Comparison of Retinal Microvasculature in Patients With Alzheimer’s Disease and Primary Open-Angle Glaucoma by Optical Coherence Tomography Angiography

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PURPOSE. Comparison of retinal microvasculature within the macula and the optic nerve head in the eyes of patients with Alzheimer’s disease (AD), primary open-angle glaucoma (POAG), and in a healthy control (HC) group, using optical coherence tomography angiography (OCTA).

METHODS. In this cross-sectional study, 27 patients with AD, 27 with POAG, and 27 healthy controls were enrolled. The Mini-Mental State Examination test was used to assess cognitive function. Ophthalmic examination included OCTA, which was used for the imaging of vascular flow within the layer of radial peripapillary capillaries (RPCs), and also in the superficial vascular plexus (SVP) and deep vascular plexus (DVP) of the retina.

RESULTS. In the AD group, the density of vessels in DVP was significantly reduced and the foveal avascular zone was increased when compared to POAG and HC groups (P < 0.001). Patients with POAG had a significantly reduced vessel density in RPCs and SVP as compared to AD and HC groups (P < 0.001). The average thickness of peripapillary retinal nerve fiber layer was correlated with the vessel density in SVP in patients with POAG (Pearson’s r = 0.66; P = 0.0002) and was significantly lower in POAG and AD groups than in the HC group (P < 0.001).

CONCLUSIONS. AD and POAG are neurodegenerative diseases associated with apoptosis of nerve cells and impairment of microvasculature. Despite the fact that in both diseases there are abnormalities of the entire retinal vascular system, significant microcirculatory impairment in POAG patients affects superficial vessels, whereas in AD patients it affects vessels located in the deeper retinal layers.

Keywords: Alzheimer’s disease, primary open-angle glaucoma, retinal microvasculature, peripapillary retinal nerve fiber layer, optical coherence tomography angiography

Alzheimer’s disease (AD) is the most frequent cause of dementia worldwide, accounting for approximately 60% to 70% of all cases. Its incidence increases with age and it is estimated that the number of patients globally will quadruple by 2050.1,2 AD is a progressive, irreversible impairment of cognitive function due to apoptosis of nerve cells and brain atrophy.3 The main cause of development and progression of dementia is extracellular β-amyloid (Aβ) plaque formation, a consequence of amyloid precursor protein proteolysis in the vicinity of synapses, and the formation of intracellular neurofibrillary tangles composed of tau-protein (p-tau).4,5 There is evidence that abnormalities in the central nervous system (CNS) manifest up to 20 years before clinical symptoms appear, emphasizing the importance of identifying a biomarker for early diagnosis.6

The retina is considered to be a CNS nodule connected to the CNS through the optic nerve (also known as cranial nerve II, CNII). In 1986, Hinton et al.7 were the first to describe how CNII and the retina are affected by neurodegenerative changes in patients with AD. Previous studies have shown that the peripapillary retinal nerve fiber layer (pRNFL) is significantly thinner in AD patients than healthy subjects. In spectral-domain optical coherence tomography (SD-OCT), the difference is from 16.64 μm to 8.25 μm and is highest in the superior quadrant.8 However, changes in the thickness of pRNFL occur not only in AD but also in other neurodegenerative diseases, especially glaucoma.9,10 Recently, Aβ deposits have been identified on histopathologic examination in the retina of patients with AD. Similarly to neurological tissue, these deposits accumulate primarily in the vicinity of blood vessels.11 A potential relationship between deposition of abnormal Aβ in the vicinity
of vessels and the disturbance of the blood flow, as well as the diameter of retinal vessels, has been identified in AD patients. The pathologic mechanism underlying primary open-angle glaucoma (POAG) involves apoptosis of retinal ganglion cells (RGCs) and gradual decrease in thicknesses of both pRNFL and the retinal ganglion cell layer (GCL). In addition to increased intraocular pressure (IOP), vascular mechanisms play an important role in this process. It has been observed that patients with glaucoma have reduced blood flow in retina and choroid, which contributes to neurodegenerative processes in the eye.

