Retinal arteriolar geometry is associated with cerebral white matter hyperintensities on MRI.

FN Doubal[1*] MRCP, R de Haan[2], TJ MacGillivray[3,5] PhD, P Cohn-Hokke[2] MD, B Dhillon[4] FRCOphth, MS Dennis[1] FRCP, JM Wardlaw[1, 5] FRCR.

[1]Division of Clinical Neurosciences, University of Edinburgh.

[2]Academic Medical Centre, University of Amsterdam,

[3]Wellcome Trust Clinical Research Facility, University of Edinburgh

[4]Princess Alexandra Eye Pavilion, University of Edinburgh

[5]Scottish Imaging Network, A Platform for Scientific Excellence (SINAPSE) Collaboration

* corresponding author – Division of Clinical Neurosciences, Bramwell Dott Building, Western General Hospital, Edinburgh UK EH4 2XU.

fergus.doubal@ed.ac.uk , fax 00 44 131 332 5150, tel 00 44 131 537 2909.

Total word count: 4,589

Abstract word count: 248

Abstract

Background. Cerebral small vessel disease (lacunar stroke and cerebral white matter hyperintensities) is caused by vessel abnormalities of unknown aetiology. Retinal vessels show developmental and pathophysiological similarities to cerebral small vessels and microvessel geometry may influence vascular efficiency.

Hypothesis. We hypothesized that retinal arteriolar branching angles or co-efficients (the ratio of the sum of the cross sectional areas of the two daughter vessels to the cross sectional area of the parent vessel at an arteriolar bifurcation) may be associated with cerebral small vessel disease.

Methods. We performed a cross-sectional observational study in a tertiary referral hospital, United Kingdom. An experienced stroke physician recruited consecutive patients presenting with lacunar ischaemic stroke with a control group consisting of patients with minor cortical ischaemic stroke. We performed brain magnetic resonance imaging to assess the recent infarct and periventricular and deep white matter hyperintensities. We subtyped stroke with clinical and radiological findings. We took digital retinal photography to assess retinal arteriolar branching co-efficients and branching angles using a semi-automated technique.

Results. We recruited 205 patients (104 lacunar stroke, 101 cortical stroke), mean age 68 (Standard Deviation 12) years. With multivariate analysis, increased branching coefficient was associated with periventricular white

matter hyperintensities (p=0.006) and ischaemic heart disease (p<0.001); decreased branching co-efficient with deep white matter hyperintensities (p=0.003) but not with lacunar stroke subtype (p=0.96). We found no associations with retinal branching angles.

Conclusions. Retinal arteriolar geometry differs between cerebral small vessel phenotypes. More research is needed to ascertain the clinical significance of these findings.

Introduction

Lacunar or small subcortical ischaemic strokes are common (25% of ischemic strokes[1]) and arise from disease of small perforating arterioles although their exact aetiology remains uncertain.[2] Lacunar strokes are associated with white matter hyperintensities (WMH)[3] which are associated with ageing,[4] cognitive impairment and dementia[5] but the exact aetiology of these WMH is unknown.

The retinal arterioles are of similar size and physiology to the cerebral arterioles.[6] Cerebral arterioles' sizes are below that which can be visualised reliably with current human imaging techniques but the retina can be photographed directly. Retinal vascular abnormalities are associated with both stroke and white matter disease presence and progression[7-10] and retinal venular[11, 12] and arteriolar[12] widths differ between stroke subtypes. Retinal vessel abnormalities may act as markers for cerebral small vessel disease although retinal vascular geometry has not been studied in ischaemic stroke subtypes.

The geometry of arterioles may affect the efficiency of the circulation,[13] that is, the ability of the arteriolar tree to deliver blood to tissue with a minimum total blood volume and with minimal loss of energy at each bifurcation. The branching coefficient of an arteriolar bifurcation measures the change in total cross sectional area across the bifurcation. An increased branching co-efficient represents wider daughter vessels and a decreased branching coefficient indicates narrower daughters compared to the parent vessel. Each

may affect the energy required to deliver blood around the body and hence the efficiency of the circulatory system. A few studies suggest that this theory may hold true in biology, for example abnormalities in branching co-efficient have been associated with cognitive impairment,[14] peripheral vascular disease[15] and ischaemic heart disease (IHD).[16]

Retinal arteriolar branching angles represent the angle subtended by the two daughter vessels. A change in absolute angles or deviation away from a theoretical optimum branching angle may also affect the circulatory efficiency and studies have also shown associations between branching angles and hypertension[17] and cognitive function.[14]

Hypothesis

We hypothesised that if cerebral small vessel disease is due to an intrinsic small vessel abnormality then patients with cerebral small vessel disease (either lacunar stroke or WMH) would have altered retinal arteriolar branching co-efficients and branching angles compared with patients with large artery atheromatous stroke.

