Changes in the Radial Peri-papillary Capillaries (RPCs) in human retina during ‘physiological aging’: the forgotten vascular bed in glaucoma pathogenesis
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Purpose: To characterize the structure, distribution, caliber & glial ensheathment of radial peri-papillary capillaries (RPCs) in human retina & their changes during ‘physiological ageing’.

Methods: A total of 53 human eyes including 1 young (aged 9) and 52 adults (aged 17-84) were examined. Multiple marker immunohistochemistry was performed with markers CD34, CD39, GFAP, NG2, α-SMA, S-100b, NF, Neu-N and UEA-1 Lectin to examine vascular density, astrocyte & pericyte ensheathment, RPC distribution, vascular branching patterns and CD39 expression levels. RPCs ultrastructure was examined using TEM.

Results: RPCs are located within 6μm of the inner limiting membrane and have unique ultrastructural features including thin basal lamina, sparse astrocyte & pericyte ensheathment, and importantly, luminal diameter of 4-5μm as compared to capillaries in the superficial vascular plexus. The outer limit of RPCs extended significantly further than previously reported by Henkind (1967) & showed a higher anastomosis, suggesting higher perfusion efficiency in healthy young adults. Mapping of the outer limit of the RPCs & quantitative morphological analysis in 48 adult retinas showed that their extent, caliber & vessel density decreased significantly with age. CD39 expression intensity & vascular density were also found to decrease. Importantly, not only did the extent & density of RPCs decrease with age, many RPC segments showed marked decrease in caliber with some segments appearing collapsed, suggestive of a compromised rheology. Vessel caliber showed a decrease from 7-7.5μm in healthy young adults to 6μm in the 8th decade.

Conclusions: The superficial location and thin basal lamina of RPCs make them susceptible to lumen collapse under high intraocular pressure, while their restriction to the nerve fiber layer makes them responsible for the metabolic needs of the ganglion cell axons, making their functional capacity particularly relevant to glaucoma pathogenesis. Further, CD39 plays a major role in ATP-dependent processes as well as preventing thrombotic & inflammatory processes. The significant reduction in CD39 expression will further compromise RPCs function during ‘physiological ageing’. These findings are supportive of the utility of quantitative analysis of RPCs as an indicator of glaucoma predisposition, requiring further study in glaucomatous population.

Commercial Relationships: Tailoi Chan-Ling, None; Samyoul Ahn, None; Mark Koina, None; Mohammad Nasir Uddin, None; Ted Maddess, None; Samuel Adamson, None; Marconi Barbosa, None
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Investigation of optic nerve head microvascular changes in primary open angle glaucoma and chronic angle closure glaucoma using OCT-angiography
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Purpose: To identify optic nerve head (ONH) microvascular abnormalities by OCT-angiography (OCT-A) in patients with primary open angle glaucoma (POAG) or chronic angle closure glaucoma (CAGC), compared to controls without significant ocular pathology. Previous studies have reported reductions in the flow index and vascular density within the optic nerve head of patients with POAG. However, it is not known whether similar changes are present in patients with CAGC.

Methods: Patients with POAG or CAGC and reproducible VF loss, as well as control subjects, were recruited for a prospective, cross-sectional study. One eye per subject was included. Angiograms were generated from swept source OCT (Topcon) covering a 3x3mm region centered on the ONH, based on a previously described SSSA algorithm. Blood vessels were quantified by generating en face images from the internal limiting membrane (ILM) to a depth of 130μm (superficial vasculature) or from 130-390μm below the ILM (deep vasculature). The ONH area for quantification was defined based on Bruch’s membrane opening.

Results: Preliminary data are presented for 5 control, 5 POAG and 4 CAGC patients. All groups were similar in age (mean age 69.7±2.5 overall; Table 1A). Patients with POAG and CAGC had similar Humphrey Visual Field mean deviations (mean -5.21±1.73dB overall), patients with POAG and CAGC had similar Humphrey Visual Field mean deviations (mean -5.21±1.73dB overall). The superficial ONH flow index was reduced by 16% in POAG patients in comparison to control patients (p=0.008), and vascular density was reduced by 7.8% (p=0.04) consistent with previously published studies (Figure 1; Table 1B-C). In contrast, there was no significant reduction in either ONH flow index or vascular density in CAGC patients relative to control patients, and measurements for these two groups were within 2% of each other. The comparison between POAG and CAGC groups showed reduced superficial ONH flow index (p=0.03) and vascular density (p=0.05) in POAG patients. Notably, no significant differences in the deep ONH vasculature were identified across the three groups.

Conclusions: Our preliminary results suggest that POAG might be associated with more pathology of the ONH microvasculature than CAGC. These differences highlight that distinct pathophysiologic processes underlie damage to the optic nerve in POAG and CAGC. Further enrollment is ongoing to verify these findings.