Both AD and POAG affect similar populations of older people and both diseases occur more frequently with age. Previous studies have confirmed that in patients with AD, POAG, and even with preperimetry glaucoma, similar retinal changes exist, manifesting as thinning of the pRNFL. However, there are no studies that have attempted to directly compare these diseases with respect to retinal vascular changes, which would make it possible to identify new, more specific markers. OCT angiography (OCTA) is a new, noninvasive method for the quantitative and qualitative assessment of vasculization status within the macula and optic nerve head (ONH). A direct comparison of OCTA imaging results carried out in AD patients and POAG patients could make a significant contribution to understanding the pathophysiology of these diseases and may help elucidate the underlying cause of damage to the pRNFL.

The aim of our study was to compare the changes in retinal microvasculature in patients with AD and POAG and to find a biomarker that will distinguish these diseases. We used OCTA for this purpose. It is important to note that AD is a neurodegenerative disease in which abnormal proteins are deposited in the CNS, as well as in the retina and its vessels, whereas in POAG, pathologies are found mainly in the inner layers of the retina. We hypothesized that patients with AD have a decreased microvascular density in both deep and superficial vascular plexus (DVP and SVP), whereas patients with POAG have microvascular damage mainly in the SVP.

**METHODS**

**Study Design and Patient Recruitment**

This cross-sectional study was carried out between September 2017 and December 2018 in the Oftalmika Eye Hospital in Bydgoszcz, Poland. The study protocol was approved by the local bioethics committee and each subject signed a consent for participation. The study was conducted in accordance with the principles of the Helsinki Declaration and informed consent was obtained from the subjects after explanation of the nature and possible consequences of the study. Each patient enrolled in the study had been examined by a psychiatrist to assess cognitive function with the Mini-Mental State Examination (MMSE) screening test. All participants (100%) in the POAG group were treated with at least one type of ocular antihypertensive drops. Beta blockers were used most often (39%), carbonic anhydrase inhibitors in 56%, prostaglandin analogues in 51%, and α2-adrenergic receptor agonist in 22% of cases. The average number of antihypertensive eye drops was 2.0 ± 0.83.

Participants in the healthy control (HC) group were members of the Senior Club in Bydgoszcz and after consenting, were subjected to an ophthalmologic and psychological examination. The criteria for inclusion in the control group were a normal score on MMSE (≥27 points), the absence of glaucoma or any other eye disease, normal appearance of the ONH and normal thickness of pRNFL, IOP below 21.0 mm Hg, and no losses characteristic for glaucoma in visual field testing. Exclusion criteria were subjects younger than 55 years and older than 85 years, best corrected visual acuity (BCVA) ≤ 0.6, refraction abnormality above ± 3.0 Dsph and below -3.0 Dsph, and history of eye surgery except for uncomplicated cataract phacoemulsification. The study also excluded persons with diabetes, unregulated arterial hypertension (>145/85 mm Hg), a body mass index ≥ 30 kg/m², damage to CNII with an etiology other than glaucoma, and the presence of any disease of the macula and neurodegenerative diseases other than AD.

