Retinal vessel phenotype in patients with a history of retinal vein occlusion

Semecas, Rachel; Arnould, Louis; Aptel, Florent; Gavard, Olivier; Mautuit, Thibaud; Creuzot-Garcher, Catherine

Published in:
Ophthalmic Research

DOI:
10.1159/000516235

Publication date:
2021

Ophthalmic Research

Ophthalmic Res , DOI: 10.1159/000516235
Received: September 14, 2020
Accepted: February 26, 2021
Published online: April 28, 2021

Retinal vessel phenotype in patients with a history of retinal vein occlusion

Semecas R, Arnould L, Aptel F, Gavard O, Mautuit T, Creuzot-Garcher C, Bron A, MacGillivray TJ, Hogg S, Trucco E, Chiquet C

ISSN: 0030-3747 (Print), eISSN: 1423-0259 (Online)
https://www.karger.com/ORE
Ophthalmic Research

Retinal vessel phenotype in patients with a history of retinal vein occlusion

Rachel Semecas[1,2,3], Louis Arnould[4,5], Florent Aptel[1,2,3], Olivier Gavard[1,2,3], Thibaud Mautuit [1,2,3], Catherine Creuzot-Garcher[4,6], Alain Bron[4,6], Tom MacGillivray,[7] Stephen Hogg,[8] Emmanuel Trucco[8], Christophe Chiquet[1,2,3]

[1] Department of Ophthalmology, University Hospital of Grenoble, France
[2] Grenoble Alpes University, Grenoble, France
3 HP2 Laboratory, INSERM U1042, Univ. Grenoble Alpes, Grenoble, France
[4] Department of Ophthalmology, University Hospital of Dijon, France
[5] INSERM, CIC1432, clinical epidemiology unit, Dijon, France; Dijon University Hospital, Clinical investigation Center, Clinical epidemiology/clinical trials unit, Dijon, France
[6] Eye and Nutrition Research group, CSGA, UMR 1324 INRA, Dijon, France
[7] VAMPIRE project, Centre for Clinical Brain Sciences, University of Edinburgh, Edinburgh, UK
[8] VAMPIRE project, Computing, School of Science and Engineering, University of Dundee, Dundee, UK

Short title: Retinal vascular phenotype in contralateral eyes of RVO

Corresponding author:
Prof C. CHIQUET, Department of Ophthalmology, University Grenoble Alpes Hospital, 38000 Grenoble, France, email: cchiquet@chu-grenoble.fr, tel : +33 476767575

Number of Tables: 5
Number of Figures: 1
Word count: 2660

Key Words: Retinal vein occlusion, retinal image analysis, arterial diameter, vein diameter, fractal dimension, tortuosity, VAMPIRE software

Abstract
Introduction: The aim of the study was to estimate the phenotype of retinal vessels using CRAE (central retinal artery equivalent), CRVE (central retinal vein equivalent), tortuosity and fractal analysis in the unaffected contralateral eye of patients with central (CRVO) or branch (BRVO) retinal vein occlusion.
Methods: 34 patients suffering from CRVO, 15 suffering from BRVO and 49 controlled-matched subjects had a fundus image analyzed using the VAMPIRE software. The intraclass correlation coefficient and a Bland-Altman plot were done for the reproducibility study.
Results: There was a lack of evidence of difference between the control group and the CRVO group for CRAE (p=0.06), CRVE (p=0.3) and arterio-venule ratio (AVR, p=0.6). Contralateral eyes of CRVO exhibited a significantly higher arterial and minimum arterial tortuosity values (p=0.012), as compared with control eyes. Contralateral eyes of patients with a history of BRVO had a significantly higher CRAE (p=0.02), AVR (p=0.006) and minimal arterial tortuosity (p=0.05). Fractal analysis showed that contralateral eyes of BRVO had higher values of fractal parameters (D0a, p=0.005).
Conclusion: This study suggests that CVRO or BRVO are not triggered by the same retinal vascular phenotypes in the contralateral eye. The morphology of retinal vasculature may be associated with the occurrence of RVO, independently of known risk factors.