Methods

Patients

We prospectively recruited consecutive patients with a clinical syndrome of acute lacunar or mild cortical stroke presenting to our university hospital stroke service between April 2005 and December 2007. We excluded patients with contraindications to MR, hemorrhage, severe stroke and non-stroke diagnoses. An experienced stroke physician examined patients, assessed stroke severity with the National Institute for Health Stroke Scale (NIHSS)[18] and classified patients into lacunar or cortical stroke clinical syndromes according to the Oxfordshire Community Stroke Project classification.[19]   Patients underwent investigations for stroke as indicated including MRI at presentation. We recorded history of diabetes, hypertension, ischaemic heart disease and peripheral vascular disease. We defined symptomatic carotid stenosis as a relevant stenosis greater than 50% measured with the North American Symptomatic Carotid Endarterectomy Trial (NASCET) [20] We defined atrial fibrillation as either a history of paroxysmal or continuous atrial fibrillation or atrial fibrillation on electrocardiogram.

Brain imaging

Patients had diagnostic brain MRI at presentation, on a 1.5-T MR scanner (Signa LX; General Electric) with 22 mT m^{-1} maximum strength gradients. Diagnostic MRI included axial diffusion-weighted, T2-weighted, fluid-attenuated inversion recovery (FLAIR), and gradient echo sequences.

MRI analysis

MRI scans were coded for the presence, location and size of the recent infarct and any old infarcts or haemorrhages by a neuroradiologist. A recent infarct was defined as a hyper-intense area on diffusion imaging (with corresponding reduced signal on Apparent Diffusion Co-efficient (ADC) image processing), with or without increased signal on FLAIR or T2 weighted imaging, in a distribution compatible with an arterial territory. Lacunar infarcts were in the cerebral hemispheric white matter, basal ganglia or brain stem and <2cm diameter if recent (subcortical lesions >2cm were classed as striatocapsular or cortical as they have large artery disease causes). MRI scans were coded for deep (lesions not contiguous with the ventricles) and periventricular (lesions contiguous with the ventricles) WMH with the Fazekas scale which rates lesions in both regions from 0-3.[21]

Stroke Subtyping

We defined mild cortical stroke syndrome as a maximum clinical deficit of either: weakness or sensory loss in the face, arm or leg; loss of higher cerebral dysfunction (eg, dysphasia or neglect); weakness or sensory loss in the presence of loss of higher cerebral function or homonymous hemianopia suggestive of occipital cortical infarct.[19] We defined lacunar stroke syndrome as one of the classical lacunar syndromes.[19] We also classified stroke subtype using radiological criteria (ie whether the recent infarct on MRI was cortical or lacunar) and used both the clinical and radiological classification to assign a final stroke subtype classification.[19] Where the clinical differed from the radiological classification, the radiological classification took precedence

as clinical diagnosis can misclassify up to 20%.[22] If no definite recent lesion was visible on the scan, the clinical classification was used. We recorded old lesions but subtyped based upon the acute lesion.

Retinal Assessment

Patients had six field retinal photography (centred on the disc, macula, lateral macula, nasal to the disc, upper arcade and lower arcade) of both eyes, with 1% tropicamide eye, using a Canon CR-DGi digital retinal camera (Canon USA Inc.). We selected photographs centred on the optic disc for each eye. Images were analyzed within custom written Matlab software (The Mathworks Inc, Natick, NA) blind to all clinical and imaging details.

Branching co-efficient assessment.

