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Quantification of Optical Coherence Tomography Angiography in Age and Age-Related Macular Degeneration Using Vessel Density Analysis

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Purpose: The aim of this study was to determine whether vessel density (VD) as measured by optical coherence tomography (OCT) angiography provided insights into retinal and choriocapillaris vascular changes with aging and intermediate dry age-related macular degeneration (AMD).

Design: Non-randomized observational study.

Methods: Seventy-five participants were recruited into 3 cohorts: young healthy group, old healthy, and those at high-risk for exudative AMD. Raw OCT and OCT angiography data from TOPCON DRI OCT Triton were exported using Topcon IMAGENET 6.0 software, and 3D datasets were analysed to determine retinal thickness and VD.

Results: Central macular thickness measurements revealed a trend of overall retinal thinning with increasing age. VD through the full thickness of the retina was highest in Early Treatment Diabetic Retinopathy Study (ETDRS) sector 4 (the inferior macula) in all the cohorts. Mean VD was significantly higher in the deep capillary plexus than the superficial capillary plexus in all ETDRS sectors in all cohorts, but there was no significant difference noted between groups. Choriocapillaris VD was significantly lower in all ETDRS sectors in the AMD group compared with the young healthy and the old healthy groups.

Conclusions: Retinal VD maps, derived from the retinal plexi, are not reliable biomarkers for assessing the aging macular. Our nonproprietary analysis of the vascular density of the choriocapillaris revealed a significant drop off of VD with age and disease, but further work is required to corroborate this finding. If repeatable, choriocapillaris VD may provide a noninvasive biomarker of healthy aging and disease.

Key Words: age-related macular degeneration, optical coherence tomography angiography, retinal blood flow, vessel density

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Currently we lack a reliable, noninvasive vascular biomarker to monitor both the "healthy" aging and disease progression of age-related macular degeneration (AMD). Optical coherence tomography angiography (OCT-A) represents a novel, noninvasive, dye-less retinal vascular imaging technique that can be rapidly acquired during a clinical consultation. OCT-A is a progression of OCT, using repeated B-scans to detect motion contrast.[1] Based on the assumption that this represents the movement of erythrocytes travelling through the retinal blood vessels, the retinal vasculature can therefore be visualized.[2] Previous studies have shown the value of this imaging technique in the evaluation of glaucoma, diabetic retinopathy (DR), retinal vein occlusions, and AMD[3].

The laminar structure of the retina lends itself to segmentation analysis. Histologically, the retina has four retinal capillary plexuses, within the macular only 3 of these are considered: the superficial, deep, and intermediate capillary plexuses.[4,5] When the macular vasculature is imaged using OCT-A, the vascular layers are segmented automatically by the software. This results in the merging of the intermediate with the deep capillary plexus.[3] The regions of interest identified on the OCT-A therefore include the superficial capillary plexus (SCP) and the deep capillary plexus (DCP).[3] The SCP consists of the vasculature within the retinal nerve fibre layer and ganglion cell layer. The DCP represents the vascular plexuses present at two locations: these plexuses are at the border of the inner nuclear layer and the outer plexiform layer, and the border of the inner plexiform layer and inner nuclear layer.[6]

OCT-A images can be subjectively appraised for the presence of disease or post processed to produce quantitative data that can be evaluated objectively. A number of studies[7,8] have used perfusion indices such as vessel density (VD) and flow index[9] as a method of quantitatively analysing OCT-A images. VD is defined as the "percentage area occupied by vessels in the segmented area."[9] Flow index is defined as "the average decorrelation values in the segmented area."[9] It has been proposed that these indices could be of value in monitoring disease progression in AMD and DR.[7,10,11] When measuring VD, both vessel length and diameter need to be considered. Poor perfusion can also result in vessel dilation; therefore, VD may not give complete information about the retinal vascular status.[12]