INTRODUCTION
Retinal vein occlusions [RVO) have prevalence rates ranging from 0.1% to 1.1% of the ageing population and bilateralization occurs in 5% of the cases over a 1- to 3-year period. Virchow's triad includes the three broad categories of factors that are thought to contribute to thrombosis, as in central retinal vein occlusion (CRVO): hypercoagulability, hemodynamic changes (stasis, turbulence) and endothelial injury/dysfunction. Two main risk factors have been identified: systemic hypertension and open-angle glaucoma [1]. The pathogenesis of branch retinal vein occlusion (BRVO) is multifactorial in origin and not completely defined; with a possible combination of mechanical compression, degenerative changes in vessel walls, and/or hypercoagulable factors [1]. The

arteriosclerotic changes (at the sites of arteriovenous crossing) are believed to result in venule occlusion through endothelial cell damage, thrombosis, and focal venous narrowing at sites of arterio-venous nicking [2].
The study of vessel caliber and tortuosity merits further consideration since it is not known whether a patient's vessel network promotes the onset of RVO, independently of association with cardiovascular risk factors. We hypothesize that the retinal phenotype (vasculature morphometry) may be associated with the occurrence of RVO. The analysis of fundus camera images offers a non-invasive measurement method to study the vascular retinal network. The vascular morphological phenotype (including tortuosity and fractal dimension) provides information on the architecture and geometry of the vessel network, which determines the efficiency of blood circulation. We used the VAMPIRE (Vessel Assessment and Measurement Platform for Images of the Retina, Universities of Edinburgh and Dundee) software, which enables a detailed quantitative analysis of the vascular morphometry [3]. VAMPIRE has been used in several studies on retina biomarkers for lacunar stroke, cognition, dementia, hypertension and cardiovascular diseases among others [4]. More recently, VAMPIRE has been used to investigate the retinal vessel phenotype in ocular disease, such as non-arteritic ischemic optic neuropathy and glaucoma [5,6].
There is evidence that vascular parameters such as central retinal vein equivalent (CRVE), central retinal artery equivalent (CRAE), arterio-venule ratio (AVR) and tortuosity are considered to be correlated between right and left eyes. We studied the vascular phenotype of the unaffected contralateral eyes of patients with retinal vein occlusion and compared it to a control group. Previous studies evaluated the contralateral eyes of RVO and showed the subtle abnormalities in eyes with normal appearance: late peripheral retinal leakage using peripheral fluorescein angiography [7], non-perfused foveal capillaries using adaptive optics scanning light ophthalmoscope [8], and decreased retinal nerve fibre thickness in the inferior and superior-temporal quadrants using optical coherence tomography [9].
Therefore, we aimed to characterize the phenotype of retinal vessels using CRAE, CRVE, tortuosity and fractal dimension in the non-affected contralateral eye of patients with a history of CRVO and BRVO, and to compare this phenotype with that of a control group matched for age, sex, systemic hypertension and diabetes.

METHODS

Retinal vein occlusion (RVO) population

Thirty-four patients suffering from CRVO and 15 patients from BRVO were included in this multi-centric case-control study in 2016 and 2017 (17 in the Ophthalmology Department of Grenoble Hospital and 22 in Dijon University Hospital). The inclusion criteria of patients were: age over 18 years, diagnosis of CRVO or BRVO, patients with ametropia (spherical equivalent) ≤ 3 diopters (D). Exclusion criteria for the contralateral eye of the RVO eye were the presence of additional ocular diseases, and fundus pictures with low quality (n=14) due to cataract or poor fixation.

Control group

Forty-nine controlled-matched subjects (1:1) were prospectively included at the Ophthalmology Department of Grenoble University Hospital. The inclusion criteria were: age over 18 years, patient without any ophthalmologic medical history, patients with ametropia ≤ 3 D. Exclusion criteria were: adult under guardianship or unable to consent, patients with ametropia > 3 D and ocular disease. This control population was matched to the RVO population for age (5-year interval), sex and systemic hypertension and diabetes.

Acquisitions and analysis using 30 or 45-degree funduscopic color photograph

30 or 45-degree fundus camera images of the right-eye were acquired, centered on the optic nerve and the macula, using a non-mydriatic camera: Visucam 200 (Carl Zeiss Meditec™ France, resolution of 2124 x 2056 pixels) or CR2 (Canon™ Europa, Amstelveen, The Netherlands, resolution of 4752 × 3168 pixels).

Image analysis

VAMPIRE measures semi-automatically morphological parameters of the retinal vessels. First, the optic disc contour and the macula center are located. This enabled the definition of the usual retinal coordinates (x axis through optic disc (OD) and macula centers, origin in the OD center) and circular zones around the OD, namely zone A (between OD center and 0.5 optic disc diameter (ODD)), zone B (between 0.5 and 1 ODD), and zone C (between 0.5 and 2 ODD), shown in Figure 1. Manual correction can be performed efficiently when the optic disc or fovea have been incorrectly identified automatically. Vessels are subsequently detected and labelled as arterioles or venules semi-automatically (Figure 1).

