vs. −31% unaffected; RET −50% vs. −36%). However, as opposed to the findings for RNFL-T and RET, the greatest difference between affected and unaffected RNFL, regarding BIR reduction relative to healthy eyes, was not found in patients with affected inferior RNFL (BIR: inferior −8% affected vs. superior −4% unaffected), but in eyes with affected superior RNFL (BIR: inferior −8% affected vs. superior −14% unaffected). In other words, the BIR of the inferior quadrant was not depending on the designated status of the inferior RNFL (affected vs. unaffected, P = 0.994), although the BIR of the superior quadrant was significantly reduced in patients with designated affected superior RNFL when compared with those patients with unaffected superior RNFL (P = 0.021).

When we take the reduction of RNFL-T as a crude measure of the amount of damage, we note that the affected inferior quadrant displayed a tendency towards more damage than the affected superior quadrant (−48% vs. −42%, independent t-test P = 0.152), whereas the desig-
nated unaffected inferior quadrant had significantly more damage than the unaffected superior quadrant (−31% vs. −23%, independent t-test \( P < 0.001 \)). This demonstrates that also apparently unaffected quadrants (according to VF results) displayed some reduction in RNFL-T and RET and that this reduction was more pronounced for patients with affected superior RNFL and designated unaffected inferior RNFL.

Pearson correlation coefficients between age, BIR, RNFL-T, RET, and VF MD are shown in Table 4. As expected, BIR exhibited the best correlation with RET. The MD of the VF showed a good correlation with RNFL-T and RET but no
significant correlation with BIR. A strong correlation was only found between RNFL-T and RET. Age did not correlate significantly with any of the parameters.

Diagnostic accuracy was slightly better for global RNFL-T with an area under the receiver operating characteristic curve (AROC) of 0.950 (95% CI 0.909–0.992), compared to global RET with an AROC of 0.943 (0.901–0.985) but was excellent in both cases. AROC of global BIR was only fair with 0.657 (0.527–0.748).

Repeatability was excellent for all our primary outcome measures (calculated with global values of the circle) with an intraclass correlation coefficient (ICC and 95% CI) and pooled standard deviation (SD) of 0.929 (0.845–0.971) and 0.0035°/μm for BIR, 0.999 (0.997–0.999) and 0.87μm for RNFL-T, and 0.97 (0.997–0.999) and 0.73 μm vs. 0.94 μm; (2) RET: 0.986 (0.916–0.998) vs. 0.989 (0.967–0.997) and 0.29° vs. 0.21°; versus (3) BIR: 0.935 (0.709–0.990) vs. 0.893 (0.724–0.966) and 0.0031°/μm vs. 0.0037°/μm, respectively.

**DISCUSSION**

To the best of our knowledge, this is the first study in a larger subject population presenting evidence of an altered BIR in the RNFL of early glaucoma eyes in comparison to healthy controls as measured with PS-OCT. Although on average the global BIR was lower by only 4%, the difference was larger in the superior and inferior quadrants where BIR was 8% lower in a group of early glaucoma eyes compared to age-matched healthy eyes.

Previous PS-OCT based studies have reported these findings only in single cases or have compared a limited data set of glaucoma cases with healthy subjects. Furthermore, in the latter study, the quantitative analysis of BIR was calculated only for areas where the RNFL thickness was above 100 μm, which excluded a part of the healthy RNFL (temporal and nasal) and even more parts of pathologically reduced RNFL in glaucomatous eyes. In contrast to this we here present results based on the evaluation of the full 360° of the measurement circle without excluding thinner locations and present a larger data set with age-matched samples of healthy and glaucomatous eyes. Nevertheless, our results are compatible with the above-described cases presented...
Table 3.

