

# Retinal vasculature in glaucoma: a review

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## ABSTRACT

Despite the critical impact of glaucoma on global blindness, its aetiology is not fully characterised. Elevated intraocular pressure is highly associated with glaucomatous optic neuropathy. However, visual field loss still progresses in some patients with normal or even low intraocular pressure. Vascular factors have been suggested to play a role in glaucoma development, based on numerous studies showing associations of glaucoma with blood pressure, ocular perfusion pressure, vasospasm, cardiovascular disease and ocular blood flow. As the retinal vasculature is the only part of the human circulation that readily allows non-invasive visualisation of the microcirculation, a number of quantitative retinal vascular parameters measured from retinal photographs using computer software (eg, calibre, fractal dimension, tortuosity and branching angle) are currently being explored for any association with glaucoma and its progression. Several population-based and clinical studies have reported that changes in retinal vasculature (eg, retinal arteriolar narrowing and decreased fractal dimension) are associated with optic nerve damage and glaucoma, supporting the vascular theory of glaucoma pathogenesis. This review summarises recent findings on the relationships between quantitatively measured structural retinal vascular changes with glaucoma and other markers of optic nerve head damage, including retinal nerve fibre layer thickness. Clinical implications, recent new advances in retinal vascular imaging (eg, optical coherence tomography angiography) and future research directions are also discussed.

## INTRODUCTION

Despite the critical impact of glaucoma on global blindness, its aetiology is not fully characterised. It has been recognised that elevated intraocular pressure (IOP) exerts direct mechanical damage to the optic nerve head (ONH).<sup>1 2</sup> However, among glaucoma patients, only one-third to half have elevated IOP at the initial stages.<sup>3–5</sup> In some, visual field loss continues despite adequate IOP control to normal levels.

Consequently, non-IOP-dependent mechanisms have been proposed. The ‘vascular theory’ of glaucoma hypothesises retinal ganglion cell (RGC) loss as a consequence

of insufficient blood supply.<sup>6 7</sup> Vasospasm and autoregulatory dysfunction have been postulated to reduce ocular blood flow. This role is further supported by the association of glaucoma with vascular diseases, such as hypertension and diabetes,<sup>8–10</sup> though discrepancies exist,<sup>11 12</sup> and inclusion as part of the primary vasospastic syndrome following its relationship with Raynaud’s phenomenon, autoimmune diseases and migraine.<sup>13–16</sup> Nevertheless, ongoing discussion over the influence of ocular perfusion pressure (OPP) on glaucoma recognises the inconsistent findings of the influence of diastolic and systolic OPP in the incidence and progression of glaucoma in large epidemiological studies,<sup>5 17–21</sup> which is further complicated by the dynamic relationship between OPP, blood pressure and IOP.<sup>22</sup>

Both static and dynamic properties of the retinal microcirculation may be implicated in the vascular phenomenon in glaucoma. Study of the retinal microcirculation is thus made possible by the accessibility of retinal vasculature via non-invasive means. Over the past two decades, semiautomated software systems have enabled objective and reliable quantification of geometric components of the retinal vasculature from retinal photography, including retinal vascular calibre, tortuosity, branching angle and fractal dimensions.<sup>23</sup> In effect, multiple studies have linked geometric retinal vascular parameters with vascular diseases including ischaemic heart disease, hypertension, stroke and diabetes.<sup>24–33</sup>

In this review, we summarise recent findings on the relationships between quantitatively measured structural retinal vascular changes with glaucoma and other markers of ONH damage. We further discuss the recent new advances in retinal vascular imaging (eg, optical coherence tomography angiography) and future research directions.



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## METHODS AND MATERIALS

A comprehensive literature search on PubMed was performed for studies published until August 2016 with keywords 'glaucoma', 'retinal nerve fibre layer thickness', 'geometry', 'retinal vascular calibre', 'tortuosity', 'branching angle' and 'fractal dimensions'. Combinations of these terms were used as well. Search results were limited to studies published in English and in human subjects only. Selected papers were then reviewed thoroughly and evidence was summarised.