Here we used CRAE, the mean of the widths of the six largest arteries (using the revised Knudtson formulas)[10]; CRVE, as above but for the six largest venules;[9] arterio-venule ratio (AVR = CRAE/CRVE); FD of the vascular network in Zone C, a measure of geometric complexity of the pattern of the vessels in zone C, including the degree of branching complexity, and vascular tortuosity. AVR, CRAE and CRVE were computed in zone B, fractal measure and vascular tortuosity analysis in zone C. Raw measurements of CRAE and CRVE were in pixels. Tortuosity may be associated with high blood flow, angiogenesis and blood vessel congestion.
Following previously reported studies, the pixel-to-mm conversion factor was obtained by dividing the average vertical ODD (over all images, acquired with the same camera at the same resolution) by the assumed average of the disc diameter in microns (1850 μm)[10–12].
Excellent intra- and inter-operator reproducibility (above 0.82 and 0.91 respectively) for the two operators (RS and OG) participating in the study was obtained with 100 fundus images from healthy subjects (n = 30) or patients suffering from glaucoma or contralateral eyes from retinal vein occlusion (n=70) twice by two different operators (RS and OG).

Statistical analysis
Statistical analysis was performed using the Statistical Package for the Social Sciences program (SPSS 17.0 for Windows, Chicago, IL, USA). Quantitative data were expressed as mean and standard deviation (SD), after checking the normality of data. The intraclass correlation coefficient with a confidence interval of 95% and a Bland-Altman plot were done for the reproducibility study. Mean comparisons were studied using two-tailed t-test. Statistical significance was set at $p<0.05$.

RESULTS
Reproducibility study (n=100)
The inter- and intra- operator reproducibility (ICC) was calculated from a series of 100 fundus images (Table 1) and was considered as excellent. Inter-operator ICC ranged from 0.918 to 0.992 and intra-operator ICC from 0.823 to 0.98.

CRVO population (n=34)
Patients were male in 22 out the cases, had systemic hypertension in 41% out of the cases. The vascular parameters of the contralateral eye were compared with the control eyes (Tables 2 and 3). No significant difference was found between the control group and the CRVO group for age (p=0.75), refraction (p=0.08), CRAE (p=0.06), CRVE (p=0.3) and AVR (p=0.6). Contralateral eyes of CRVO exhibited a significantly higher arterial and minimum arterial tortuosity values (p=0.012). There was no statistically significant difference between the two groups for fractal dimension analysis (p=0.26).

BRVO population (n=15):
Patients were male in 8 out the cases, had systemic hypertension in 35% out of the cases. The vascular parameters of the contralateral eye of 15 patients were compared with a group of 15 matched control eyes (Table 4 and 5). Contralateral eyes of patients with a history of BRVO had a higher CRAE (p=0.02), AVR (p=0.006) and minimal arterial tortuosity (p=0.05). Fractal analysis showed that contralateral eyes of BRVO had significantly higher values of fractal dimension, such as D0a (p=0.005), D1a (p=0.008), D2a (p=0.008), D0tot (p=0.01), D1tot (p=0.01), D2tot (p=0.01).

DISCUSSION
This original study showed that contralateral eyes exhibit (a) in cases of CRVO, a greater arterial tortuosity values and (b) in cases of BRVO a greater CRAE, AVR, arterial tortuosity and higher values of fractal parameters, as compared with control group matched for age, sex, systemic hypertension and diabetes. These exploratory results strongly suggest that the morphology of retinal vasculature may be associated with the occurrence of RVO, independent of known risk factors.
PATIENTS WITH A CRVO HISTORY
Epidemiological studies have shown that contralateral eyes of patients with a history of CRVO in one eye have (a) a significantly increased risk of developing RVO compared with the general population (7% probability within 4 years)[13](14], (b) an increased number of non-perfused capillaries near the foveal avascular zone and decreased

perfused foveal microvascular density [8]. Therefore, studying contralateral eyes may elucidate the early pathological changes signaling a future occlusive event.