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Peripapillary Capillary Density in Anterior Ischemic Optic Neuropathy Compared to that in Severe Primary Open-Angle Glaucoma
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Purpose: Non-arteritic anterior ischemic optic neuropathy (NAION) and primary open-angle glaucoma (POAG) both damage retinal ganglion cell axons, which are perfused by the radial peripapillary capillaries. To further evaluate the ischemic nature of NAION, we compared peripapillary capillary density (PCD) in NAION eyes to POAG eyes matched for visual field mean deviation (MD) and retinal nerve fiber layer thickness (RNFLT).
Methods: 31 chronic NAION (>6 months after acute event) and unaffected fellow eyes (31 subjects) 30 severe POAG eyes (20 subjects), and 77 control eyes (46 healthy subjects) were imaged on a commercial optical coherence tomography angiography system (AngioVue, Avanti RTVue-XR, Optovue, CA), producing 4.5mm×4.5mm images centered on the optic nerve head. Two concentric circles of diameters 1.95mm (inner) and 3.45mm (outer) were manually placed, producing an annular region-of-interest of width 0.75mm. Image analysis with major vessel removal was performed using a custom MATLAB program (The Mathworks, Inc., Natick, MA). Whole-image PCD, whole-anulus PCD, and sectoral PCD were measured. A linear mixed model was used to compare PCD among groups and account for inter-eye correlation and multiple comparisons.

Results: Whole-image and whole-anulus PCD in NAION (30.1±6.0% and 29.7±6.5%, respectively) and severe POAG (28.9±4.8% and 29.7±5.2%, respectively) eyes were significantly decreased compared to unaffected fellow eyes (41.6±4.5 and 42.3±4.3%) and control eyes (42.3±2.3% and 43.9±2.1%) (all P<0.001). Whole-image and whole-anulus PCD were not statistically different between NAION and severe POAG eyes (both P=0.99). However, POAG eyes exhibited greater superior PCD loss compared to NAION eyes (P<0.01). In contrast, NAION eyes exhibited greater inferior PCD loss compared to POAG eyes (P<0.01). In all study eyes, whole-image PCD was significantly correlated to both MD and RNFLT (r=0.79 and r=0.72, respectively, both P<0.001).

Conclusions: Whole-image and annular PCD were affected to similar degrees in chronic NAION and severe POAG, suggesting that independent of the type of optic nerve damage, PCD loss may mirror retinal ganglion cell axonal damage.
Commercial Relationships: Masoud A. Fard, None;
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Presentation Time: 4:30 PM–4:45 PM
Radial Peripapillary Capillary Plexus Perfusion and Regional Visual Field Loss in Glaucoma
Liang Liu, Yali Jia, Ou Tan, JIE WANG, Beth Edmunds, Hana L. Takusagawa, Mansi Parikh, John C. Morrison, David Huang - casey eye institute, Oregon Health & Science University, Portland, OR.

Purpose: To analyze the relationship between radial peripapillary capillaryplexus (RPCP) capillary density and visual field (VF) in glaucoma using optical coherence tomography angiography (OCTA).

Methods: 41 primary open angle glaucoma (POAG) and 38 age-matched normal participants were analyzed in this observational study. One eye of each participant was imaged by 4.5×4.5 mm peripapillary OCTA scan using the AngioVue (Optovue, Inc.). The RPCP en face angiogram, which was obtained by the maximum flow projection in retinal nerve fiber layer (NFL), was divided into 8 sectors that follow the trajectory of nerve fibers and corresponds to a VF sectoral division scheme (Fig.1). The capillary density (CD), defined as the percentage area occupied by flow pixels after excluding large vessels, was calculated from a 4×4 mm square excluding optic disc using a reflectance-compensated algorithm. RPCP CD and retinal NFL thickness were converted to a percent loss relative to the normal average. The VF total deviation dB values at each sector were converted to percent loss (% loss = 100*(1-10^{-\text{dB/10}})).

Results: The participants in glaucoma group were 64±10 years old, with VF MD -5.5±4.4 dB. The overall average loss for VF, RPCP and NFL were 38%±28%, 34%±21%, and 29%±16%, respectively. Focal capillary dropout could be visualized in the RPCP angiogram of glaucomatous eyes (Fig. 1). Sector 4 had the greatest mean percent loss for all parameters. VF was highly correlated with both NFL thickness and RPCP CD in sector 4 (Fig. 2A, 2B, linear regression, the intercept was fixed at origin). In other sectors, RPCP CD correlated with VF from 0.408 to 0.816 (Pearson r, all P<0.008) (Fig. 2).