| Healthy \( (n=49) \) Inferior RNFL Affected \( (n=27) \) Superior RNFL Affected \( (n=19) \) | Diff. Infer. Vs. Sup. Affected | Mean ± SD | Difference to Healthy | P Value | Mean ± SD | Difference to Healthy | P Value | Diff. Infer. Vs. Sup. Affected | P Value |
|---|---|---|---|---|---|---|---|---|---|
| RNFL-Thickness (μm) | | | | | | | | | |
| Superior | | | | | | | | | |
| 122 ± 16 | 190 ± 21 | 15.8 ± 3.2 | -4% | 0.011 ± 0.011 | 0.111 ± 0.010 | <0.001 | 0.001 | 0.001 |
| Inferior | | | | | | | | | |
| 120 ± 16 | 190 ± 21 | 15.8 ± 3.2 | -4% | 0.121 ± 0.011 | 0.111 ± 0.010 | <0.001 | 0.001 | 0.001 |
| Retardation (°) | | | | | | | | | |
| Superior | | | | | | | | | |
| 15.7 ± 3.2 | 11.5 ± 3.4 | -27% | -8% | 0.121 ± 0.013 | 0.121 ± 0.013 | <0.001 | <0.001 | 0.001 |
| Inferior | | | | | | | | | |
| 15.7 ± 3.2 | 7.5 ± 2.1 | -52% | -8% | 0.126 ± 0.013 | 0.121 ± 0.016 | <0.001 | <0.001 | 0.001 |
| Birefringence (°/μm) | | | | | | | | | |
| Superior | | | | | | | | | |
| 0.126 ± 0.013 | 15.7 ± 3.2 | 15.7 ± 3.2 | -4% | 0.126 ± 0.013 | 0.121 ± 0.013 | <0.001 | <0.001 | 0.001 |
| Inferior | | | | | | | | | |
| 0.121 ± 0.011 | 7.5 ± 2.1 | 7.5 ± 2.1 | -4% | 0.121 ± 0.011 | 0.121 ± 0.011 | <0.001 | <0.001 | 0.001 |

Table 4.

| Parameters | BIR | RNFL-T | RET | VF MD |
|---|---|---|---|---|
| RNFL-T | 0.272 | | | |
| RET | 0.504* | 0.963* |
| VF MD | 0.128 | 0.689* | 0.647* |
| Age | -0.067 | -0.114 | -0.113 | -0.177 |

*Correlation is significant at the 0.01 level (two-tailed).

by Desissaire et al., which showed a rather similar difference in BIR (0.135°/μm in seven healthy eyes compared to 0.129°/μm in six glaucoma eyes).

Comparing the BIR to previous literature, we note that the mean circumpapillary BIR of healthy eyes was lower in the present study at 0.112°/μm compared to 0.143°/μm in the study of Pollreisz et al. and 0.135°/μm in the study of Desissaire et al. To explain this difference, it is necessary to address several aspects. In the two studies mentioned above, true circular scans with a smaller diameter (3.0 mm vs. 3.5 mm in the current study) using the slope method for BIR calculation were analyzed, and the BIR was only calculated for areas where the RNFL was at least 100 μm thick, resulting in a higher BIR. Nevertheless, after the publication of these studies, further refinements were implemented in the post-processing of the PS-OCT images. These included an improved corneal compensation and a refined spectrum correction (better adaption of the spectral matching of the two spectrometer channels), which led to a systematic reduction of the BIR. To assess changes caused by these implementations, we used the fact that some of our healthy subjects also participated in the analysis of Pollreisz et al. (n = 5). Therefore we compared the BIR of the current study with the true circular scans from the study of Pollreisz et al. analyzing the same subjects by using the same virtual B-scan diameter (3.0 mm) and the same method for BIR calculation (quotient method) and obtained almost identical results (BIR of current study = 0.110°/μm ± 0.003°/μm, 95% CI = 0.105–0.115 vs. Pollreisz = 0.112°/μm ± 0.004°/μm, 95% CI = 0.106–0.119) (Table 5).

As can be seen in Table 5, the BIR was higher before the post-processing was refined, indicating an overestimation of the BIR in the old post-processing method. Exclusion of RNFL-T < 100 μm in the five patients also resulted in an increase of BIR. However, this was an obvious fact as the thin temporal part of the circumpapillary RNFL, which has low BIR, was excluded by this method. An increased diameter of the circle had only a small increasing influence on the BIR in this subset, calculated with the data from this study (Diameter 3.0 mm: BIR = 0.110°/μm ± 0.003°/μm, 95% CI = 0.105–0.115 vs. 3.5 mm: BIR = 0.113°/μm ± 0.002°/μm, 95% CI = 0.110–0.116). These findings indicate that BIR values can only be compared well if the same methods are used for the BIR calculation.