The significant finding in our CRVO population was the abnormal higher arterial tortuosity in patients with a history of CRVO. This abnormality could increase the blood resistance and modify the blood rheology in some retinal vessels and may indicate a predisposition for occurrence of RVO. In the literature, reduced arterial tortuosity is usually reported with age, hypertension and BMI whereas increased venular tortuosity is associated with a younger age, higher BP and lower HDL [15].

The other important finding was the absence of changes of CRAE, CRVE, AVR and fractal dimension in patients as compared with the matched control group. Notwithstanding the pilot size of our cohort, this could be a major finding since these parameters are correlated with age, BMI and history of hypertension [16], which were all taken into account with matching populations.

PATIENTS WITH A BRVO HISTORY

Patients with a history of BRVO had higher CRAE and AVR, whereas CRVE was not significantly different when compared with the control group. A larger arteriolar calibre has been previously reported with current cigarette smoking [17], the activity of plasma GPx-3, a major player in oxidative stress regulation [17], calcium channel blockers, combined use of aspirin and antihypertensive agents [18], higher levels of serum glucose (glycated haemoglobin HbA1c [19]. These factors should be therefore considered in future studies. In the present study, only 6 patients out of 49 took calcium channel blockers (4 CRVO, 2 BRVO).

The increase of AVR is probably due to the significant increase in CRAE whereas CRVE is comparable in both groups. On the other hand, matching both groups with the presence or absence of hypertension may explain why we did not measured the well-known decrease in CRAE associated with hypertension [20,21]. One previous study investigated the vascular phenotype of eyes (n=25) before development of BRVO [22] and found that AVR was significantly smaller in eyes whereas CRAE and CRVE did not vary significantly as compared with the fellow eyes which did not develop BRVO. AVR of contraleteral eye of BRVO in our study (0.76± 0.05) was close to that found (0.73) in fellow eyes in the study of Kawasaki et al. On the other hand, severe arterio-venous nicking, isolated retinopathy and a smaller angle at the crossing site were more prevalent in eyes which developed BRVO compared with fellow eyes. Our study is complementary to this latter study since we studied only fellow eyes at the time of BRVO, which is different from a longitudinal study. We also compared the fellow eyes to a control group matched for hypertension, age and diabetes.

We found that contralateral eyes of BRVO have higher fractal dimension of the vasculature network. The FD index quantifies the complexity and density of the vessel branching pattern visible in a fundus image. FD variations are an indicator of deviations from the normal or optimized network and, when reduced, a potential marker of cardiovascular (coronary disease) or neurologic disease (stroke). Our results suggest that these patients maintain a high level of complexity even if hypertension and age are considered.

We did not find an overall increase in tortuosity whereas the minimal arterial tortuosity was increased significantly in these patients. This suggests a change in the distribution of arterial tortuosity among arterioles within unaffected contralateral eyes in both CRVO and BRVO populations. This difference is not likely associated with diabetes and systemic hypertension since these factors were well balanced with the control group. Factors known to have an impact on tortuosity were not significantly involved in our series (refraction, race). The significance of this result should be confirmed in further studies and other associated factors should be investigated, such as cardiovascular diseases (such as ischemic heart disease [16], obesity [15], dyslipidemia [22] and renal dysfunction [23].

Strengths on this study were (a) the excellent inter- and intra-observer agreement (above 0.9 and 0.8 respectively) using VAMPIRE, consistent with that described in other studies using this software [24,25], and (b) matching of the control group on potential confusion factors (hypertension, age, diabetes). This control group was matched not only for well-known factors influencing vascular parameters (age)[15] and systemic hypertension but also for risk factors of retinal vein occlusion (diabetes, systemic hypertension) [26]. No RVO subject in our cohort had glaucoma.

We acknowledge several limitations of this pilot. First, the modest (pilot-size) size of the cohort. Second, the absence of measurements of blood pressure or the assessment of the severity of hypertension. Many studies reported association between lower monofractal dimensions and higher mean arterial blood pressure [27,28]. Association between lower fractal dimensions and diastolic blood pressure were also reported [28,29]. Other clinical variables such as history of cardiovascular or neurovascular disease, body mass index and dyslipidemia, may alter the retinal vascular phenotype in the RVO and control groups [30].Third, the use of images taken at