**OCTA Imaging**

OCTA imaging was carried out with an Avanti RTVue XR (Optovue, Inc., Fremont, CA, USA) capable of scanning at 70,000 A-scans/s, permitting measurements with an axial resolution of 5 μm using a source of light with a wavelength of 840 ± 10 μm. We used a newly developed system (software
version 2017.1.0.151) equipped with three-dimensional Projection Artifact Removal (3D PAR); therefore, the projection artifacts are reduced in all the deeper layers while maintaining their authentic layout, and the foveal avascular zone (FAZ) parameters have been improved.25,26 Both eyes of all patients were examined on the same day between 8.00 AM and 12.00 AM after their pupils were dilated. The macula was analyzed by using B-scans covering an area of 6 × 6 mm² repeated horizontally and vertically. Each of the B-scans consisted of 400 A-scans (versus traditional 304 A-scans) centered on the fixation point. On an area of 4.5 × 4.5 mm² centered on the ONH, the peripapillary vessels were analyzed. The images consisted of two sets of B-scans repeated horizontally and vertically, each consisting of 400 A-scans. Only measurements of good technical quality with a signal quality (SOQ) of 6 or more on a 10-degree scale, with which a commercial camera is equipped, qualified for further analysis. Measurements with motion artifacts present on the en face images (irregular patterns of vessels or a blurred boundary of the ONH) were also rejected. The data were analyzed with commercially available software consisting of automatic segmentation of the SVP and DVP, and then automatic measurement of the density of vessels in both these plexuses as well as in the FAZ in the macular area. The 3D PAR algorithm developed by Optovue reduces projection artifacts from the entire OCTA volume on a per voxel basis, using information from the OCT and OCTA volume to distinguish the OCTA signal in situ from projection artifacts, based on parameters acquired from the OCTA and OCT intensity profiles around the voxel of interest.25,26 The scan covering the ONH was used to measure the density of vessels throughout the en face image with dimensions of 4.5 × 4.5 mm² and the density of vessels in the peripapillary area extending between the 2- and 4-mm-diameter elliptical contour lines around the disc margin. The density of the radial peripapillary capillaries (RPCs) was defined as extending from the internal limiting membrane to the posterior boundary of the RNFL. Macular vessel density was analyzed throughout the examined area of 6.0 × 6.0 mm², the area of fovea within the annulus of 1 mm in diameter, the parafoveal area between the annuli at 1 mm and 3 mm from the center of the fovea, and the perifoveal area between the annuli at 3 mm and 6 mm from the center of the fovea.

**SD-OCT Imaging**

All patients underwent SD-OCT imaging with the objective of measuring the thickness of pRNFL using the Spectralis OCT device (Heidelberg Engineering, Heidelberg, Germany). The pRNFL was measured within a circular scan that consisted of 768 A-scans. The scanned circle was 3.46 mm in diameter and was centered with the ONH. The global thickness of the pRNFL was analyzed over 360°. The Avanti RTVue XR system (Optovue) was used to perform SD-OCT measurements and to analyze ONH parameters such as disc area, rim area, C/D area, and cup volume.

**Statistical Analysis**

One eye of each patient was included in the analysis. In cases where both eyes met the assumed criteria, the eye was selected according to the higher SQ score of the macula in OCTA imaging (when the SQ of OCTA images was the same in both eyes, the quality of ONH images was decisive). Scores and variability, arithmetic mean, and the coefficient of variation (%) were calculated. Among the groups, OCTA and SD-OCT results were compared by using multifactor analysis of variance (ANOVA), allowing analysis of the influence of several factors interfering with OCTA results on a dependent variable. To reduce error variance during the analysis, the total variability of the examined feature was divided into variability caused by the influence of sex, age, and SQ. Tukey’s post hoc test was used to determine any significant differences between the groups. The independence between the variables was analyzed with χ² test. The surface area under the receiver operating characteristic curve (AROC) was used to determine diagnostic accuracy of the analyzed parameters discriminating between AD, POAG, and HC patients. An AROC of 1.0 represents perfect discrimination, whereas an AROC of 0.5 represents accidental discrimination. Pearson’s correlation was used to determine the effect of MMSE and pRNFL on the measurements of vessel density in individual retinal plexuses.

**RESULTS**

Initially, 86 patients were enrolled in the study. Owing to the poor quality of OCTA images of both eyes (movement artifacts, segmentation errors, SQ < 6, a significant amount of floaters), three people from the AD group and two from the POAG group were excluded. As a result, 81 people qualified for the analysis and were assigned to three groups. Twenty-seven patients with AD, 27 patients with POAG, and 27 healthy subjects, a control group, were enrolled in the study. There was no significant difference in age or sex between the study groups. The AD group was characterized by a significantly lower MMSE score (20.57 ± 2.73) than the other groups (P < 0.001). In all eyes of the patients with glaucoma, the disease was of a perimetric nature and medium degree of severity (MD: −8.77 ± 7.85 dB), whereas patients in the control group had normal results on visual field testing (MD: −0.34 ± 1.47). The mean IOP was significantly higher in the group of patients with glaucoma (Table 1).