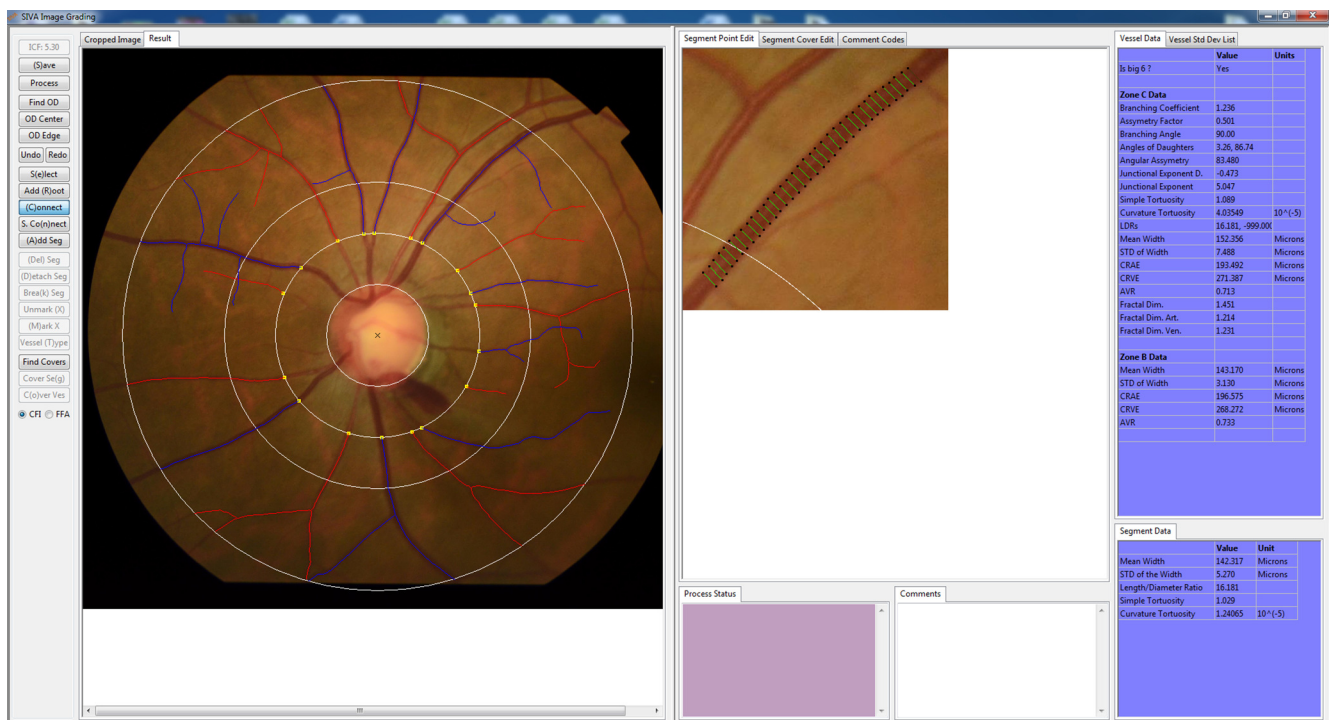
## OCULAR MICROCIRCULATION IN GLAUCOMA

Owing to the increasing recognition of involvement of vascular phenomena in glaucoma, interest in the presence of retinal microcirculatory changes in glaucoma patients has been raised. Improvement in blood flow and visual field measurements in some eyes following treatment with vasodilating calcium channel blockers<sup>34</sup> or carbon dioxide inhalation<sup>35</sup> present evidence of vascular autodyregulation. In addition, a recent study showed multiple comparable ocular and systemic vascular alterations in the early stages of patients both with primary open-angle glaucoma (POAG) and normal tension glaucoma (NTG), which were not replicated in controls.<sup>36</sup> The idea that a continuum of disturbed circulation exists between the two previously 'distinct' disease entities is proposed and further extends the need for evaluation of vascular properties (eg, ocular blood flow).

ONH blood flow is tightly autoregulated to meet the functional and metabolic demands of the retina,

including RGC. Technological advancements have made visualisation, direct measurements and quantification of in- vivo ocular blood flow possible, though a gold standard that provides all the relevant information in one reading has yet to be established. Current modes of analysis of this dynamic parameter include, but are not limited to, angiography, laser Doppler techniques, Heidelberg Retina Flowmeter, laser speckle phenomenon and retinal vessel analyser.<sup>37-38</sup> MRI can provide not only dynamic blood flow measurement within deep orbital structures but also a non-invasive measurement of intracranial structures.<sup>39</sup> Nevertheless, the vast variety of instruments create difficulty for data unification, though a consistent demonstration of decreased average blood flow in some glaucoma patients was found in the retinal,<sup>40-41</sup> ONH<sup>42-43</sup> and choroidal<sup>44</sup> circulations.

Murray's Principle of Minimum Work established that the vascular network conforms to an 'optimally' designed topographical geometry.<sup>45</sup> This minimises shear stress and work across vascular network and allows sufficient blood distribution to tissue with the least amount of energy. As blood flow is a function of cardiac output and regulated by relative local resistance, deviations to ideal structure and function of the microcirculation will lead to reduced efficiency and impaired circulatory transport.<sup>46</sup> In view of the challenges in dynamic analysis, interest has turned in the direction of the vascular network's static components,



**Figure 1** Quantitative measurement of retinal vasculature from retinal fundus photograph using a computer-assisted program (Singapore I Vessel Assessment (SIVA)).

including its design, since they reflect resistance to ocular blood flow and affect function.

## QUANTITATIVE MEASUREMENTS OF RETINAL VASCULATURE

With the introduction of modern digitalised retinal photography, semiautomated computer-assisted programmes have been developed to objectively and reliably quantify subtle retinal vascular changes from retinal photographs, with focus on calibre measurement. Optimate (Department of Ophthalmology and Visual Science, University of Wisconsin—Madison) and IVAN (Department of Ophthalmology and Visual Science, University of Wisconsin—Madison) softwares analyse digitalised retinal photographs and measure the retinal vessel widths.<sup>47 48</sup> With the development of digital retinal photography, newer programs such as Singapore I Vessel Assessment (SIVA)<sup>25</sup> and Vessel Assessment and Measurement Platform for Images of the REtina (VAMPIRE)<sup>49</sup> softwares have evolved to evaluate novel classes of retinal vascular geometric parameters, including tortuosity, fractal dimension and branching angle, providing comprehensive assessment of retinal vasculature (figure 1). Such development provides an accessible, non-invasive model to study correlations and consequences of microvascular dysfunction in both systemic and ocular diseases.<sup>50–52</sup> For example, systemic review has confirmed that wider retinal venular calibres predict stroke,<sup>53</sup> and meta-analysis showed independent associations between wider retinal venules and narrower arterioles with increased risk for cardiovascular events in women.<sup>54</sup>

### Retinal vascular calibre

Retinal vascular calibre is measured in terms of central retinal artery equivalent (CRAE), central retinal vein equivalent (CRVE) and arteriovenous ratio (AVR).<sup>47 48</sup> CRAE is a summary index reflecting the average width of retinal arterioles, and CRVE is a summary index reflecting the average width of retinal venules. It has been recognised that CRAE and CRVE should be analysed independently, as they reflect distinct systemic vascular disease pathways. AVR is a dimensionless ratio that is used to compensate for magnification differences and refractive error, and its value is non-specific to changes in arterioles, venules or both.