different resolutions. To partially correct for these magnification differences between cameras, a calibration factor was obtained by measuring the average vertical height of the optic disc (in pixels) in all retinal images with the same resolution and the same camera, and dividing the assumed average of the disc diameter (1850 µm, average disc diameter measured for the Caucasian subjects) with the average value ODD measurements in pixels in the same series. This technique has been extensively used in different studies using SIVA [12] and IVAN [31].
Fourth, associations between smaller retinal vessel diameters and longer axial length and more myopic refraction were reported previously [32,33]. The effect of refraction was limited in our study since the spherical equivalent was 0.5 ±1.5D in the RVO group and 0.5 ±1.5D in the control group. Ideally it would be useful to measure axial length in order to convert pixels into microns using Bennet's formula [34].
Fifth and finally, we assumed that contralateral eyes reflected the phenotype of both eyes. We based this hypothesis on previous studies showing good correlations for vascular parameters between right and left eyes. Using VAMPIRE (Kirin, Mirna: Genetic Analysis of Retinal Traits. University of Edinburgh, Ph Thesis, 2013, https://era.ed.ac.uk/handle/1842/9619?show=full), monofractal and multifractal dimensions of the retinal vasculature were also tested for left and right eye correlation (n= 130 images of heathy eyes). Correlation values were higher compared to the branching parameters. Left-right eye correlations were 0.78 for monofractal dimensions and 0.50 for multifractal dimensions. We note however that the degree of symmetry of the morphology, and its quantitative characterisation, are the object of current discussion [35].

CONCLUSION

This pilot study in patients with a history of CVRO or BRVO suggests that both diseases are not associated with the same retinal vascular phenotypes in the contralateral eye. Contralateral eyes of CRVO eyes exhibited a higher tortuosity whereas contralateral eyes of BRVO eyes had higher CRAE, AVR and fractal dimension. These factors should be studied further in a longitudinal, larger study to better understand the potential risk factors associated with the retinal phenotype.

STATEMENTS OF ETHICS:

Written informed consent was obtained from the subjects after explanation of the experiment. The study followed the Declaration of Helsinki guidelines for research involving human subjects and was approved by the local Institutional Review Board (IRB# 5921).

CONFLICT OF INTEREST STATEMENT:

C CHIQUET: Thea, Allergan, Horus
A BRON: Aerie, Allergan, Bausch Lomb, Santen, Théa
F APTEL: Aerie, Allergan, BaushLomb, Glaukos, Horus, Quantel, Santen, Thea
C CREUZOT: Allergan, Bayer, Alcon, Novartis, Roche, Théa, Bausch and Lomb

R SEMECAS, L ARNOULD, O GAVARD, T MAUTUIT , T MAC GILLIVRAY, S HOGG, E TRUCCO: none

FUNDINGS: The study was funded by the Association de Recherche et de Formation en Ophtalmologie (ARFO) and Fondation de France (Berthe Fouassier grant).

AUTHOR CONTRIBUTION: all authors contribute significantly to:

- Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work: all authors
- Drafting the work or revising it critically for important intellectual content: all authors
- Final approval of the version to be published: all authors
- Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

REFERENCES

1. Hayreh SS. Prevalent misconceptions about acute retinal vascular occlusive disorders. Prog Retin Eye Res. 2005 Jul;24(4):493–519.
2. Koch E, Rosenbaum D, Brolly A, Sahel J-A, Chaumet-Riffaud P, Girerd X, et al. Morphometric analysis of small arteries in the human retina using adaptive optics imaging: relationship with blood pressure and focal vascular changes. J Hypertens. 2014 Apr;32(4):890–8.