A trained grader identified the five most proximal measurable arteriolar junctions to the optic disc and used semi-automated computer software to measure the branching co-efficient of each bifurcation. The software tracked down each vessel from the centre point of each bifurcation fitting a profile of signal intensity at right angles to the longitudinal axis of the vessel with a Gaussian curve to determine the width of each vessel (See figure 1). Each profile was manually inspected and rejected if the Gaussian line did not fit well (r correlation <0.7).   We validated this process with Bland Altman plots comparing software performance to best human measurement (with a caliper on enlarged images) and found no systematic bias and a mean difference for 50 randomly chosen vessels of 0.006 pixels (95% CI -3.3 to 3.3 pixels). We

calculated the branching co-efficient with the following formula where D_0 =

parent diameter and D_1 and D_2 = the daughter diameters:[14]

Branching coefficient = $(D_1^2 + D_2^2)/ D_0^2$

We aimed to measure the branching co-efficients of the 5 most proximal

measurable bifurcations to the optic disk but included patients in whom 3 or

more were measurable in our primary analysis. In a pre-specified sensitivity

analysis, we also analyzed patients who had at least 5 branching co-efficient

measurements. We avoided assuming that branching co-efficients within

each eye were normally distributed by taking the median branching co-

efficient for each eye. There was good within patient correlation between left

and right eyes (Pearsons correlation co-efficient 0.53 for 32 randomly chosen

patients) and we randomly chose an eye to measure. The intra-rater intra

class correlation co-efficient for a random sample of 10 images assessed 2

weeks apart was 0.82.

We also assessed deviation from a theoretical optimum branching coefficient

of 1.26.[14, 23]   The optimum branching co-efficient for each bifurcation varied

between 1.00 and 1.26 according to the asymmetry index which is the ratio of

the smaller daughter diameter over the larger daughter diameter.[24]

Arteriolar branching angles.

A trained grader identified the 5 most proximal measurable bifurcations to the

optic disc and the software tracked down each vessel to a point two parent

vessel widths from the bifurcation (where turbulent flow becomes laminar after the bifurcation[25]), and drew a line reflecting the course of the vessel. The branching angle was calculated using the Cosine rule (See figure 2). Due to a large variation in angles within each eye, we only included patients in the analysis in whom we were able to perform 5 angle measurements to provide a reliable average. As we could not assume normal distribution of angles within each eye we took the median of the five angles from each eye. The correlation between angles in the left and right eye was poor (Pearson correlation co-efficient 0.23 for 27 randomly chosen patients) and we therefore measured angles in both eyes. We then took the mean of these two values to give an angle measurement for each patient.

We also measured deviation from the optimum branching angle (theoretically calculated as 75 degrees)[14, 23] for each bifurcation and we assessed the median deviation from the optimum for each eye. In a random sample of 10 photographs graded 2 weeks apart the intra rater class correlation coefficient for median angle was excellent at 0.961.

Statistical Analysis

All analyses were performed within Minitab (version 14, Minitab Inc, PA, USA). We compared baseline characteristics between the two stroke groups with t-tests, Mann-Whitney U tests and differences in proportions. The branching coefficients and branching angles were normally distributed between patients, as were the deviations from the optimum branching coefficient and angles (after square root transformation) and we therefore

performed multivariable linear regression with branching co-efficient and branching angles as the continuous outcomes and vascular risk factors, stroke subtype and WMH as the independent explanatory variables. We set an alpha level for significance of 0.05.

This study was approved by the Local (Lothian) Research Ethics Committee and all patients provided written informed consent.

Results

We recruited 205 patients (mean age 68.0 years SD 11.6). There were 104 lacunar strokes (51%) and 101 cortical strokes (49%) and 135 patients were male (66%). We could not measure at least 3 branching co-efficients in 24 patients (due to poor quality of the photograph, a paucity of bifurcations in the field of view, or local anatomical variations precluding computer measurements of vessel widths). Therefore 181 patients were included in the analysis of branching co-efficients. We were not able to measure 5 branching angles in at least one eye of 61 patients and therefore included 144 patients in the analysis of branching angles. The 24 patients excluded from the branching co-efficient analysis were older (74.5 SD 8.52 years v 67.1 SD 11.7 years) and the 61 patients excluded from the branching angle analysis more often had hypertension (75% v 56%) but did not differ in other respects. The baseline characteristics of the 181 patients with at least 3 branching co-efficients are shown in table 1.