The OCTA cross sections of the macula with visible flow signals both through the center of the fovea and in the circumferential part of the macula were similar in all groups. En face angiograms were also similar; however, in patients with AD, the flows in SVP were narrower and more interrupted than...
in other groups. In 13 eyes with glaucoma, areas with reduced flow within SVP were visible. On en face images, DVP was similar in all groups (Fig. 1).

Comparison of mean vessel density is presented in Table 2. In the macula the lowest density in SVP occurred in patients with POAG compared to patients with AD and to the HC group \( (P < 0.001) \). Patients with AD had the lowest vessel density in DVP, compared to other groups (Fig. 2). Differences in vessel density within SVP and DVP persisted in both parafovea and perifovea (Fig. 3). The largest difference in vessel density in DVP between the group of patients with AD and other groups was in the perifoveal area. The ratio of DVP to SVP whole density was 0.93 in the AD group and differs significantly from that of the POMG and HC groups where this ratio gained values of 1.20 and 1.03, respectively \( (P < 0.001) \). Patients with AD had the largest mean FAZ area and that was significantly different from that of other groups \( (P < 0.001) \). Patients with POAG also had a significantly increased mean FAZ area in comparison to the control group \( (P = 0.015) \) but smaller in comparison to the AD group \( (P = 0.012) \). Analysis of vessel density in the RPC layer on the entire surface of the en face image and in the peripapillary area revealed that patients with glaucoma had a significantly reduced capillary network density \( (P < 0.001) \). The density in this area did not differ significantly between patients with AD and healthy subjects.

The thickness of pRNFL was significantly reduced among patients with POAG in comparison to the other groups \( (P < 0.001) \). However, in patients with AD, pRNFL was also significantly thinner than in the control group \( (P < 0.05) \). In the eyes of patients with glaucoma, the parameters characterizing the ONH such as rim area, C/D area, and cup volume differed significantly as compared to the two other groups (Table 3).
No significant correlation was found between the MMSE score and vessel density in SVP or DVP and the FAZ area in patients with AD. In the group of patients with glaucoma, there was a significant correlation between vessel density in SVP and the thickness of pRNFL (Pearson’s $r = 0.66; P = 0.0002$). This relationship was not found in the DVP. Table 4 shows that the AROC was used to reflect the diagnostic accuracy for each parameter to provide distinction between AD and other surveyed participants. The use of a logistic regression model with three parameters—the density of the peripapillary RPCs, DVP whole, and area of the FAZ—allowed us to obtain an AROC of 0.93 to separate AD patients from combined groups of POAG patients and HCs.

**DISCUSSION**

AD and POAG are multifactorial neurodegenerative diseases associated with aging. During embryogenesis, the CNII and retina develop as a direct extension of the diencephalon, so that abnormalities occurring in the CNS can also be observed in the fundus of the eye in the case of AD. It is believed that nerve cell damage can have a common pathogenesis for both AD and POAG, and there is increasing discussion about common risk factors and mediators responsible for the onset of these diseases.

**FIGURE 2.** The graphical representation of vessel density within the superficial retinal vascular plexuses and deep retinal vascular plexuses in sample optical coherence tomography angiography images in the groups studied. **(a)** Density in the superficial retinal vascular plexuses in a patient with Alzheimer’s disease. **(b)** Density in the deep retinal vascular plexuses in a patient with Alzheimer’s disease. **(c)** Density in the superficial retinal vascular plexuses in a patient with primary open-angle glaucoma. **(d)** Density in the deep retinal vascular plexuses in a patient with primary open-angle glaucoma. **(e)** Density in the superficial retinal vascular plexuses in a healthy control group. **(f)** Vascular density in the deep retinal vascular plexuses of a healthy control group. Cloudiness in the vitreous body causes an artifact visible as the dark blue area of the reduced vascular density in the upper nasal part of the macula.
and progression of both diseases. Yoneda et al. in their studies on the pathogenesis of glaucoma have shown a significant decrease in $A\beta$ as well as an increase in the level of the tau protein in the vitreous body. Similar changes in the amount of these proteins occur in the cerebrospinal fluid of subjects with AD. McKinnon in animal model studies has suggested that the cause of death of RGCs in patients with ocular hypertension may be the chronic neurotoxicity caused by deposition of $A\beta$ induced by an increase in IOP, as well as reduction in vascular endothelial growth factor (VEGF), which resembles AD at the molecular level. These results confirm the hypothesis that degenerative changes in the eye with glaucoma may have the same pathogenesis as in the case of AD. In addition to specific neuropathologic changes caused by