There are a wide range of OCT-A devices available, each using a variety of algorithms to capture retinal images. These include OCT-based optical microangiography; split-spectrum amplitude decorrelation angiography; OCT angiography ratio analysis; speckle variance; phase variance; and correlation mapping.[13] Specific OCT-A models such as the AngioVue (Optovue,

Inc., Fremont, CA) have inbuilt software for perfusion indices calculation.[14] However, the majority of studies have applied post image analysis in a variety of methods after the images are exported from the OCT-A instrument.[2]

This heterogeneity in the image capture and analysis means that data calculated using different algorithms are not directly comparable, due to systemic difference and poor agreement.[2] Therefore, external normative databases are unreliable and longitudinal monitoring of disease using VD requires scans to be performed using the same instrument, scan location, and algorithm.[2]

The aims of this study are to compare the macular VD values for young healthy (YH), old healthy (OH), and patients with intermediate dry AMD at high risk of progression to exudative AMD, and to determine whether these perfusion indices could usefully serve as a biomarker for both "healthy" aging and the identification of disease risk.

MATERIALS AND METHODS

Seventy-five participants were recruited through Auckland Optometry Clinic and Milford Eye Clinic, Auckland, New Zealand. All participants provided written informed consent before imaging. Ethics approval (#018241) from the University of Auckland Human Participants Ethics Committee was obtained for this study. This research adhered to the tenets of the Declaration of Helsinki.

Participants were divided into 3 groups: YH group, OH group and the high-risk intermediate AMD group. Twenty participants were recruited into the YH group, 21 were recruited into the OH group, and 34 recruited into the AMD group. A comprehensive eye examination was conducted on each participant before the OCT and OCT-A scans to determine high contrast best corrected visual acuity and the ocular health status of the fundus. Patients with any posterior eye disease that could potentially affect the choroidal or retinal vasculature including, but not limited to glaucoma, polypoidal choroidal vasculopathy, DR, hypertensive retinopathy, and high myopia ($\geq$6D), were excluded from the study. Patients with any history of neurological disorders were also excluded. The YH group consisted entirely of individuals between the ages of 20 and 26 a best corrected visual acuity of $\geq$6/9 in the eye under test. The "OH" group consisted of individuals over the age of 55 years and who had a best corrected visual acuity of $\geq$6/9 in the eye under test. The "AMD group" consisted entirely of patients with high risk intermediate dry AMD. This being diagnosed if the individual had at least two of the following risk factors; reticular pseudodrusen, established neovascular AMD in the fellow eye, confluent soft drusen with accompanying changes within the retinal pigment epithelium. To ensure that all patients in the "AMD cohort" could maintain fixation during OCT-A imaging, only those patients with a best-corrected visual acuity of 6/15 or better were enrolled. The mean age of the participants in the YH group, OH, and AMD participants were 23 ± 3, 65 ± 10, and 75 ± 8 years, respectively. Only 1 eye of each patient was used for the analysis, and if the patient had both eyes scanned, the OCT-A scan that had the better quality (assessed subjectively by the clinical grader) of the two was used. Mean best-corrected visual acuity for the YH, OH, and AMD group were 6/6, 6/9, and 6/12, respectively.

The ocular health of all participants was assessed at Auckland Optometry Clinic, by a registered optometrist, before enrollment in the study. The macular status of patients enrolled into the AMD group was assessed separately by an experienced retinal specialist (DS).

Data Collection Protocol

Participants were dilated with 1.0% tropicamide if the pupils were deemed to be too small for adequate OCT scans. Intraocular pressures were measured before and after dilation by iCare tonometry. OCT/OCT-A scans, fundus photography, and clinical measurements were all performed at the University of Auckland Optometry Clinic, in a single session.

SS-OCT-A Device and Scanning Protocol

The swept source OCT-A device (Topcon DRI OCT Triton, Topcon Corporation, Japan) was used to obtain the OCT and OCT-A scans. A macular line and $3 \times 3\,mm^2$ OCT, and $3 \times 3\,mm^2$ OCT-A scan were performed on each participant. All scans were centered on the fovea, and retinal layers were identified using the IMAGENET 6.0 automated layer detection tool so that the SCPs and DCPs could be evaluated. The quality of the generated layers was checked manually. Scan quality was evaluated at the time of acquisition, and repeated if required.