### Retinal vascular tortuosity

Retinal vascular tortuosity reflects vessel curvature and is summarised as the ratio between the actual distance a vessel travels from points A to B and the shortest straight-line distance between points A and B.<sup>55</sup> A larger tortuosity index indicates more curves in a retinal vessel. Retinal vascular tortuosity can be computed as the integral of the curvature square along the path of the vessel, normalised by the total path

length.<sup>24</sup> Since this measure is represented as a ratio, its value is dimensionless.<sup>56</sup>

### Retinal vascular bifurcation angle

Retinal vascular bifurcation angle is defined as the first angle subtended between two daughter vessels at a vascular junction. Both retinal arteriolar branching angle and retinal venular branching angle could be derived, and they represent the average branching angle of arterioles and venules, respectively.<sup>57</sup>

### Fractal dimension

Fractal dimension describes how thoroughly a pattern fills two-dimensional spaces and represents a 'global' measure that summarises the whole branching pattern of the retinal vascular tree.<sup>58 59</sup> It is calculated from a skeletonised line tracing using a box-counting method. Larger values indicate a more complex branching pattern.

## RETINAL VASCULAR CHANGES ASSOCIATED WITH GLAUCOMA

Generalised narrowing of the retinal vessels is characteristic of advanced glaucomatous optic nerve damage.<sup>60</sup> A number of epidemiological studies have shown association between retinal vascular changes, particularly narrowing in retinal vascular calibre, with glaucoma. Table 1 presents the associations between quantitative retinal vascular parameters with glaucoma in population-based and hospital-based cross-sectional studies.

Prior to availability of semiautomated machines, retinal vessel calibres in eyes with glaucoma were explored via manual means.<sup>60–62</sup> Evidence of decreasing retinal vessel calibre with increasing glaucoma stage was demonstrated, with stronger correlation for arteries than veins. Quadrants with greater ONH damage corresponded with narrower retinal arteries. Regarding this association, two schools of explanations have been postulated. On the one hand, RGC loss has been suggested to lead to vasoconstriction as an adjustment to decreased metabolic needs. This is in line with the observation of retinal arterial narrowings in eyes with non-glaucomatous optic atrophy.<sup>62–64</sup> Alternatively, the underlying pathological process leading to RGC loss has been proposed to be related to impaired local autoregulation, vasoactive substance leakage and consequently vasoconstriction.<sup>65</sup> On the molecular level, this is supported by elevated biomarkers of oxidative stress in aqueous humour, serum and trabecular meshwork samples of glaucoma patients.<sup>66 67</sup> Reactive free radicals scavenge nitrous oxide, an innate vasodilator secreted by smooth muscles that alter vascular tone. Short posterior ciliary artery (SPCA), in particular, has been found to exhibit transient vasospasm on radical exposure in in-vitro models,<sup>68</sup> and reduced SPCA

**Table 1** Associations between quantitative retinal vascular parameters with glaucoma in population-based and hospital-based cross-sectional studies

Study and year	Study type	Sample size	Method of assessment	Changes in parameters in association with glaucoma				
				Arteriolar calibre	Venular calibre	Fractal dimension	Tortuosity	Branching angle
Ciancaglini <i>et al</i> <sup>71</sup> (2015)	Hospital-based cross-sectional study	N/A	Heidelberg Doppler flowmetry	–	–	Reduced	–	–
De Leon <i>et al</i> <sup>72</sup> (2015)	Hospital-based, cross-sectional study	Any glaucoma: 158	IVAN	Reduced	Reduced	–	–	–
Gao <i>et al</i> <sup>78</sup> (Handan Eye Study) (2015)	Population-based cross-sectional study	Healthy: 5788 POAG: 54 PACG: 19 PACS: 731 PAC: 64	Optimate	Reduced	Reduced	–	–	–
Yoo <i>et al</i> <sup>79</sup> (2015)	Hospital-based case-control study	Healthy: 60 HPG: 63 NTG: 82	IVAN	Reduced	Not significant	–	–	–
Wu <i>et al</i> <sup>82</sup> (Singapore Malay Eye Study) (2013)	Population-based, cross-sectional study	Healthy: 2666 POAG: 87 OHT: 58	SIVA	–	–	Reduced	Reduced	Reduced
Amerasinghe <i>et al</i> <sup>76</sup> (Singapore Malay Eye study) (2008)	Population-based, cross-sectional study	Healthy: 2892 POAG: 88 PACG: 5 NTG: 74	IVAN	Reduced	Reduced	–	–	–
Wang <i>et al</i> <sup>77</sup> (Beijing Eye Study) (2007)	Population-based, cross-sectional study	Total: 2418	Manual	Reduced	Not significant	–	–	–
Klein <i>et al</i> <sup>80</sup> (Beaver Dam Eye Study) (2004)	Population-based, cross-sectional study	Total: 4613 Any glaucoma: 199	Optimate	Not significant	Not significant	–	–	–
Mitchell <i>et al</i> <sup>75</sup> (Blue Mountains Eye Study) (2004)	Population-based, cross-sectional study	Healthy: 3065 HPG: 38 NTG: 21 OHT: 163	Optimate	Reduced	Reduced	–	–	–

Continued



Table 1 Continued

Study and year	Study type	Sample size	Method of assessment	Changes in parameters in association with glaucoma				
				Arteriolar calibre	Venular calibre	Fractal dimension	Tortuosity	Branching angle
Angelica <i>et al</i> <sup>74</sup> (2001)	Hospital-based, cross-sectional study	Total: 143	HRT software 1.11, Interactive Means program	Not significant	–	–	–	–
Rankin <i>et al</i> <sup>62</sup> (1996)	Hospital-based, case-control study	Healthy: 7 POAG: 32 NTG: 48 OHT: 19 Suspects: 4	Manual	Reduced	–	–	–	–
Rader <i>et al</i> <sup>61</sup> (1994)	Hospital-based, case-control study	Healthy: 206 Any glaucoma: 226	Manual	Reduced	–	–	–	–
Jonas <i>et al</i> <sup>60</sup> (1989)	Hospital-based, cross-sectional study	Healthy: 173 POAG: 281	Manual	Reduced	Reduced	–	–	–