3. Giachetti A, Ballerini L, Trucco E. Accurate and reliable segmentation of the optic disc in digital fundus images. J Med Imaging (Bellingham). 2014 Jul;1(2):024001.
4. McGrory S, Cameron JR, Pellegrini E, Warren C, Doubal FN, Deary IJ, et al. The application of retinal fundus camera imaging in dementia: A systematic review. Alzheimers Dement (Amst). 2017;6:91–107.
5. Remond P, Aptel F, Cunnac P, Labarere J, Palombi K, Pepin J-L, et al. Retinal Vessel Phenotype in Patients with Nonarteritic Anterior Ischemic Optic Neuropathy. Am J Ophthalmol. 2019;208:178–84.
6. Chiquet C, Gavard O, Arnould L, Mautuit T, Macgillivray TJ, Bron AM, et al. Retinal vessel phenotype in patients with primary open-angle glaucoma. Acta Ophthalmol. 2020 Feb;98(1):e88–93.
7. Suzuki N, Hirano Y, Tomiyasu T, Esaki Y, Uemura A, Yasukawa T, et al. Retinal Hemodynamics Seen on Optical Coherence Tomography Angiography Before and After Treatment of Retinal Vein Occlusion. Invest Ophthalmol Vis Sci. 2016 Oct 1;57(13):5681–7.
8. Pinhas A, Dubow M, Shah N, Cheang E, Liu CL, Razeen M, et al. Fellow eye changes in patients with nonischemic central retinal vein occlusion: Assessment of Perfused Foveal Microvascular Density and Identification of Nonperfused Capillaries. Retina (Philadelphia, Pa). 2015 Oct;35(10):2028–36.
9. Kim S, Sung KR, Joe SG, Kim JT, Lee DH, Lee JY, et al. Comparison between glaucomatous and non-glaucomatous eyes with unilateral retinal vein occlusion in the fellow eye. Korean J Ophthalmol. 2013 Dec;27(6):440–5.
10. Knudtson MD, Lee KE, Hubbard LD, Wong TY, Klein R, Klein BEK. Revised formulas for summarizing retinal vessel diameters. Curr Eye Res. 2003 Sep;27(3):143–9.
11. Varma R, Tielsch JM, Quigley HA, Hilton SC, Katz J, Spaeth GL, et al. Race-, age-, gender-, and refractive error-related differences in the normal optic disc. Arch Ophthalmol. 1994 Aug;112(8):1068–76.
12. Hubbard LD, Brothers RJ, King WN, Clegg LX, Klein R, Cooper LS, et al. Methods for evaluation of retinal microvascular abnormalities associated with hypertension/sclerosis in the Atherosclerosis Risk in Communities Study. Ophthalmology. 1999 Dec;106(12):2269–80.
13. Natural history and clinical management of central retinal vein occlusion. The Central Vein Occlusion Study Group. Arch Ophthalmol. 1997 Apr;115(4):486–91.
14. McIntosh RL, Rogers SL, Lim L, Cheung N, Wang JJ, Mitchell P, et al. Natural history of central retinal vein occlusion: an evidence-based systematic review. Ophthalmology. 2010 Jun;117(6):1113-1123.e15.
15. Cheung CY-L, Zheng Y, Hsu W, Lee ML, Lau QP, Mitchell P, et al. Retinal vascular tortuosity, blood pressure, and cardiovascular risk factors. Ophthalmology. 2011 May;118(5):812–8.
16. Witt N, Wong TY, Hughes AD, Chaturvedi N, Klein BE, Evans R, et al. Abnormalities of retinal microvascular structure and risk of mortality from ischemic heart disease and stroke. Hypertension. 2006 May;47(5):975–81.
17. Daien V, Carriere I, Kawasaki R, Cristol J-P, Villain M, Fesler P, et al. Retinal vascular caliber is associated with cardiovascular biomarkers of oxidative stress and inflammation: the POLA study. PLoS ONE. 2013;8(7):e71089.
18. Yoo E, Yoo C, Lee B-R, Lee T-E, Kim YY. Diagnostic Ability of Retinal Vessel Diameter Measurements in Open-Angle Glaucoma. Invest Ophthalmol Vis Sci. 2015 Dec;56(13):7915–22.
19. Wong TY, Islam FMA, Klein R, Klein BEK, Cotch MF, Castro C, et al. Retinal vascular caliber, cardiovascular risk factors, and inflammation: the multi-ethnic study of atherosclerosis (MESA). Invest Ophthalmol Vis Sci. 2006 Jun;47(6):2341–50.
20. McGowan A, Silvestri G, Moore E, Silvestri V, Patterson CC, Maxwell AP, et al. Evaluation of the Retinal Vasculature in Hypertension and Chronic Kidney Disease in an Elderly Population of Irish Nuns. PLoS ONE. 2015;10(9):e0136434.
21. Triantafyllou A, Doumas M, Anyfanti P, Gkaliagkousi E, Zabulis X, Petidis K, et al. Divergent retinal vascular abnormalities in normotensive persons and patients with never-treated, masked, white coat hypertension. Am J Hypertens. 2013 Mar;26(3):318–25.
22. Kawasaki R, Nagano E, Uno M, Okada M, Kawasaki Y, Kitamura A. Retinal vascular features associated with risk of branch retinal vein occlusion. Curr Eye Res. 2013 Sep;38(9):989–93.
23. Owen CG, Rudnicka AR, Nightingale CM, Mullen R, Barman SA, Sattar N, et al. Retinal arteriolar tortuosity and cardiovascular risk factors in a multi-ethnic population study of 10-year-old children; the Child Heart and Health Study in England (CHASE). Arterioscler Thromb Vasc Biol. 2011 Aug;31(8):1933–8.
24. Sasongko MB, Wong TY, Donaghue KC, Cheung N, Jenkins AJ, Benitez-Aguirre P, et al. Retinal arteriolar tortuosity is associated with retinopathy and early kidney dysfunction in type 1 diabetes. Am J Ophthalmol. 2012 Jan;153(1):176-183.e1.