Quantitative Analysis of OCT-A Images

Raw OCT and OCT-A data were exported using Topcon IMAGENET 6.0 software. As OCT-A images are only qualitative, we chose to normalize the OCT-A datasets, before further analysis. These datasets were then correlated with VD in each retinal sector. OCT-A 3D datasets were analyzed in 3 different ways (outlined below), to investigate the retinal and choroidal thickness and VD. To ensure that any differences observed between cohorts were real, we repeatedly performed a VD analysis on a single individual (repeated 15 times over a period of 2 hours). The proprietary Triton optical coherence tomography angiography (OCTA) software (IMAGENET6) was used to generate vessel densities for each scan, using the same Early Treatment Diabetic Retinopathy Study (ETDRS) regions as dictated by our study protocol. Using these data the intraclass correlation and concordance correlation coefficient were calculated. The mean ($\pm$SD) of the absolute difference between each test was also derived.

ETDRS-specific

The 9 ETDRS sectors were computationally generated and centered manually by the clinical examiner, on the fovea (using enface OCT-A) for each participant. In some instances, the macular region was not in the center of the enface $3 \times 3\,mm^2$ OCT-A scan. Hence, after manual adjustment of the ETDRS central positioning on the fovea, the outer ETDRS regions (ie, 6–9) could have been positioned outside of the imaging area. Therefore, in this study in which we were interested in assessing the role VD may play as a potential biomarker for aging and disease of the central macular, we only used ETDRS regions 1 to 5 for consistency in our analysis. In this process, the OCT-A signal is extracted and saved for each ETDRS region, superimposed on every OCT-A enface layer. In other words, all the $3 \times 3\,mm^2$ OCT-A layers through the retinal thickness are combined to create an OCT-A volume dataset, 920 of them per $3 \times 3\,mm^2$ scan, using

the Topcon DRI OCT. Finally, the ETDRS regions are manually applied to each of the 920 enface OCT-A images and OCT-A normalized data per region are extracted (Fig. 1). This method has been recently published.[15] The averaged normalized VD of the full thickness retina and choriocapillaris of each ETDRS region was then extracted from every participant for further analysis. Normalization was performed by converting the raw data to percentage of VD in all ETDRS regions, throughout the thickness of the retina.

SCP, DCP, and Choriocapillaris

Using the same ETDRS regions as above, the VD was extracted from layers 1 and 2 for the SCP calculation, layers 3 and 4 for the DCP, and layer 8 for the choriocapillaris. Averaged normalized VD from the SCP, DCP, and choriocapillaris was then extracted for each participant, in the 3 groups.

Retinal and Choroidal Thickness

In addition to OCT-A data, we also recorded the foveal thickness and subfoveal choroidal thickness from the OCT B-scans.

Statistical Analysis

MATLAB programming software and custom-written code was used to import and analyze the data, as detailed above. Statistical analysis was performed using MATLAB Statistics Toolbox. Due to unequal sample sizes between the sample groups, we performed the 2 sample nonparametric Kolmogorov and Smirnov test, which is one of the most general nonparametric methods for comparing 2 samples. The Kolmogorov and Smirnov method assumes that the data in both groups follow Gaussian distributions. Significant differences between groups were defined as $D < 0.565$ for the Kolmogorov and Smirnov test.