AVR, arteriovenous ratio; COAG, chronic open-angle glaucoma; HPG, high-pressure glaucoma; NTG, normal tension glaucoma; OHT, ocular hypertension; PAC, primary angle closure; PACG, primary angle closure glaucoma; PACS, primary angle closure suspect; POAG, primary open-angle glaucoma; SIVA, Singapore 'I' Vessel Assessment.

blood flow velocities were associated with glaucoma progression.<sup>69</sup> Altered systemic vasoreactivity with endothelial cell dysfunction was also confirmed in NTG patients,<sup>70 71</sup> while population-based trials have demonstrated lower diastolic perfusion pressure, a measure of ocular blood flow, as a significant factor in the glaucoma incidence.<sup>5 17 19</sup> However, objective evidence for underlying mechanisms have yet to be further clarified in the future.

Though these studies were limited by use of manual, subjective methods in measurement of retinal vessel diameters, their results were consistent with recent findings employing computer-assisted programs. De Leon *et al* investigated intereye differences in retinal vascular calibre in persons with asymmetrical glaucoma using the IVAN system.<sup>72</sup> Once again, CRAE and CRVE were narrower for eyes with more severe disease. This relationship held after adjustment for age, gender, vascular risk factors and IOP, suggesting the difference in calibre to be due to severity discrepancy or other unknown factors, instead of systemic vascular diseases. Similarly, using the IVAN system, Yoo *et al*<sup>73</sup> analysed CRAE of glaucomatous suspects who showed unilateral glaucomatous conversion and noted narrower CRAE at baseline and at the point of glaucoma conversion. Angelica *et al*<sup>74</sup> dismissed the

usage of retinal vessel calibre as a predictor in glaucoma in a hospital-based cross-sectional study, as no significant association could be drawn, though no explanation was given.

Population-based studies have further supported the above findings. The Blue Mountains Eye Study (BMES) showed that eyes with POAG were 2.7 times more likely to have generalised retinal arteriolar narrowing than eyes without glaucoma.<sup>75</sup> This remained true after adjusting for risk factors for glaucoma and is independent of IOP and OPP. The Singapore Malay Eye Study found consistent association of quantitatively measured retinal vascular calibre with prevalence of glaucoma and larger vertical cup–disc ratio (CDR).<sup>76</sup> The Beijing Eye Study showed significantly thinner retinal arteries but insignificant difference in retinal vein diameters.<sup>77</sup> In the Handan Eye Study, both narrower retinal arterioles and venules were observed in primary angle closure glaucoma and POAG than those in normal controls, primary angle closure or primary angle closure suspect,<sup>78</sup> suggesting that the narrowing of retinal vessels resulting from the glaucoma process is irrespective of status of angle closure. More recently, Yoo *et al* reported similar findings of retinal arteriolar narrowing in glaucoma, and further found that the diagnostic ability of retinal arteriolar calibre was

Table 2 Relationship between retinal vascular parameters with glaucoma-associated outcomes

Study and year	Study type	Sample size	Method of assessment	Outcome	Changes in parameters in association with glaucoma				
					Arteriolar calibre	Venular calibre	Fractal dimension	Tortuosity	Branching angle
Tham <i>et al</i> <sup>67</sup> (Singapore Malay Eye study) (2013)	Population-based, cross-sectional study	Healthy: 352	SIVA	RNFL thickness	Reduced	Reduced	Reduced	Reduced	–
Kim <i>et al</i> <sup>69</sup> (2012)	Hospital-based, case-control study	Healthy: 48 NTG: 67	Visupac	RNFL thickness	Reduced	Not significant	–	–	–
Koh <i>et al</i> <sup>68</sup> (Singapore Malay Eye Study) (2010)	Population-based, cross-sectional study	Healthy: 2641	SIVA	Neuroretinal rim area	–	–	–	Reduced	–
Zheng <i>et al</i> <sup>65</sup> (Singapore Malay Eye Study) (2009)	Population-based, cross-sectional study	Healthy: 2599 Any glaucoma: 107	IVAN	RNFL thickness	Reduced	Reduced	–	–	–
Cheung <i>et al</i> <sup>68</sup> (Sydney Childhood Eye Study) (2008)	Population-based, cross-sectional study	Healthy: 1204	Optimate	RNFL thickness	Reduced	Reduced	–	–	–
Lim <i>et al</i> <sup>90</sup> (2009)	Hospital-based, cross-sectional study	Healthy: 104	Optimate	RNFL thickness	Reduced	Reduced	–	–	–
Samarawickrama <i>et al</i> <sup>61</sup> (Sydney Childhood Eye Study) (2003)	Population-based, cross-sectional study	Healthy: 2038	Optimate	RNFL thickness	Not significant	Reduced	–	–	–
Hall <i>et al</i> <sup>62</sup> (2001)	Hospital-based case series	POAG: 64	Manual	VFD	Reduced	Not significant	–	–	–
Jonas and Naumann <sup>66</sup> (1989)	Hospital-based, case-control study	Healthy: 173 POAG: 281	Manual	CDR	Reduced	Reduced	–	–	–
				RNFL thickness	Reduced	Reduced	–	–	–
				VFD	Reduced	Reduced	–	–	–

CDR, cup–disc ratio; NTG, normal tension glaucoma; POAG, primary open-angle glaucoma; RNFL, retinal nerve fibre layer; VFD, visual field defect; SIVA, Singapore 'I' Vessel Assessment.

comparable to retinal nerve fibre layer (RNFL) thickness in detecting OAG, which is an optimistic introduction to its potential use in clinical settings.<sup>79</sup>

Nevertheless, the Beaver Dam Eye Study, a Caucasian population-based cohort study, did not find any associations of retinal vascular calibre related to prevalent glaucoma, large cup-to-disk ratio or elevated IOP.<sup>80</sup> The authors attributed this deviation of their findings to the difference in the methodology of selection of arterioles for evaluation. A number of previous studies have focused solely on peripapillary vessel calibres<sup>62–74</sup> however, Klein *et al* excluded peripapillary vessels because of the variability in retinal nerve fibre layer thickness in this area.