![Figure 3](image_url)

**Figure 3.** Comparison of vascular density in the foveal, parafoveal, and perifoveal areas in the studied groups within the superficial retinal vascular plexuses (a) and deep retinal vascular plexuses (b). The average density in the parafoveal and perifoveal part of the macula was significantly different between Alzheimer’s disease and primary open-angle glaucoma groups.
abnormal proteins, AD and POAG are associated with vascular changes.32,33

In our study, we used OCTA technology to assess the retinal microvasculature of the macula and ONH in patients with AD, POAG, and in HCs. We compared the results of the groups, analyzed to each other, in order to find distinctive differences. We found that the density of vessels in individual retinal plexuses differed significantly between the study groups. We showed that patients with AD have a significantly lower vascular density in DVP and enlarged FAZ area in comparison to the other groups. In addition, we observed that SVP also exhibits a reduction in vessel density as compared to the HC group, but this difference was not statistically significant (P = 0.189). For the POAG group, a decrease in vessel density could also be observed in all retinal vascular plexuses. However, statistically significant changes occurred only in the RPC layer and in SVP, which correlated with the loss of the pRNFL thickness. No correlation between the MMSE score and the retinal vascular density was demonstrated.

To date, only three studies34–36 have been published in which OCTA has been used to evaluate the microvasculature in patients with AD. Bulut et al.34 were the first to use OCTA to evaluate retinal vascular changes in AD. They have found that vessel density in SVP is significantly reduced in the AD group (P < 0.05), which correlates with MMSE scores, while the FAZ area is enlarged (P < 0.001) compared to the HC group. The positive correlation between vessel density in SVP and FAZ area with MMSE may be due to the fact that the mean MMSE score (16.97 ± 6.39) in AD patients is significantly lower than in our research, which could affect the disclosure of this correlation. A lower degree of dementia is associated with a less advanced stage of the disease in which degenerative changes may be less pronounced. The authors34 also suggest that vascular impairment may be related to the reduced angiogenesis caused by VEGF's being bound and blocked by Aβ. In addition, as the Aβ deposits settle inside the walls of blood vessels, they are likely to lead to occlusion and reduced blood flow, which has also been reported in previous work.37,38 The authors have not analyze the density of vessels in DVP probably because the software available to them was in its early version.

Lahme et al.35 have used OCTA to evaluate vascular density of the macula and ONH in patients with AD. Their results demonstrate a decrease in vascular flow density in each retinal plexus, yet significant changes are found in SVP (P < 0.001) and RPCs (P < 0.05), which in patients with AD, correlate with the Fazekas scale for white substance changes of vascular origin. Vascular brain damage was associated with reduced flow density in the superficial OCT angiogram of the retina. These authors have not observed significant changes in the FAZ area and in the deep retinal OCT angiogram of the macula (P = 0.09) in AD patients compared to HC group. Moreover, they have not found a correlation between the density of vascular flow and the level of Aβ, tau protein, or MMSE score.35 This can be explained by the fact that the authors examined a macular area of 5 × 5 mm2, while the vascular changes in the population under study are most visible in the peripheral part of macula. Moreover, the patients included in their study have a lower degree of dementia (MMSE score 22.32 ± 4.45) than the group analyzed in our investigation.

In turn, Jiang et al.36 using OCTA and fractal analysis (box counting, Dbox) for the assessment of vessel network density in SVP and DVP, have investigated the relationship between microvasculature and the thickness of GCL-inner plexiform layer (IPL) in patients with AD and MCI. Their findings are similar to ours, namely, the vessel density decreases in DVP and SVP in the AD group, whereas the GCL-IPL thickness is only correlated to DVP.