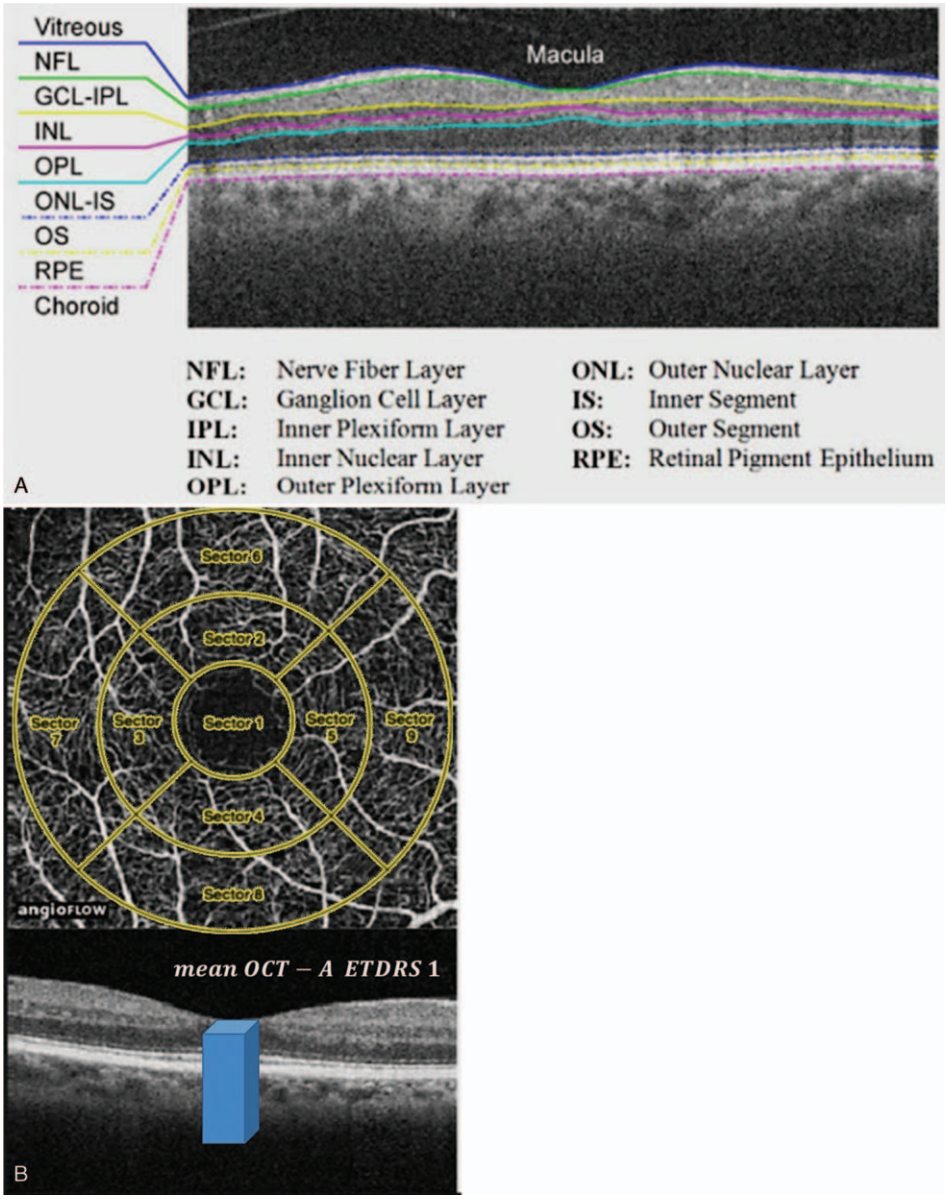

FIGURE 1. Retinal layers were automatically identified by IMAGENET 6 software (A), the SCP and DCP were then isolated from layers 1 and 2, and 3 and 4, respectively. ETDRS regions 1-9 are superimposed onto the enface OCT-A images and manually adjusted so that sector 1 was centered on the fovea. In this study we selected sectors 1-5 (B). ETDRS indicates Early Treatment Diabetic Retinopathy Study; DCP, deep capillary plexus; OCT-A, optical coherence tomography angiography; SCP, superficial capillary plexus.