Overall, population-based and hospital-based cross-sectional studies largely supported the association of narrower vessel calibre with glaucoma, though individual studies focused on CRAE alone or only found significant reduction in CRAE and not CRVE.

Owing to the relatively new availability of technology in advanced geometry measurements, only two studies have evaluated retinal vascular geometric parameters other than calibre size. In a hospital-based study, Ciancaglini<sup>81</sup> *et al* found correlation between ONH damage with a reduced retinal vascular fractal dimension. The Singapore Malay Eye Study also had a consistent finding of lower retinal vascular fractal dimension in glaucoma.<sup>82</sup> In this study, Wu *et al* also evaluated vessel tortuosity and branching angle, and noted significantly smaller vessel tortuosity and retinal venular branching angle in eyes with glaucoma. Taken together, these findings suggest that circulatory optimality of vessels in glaucoma eyes may be compromised due to proven changes in the design of the geometrical pattern. However due to the cross-sectional nature of data, information on the temporality of retinal vascular changes with glaucoma incidence is limited.

### RETINAL VASCULAR CHANGES WITH GLAUCOMA-ASSOCIATED OUTCOMES

Reduced RNFL thickness, greater CDR and characteristic visual field defects are hallmarks of glaucomatous optic neuropathy. Table 2 summarises cross-sectional studies that defined the relationship between retinal vascular parameters with these glaucoma-associated outcomes.

The correlation between narrower retinal vessel calibre and thinner RNFL thickness has been consistent since the 1980s.<sup>83–85</sup> Studies analysed included hospital-based or population-based cross-sectional data, measurements carried out by manual means or computer programs, and populations of children, adolescents and adults. Although the biological mechanisms remain uncertain, these findings support the hypothesis that the loss of RGCs in thinned RNFL lowers metabolic and vascular demands, leading to narrower vascular calibre as part of an autoregulatory response.<sup>65–86–91</sup> This is supported by a similar finding

of decreased vessel diameter in non-glaucomatous optic neuropathies such as non-arteritic ischaemic optic neuropathy and descending optic nerve atrophy.<sup>63</sup> Regardless, the temporal relationship of whether peripapillary vessel narrowing causes damage to the optic nerve, or the reverse, is true, has yet to be demonstrated definitively.

Discrepancy in the strength of association between arterioles and venules with RNFL was noted. The Singapore Malay Eye Study noted stronger association in venules than arterioles,<sup>65–87–92</sup> while Kim *et al*<sup>89</sup> only associated RNFL thickness with arteriolar calibre, but not venular. The contrasting findings may be explained by the complex interaction between various mediators for vasodilatation and vasoconstriction on arterioles and venules. Retinal venular calibre is more strongly influenced by diabetes mellitus, while arteriolar calibre is more related to hypertension.<sup>89</sup> It has also been proposed that narrower venular calibre may indicate venous congestion and cytotoxic damage, with subsequent secondary constriction of arteriole.<sup>93–96</sup> The different spectrum of baseline systemic diseases in studies may therefore contribute to the discrepancy in findings. Nevertheless, compatible association between thinner RNFL thickness with narrowed calibre in healthy children and adolescents indicate that the relationship in adults with pathological eyes are at least in part physiological in origin.<sup>91</sup>

Apoptosis of RGCs lead to increased CDR, which is a pathognomonic feature of glaucoma. Studies have been inconsistent in demonstrating its relationship with vessel calibre.<sup>88–90–91</sup> Lim *et al*<sup>90</sup> described the association between narrower retinal venular diameter with CDR, which was lacking for arteriolar calibre. This was attributed to retinal veins' lower resistance to deformation due to their non-existent tunica media.<sup>90</sup> Nevertheless, while increase in CDR is a clinical indicator for glaucoma progression, the reliability of CDR to detect glaucoma is limited by the wide variability in cup sizes, and interobserver and intraobserver variability. Poor correlation between RGC counts and CDR has also been demonstrated, suggesting that CDR is an insensitive method for evaluation of glaucomatous structural damage.<sup>97</sup>

Consistency is seen for the correlation between arteriole calibre with visual field defect. Hall *et al* compared calibre in POAG patients with marked difference in visual field defects between hemifields, and found significant correlation between arteriolar calibre with visual field defect.<sup>92</sup> Similarly, Jonas and Naumann<sup>86</sup> correlated visual field defects with both arteriole and venule calibres. Koh *et al* was the only study that evaluated vessel tortuosity and correlated decreased tortuosity with a thinner neuroretinal rim, which was more significant in arterioles.<sup>98</sup> This was in line with studies that linked straighter retinal vessels with ischaemic heart disease and higher blood pressure.<sup>99</sup>