Zotter et al. measured a circumpapillary BIR ranging from 0.101°/μm to 0.141°/μm superiorly and inferiorly, Görzinger et al. circumpapillary BIR of 0.02°/μm to 0.140°/μm, Cense et al. reported around the ONH of 0.05°/μm to 0.175°/μm (after conversion to single pass values), Yamanari et al. single-pass BIR of 0.05° to 0.27°/μm, whereas we observed a range of the averaged circumpapillary BIR of 0.069°/μm to 0.148°/μm. Taking these factors into account, our measured values tend to be
in the lower range compared to previous studies but are still compatible.25,25

Another finding of our study was that the averaged circular BIR scans did not show a double-hump pattern as in previous studies,13,15,23,25 but a single dip in the temporal quadrant. In the previous studies, with exception of the study by Huang et al.13 where a scanning laser polarimeter was used, the slope method was used to calculate the BIR because it has the advantage of working well in thicker RNFL areas and is rather independent of segmentation errors. However, if the RNFL is thin, it is more difficult to detect a distinct RET slope in the RNFL, and the BIR is therefore unreliable. Because of these uncertainties of the slope method in thin RNFL locations, we opted for the quotient method in this study, which is more prone to segmentation errors, but segmentation was in every case manually checked and corrected.

To evaluate differences between the slope and quotient method, we recalculated and compared the averaged circular BIR scans of the seven healthy eyes from the study by Desissaire et al.35 This revealed a lower BIR and a wider 95% CI of the slope method as compared to the quotient method (Global BIR slope method = 0.102°/μm ± 0.010°/μm, 95% CI = 0.092°/μm to 0.112°/μm vs. BIR quotient method = 0.123°/μm ± 0.065°/μm, 95% CI = 0.116°/μm to 0.129°/μm). As can be seen in Figure 4, the quotient method shows only a single dip temporal, whereas the slope method shows the double-hump pattern. In the thicker superior and inferior RNFL sections, both methods provide similar results.

The above re-evaluations of data suggest that the single-dip pattern of BIR may be more plausible, whereas the nasal dip of the double-hump pattern instead would be an artifact of using the slope method to the thinner nasal RNFL. Because it has been shown that parallel microtubules in the RNFL are the primary source of BIR in non-human primates and the packing density of microtubules was lower temporally than nasally, this could also explain the lower BIR temporally in our cohort.11,30 Also the uneven peripapillary distribution of ganglion cell densities and different RNFL diameters might contribute to this observation.31–33

Initially, we did not expect an increased BIR of glaucomatous eyes in the temporal quadrant. This difference was observed exclusively in the range ±40° from the disc-fovea line, which represents RNFBs serving the fovea, the perifovea, and the papillomacular bundle. These axons differ from those originating elsewhere by size and ganglion-cell type34 and importantly also by a greatly reduced BIR.13,15 There is some evidence suggesting that the distribution of axon diameters changes within the papillomacular bundle as the RNFBs approach the OD,33 which may be interpreted as a sign of larger diameters of the axons joining the papillomacular bundle closer to the OD. It remains unclear whether our surprising finding might be related to a differential loss of different RGC populations with diverse intrinsic BIR of their axons.

Additionally, to the main result of reduced BIR in early glaucoma, we also report on statistically significant and relevant reductions of RNFL-T and RET. Since our early glaucoma eyes had on average a VF MD of about −3 dB this finding is all but surprising.

We analyzed the diagnostic value of RNFL-T, RET, and BIR in our data set using the ROC. RET was found to have excellent diagnostic accuracy, similar to RNFL-T, while the diagnostic accuracy of the BIR was found to be at best fair. However, it should be noted that BIR is a measure of the tissue-specific (material) properties of the RNFL. BIR may be a valuable marker at a very early stage of the disease or may act as an indicative marker of areas that could show future glaucoma progression, but both remain to be shown in future studies. While our glaucoma eyes were conventionally classified as early glaucoma with VF defects with a MD of better than −6 dB, it is known that by the time the first VF defects become detectable in routine VF examination, a considerable amount of RNFL damage has already occurred.35

Possibly there might be a time window in the disease process where the BIR of the RNFL is already reduced, but the thickness is still unchanged. At least for experimental glaucoma in a nonhuman primate model, it has been shown that the BIR of the RNFL, as determined by a combination of conventional OCT and scanning laser polarimetry, is reduced earlier than RNFL thickness.16,17 However this time window would likely be in the pre-perimetric stage of the disease.

**Table 5.** Comparison of RNFL-Birefringence*

|                         | Polleirsz Old Post-Processing (n = 5) | Polleirsz New Post-Processing (n = 5) | Current Study New Post-Processing (n = 5) |
|-------------------------|--------------------------------------|---------------------------------------|------------------------------------------|
| No RNFL excluded        | 0.126 ± 0.003 0.122–0.130            | 0.112 ± 0.004 0.106–0.119             | 0.110 ± 0.003 0.105–0.115                |
| RNFL <100 μm excluded   | 0.137 ± 0.003 0.132–0.141            | 0.119 ± 0.003 0.114–0.125             | 0.119 ± 0.004 0.113–0.124                |

SD, standard deviation.