Arteriolar Branching co-efficients.

In the 181 patients, the mean branching co-efficient was 1.44 (SD 0.19). There was no difference in mean branching co-efficients between lacunar (1.43, SD 0.17) and cortical stroke (1.44, SD 0.20). On multivariable linear regression (Table 2), the presence of IHD and increased periventricular WMH score were both significantly and independently associated with an increased branching co-efficients (representing wider daughters in relation to the parent vessel) and increased deep WMH score was significantly and independently associated with a decreased branching co-efficients (representing narrower

daughter vessel diameters in relation to the parent vessel). In our prespecified analysis of patients with 5 branching co-efficients measured in an eye (n=119) the relationships between branching co-efficient and IHD and deep WMH remained but the association between periventricular WMH and branching co-efficient was attenuated and became non-significant (data not shown). When we looked at deviation from the optimum branching co-efficient we found that the results did not change from those in Table 2.

Arteriolar branching angles.

In the 144 patients with 5 angles measured per eye, we found that the mean branching angle was 84.1º with a standard deviation of 7.1º. Arteriolar branching angles did not significantly differ between lacunar (mean 85.2º SD 7.3º) and cortical stroke (mean 83.0º SD 7.3º, difference = 2.3 95% CI 0.0 to 4.6 p=0.054). On univariable and also on multivariable analysis, only a history of PVD was associated with increased branching angle (Table 3), but note there were very few patients with PVD. Retinal branching angles were not associated with either deep or periventricular WMH. We assessed deviation from an optimum branching angle of 75º but the associations shown in Table 3 did not change.

Discussion

We have shown that increased retinal arteriolar branching co-efficients are associated with increased periventricular WMH and ischaemic heart disease in patients presenting with mild stroke. Decreased retinal arteriolar branching co-efficients are associated with increased deep WMH. Branching co-efficients are not associated with ischaemic stroke subtype. We have not demonstrated significant associations between retinal arteriolar branching angles and ischaemic stroke subtype, WMH or most other vascular risk factors. No previous studies have assessed retinal vascular geometry within ischaemic stroke subtypes or associations with WMH.

The strengths of this study include prospective recruitment and careful patient assessment at the time of the stroke by an experienced physician with diagnostic MRI graded by an experienced neuroradiologist. Assessment of retinal images was blind to clinical and imaging details. We used a specifically written semi-automated software program to assess retinal vessels to minimize human operator variability resulting in excellent intra-rater repeatability scores. We found that angles did not correlate well between left and right eyes so measured both eyes where possible. We used patients with cortical stroke as controls to avoid confounding by secondary preventative medications, common vascular risk factors and the presence of stroke, all of which might theoretically affect the appearance of small vessels. Comparison with normal age-matched controls without stroke would not have been appropriate, as then we would only be able to conclude that any differences were due to the presence of risk factors and having any stroke.

We also acknowledge weaknesses. The semi-automated software limited the number of patients that we were able to include because, unlike a human operator, the semi-automated software is not able to make allowances for anatomically difficult vessels ie those with indistinct edges or where a venule is in close proximity to an arteriole. The sample size may not have been large enough to account for interactions between key variables.

It is intriguing that deep and periventricular WMH are associated with opposing directions of altered branching co-efficient. A decreased branching co-efficient indicates that the daughter vessels are narrower with respect to the parent vessel and an increased branching co-efficient that the daughters are wider. Pathological studies have indicated that the mechanism of tissue damage in deep and periventricular WMH may differ;[26] as deep lesions may have more "ischemic" causes whilst periventricular changes may occur following disruption of the ependymal lining of the ventricles.[27] Deep and periventricular WMH may have slightly different associations with vascular risk factors[28] so, at least for the present, should be considered separately in the assessment of white matter disease.[29]

The association between IHD and increased branching co-efficients validates previous findings that increased branching co-efficients predicted death with ischaemic heart disease. The exact explanation for this is unclear. It is not simply attributable to medication as both our patient groups were taking similar medications and medication is not known to affect retinal vessel

widths.[30] The true pathophysiological significance of branching coefficients is not known, nor whether these are fixed from birth, alter with age, predispose to or change in the presence of disease. It is therefore difficult to speculate on whether increased branching coefficients might predispose to or be a response to large artery disease. Further studies are needed to examine this finding.