**Table 3** Relationship between vascular geometry with the incidence or progression of glaucoma

Study and year	Study type	Follow-up duration	Sample size	Method of assessment	Outcome	Changes in parameters in association with glaucoma	
						Arteriolar calibre	Venular calibre
Lee <i>et al</i> <sup>102</sup> (2014)	Hospital-based, prospective study	24.3 months	NTG: 27	IVAN	Progression of glaucoma: 27	Reduced	Not significant
Kawasaki <i>et al</i> <sup>100</sup> (The Blue Mountains Eye Study) (2013)	Population-based, prospective study	10 years	Total: 2417	Optimate	Incidence of glaucoma: 82	Reduced	Not significant
Ikram <i>et al</i> <sup>101</sup> (Rotterdam Eye Study) (2005)	Population-based, prospective study	6.5 years	Total: 3464	Optimate	Incidence of glaucoma: 74	Not significant	Not significant
Papastathopoulos & Jonas <sup>64</sup> (1999)	Hospital-based, prospective study	37 months	OHT: 31 POAG: 59 NTG: 22 SOAG: 11	Colour stereo optic disc photographs	Progression of glaucoma: 37	Reduced	–

CRAE, central retinal artery equivalent; CRVE, central retinal vein equivalent; NTG, normal tension glaucoma; OHT, ocular hypertension; POAG, primary open-angle glaucoma; SOAG, secondary open-angle glaucoma; –, parameter not investigated.

### LONGITUDINAL RELATIONSHIP BETWEEN RETINAL VASCULAR CHANGES WITH GLAUCOMA

Prospective studies provide information on the causative relationship between the parameters in question and glaucoma. This is relevant in determining whether vascular dysfunction preceded development of glaucoma or is a consequence of optic neuropathy progression. Table 3 lists longitudinal studies that evaluated the relationship between vascular geometry with the incidence or progression of glaucoma.

Two studies evaluated glaucoma incidence. In an urban Caucasian population, 10-year follow-up data from the BMES revealed that narrower retinal arterioles were associated with higher OAG incidence, and suggest the potential use of retinal vessel calibre to identify patients with increased risk for glaucoma development.<sup>100</sup> This finding supports previous cross-sectional studies' concept that vascular changes are involved in the early course or pathogenesis of glaucoma. However, the Rotterdam Study, another Caucasian population-based study of 6.5 years of follow-up, had contradicting results.<sup>101</sup> Both retinal arteriolar and venular baseline diameters were not found to be associated with incident OAG and incident optic disc changes. The discrepancy in findings may be

due to the difference in duration of follow-up and higher incidence of POAG in BMES. Moreover, due to the elderly skewed cohort, the Rotterdam Study had a substantial number of participants (n=838) who passed away during the follow-up.

Progression of glaucoma was evaluated in two prospective studies. Papastathopoulos and Jonas performed a minimum 8-month follow-up for a group of patients with progressive glaucomatous optic nerve damage and noted significant focal narrowing of retinal arterioles associated with neuroretinal rim loss.<sup>64</sup> This was not found in patients with static optic discs. Retinal venules were not analysed. Nevertheless, the authors concluded that focal narrowing does not necessarily involve progression of glaucoma, and is not pathognomonic for any particular subtype.

More recently, Lee *et al* compared 27 eyes with bilateral NTG who showed asymmetrical glaucoma progression after a mean follow-up of 24.3 months and found significant narrowing of retinal arteriolar calibre in progressed eyes but not in contralateral stable eyes.<sup>102</sup> No correlation was found for retinal venular calibre, however, they hypothesised this may be due to clinically asymptomatic engorgement of venous blood flow in glaucoma, together with different regulatory



mechanisms governing changes in retinal artery and vein diameters.<sup>102</sup> No significant intereye difference was observed in the mean baseline vessel calibre between progressed and stable eyes.

### DYNAMIC RETINAL VASCULAR CHANGES WITH GLAUCOMA

The vascular theory of glaucoma considers optic nerve damage as a consequence of insufficient blood supply due to either increased IOP or other dysregulatory factors reducing ocular blood flow. Thus apart from associating structural vessel properties with glaucoma, functional performance reflects abnormalities and dysregulations in pathogenic eyes. Technological advancements have allowed quantitative evaluation of ocular blood flow and perfusion, and could serve as an imaging target for early diagnosis and monitoring of glaucoma.

ONH blood flow could be determined from simultaneous measurements of the blood column diameter and the centreline blood speed. Scanning laser Doppler flowmetry with automated perfusion imaging analysis evaluates frequency shift of perfused vessels and capillaries. Vessels are identified, segmented, and velocity then derived from the rate of flow shift. Laser speckle flowgraphy also calculates the speckle pattern that arises from the scatter of the laser irradiation from an illuminated fundus. Changes in the velocity of the blood flow blur the speckle pattern and the mean blur rate is then derived. Both methods have shown reduced ONH and peripapillary blood flow dynamics in glaucoma.<sup>103–105</sup> Diminished flow in POAG suspect eyes before the development of clinically detectable visual field loss was confirmed as well.<sup>106 107</sup> However, laser Doppler flowmetry only evaluates a small area of the retina, while absorbance and reflectance of disc tissue limits repeatability of laser speckle flowgraphy.

Ocular perfusion, another reflection of ocular blood flow, can be estimated by retinal arteriovenous passage time via digital scanning laser fluorescein angiograms. It characterises the passage of blood from the retinal artery, through capillaries, to the retinal vein. Prolonged passage time has been found to be reduced in both NTG and POAG patients,<sup>108–110</sup> which was attributed to reduction of the capillary diameter potentially due to vasospasms or arteriosclerosis. Nevertheless, routine usage of fluorescein angiography (FA) is limited by its invasiveness, difficulty in accurate quantification and potential adverse systemic effects.

