365 Capillaries, Blood Flow, OCT Angiography
Tuesday, May 09, 2017 3:45 PM–5:30 PM
Ballroom 4 Paper Session
Program #/Board # Range: 3391–3397
Organizing Section: Glaucoma

Program Number: 3391
Presentation Time: 3:45 PM–4:00 PM
Changes in the Radial Peri-papillary Capillaries (RPCs) in human retina during 'physiological aging': the forgotten vascular bed in glaucoma pathogenesis
Tailoi Chan-Ling[1], Samyoul Ahn[1], Mark Koina[2], Mohammad Nasir Uddin[1], Ted Maddess[3], Samuel Adamson[1], Marconi Barbosa[3].
[1]Department of Anatomy & Histology, University of Sydney, Sydney, NSW, Australia; [2]ACT Pathology, Garran, ACT, Australia; [3]Diagnostics for Eye Diseases Group, Australian National University, Canberra, NSW, Australia.
Purpose: To characterize the structure, distribution, caliber & glial ensheathment of radial peri-papillary capillaries (RPCs) in human retina & their changes during 'physiological aging'.
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 ensheathement, 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 aging'. 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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Program Number: 3392
Presentation Time: 4:00 PM–4:15 PM
Investigation of optic nerve head microvascular changes in primary open angle glaucoma and chronic angle closure glaucoma using OCT-angiography
Luciano C. Custo Greig[1], Kaikai Qiu[1, 2], Soumya Awasthi[1], John B. Miller[1], Stacey Brauner[1], Scott H. Greenstein[1], Angela V. Turalba[1], Louis R. Pasquale[1, 3], Lucy Q. Shen[1].
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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 (CACG), 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 CACG.
Methods: Patients with POAG or CACG 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 SSDA 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 CACG patients. All groups were similar in age (mean age 69.7±2.5 overall; Table 1A). Patients with POAG and CACG 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 CACG patients relative to control patients, and measurements for these two groups were within 2% of each other. The comparison between POAG and CACG 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 CACG. These differences highlight that distinct pathophysiologic processes underlie damage to the optic nerve in POAG and CACG. Further enrollment is ongoing to verify these findings.

A. Baseline patient characeristics			
	Control (N=5)	POAG (N=5)	CACG (N=4)
Age (years)	73.4 ± 3.8	67.6 ± 2.7	67 ± 6.3
Ethnicity (% white)	100.0	100.0	75.0
Best corrected visual acuity (logMAR)	0.019 ± 0.022	0.035 ± 0.039	0.044 ±0.050
Intraocular pressure (mm Hg)	13.8 ± 1.6	13.8 ± 0.9	12.5 ± 2.1
Cup-to-disc ratio	0.34 ± 0.03	0.8 ± 0.04	0.68 ± 0.05
Mean deviation (dB)		-4.66 ± 4.18	-6.34 ± 1.79
Pattern standard deviation (dB)		7.38 ± 2.78	4.95 ± 1.40
B. Quantification of ONH microvasculature			
	Control N=5	POAG N=5	CACG N=4
Flow Index (normalized to control) - Superficial Vasculature (ILM-130um)	1 ± 0.019	0.84 ± 0.061	0.98 ± 0.039
Flow Index (normalized to control) - Deep Vasculature (130-390um)	1 ± 0.019	0.99 ± 0.024	1.02 ± 0.070
Vessel Density (%) - Superficial Vasculature (ILM-130um)	65.9 ± 1.41	60.7 ± 2.14	66.6 ± 2.00
Vessel Density (%) - Deep Vasculature (130-390um)	90.7 ± 1.39	87.0 ± 1.76	88.1 ± 2.60
C. Statistical analysis (t-test)			
	Control vs POAG (p-value)	Control vs CACG (p-value)	POAG vs CACG (p-value)
Flow Index (normalized to control) - Superficial Vasculature (ILM-130um)	**0.008***	0.344	**0.034**
Flow Index (normalized to control) - Deep Vasculature (130-390um)	0.350	0.367	0.327
Vessel Density (%) - Superficial Vasculature (ILM-130um)	**0.037**	0.388	**0.046**
Vessel Density (%) - Deep Vasculature (130-390um)	0.071	0.196	0.366

Table 1: A. Baseline patient characteristics. Values are expressed as mean value ± standard error of the mean. **B. Quantification of ONH microvasculature.** Flow index data are normalized to control eyes. Vessel density was obtained by applying the same threshold across all images, and the percentage of the ONH *en face* projection occupied by blood vessels was then calculated. Mean values ± standard error of the mean are provided for each group. **C. Statistical analyses.** Unpaired two-tailed t-tests were carried out, with a significance threshold of $p < 0.05$. Comparisons that met significance threshold are shown in bold.
* significant with Bonferroni correction