We found no associations with branching angles (the association with PVD is based on few patients), consistent with some previous studies finding no link between angles and hypertension,[31] peripheral vascular disease,[15] and death with IHD and stroke.[16] It is possible that angles may not predispose to or change in response to systemic disease. The poor correlation between left and right eye further questions whether angles have anything to do with systemic disease. Previous studies either looked at one eye only or combined eyes to achieve 5 measurements[16] thereby assuming that angles do not differ between left and right, or did not specify which eye was measured.

In our cohort, the observed mean of 1.43 (SD 0.19) was higher than the optimum theoretically derived branching co-efficient of 1.26. As the majority of our patients had positive deviations from the theoretical optimum, our results did not alter when we assessed deviation from the optimum branching co-efficient rather than absolute values, consistent with other studies.[16] In study populations where the mean branching co-efficient (or branching angle) is nearer to the theoretical optimum, assessing deviation leads to more

diverse results.[14]. However, not all theories attempting to explain biological systems hold true in vivo. Data are too sparse to know whether optimality of branching coefficients and angles differ between arteriolar beds or patient populations. Further studies should assess both absolute and deviation from optimum values focusing on different vascular beds, in response to pharmacological challenges, at different ages, and in the presence of different diseases to assess the real implications of vascular geometry.

Acknowledgements

Funding

Disclosures

The authors have no financial disclosures to report

Reference List

(1) Sudlow CLM, Warlow CP. Comparable studies of the incidence of stroke and its pathological types. Results from an international collaboration. *Stroke* 1997;28:491-9.

(2) Wardlaw JM. What causes lacunar stroke? *J Neurol Neurosurg Psychiatry* 2005;76(5):617-9.

(3) Wiszniewska M, Devuyst G, Bogousslavsky J, Ghika J, van Melle G. What Is the Significance of Leukoaraiosis in Patients With Acute Ischemic Stroke? *Arch Neurol* 2000;57(7):967-73.

(4) Gunning-Dixon FM, Raz N. The Cognitive Correlates of White Matter Abnormalities in Normal Aging: A Quantitative Review. *Neuropsychology* 2000;14(2):224-32.

(5) Sitoh YY, Sitoh YY, Sahadevan S. Clinical significance of cerebral white matter lesions in older Asians with suspected dementia. *Age Ageing* 2004;33(1):67-71.

(6) Patton N, Aslam T, Macgillivray T, Pattie A, Deary IJ, Dhillon B. Retinal vascular image analysis as a potential screening tool for cerebrovascular disease: a rationale based on homology between cerebral and retinal microvasculatures. *J Anat* 2005;206(4):319-48.

(7) Ikram MK, De Jong FJ, Van Dijk EJ, et al. Retinal vessel diameters and cerebral small vessel disease: The Rotterdam Scan Study. *Brain* 2006;129(1):182-8.

(8) Wong TY, Klein R, Sharrett AR, et al. Cerebral white matter lesions, retinopathy, and incident clinical stroke. *Journal of the American Medical Association* 2002;288(1):67-74.

(9) Wong TY, Klein R, Couper DJ, et al. Retinal microvascular abnormalities and incident stroke: The Atherosclerosis Risk in Communities Study. *Lancet* 2001;358(9288):1134-40.

(10) Doubal FN, Hokke PE, Wardlaw JM. Retinal microvascular abnormalities and stroke: a systematic review. *J Neurol Neurosurg Psychiatry* 2009;80(2):158-65.

(11) Doubal FN, MacGillivray TJ, Hokke PE, Dhillon B, Dennis MS, Wardlaw JM. Differences in retinal vessels support a distinct vasculopathy causing lacunar stroke. *Neurology* 2009;72(20):1773-8.

(12) Lindley RI, Wang JJ, Wong MC, et al. Retinal microvasculature in acute lacunar stroke: a cross-sectional study. *Lancet Neurol* 2009;8(7):628-34.

(13) Murray CD. The Physiological Principle of Minimum Work. I. The Vascular System and the Cost of Blood Volume. *Proceedings of the National Academy of Sciences of the United States of America* 1926;12(3):207-14.