### LIMITATIONS OF THE CURRENT STUDIES AND FUTURE DIRECTIONS

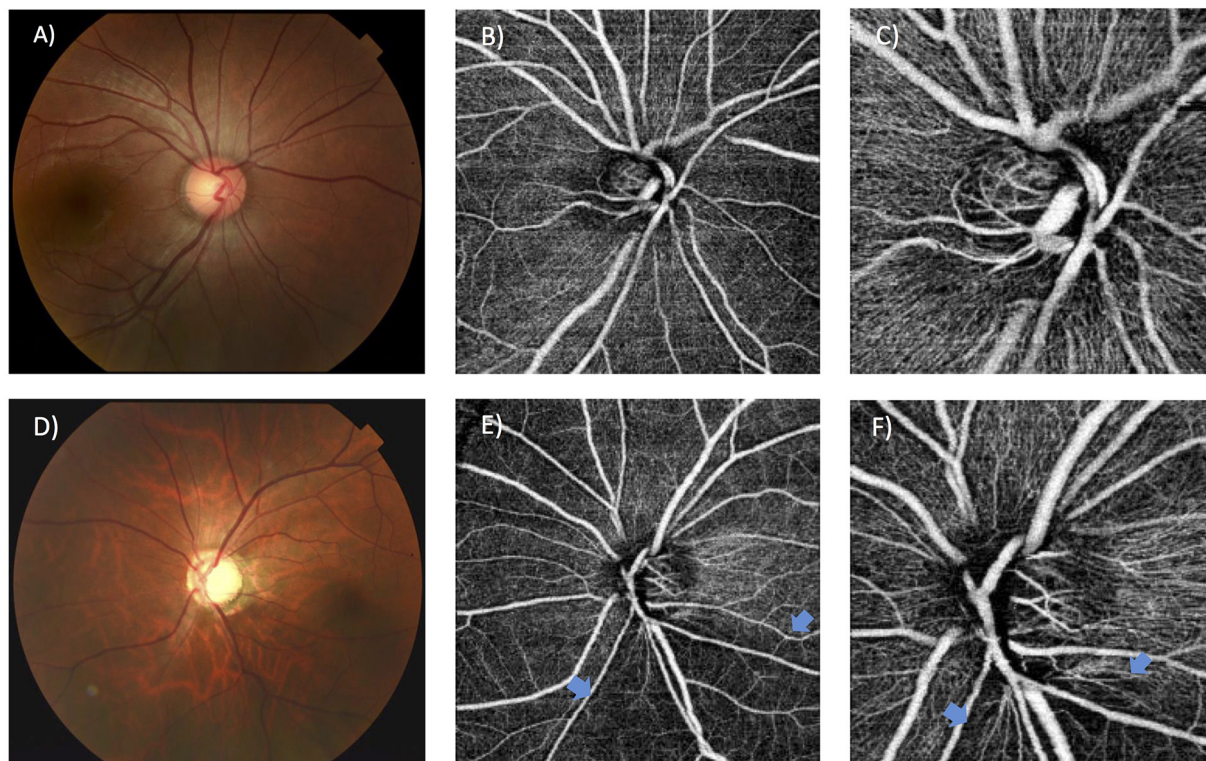
Despite the promising potential of retinal vascular imaging in glaucoma, there are still gaps in translating research into clinical practice. A shortcoming is the lack of knowledge about the normative data and reference levels for measurement. The majority of clinical trials compared pathological eyes with healthy eyes and

derived conclusions based on comparison. Baseline reference values have yet to be concluded, which is further complicated by the influence of systemic, genetic and environmental factors on the variations of retinal vascular calibre size.<sup>111</sup> Widespread implementation is also limited by availability of expertise. Current software is not fully automated and will require input from trained technicians to operate standardised protocols and provide expert manipulation and handling of specialised computer software. In addition, most studies in the current review did not specify subtypes in the associations, though the relationship between altered structural parameters with glaucoma held irrespective of IOP or angle closure. This may support the idea that vascular mechanisms underlie all subtypes of POAG. Further work could focus on elucidating differences in vessels in normal and high pressure glaucoma. Another limitation in the evaluation of retinal vascular calibre lies in its multifactorial influence by other systemic and individual characteristics. While most studies have taken into account patients' age, gender, systemic vascular diseases and IOP, other variabilities, such as caffeine consumption and smoking habits, have not been considered.<sup>111 112</sup> Hao *et al* reported significant changes in individual vessel calibre over a cardiac cycle but not in vessel calibre summaries (including CRAE and CRVE) and geometric measures, suggesting a mild correlation of pulse cycle and vessel diameter that need to be taken into account during sampling.<sup>113</sup> In addition, although multiple advanced analytic tools enable quantification of retinal vascular imaging, there are technological challenges that may compromise precision. Refractive errors and axial length variabilities cause discrepancies in magnification, while image display quality (contrast, brightness and focus) may be compromised by media opacities and pupil size.<sup>23</sup> The lack of automated imaging of retinal vascular also leads to unavoidable intragrader and intergrader variability that has yet to be refined.<sup>114</sup>

Future directions include focused analysis of the chronological nature of retinal vessel changes via means of longitudinal studies so as to better delineate the chicken–egg relationship between glaucomatous changes and narrowed vessel calibre. Detailed subtype analysis is also warranted to delineate whether the vascular phenomenon is more profound in NTG eyes, as conventionally believed, or actually exists as a spectrum among all glaucoma subtypes. Effort in clinical application of current data and ease of software use in daily practice should also be explored to close the gap between clinical and experimental investigations.

### NEW ADVANCES IN RETINAL IMAGING

Advances in technology have attempted to supplement the shortcomings of existing instruments. Peripapillary capillaries have been recognised to be a highly specialised vasculature that supply the nerve fibre layer,<sup>115</sup>



**Figure 2** Assessment of retinal capillary network around optic nerve head using optical coherence tomography angiography in a normal eye (A–C) and a glaucomatous eye (D–F). Decreased peripapillary capillary density is indicated by blue arrows.

and a better understanding of this network may reflect focal or contiguous disc capillary network defects or act as a supplementary indicator of RGC damage.