There are many more reports on POAG in the literature in which OCTA parameters have been evaluated. Most studies to date have shown that the disease affects all vessels and a decrease in density can be observed in each plexus, and statistical significance has been demonstrated in SVP, RPC, or in full-thickness scans.39–43 This is consistent with our results. In two other published works,44,45 a significant decrease in retinal vessel density has been found both in SVP and DVP. The difference in the results obtained in each of these studies may be related to the quality of the images obtained, the artifacts casting shadows especially on the deeper layers of the retina, as well as a difference in the software used in OCTA, which has a particularly large impact on DVP.

The results of this study confirm that AD and POAG are neurodegenerative diseases that are associated with retinal

### Table 3. Differences Between the Groups in Morphologic Parameters of the ONH and the Macula in SD-OCT Imaging

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AD</th>
<th>POAG</th>
<th>HC</th>
<th>P Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cup/disc area ratio†</td>
<td>0.17 ± 0.11</td>
<td>0.39 ± 0.23</td>
<td>0.12 ± 0.25</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Rim area, mm²†</td>
<td>1.71 ± 0.37</td>
<td>1.16 ± 0.45</td>
<td>1.79 ± 0.39</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Disk area, mm²†</td>
<td>1.96 ± 0.52</td>
<td>1.97 ± 0.51</td>
<td>1.96 ± 0.39</td>
<td>0.8443</td>
</tr>
<tr>
<td>FAZ area, mm²†</td>
<td>66.11 ± 16.79</td>
<td>102.85 ± 8.87</td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Rim area, mm²†</td>
<td>89.74 ± 6.58</td>
<td>66.11 ± 16.79</td>
<td>102.85 ± 8.87</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Peripapillary RPCs</td>
<td>0.9609 (0.919–1.00)</td>
<td>0.6193 (0.462–0.777)</td>
<td>0.7901 (0.680–0.891)</td>
<td></td>
</tr>
<tr>
<td>SVP whole</td>
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<td>0.5912 (0.435–0.748)</td>
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<td>DVP whole</td>
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<td>FFA</td>
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<td>0.9369 (0.877–0.997)</td>
<td>0.7874 (0.660–0.915)</td>
<td>0.8621 (0.778–0.946)</td>
<td></td>
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</tbody>
</table>

*ANOVA test, P value < 0.05 was considered to be statistically significant.
† Mean ± SD.

### Table 4. Diagnostic Accuracy of OCTA Parameters in Discriminating Between Patients With AD, POAG, and HC

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AD vs. POAG</th>
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<th>AD vs. POAG + HC</th>
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<td>DVP whole/SVP whole</td>
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</table>

CI, confidence interval.
microvasculature changes, and the degree and level of vascular damage depend on the disease. In the case of POAG, the disease process involves RGCs in the GCL and RNFL, hence vascular damage is more selective and affects SVP and RPC. AD is the disease with the greatest degree of vascular damage in DVP, which is significantly correlated with the decrease in the thickness of GCL-IPL. Most alterations localize in the peripheral parts of the larger area of the retina because we have reason to believe that antiglaucoma eye drops at the time of the examination. 48 glaucomatous patients in the current study did not stop using extensive eye drops on the result of the study, they should be autoregulation. To eliminate the potential effect of antihyperemic agents, which may have a potential confounding effect on the hemodynamics of ocular blood flow and retinal vascular damage is more selective and affects SVP and RPC. AD disease process involves RGCs in the GCL and RNFL, hence microvasculature dysfunction, which can be effectively evaluated with OCTA. In summary, this is the first study comparing OCTA angiograms in patients with AD, POAG, and in HCs. The results showed that AD and POAG are associated with retinal microvasculature dysfunction, which can be effectively evaluated with OCTA. Depending on the disease, significant vascular damage can affect different retinal plexuses. Despite the fact that in both diseases there are abnormalities in the entire retinal vascular system, the microvasculature impairment in POAG affects superficial vessels to the greatest extent, whereas in AD, it affects vessels located in the deeper layers of the retina. The ratio of DVP to SVP whole density suggests a different vascular phenotype in AD than in POAG. The results are promising, and further study is warranted because this can be a useful method for diagnosing neurodegenerative diseases. We consider that the use of OCTA may help to distinguish the cause of pRNFL damage and can be used in the future as a new biomarker in the early diagnosis of AD and POAG.

References