Conclusions: There is excellent correlation between RPCP, NFL, and VF loss when measured in corresponding sectors and converted to the same linear scale. Structural and angiographic OCT parameters may be useful in assessing the location and severity of glaucoma damage.

Figure 1. Map of corresponding sectors between RPCP capillary density (CD) loss and VF sensitivity loss in a glaucomatous eye.

Figure 2. Relationship between VF loss, NFL thickness and RPCP capillary density in each sector. Table cells display Pearson’s r (P value). Individual POAG eye values were plotted for sector 4, which had the worst damage.

Commercial Relationships: Liang Liu, None; Yali Jia, Optovue (P); Ou Tan, None; JIE WANG, None; Beth Edmunds, None; Hana L. Takusagawa, None; Mansi Parikh, None; John C. Morrison, None; David Huang, Optovue (I), Optovue (F), Optovue (P)

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Presentation Time: 4:45 PM–5:00 PM
Measurement of macular capillary network in glaucoma: An optical coherence tomography angiography (OCT-A) study
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Purpose: Retinal vascular abnormalities have been implicated in glaucoma although less is known about its association with retinal ganglion cell degeneration. This study compared (1) the diagnostic performance between vessel density (VD) of the superficial capillaryplexus and ganglion cell inner plexiform layer (GCIP) thickness measured at the macula for detection of glaucoma and (2) their association with visual function.

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**Methods:** OCT-A (Triton OCT) of the macula, OCT macular imaging (Cirrus HD-OCT) and standard automated perimetry were performed in 147 eyes from 96 glaucoma patients, 26 pre-perimetric glaucoma subjects, and 25 healthy participants. VD of the superficial capillary plexus and GCIPL thickness were measured (Fig. 1). The diagnostic performance for detection of glaucoma was determined by the area under receiver operating characteristic curve (AUC). The structure function association was evaluated with linear regression analysis. Bootstrap resampling was performed to compare the strength of association between visual sensitivity and GCIPL thickness/VD at the macula (3x3mm²).

**Results:** VD was smaller in glaucomatous eyes compared with healthy eyes (p<0.001) although there were no significant differences in VD between eyes with pre-perimetric glaucoma and healthy (p=0.498) or glaucoma (p=0.071) (Fig. 1). This is in contrast to GCIPL thickness in which significant differences were detected not only between the glaucomatous and normal eyes but also between eyes with pre-perimetric glaucoma and healthy or glaucomatous eyes (p≤0.001). The AUC (±SD) for discriminating glaucomatous/ pre-perimetric glaucomatous eyes from healthy eyes was highest for GCIPL thickness (0.96±0.03/0.74±0.16), followed by VD (0.66±0.08/0.50±0.14). The strength of association between GCIPL thickness and visual sensitivity (R²=0.51) was stronger than that between VD and visual sensitivity (R²=0.20) (p<0.001).

**Conclusions:** Macular GCIPL thickness had a higher diagnostic performance for discrimination between normal eyes and eyes with glaucoma/pre-perimetric glaucoma and demonstrated a stronger association with visual function compared with VD measurement at the corresponding location. Our finding suggests that loss in the superficial macular capillary network in glaucoma is likely to be consequential to retinal ganglion cell loss.

VD maps (%, upper panel) and GCIPL thickness maps (μm, lower panel).

**Commercial Relationships:** Kelvin H. Wan, None; Alexander Lam, None; Christopher K. Leung, Alcon (R), Santen (R), Merck (R), Oculus (F), Alcon (C), Carl Zeiss Meditec (F), Glaukos (F), Carl Zeiss Meditec (P), Allergan (R), Optovue (F), Global Vision (R), Tomey (F), Tomey (R), Novartis (R), Lumenis (R), Topcon (F), Allergan (C)

**Program Number:** 3396

**Presentation Time:** 5:00 PM–5:15 PM

**The Rate of Macular Microvascular Dropout is Faster in Glaucoma Eyes than Glaucoma Suspect and Healthy Eyes: A Longitudinal Study**

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**Purpose:** Decline in retinal microvasculature is suspected to be a key factor in the mechanisms underlying glaucomatous disease progression and can now be approximated non-invasively using optical coherence tomography angiography (OCT-A) measurements of vessel density. This study aimed to evaluate the association between glaucoma status and rate of change in macula vessel density measurements using OCT-A.

**Methods:** A total of 83 eyes from 22 healthy participants, 30 glaucoma suspects and 31 glaucoma patients enrolled in the Diagnostic Innovations in Glaucoma Study who had at least two visits and 12 months of follow up with OCT-A (Angiovue; Optovue, Fremont, CA) imaging were included. Vessel density was summarized as whole, superior and inferior en face image regions of the superficial macula. The rate of change in OCT-A vessel density was compared across diagnostic groups using generalized estimating equations (GEE) in this study.