TABLE 1. Mean Vessel Density From Full Retinal Thickness Per ETDRS Sector, for Each Group

ETDRS Sectors	Foveal % (1)	Superior % (2)	Nasal % (3)	Inferior % (4)	Temporal % (5)
Young healthy n = 20	7.5 + 6.6	18.1 + 7.3	16.0 + 7.0	20.5 ± 8.3	18.2 + 8.1
Old healthy n = 21	14.7 + 9.8	21.1 + 8.3	20.8 + 9.9	21.5. + 11	21.1 + 9.6
AMD n = 34	29.6 + 19.8	48.5 + 31.4	43.8 + 31.9	47.7 + 27.9	48.6 + 24.8

AMD indicates age-related macular degeneration; ETDRS, Early Treatment Diabetic Retinopathy Study.

RESULTS

Test-retest Variability Assessment

The intraclass correlation factor was found to be 0.993. The concordance correlation coefficient was found to be 0.989. The mean of the absolute difference between each test was 3.72 ± 2.57 μm.

Mean Age, Central Macular Thickness, and Foveal Avascular Zone Diameter

Seventy-five participants were included in this study of which 20 were in the YH group (23 ± 3 years old), 21 in the OH group, (65 ± 10 years old) and 34 in the AMD group (75 ± 8 years old). OCT data were segmented by IMAGENET 6.0 software, and the mean central macular thickness measurements for the 3 participating cohorts was extracted: YH, 217.4 ± 9.28 μm; OH, 209.8 ± 6.13 μm; AMD, 174.2 ± 20.3 μm. Horizontal diameter of the foveal avascular zone (FAZ) was also measured from each OCT and mean FAZ measurements were calculated: YH, 672.5 ± 73.4 μm; OH, 577.7 ± 98.8 μm; AMD, 639 ± 126.2 μm.

VD Measurements: Full Retinal Thickness

Table 1 shows the mean VD throughout the full retinal thickness, for each ETDRS sector. In all groups, the highest VD was found in sector 4, the inferior macula. Lowest VD in all groups was in sector 1 the foveal/parafoveal region. A statistical difference between the VD in Sector 1 was found between all the groups, with the VD being higher in the AMD cohort (29.6 ± 19.8) compared with the YH (7.5 ± 6.6) and OH groups (14.7 ± 9.68). Analysis of the VD of ETDRS sectors 2 to 5, revealed that there was a statistical difference in the VD between the AMD group and both the YH and OH groups, but not between the YH and OH groups.

VD measurements: SCP Segment

Table 2 shows the mean VD of the SCP segment within each ETDRS sector in each group. Although there was a trend towards the mean VD derived from the SCP in all ETDRS sectors being higher in the AMD cohort compared with the 2 healthy cohorts, no significant difference was found in the VD's recorded in any of the 5 ETDRS sectors between the 3 cohorts.

VD measurements: DCP Segment

Table 3 shows the mean VD of the DCP segment within each ETDRS sector for all groups.

The mean VD was found to be significantly higher (<0.01) in the DCP than the SCP in all ETDRS sectors and in all groups. Although there was a trend toward the mean VD derived from the DCP in ETDRS sector 1 being higher in the AMD cohort compared with the 2 "healthy" cohorts, the difference was not

TABLE 2. Mean Vessel Density in Superficial Capillary Plexus Defined in Sectors Based on the ETDRS Chart (Foveal, Superior, Nasal, Inferior, and Temporal)

ETDRS Sectors	Foveal % (1)	Superior % (2)	Nasal % (3)	Inferior % (4)	Temporal % (5)
Young healthy n = 20	19.8 ± 3.3	25.0 ± 4.7	24.5 + 4.2	27.8 + 4.8	25.5 + 4.8
Old healthy n = 21	21.3 ± 3.9	25.9. + 3.5	25.7 ± 4.1	26.7. + 4.9	24.6. + 5.0
AMD n = 34	25.4 ± 7.9	30.4 + 10.5	30.7 + 11.1	32.5 ± 11.0	32.0 + 8.5

AMD indicates age-related macular degeneration; ETDRS, Early Treatment Diabetic Retinopathy Study.