FA is the gold standard for imaging the capillary network. However, it is invasive in operation (requiring intravenous injection of fluorescein dye), time consuming, confounded by superimposition of capillaries from different retinal layers and only offers two-dimensional image analysis with lack of quantifiable parameters. All of the shortages above reduce the clinical utility of FA. Optical coherence tomography—angiography (OCT-A) offers three-dimensional, non-invasive retinal and choroidal microcirculation vasculature analysis and blood flow estimation<sup>116 117</sup> (figure 2). It is based on mapping erythrocyte movement over time by comparing sequential optical coherence tomography-B scan (OCT-B scan) ultrasounds images at a given cross-section. OCT-A is able to separately detect the superficial capillary network in the ganglion cell layer, the deep capillary network in the outer plexiform layer and choriocapillaris below retinal pigment epithelium without intravenous dye injection, providing depth-resolved visualisation of the retinal and choroid vasculature and blood flow. Moreover, OCT-A can generate data on vascular flow to quantify retinal or optic disc perfusion, independent of time and dye injection. As OCT-angiograms are coregistered with OCT-B scans from the same area, it also allows for simultaneous visualisation of structure and blood flow

for clinical interpretation. Recently, data derived from OCT-A readings have shown that peripapillary vessel density, peripapillary flow index and optic disc perfusion are reduced in glaucomatous eyes compared with aged-matched normal eyes.<sup>118–122</sup> These changes correlated to disease severity, structural changes and functional damages, including RNFL thickness, visual field mean deviation, visual field pattern SD and visual field index. In addition, OCT-A indices have outperformed RNFL thickness in having a stronger correlation with visual field loss.<sup>117 118 123</sup> These findings support the notion that OCT-A is a promising and useful imaging modality for evaluating glaucomatous microvasculopathy, which may allow earlier diagnosis and detection of nerve fibre functional loss before thinning occurs. Compared with FA, OCT-A also offers superior details in analysing radial peripapillary capillaries, which is a unique plexus within the inner nerve RNFL that provides nutritional support to the RGCs.<sup>124</sup> Reduction in the network's density has been strongly correlated with thinner RNFL thickness and poorer visual field index.<sup>125</sup> Compared with vessel measurements based on digital photography, which is more appropriate for large vessels with less sensitivity, studies that utilised OCT-A allowed more accurate measurement of the low velocities of deep plexuses. Furthermore, since OCT-A is a depth-resolved technique, it offers technical advantage in the growing interest of investigating the deep layer

microvasculature. Recently, with OCT-A imaging, Suh *et al.*<sup>126</sup> reported that decreased deep-layer vessel density within the parapapillary area, which is downstream from the SPCA perfused deep ONH, is associated with lamina cribrosa defect, visual field impairment and RNFL thinning. This finding may support the microvascular pathophysiology concepts of glaucoma, since the superficial and deep retinal layers are perfused individually by the central retinal and short posterior ciliary arteries, respectively.<sup>127</sup> However, OCT-A has several limitations. First, limited by the current scanning speed and patient comfort during the acquisition, a 6×6 mm<sup>2</sup> area is the largest scanning field that can be provided by the most updated OCT-A imaging device. This may be suboptimal for peripheral retinal vasculature. Second, data on validity of OCT-A assessment, such as intersection or intrasection reliability, comparability with gold standard and correlation with clinical outcomes is still scarce. Third, despite modern technology, automated, objective and robust methods that have evidence-based proof of accuracy for vasculature identification for quantitative assessment of capillary perfusion are still lacking.<sup>128 129</sup> In addition, image artefacts are common in OCT-A, especially motion and projection artefacts, leading to inaccurate assessment.<sup>130</sup> Advanced softwares to neutralise artefacts while maintaining adequate intensity and visibility of pathological vascular changes are required,<sup>125</sup> while media opacities and segmentation errors should be taken into account as factors that influence OCT-A interpretation.

Retinal functional imaging is another method to obtain blood flow velocity by comparing erythrocyte movement in serial retinal images. Elevated mean retinal blood flow velocity was found in peripapillary vasculature,<sup>131</sup> which may reflect a steal phenomenon when retinal vessels experience increased flow following decreased retinal capillary perfusion. Increased venous velocity has been consistently found in eyes with preperimetric glaucomatous optic neuropathy, which may reflect early vascular dysfunction.<sup>132</sup> Imaging systems that employ adaptive optics, such as retinal fundus camera, OCT and scanning laser ophthalmoscope, have also provided in-vivo, high-resolution imaging of the vasculature and nerve fibre layer that overcomes poor lateral resolution in conventional ocular optics,<sup>133</sup> and have been shown to be in precise agreement with histology in primate studies.<sup>134</sup>

## CONCLUSION

This review offers solid, consistent evidence for proof of concept that structural designs and changes in the retinal vasculature are associated with glaucoma. Most cross-sectional studies support the association between narrowed vessel calibre with glaucoma and glaucoma-associated outcomes, including thinner RNFL, increased CDR and thinner neuroretinal rim area.

Specific vessel patterns, including reduced fractal dimension, tortuosity and branching angle, have also been largely associated with glaucoma in hospital-based and population-based studies, though evidence is scarce. Further meta-analysis or pooled analysis could quantitatively evaluate their consistency. Longitudinal data bear weight in elucidating the temporal association of these findings with the incidence or progression of glaucoma. However, the small number of related studies limits the significance of the evidence, particularly when conclusions are in contradiction. More prospective, long-term follow-up data are needed.

New retinal imaging techniques confirm the pathogenetic concept of vascular dysregulation in glaucoma eyes, especially with NTG. Clear differences when compared with controls are demonstrated. Their potential usefulness in the diagnosis, staging and monitoring of glaucoma is recognised, and their function as a future imaging target should be utilised.

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