**Results:** All three diagnostic groups were followed for between 2.9-3.9 visits over 13 to 14 months on average. Baseline macular vessel density was higher in normal eyes followed by glaucoma suspects and glaucoma eyes for whole (52.3%, 48.9%, and 47.8%, respectively), superior (53.0%, 50.1%, and 48.5%, respectively) and inferior regions (52.6%, 49.7%, and 48.2%, respectively) (P<0.001 for all). In univariate analysis, vessel density was significantly associated with age, diagnostic category, and SSI (all P<0.05) for all regions. In multivariate analysis, the mean (95% CI) rate of macular whole enface vessel density change was faster in glaucoma eyes compared to glaucoma suspects (-2.44%/yr [-1.08% to -3.80%/yr]; p<0.001) and healthy eyes (-2.95%/yr [-0.93% to -4.97%/yr]; p=0.004). Glaucoma eyes also had a significantly faster rate of vessel density dropout than glaucoma suspect and healthy eyes in the inferior region (p=0.002 and p<0.001) and superior region (p=0.001 and p=0.083).

**Conclusions:** In this study with a short follow-up time, differences in significant macular vessel density change between groups were detected, with the rate of dropout faster in glaucoma patients compared to glaucoma suspect and healthy subjects.

**Commercial Relationships:** Takuehi Shoji, Linda M. Zangwill, Optovue Inc (F), Heidelberg Engineering (F), National Eye Institute (F), Topcon Medical Systems Inc. (F), Carl Zeiss Meditec Inc. (F); Tadamichi Akagi, Alcon (R), Senju (R), Santen (R), Pfizer (R), Kowa (R); Luke J. Saunders, None; Adeleh Yarmohammadi, None; Patricia Isabel C. Manalastas, None; Rafaela C. Penteado, None; Felipe Medeiros, Alcon (R), Reichert (R), Sensimed (F), Allergan (F), Carl Zeiss Meditec (F), Allergan (R), Carl Zeiss Meditec Inc. (C), Bausch & Lomb (F), Novartis (C), Heidelberg Engineering (F), Carl Zeiss Meditec (R), Reichert (F), Merck (F), Alcon (F), Topcon (F), National Eye Institute (R), Allergan (C); Robert N. Weinreb, Aerie Pharmaceutical (C), Heidelberg Engineering (F), Alcon (C), Carl Zeiss Meditec (F), Quark (F),
Live imaging of retinal pericytes: evidence for early calcium uptake, capillary constriction and vascular dysregulation in ocular hypertension glaucoma

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Purpose: Pericytes are contractile cells that wrap along the walls of capillaries. In the brain, pericytes play a crucial role in the regulation of capillary diameter and vascular blood flow in response to metabolic demand. The contribution of pericytes to microvascular deficits in glaucoma is currently unknown. To address this, we used two-photon excitation microscopy for longitudinal monitoring of retinal pericytes and capillaries in a mouse glaucoma model.

Methods: Ocular hypertension was induced by injection of magnetic microbeads into the anterior chamber of albino mice expressing red fluorescent protein selectively in pericytes (NG2-DsRed). Minimally invasive, multiphoton imaging through the sclera of live NG2-DsRed mice was used to visualize pericytes and capillary diameter at one, two and three weeks after glaucoma induction. In vivo fluctuations in pericyte intracellular calcium were monitored with the calcium indicator Fluo-4. Ex vivo stereological analysis of retinal tissue prior to and after injection of microbeads was used to confirm our in vivo findings.

Results: Live two-photon imaging of NG2-DsRed retinas demonstrated that ocular hypertension induced progressive accumulation of intracellular calcium in pericytes. Calcium uptake correlated directly with the narrowing of capillaries in the superficial, inner, and outer vascular plexuses (capillary diameter: naïve control=4.7±0.1 μm, glaucoma=4.0±0.1 μm, n=5-6 mice/group, Student’s t-test p<0.05). Frequency distribution analysis showed a substantial increase in the number of small-diameter capillaries (≤ 3 μm) and a decrease in larger-diameter microvesels (≥ 5-9 μm) at three weeks after induction of ocular hypertension (n=5-6 mice/group, Student’s t-test p<0.05).

Conclusions: Our data support two main conclusions. First, two-photon excitation microscopy is an effective strategy to monitor longitudinal changes in retinal pericytes and capillaries in live animals at glaucoma onset and progression. Second, ocular hypertension triggers rapid intracellular calcium increase in retinal pericytes leading to substantial capillary constriction. This study identifies retinal pericytes as important mediators of early microvascular dysfunction in glaucoma.

Clinical Trial: NCT00221897

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