*Comparison of the five overlapping participants of the current study and the study by Pollreisz et al. on a circular B-scan with a diameter of 3.0 mm using the old and new post-processing method for the all RNFL-thicknesses and RNFL-thickness <100 μm excluded.
and our results are well in agreement with this assumption.

We see one piece of evidence in our data, supporting the hypotheses of pre-perimetric reduction of BIR. It is important to note that we based the following assumptions on the fact that the inferior RNFL is more frequently involved in initial glaucomatous damage compared to the superior RNFL. Therefore we can also speculate that subjects with initial superior RNFL damage will have more subclinical damage in the inferior RNFL, whereas subjects with initial inferior RNFL damage will have less subclinical damage in the superior RNFL. Interestingly, in the subgroup with affected superior RNFL, the decrease in BIR of the apparently unaffected inferior hemisphere was about the same as in the subgroup with inferior affected RNFL (inferior affected –8% vs. superior affected –8%). This behavior was observed uniquely for the BIR of the inferior RNFL and not for RNFL-T or RET, suggesting that the relatively large reduction in BIR of the apparently unaffected inferior RNFL in eyes with affected superior RNFL may be an early sign of glaucomatous damage. These deductions will have to be proven in future longitudinal studies.

Confidence intervals computed at the 95% level for the mean RNFL-T (95% CI) of glaucoma = 69 μm (65–73 μm) versus healthy = 99 μm (96–103 μm) and RET of glaucoma = 7.5° (7.1°–8.0°) versus healthy = 11.5° (11.0°–12.1°) and thus also for BIR (95% CI) of glaucoma = 0.108°/μm (0.105°/μm to 0.110°/μm) versus healthy = 0.112°/μm (0.109°/μm to 0.114°/μm) were narrow. Surprisingly, the 95% CI for BIR seemed to be narrower compared to RNFL-T and RET. This can be explained by the fact that RNFL-T and RET are strongly correlated with each other, which likely reduces the variation in BIR. The repeatability was excellent for RNFL-T and RET and good for the BIR in both glaucoma and healthy individuals.

The presented study has some limitations. The number of patients included was still moderate and not representative of all age groups and ethnicities. Additionally, this is a cross-sectional study, and it was not possible to answer the interesting question of whether in human glaucoma, as in nonhuman primates, BIR is reduced very early in the disease while RNFL thickness is still unaltered. As described in nonhuman primates, BIR is reduced very early in the disease while RNFL thickness is still unaltered. As described in previous studies, diabetes mellitus also can reduce the BIR of the RNFL. Therefore undiagnosed diabetes mellitus, but also possibly other unknown reasons for altered BIR of RNFL, may distort our results. Another possible limitation of our study was that we did not measure RNFL reflectance. RNFL reflectance has been demonstrated to be a material property of the parallel microtubules within the RGC axons and has been shown to be reduced in glaucoma. Reflectance may influence the SNR of RNFL and thus affect the accuracy of the RNFL segmentation, but this was manually corrected if necessary. Because the RET was extracted from the photoreceptor layer, not including the RNFL, it would be difficult to explain why the reflectance of the RNFL might have influenced the RET in our setting.

All these findings support the hypothesis that in glaucoma there is not only a loss of RNFs but also intracellular remodeling of retinal ganglion cell axons characterized by the loss or alteration of microtubules, resulting in decreased BIR. Nevertheless, to determine whether BIR is altered in very early glaucoma before RNFL-T changes, prospective longitudinal studies involving subjects with ocular hypertension or suspected glaucoma are needed.

**CONCLUSION**

To the best of our knowledge, this is the largest study comparing healthy with glaucoma eyes using PS-OCT. Our data show that besides RNFL-T and RET also BIR is significantly lower in early perimetric glaucoma compared to healthy eyes, which may be interpreted as a sign of a loss of microtubules within the RNFs. Thus PS-OCT provides additional information about RNFL structure and might contribute to early glaucoma detection. Longitudinal analyses of larger data sets of pre-perimetric glaucoma patients are needed to confirm BIR changes in the very early stages of glaucoma, where RNFL-T is still unchanged.

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