TABLE 3. Mean Vessel Density in Deep Capillary Plexus Defined in Sectors Based on the ETDRS Chart (Foveal, Superior, Nasal, Inferior, and Temporal)

ETDRS Sectors	Foveal % (1)	Superior % (2)	Nasal % (3)	Inferior % (4)	Temporal % (5)
Young healthy n = 20	27.0 + 12.9	62.3 ± 23.1	60.0 ± 20.1	64.8 ± 21.5	59.9 + 21.2
Old healthy n = 21	35.9 + 20.3	57.1 ± 19.9	56.3 ± 22.6	56.6 ± 22.1	54.1 + 24.8
AMD n = 34	43.8 + 31.6	55.0 + 44	53.9 ± 41.1	62.0 ± 50.1	55.6 + 40.6

AMD indicates age-related macular degeneration; ETDRS, Early Treatment Diabetic Retinopathy Study.

TABLE 4. Mean Vessel Density in the Choriocapillaris, Based on the ETDRS Chart (Foveal, Superior, Nasal, Inferior, and Temporal)

ETDRS Sectors	Foveal Ratio (1)	Superior Ratio (2)	Nasal Ratio (3)	Inferior Ratio (4)	Temporal Ratio (5)
Young healthy n = 20	97 + 10.5	86.2 + 10.7	90.8 + 11	103.8 + 9.8	103 + 11.3
Old healthy n = 21	97 + 4.5	85.5 + 8.5	83.9 + 9.5	86.3 + 10.5	80.1 + 12.8
AMD n = 34	71.4 + 24.3	68.1 + 23.8	69.7 + 24.4	68.4 + 22.7	73.1 + 19.4

AMD indicates age-related macular degeneration; ETDRS, Early Treatment Diabetic Retinopathy Study.

statistically significant. There was a trend for the mean VD derived from the DCP in the remaining ETDRS sectors: 2 to 5, to be higher in the YH cohort compared with the other 2 cohorts. This difference was not statistically significant.

VD measurements: Choriocapillaris Segment

Table 4 shows the mean VD of the choriocapillaris segment within each ETDRS sector for all groups. In contrast to the measurements recorded in the retinal segments, statistically significantly lower mean VDs were recorded in the choriocapillaris segment layer in the AMD group across all ETDRS sectors compared with the 2 healthy groups. There was no significant difference in the VDs measured in each of the ETDRS sectors between YH and the OH groups.

DISCUSSION

Although a number of changes within the aging macular have previously been reported, a reliable, noninvasive biomarker of both "healthy" macular aging and disease risk remains elusive. Retinal thickness, both at the fovea and extra-foveal regions, has previously been shown to differ significantly between individuals with early AMD and age-matched controls with the retina being thinner in individuals with disease.[16] The results of our study also revealed that there was a trend for the overall retinal thickness to thin with age, but this difference was not statistically significant between cohorts. It is also recognized that the choroid tends to thin with age, with the nasal area being thinnest, and the subfoveal region being thickest.[17] In addition to thinning with "normal aging," choroidal thinning has also been demonstrated in individuals with early AMD compared with age matched controls[16,18–20]; however, the significance of this observation is controversial as both the extent and the pattern of thinning is significant in some[20] but not all studies.[16,18,19] The histological evidence regarding choroidal thickness is also contradictory with some studies showing reduction in late AMD[21] and others showing no change[19]; moreover, the changes in the vasculature of the choriocapillaris in early AMD that have been reported from histological studies[19,21] seem to be independent of choroidal thickness.