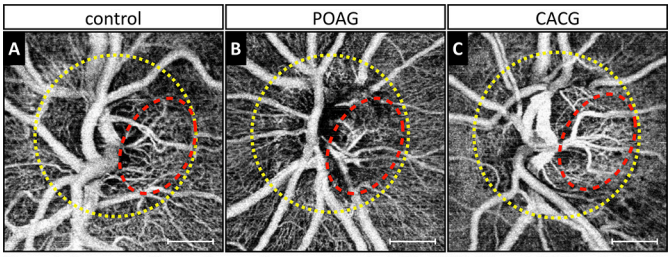

Figure 1: Representative *en face* angiograms of control (A), POAG (B), and CACG patients (C). Images were obtained by collecting data from the ILM to a depth of 260µm and generating maximal intensity projections. Dashed circled indicates ONH (yellow). Dashed ellipse indicates temporal disc area (red), where microvascular changes can best be appreciated. There is substantial attenuation of blood vessels in the POAG patient, but not the CACG patient, relative to the control patient. Scale bars are 500µm.

Commercial Relationships: Luciano C. Custo Greig, None; **Kaikai Qiu**, None; **Soumya Awasthi**, None; **John B. Miller**, None; **Stacey Brauner**, None; **Scott H. Greenstein**, None; **Angela V. Turalba**, None; **Louis R. Pasquale**, None; **Lucy Q. Shen**, None

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Program Number: 3393
Presentation Time: 4:15 PM–4:30 PM

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-annulus 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-annulus 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±5.0) and control eyes (42.3±2.3% and 43.9±2.1%) (all P<0.001). Whole-image and whole-annulus 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 PCD 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.

	AION	Fellow Eye	POAG	Control	*P1*	*P2*	*P3*
Age, yrs.	54.1±11	-	58.4±8.4	58.4±10.3	-	>0.99	0.6
Gender, female:male	15:16	-	10:20	55:22	-	<0.001	<0.001
Mean deviation, dB(µm)	-18.4±8.6	-1.0±2.7	-20.9±7.3	-0.6±1.7	<0.001	<0.001	0.4
RNFLT, µm(µm)	59±16	102±11	63±15	98±10	<0.001	<0.001	>0.99
Superior PCD, %	32.5±6.8	42.9±4.3	27.9±7.6	43.7±2.2	<0.001	<0.001	<0.01
Inferior PCD, %	31.2±7.6	42.6±4.5	35.5±7.2	43.9±2.2	<0.001	<0.001	<0.01
Nasal PCD, %	26.8±9.3	41.4±7.0	29.3±7.6	43.3±2.7	<0.001	<0.001	0.37
Temporal PCD, %	28.2±8.4	42.5±5	26.1±8.1	44.6±2.2	<0.001	<0.001	>0.99

Table. Clinical characteristics and results of testing. P1, comparison of AION & fellow eyes; P2, comparison of POAG & control; P3, comparison of POAG & AION.

Commercial Relationships: Masoud A. Fard, None; **Sasan Moghimi**, None; **Yanin Suwan**, None; **Lawrence Geyman**, None; **Toco Y. Chui**, None; **Richard B. Rosen**, NanoRetina (C), Carl Zeiss Meditech (C), Marrus Family Foundation, Bendheim-Lowenstein Family Foundation, Wise Family Foundation, New York Eye and Ear Chairman's Research Fund, Violett Fund, and Milbank Foundation (F), Advanced Ocular Technologies (C), Opticology (I), Clarity (C), ODOS (C), Regeneron (C), Genentech (F), Optovue (C), Allergan (C); **Robert Ritch**, The Ablon Family Research Fund of the New York Glaucoma Research (F), Sabatine Foundation Research Fund of the New York Glaucoma Research Institute, New York, NY (F)

Support: Dr.Rosen: Marrus Family Foundation, Bendheim-Lowenstein Family Foundation, Wise Family Foundation, New York Eye and Ear Chairman's Research Fund, Violett Fund, and Milbank Foundation

Program Number: 3394
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 capillary plexus (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[$^{dB/10}$])).
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.