(14) Patton N, Pattie A, Macgillivray T, et al. The association between retinal vascular network geometry and cognitive ability in an

elderly population. *Invest Ophthalmol Vis Sci* 2007;48(5):1995-2000.

(15)   Chapman N, Dell'omo G, Sartini MS, et al. Peripheral vascular disease is associated with abnormal arteriolar diameter relationships at bifurcations in the human retina. *Clin Sci (Lond)* 2002;103(2):111-6.

(16)   Witt N, Wong TY, Hughes AD, et al. Abnormalities of retinal microvascular structure and risk of mortality from ischaemic heart disease and stroke. *Hypertension* 2006;47(5):975-81.

(17)   Stanton AV, Wasan B, Cerutti A, et al. Vascular network changes in the retina with age and hypertension. *J Hypertens* 1995;13(12 Pt 2):1724-8.

(18)   Brott T, Adams HP, Jr., Olinger CP, et al. Measurements of acute cerebral infarction: a clinical examination scale. *Stroke* 1989;20(7):864-70.

(19)   Bamford J, Sandercock P, Dennis M, Warlow C, Burn J. Classification and natural history of clinically identifiable subtypes of cerebral infarction. *The Lancet* 1991;337(8756):1521-6.

(20)   Beneficial effect of carotid endarterectomy in symptomatic patients with high-grade carotid stenosis. North American Symptomatic Carotid Endarterectomy Trial Collaborators. *N Engl J Med* 1991;325(7):445-53.

(21) Fazekas F, Chawluk JB, Alavi A, Hurtig HI, Zimmerman RA. MR signal abnormalities at 1.5T in Alzheimer's dementia and normal aging. *Am J Roentgenol* 1987;149(2):351-6.

(22) Mead GE, Lewis SC, Wardlaw JM, Dennis MS, Warlow CP. How well does the Oxfordshire community stroke project classification predict the site and size of the infarct on brain imaging? *J Neurol Neurosurg Psychiatry* 2000;68(5):558-62.

(23) Murray CD. The physiological principle of minimum work applied to the angle of branching of arteries. *J Gen Physiol* 1926;9(6):835-41.

(24) Zamir M. Nonsymmetrical bifurcations in arterial branching. *J Gen Physiol* 1978;72(6):837-45.

(25) Tadjfar M. Branch angle and flow into a symmetric bifurcation. *J Biomech Eng* 2004;126(4):516-8.

(26) Fernando MS, Simpson JE, Matthews F, et al. White Matter Lesions in an Unselected Cohort of the Elderly: Molecular Pathology Suggests Origin From Chronic Hypoperfusion Injury. *Stroke* 2006;37(6):1391-8.

(27) Fazekas F, Schmidt R, Scheltens P. Pathophysiologic mechanisms in the development of age-related white matter changes of the brain. *Dement Geriatr Cogn Disord* 1998 July;9 Suppl 1:2-5.

(28) de Leeuw FE, de Groot JC, Bots ML, et al. Carotid atherosclerosis and cerebral white matter lesions in a population based magnetic resonance imaging study. *Journal of Neurology* 2000;247(4):291-6.

(29) Sachdev P, Wen W, DeCarli C, Harvey D. Should We Distinguish Between Periventricular and Deep White Matter Hyperintensities?. *Stroke* 2005;36(11):2342-4.

(30) Wong TY, Knudtson MD, Klein BEK, Klein R, Hubbard LD. Medication use and retinal vessel diameters. *American Journal of Ophthalmology* 2005;139(2):373-5.

(31) Chapman N, Mohamudally A, Cerutti A, et al. Retinal vascular network architecture in low-birth-weight men. *Journal of Hypertension* 1997;15(12):1449-54.

Table 1. Baseline characteristics of patients by ischaemic stroke subtype.