Similarly inconclusive observations have been reported with respect to the width of the FAZ with age. Although some studies report no difference with age,[22,23] others have found that the FAZ enlarges with increasing age.[24,25] Where reported, the foveal avascular zone at the level of the SCP has been found to be significantly smaller in >60 years old compared with younger study groups,[14] but the significance of this difference is unknown and furthermore, no difference was observed when the FAZ at the level of the DCP was analyzed.[14] Recently, a study studying the FAZ in patients with intermediate nonexudative AMD found no significant difference in the width of the FAZ between patients with nonexudative AMD and healthy controls.[26] We likewise found there to be no statically significant difference in the diameter of the FAZ between any of the 3 cohorts studied.

Where objective measures of macular anatomy have failed, OCTA may provide an opportunity to develop a reliable, noninvasive vascular biomarker of the aging macular. The proprietary OCTA software now has a VD function which, in brief uses the proportion of bright pixels to the proportion of dark pixels to derive a measure of vascular density. In the first instance we reviewed the mean VD percentage throughout the full retinal thickness, for each ETDRS sector. We then used the segmentation function of the enface OCTA to explore the relationship of the vessel densities within each of the ETDRS sectors 1 to 5 in each of the 3 vascular zones of the central macular; (SCP, DCP, choriocapillaris) across the 3 cohorts: YH, OH, and AMD. As with any instrument, OCTA measurements will have a test retest variation, but currently there is no published literature informing us of the magnitude of this variability for the Triton OCTA. Thus, to ensure that differences between groups were "real," we had to first derive this statistic and the intraclass correlation factor and the concordance correlation coefficients, 2 such measurements that describe the accuracy of the test–retest reliability of a machine like OCTA. In our preliminary study both measured 0.99, a result, which indicates that the data produced by Triton OCTA is highly reproducible. The additional finding that the mean of the absolute difference between each repeat test was small (3.72 ± 2.57um) indicates that the OCTA is capable of both precisely and accurately measuring VD and thus the differences we record between groups are indeed "real."

As one might expect, the VDs derived from the relatively avascular ETDRS sector 1 were, in the full thickness maps, significantly reduced compared with the VDs derived from ETDRS sectors 2 to 5 in the YH, OH, and AMD cohorts. However, and curiously, the VD recorded in the AMD cohort was significantly higher than in the 2 "healthy" cohorts, and although there was a trend for the VDs derived from the YH cohort to be reduced compared with the OH cohort, this difference was not significant. Aside the marked difference between the AMD cohort and the other 2 "healthy" cohorts, no other clinically relevant or statistically significant patterns were noted in the VDs derived from the full thickness retinal maps.

Overall, and when the retinal vascular complexes were analyzed as separate segments, the highest vessel densities were found in sectors 2 and 4; the superior and inferior macula. Again the VDs derived from ETDRS sector 1 were greater in the AMD

cohort compared with the 2 "healthy" cohorts. Although there was a trend for the VDs derived from the OH cohort to be lower compared with the YH cohort, this difference was not significant. The spatial variation in VDs that we observed; ie, a higher mean VD in the inferior and superior ETDRS sectors and a lower mean VD in the temporal and nasal sectors, in both the deep and superficial capillary plexi, is consistent with published data from previous studies.[14,22,23] Like others,[14,27] we also observed that in all ETDRS sectors and in all 3 study groups, the mean VD derived from the DCP was significantly higher than that derived from the SCP.

The association between age and VD remains unclear, with reports of statistically significant lower mean VD in SCP, DCP, and full thickness of retina in each ETDRS sector in patients >60 years old compared with younger cohorts[14]; however, others who have studied this have found no such correlation.[22] On the whole, our results do not further our understanding of VD changes with age, being similarly inconclusive. On the basis of these data, one has to conclude that without further work, VDs derived from the retinal segments are not a reliable biomarker for "healthy" aging.