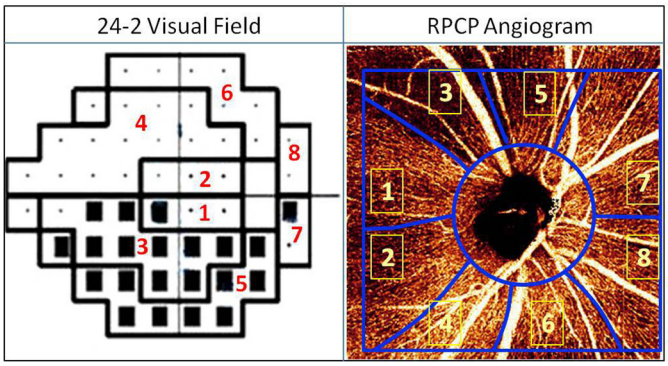

Figure 1. Map of corresponding sectors between RPCP capillary density (CD) loss and VF sensitivity loss in a glaucomatous eye.

	VF % loss							
Sector	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*
RPCP CD	0.408	0.501	0.816	0.772	0.689	0.552	0.455	0.411
% loss	(0.008)	(0.001)	(<0.001)	(<0.001)	(<0.001)	(<0.001)	(0.003)	(0.008)
RNFL thickness	0.244	0.403	0.744	0.732	0.581	0.441	0.343	0.292
% loss	(0.124)	(0.009)	(<0.001)	(<0.001)	(<0.001)	(0.004)	(0.028)	(0.064)

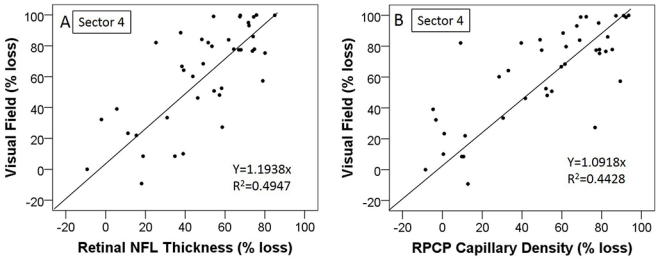

Figure 2. Relationship between VF loss, NFL thickness and RPCP capillary density in each sector. Table cells display Pearson's r (P value). Individual POAG eye values were plotted for sector 4, which had the worst damage.

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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Clinical Trial: NCT01957267

Program Number: 3395
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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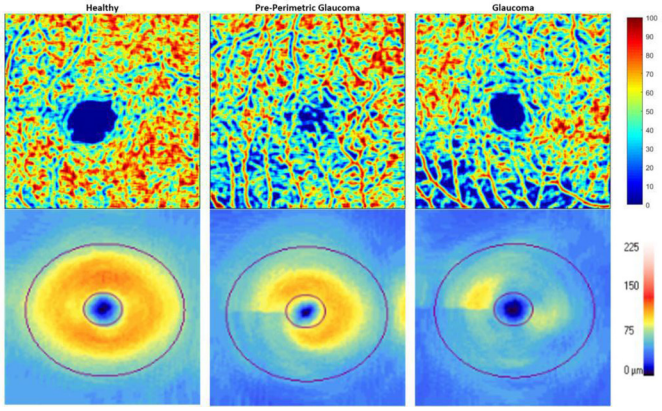
Purpose: Retinal vascular abnormalities have been implicated in glaucoma although less is known about its association with retinal ganglion cell degeneration. This study compared (1) the diagnostic performance between vessel density (VD) of the superficial capillary plexus and ganglion cell inner plexiform layer (GCIPL) thickness measured at the macula for detection of glaucoma and (2) their association with visual function.

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 (3x3mm^2).
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 ($p\leq0.001$). 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 ($R^2=0.51$) was stronger than that between VD and visual sensitivity ($R^2=0.20$) ($p<0.001$).
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
Takuhei Shoji[1, 2], Linda M. Zangwill[1], Tadamichi Akagi[1, 3], Luke J. Saunders[1], Adeleh Yarmohammadi[1], Patricia Isabel C. Manalastas[1], Rafaella C. Penteado[1], Felipe Medeiros[1], Robert N. Weinreb[1]. [1]Hamilton Glaucoma Center, Department of Ophthalmology and Shiley Eye Institute, University of California, San Diego, San Diego, CA; [2]Department of Ophthalmology, Saitama Medical University, Iruma, Japan; [3]Department of Ophthalmology and Visual Sciences, Kyoto University Graduate School of Medicine, Kyoto, Japan.
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: Takuhei 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; **Rafaella 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),

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Clinical Trial: NCT00221897

Program Number: 3397
Presentation Time: 5:15 PM–5:30 PM

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 viv*o 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.

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