Characteristic	Lacunar stroke	Cortical stroke	P value for difference
n	94	87	
Mean age (SD) years	65.2 (11.5)	69.2 (11.5)	0.02
Male n (%)	53 (56%)	62 (71%)	0.04
AF n (%)	4 (4%)	11 (13%)	0.05
Carotid stenosis>50% n (%)	4 (5%)	10 (12%)	0.08
Median Deep WMH Fazekas score (IQR)	1 (1-2)	1 (1-2)	0.97
Median periventricular WMH Fazekas score (IQR)	1 (1-2)	1 (1-1)	0.50
Past medical history of			
Hypertension n (%)	57 (66%)	53 (56%)	0.21
Diabetes n (%)	18 (19%)	10 (11%)	0.15
Ischaemic Heart Disease n (%)	13 (14%)	23 (26%)	0.03
Peripheral Vasc. Disease n (%)	3 (3%)	1 (1%)	0.62
Previous Stroke/TIA n (%)	20 (21%)	17 (20%)	0.77

AF = atrial fibrillation, WMH = white matter hyperintensity, TIA = Transient Ischaemic Attack, IQR = inter quartile range SD = standard deviation.

Table 2. Multivariable adjusted associations with absolute retinal arteriolar branching co-efficients. All values are corrected for the presence of all of the other variables in the table. TIA = transient ischaemic attack, WMH = white matter hyperintensity,

Variable	Beta co-efficient	P value
Lacunar stroke subtype	-0.001	0.96
Age	-0.001	0.70
Deep WMH	-0.076	0.003
Periventricular WMH	0.072	0.006
Past history of:		
Hypertension	-0.020	0.50
Diabetes	-0.032	0.38
Ischaemic heart disease	0.155	<0.001
Stroke/TIA	0.040	0.25
Peripheral vascular disease	0.032	0.73

Table 3. Multivariable adjusted associations with absolute retinal arteriolar branching angles. All values are corrected for the presence of all of the other variables in the table. TIA = transient ischaemic attack, WMH = white matter hyperintensity,

Variable	Beta co-efficient	P value
Lacunar stroke subtype	2.22	0.07
Age	-0.03	0.61
Deep WMH score	1.12	0.34
Periventricular WMH score	-0.52	0.65
Past history of:		
Hypertension	1.27	0.33
Diabetes	0.95	0.57
Ischaemic heart disease	0.51	0.75
Stroke/TIA	-0.33	0.83
Peripheral vascular disease	9.05	0.006*

Figure Legends

Fig 1. Illustration of vessel tracking across a bifurcation to measure widths for branching co-efficient calculation. Please note that only for illustrative purposes the vessel demonstrated in the image is a venule. We performed measurements on retinal arterioles.

Figure 2. Example of measurement of retinal vessel branching angle. The lines denoting the direction of the branches were produced by the semi-automated software which tracked down each vessel and the angle subtended by the daughter branches calculated with the cosine rule. Please note that only for illustrative purposes the vessel demonstrated in the image is a venule. We performed measurements on retinal arterioles.

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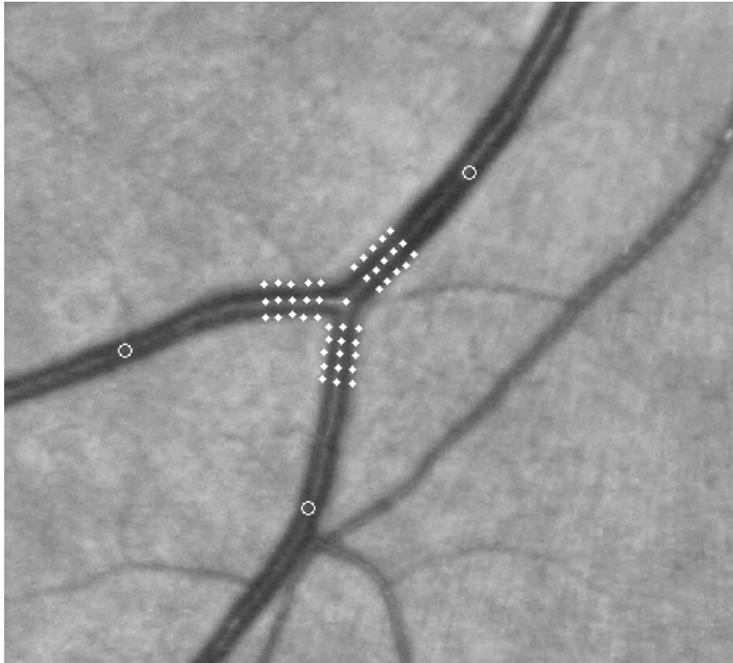
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