One consistent feature of the VDs derived from the retinal segments in the present study was the finding that the VDs recorded from ETDRS sector 1, and indeed in many other ETDRS sectors in the AMD cohort, were as a group consistently higher than those obtained from the "healthy" cohorts. One possible explanation for this surprising result is the technical challenges of obtaining high quality OCT-A scans from individuals with even modest visual impairment as accurate central fixation during the OCT-A capture can be challenging. Many of the patients in our AMD cohort struggled to maintain fixation during the image acquisition stage, despite the majority recording a Snellen acuity of 6/12. Increased acquisition time and the resultant compromise in image quality means there is a higher likelihood of projection and banding artefacts.[28] Motion artefacts can also lead to the duplication of inner retinal vascular structures.[29] As VD is derived from the percentage of the area occupied by bright pixels in a segmented area, errors such as vessel duplication and banding artefacts could be incorrectly interpreted during computational analysis as increased VD within the image. Such an error would be magnified in ETDRS sector 1 where the effects of these banding and motion artefacts would be more pronounced in what should be avascular tissue.

Although the proprietary OCTA software to assess VD is designed to measure the inner retinal plexi, a vascular bed which has a very different architecture to the choriocapillaris,[30] we were curious to know whether the principle of VD analysis could be used to assess the vascularity of the choriocapillaris. We therefore extracted the raw data, and then used a manual analysis method previously published in the literature,[15] to assess the "vascular density" of the choriocapillaris segment. Even allowing for the influence of banding and motion artefacts, issues which should increase VD measurements, we observed a significant reduction in the VD in both the AMD and OH cohorts compared with the YH cohort with the reduction in VD being most marked in the AMD cohort (Table 4). This contrasts sharply with the data derived from the retinal plexi, which broadly showed no difference between groups. Although interesting, one has to interpret these findings with caution because, to the best of our knowledge, VD analysis has not previously been used to assess the choriocapillaris. Currently, it is therefore not possible to corroborate these findings. However, a global reduction in choriocapillaris density, associated with drusen on OCT-A in dry AMD, has been demonstrated previously.[6] It is also recognized that the inner choroid and the choriocapillaris disproportionally thin with age and disease.[31] It is feasible that a thinning choriocapillaris would be less vascular and other groups have hypothesized that choroidal perfusion changes on the OCT-A may predict disease progression.[6] Debate about whether this is related to actual flow impairment[6] or projection artefact "masking" the choriocapilaris[29,32] due to large drusen or pigment epithelial detachments attenuating the OCT-A signal is still ongoing. Without further corroborative work, the importance of our finding that there is a progressive reduction in choriocapillaris VD in age and disease is difficult to assess. Furthermore, because our OH group and AMD cohorts are not age-matched, it is not possible to comment on whether any observed reduction is simply an aging phenomenon or a marker of disease. Nevertheless, if reproducible, such a measure has the potential to provide a noninvasive biomarker of both "healthy" macular aging and disease risk. Further limitations of this study include a small sample size, which might reduce the generalizability of our findings and the interpretation of the statistical significance of our data. Furthermore, the Kolmogorov and Smirnov statistical test assumes the Gaussian distribution of the tested data.

CONCLUSIONS

OCT-A mean retinal VD results for normal subjects are in line with the existing literature. Challenges of image acquisition and the significant influence of image artefact on post-acquisition analysis of patients with mild visual loss due to intermediate AMD further limits the value of VD measurements in this patient group. We believe that our data suggest that traditional measures of VD, derived from the retinal plexi, are therefore unreliable biomarkers for both "healthy" aging and quantifying risk of developing AMD. In contrast, our nonproprietary analysis of the vascular density of the choriocapillaris revealed a significant drop off of VD with age and disease. Although further work is required to corroborate this finding, if repeatable, choriocapillaris VD may provide a noninvasive biomarker of healthy aging and disease.

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Data Availability

The ophthalmic imaging data used to support the findings of this study were obtained under appropriate approval by The University of Auckland Ethics Committee and so cannot be made freely available. Requests for access to these data should be made to the corresponding author Dr Ehsan Vaghefi e.vaghefi@auckland.ac.nz, which will be then passed on The University of Auckland Ethics Committee for further process.

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