25. MacGillivray TJ, Cameron JR, Zhang Q, El-Medany A, Mulholland C, Sheng Z, et al. Suitability of UK Biobank Retinal Images for Automatic Analysis of Morphometric Properties of the Vasculature. PLoS ONE. 2015;10(5):e0127914.
26. Perez-Rovira A, MacGillivray T, Trucco E, Chin KS, Zutis K, Lupascu C, et al. VAMPIRE: Vessel assessment and measurement platform for images of the REtina. Conf Proc IEEE Eng Med Biol Soc. 2011;2011:3391–4.
27. Chen S-N, Yang T-C, Lin J-T, Lian I-B. End Stage Renal Disease as a Potential Risk Factor for Retinal Vein Occlusion. Medicine (Baltimore). 2015 Nov;94(47):e1960.
28. Cheung CY, Thomas GN, Tay W, Ikram MK, Hsu W, Lee ML, et al. Retinal vascular fractal dimension and its relationship with cardiovascular and ocular risk factors. Am J Ophthalmol. 2012 Oct;154(4):663-674.e1.
29. Kurniawan ED, Cheung N, Cheung CY, Tay WT, Saw SM, Wong TY. Elevated blood pressure is associated with rarefaction of the retinal vasculature in children. Invest Ophthalmol Vis Sci. 2012 Jan 31;53(1):470–4.
30. Liew G, Wang JJ, Mitchell P, Wong TY. Retinal vascular imaging: a new tool in microvascular disease research. Circ Cardiovasc Imaging. 2008 Sep;1(2):156–61.
31. Arnould L, Binquet C, Guenancia C, Alassane S, Kawasaki R, Daien V, et al. Association between the retinal vascular network with Singapore "I" Vessel Assessment (SIVA) software, cardiovascular history and risk factors in the elderly: The Montrachet study, population-based study. PLoS ONE. 2018;13(4):e0194694.
32. Moradi A, Sepah YJ, Ibrahim MA, Sophie R, Moazez C, Bittencourt MG, et al. Association of retinal vessel calibre and visual outcome in eyes with diabetic macular oedema treated with ranibizumab. Eye (Lond). 2014 Nov;28(11):1315–20.
33. Patton N, Maini R, MacGillivary T, Aslam TM, Deary IJ, Dhillon B. Effect of axial length on retinal vascular network geometry. Am J Ophthalmol. 2005 Oct;140(4):648–53.
34. Lim LS, Cheung CY, Lin X, Mitchell P, Wong TY, Mei-Saw S. Influence of refractive error and axial length on retinal vessel geometric characteristics. Invest Ophthalmol Vis Sci. 2011 Feb;52(2):669–78.
35. Bennett AG, Rudnicka AR, Edgar DF. Improvements on Littmann's method of determining the size of retinal features by fundus photography. Graefes Arch Clin Exp Ophthalmol. 1994 Jun;232(6):361–7.
36. Cameron JR, Megaw RD, Tatham AJ, McGrory S, MacGillivray TJ, Doubal FN, et al. Lateral thinking - Interocular symmetry and asymmetry in neurovascular patterning, in health and disease. Prog Retin Eye Res. 2017;59:131–57.

FIGURE LEGEND

Figure 1. Example of retinal coordinates, zones and vasculature detection and labeling.

1A: Example of fundus image of one eye of the control group, and segmentation of retinal vein or artery vessels. Zones A, B and C are defined in the standard reference system based on center and radius of the optic nerve disc (OD), and on the location of the fovea. The system divides the image in concentric regions around the OD center. The zones are defined as zone A (between OD center and 0.5 optic disc diameter (ODD)), zone B (between 0.5 and 1 ODD), and zone C (between 0.5 and 2 ODD). Periphery is defined as the area outside Zone C.

1B: example of the VAMPIRE interface, showing a binary vessel map.

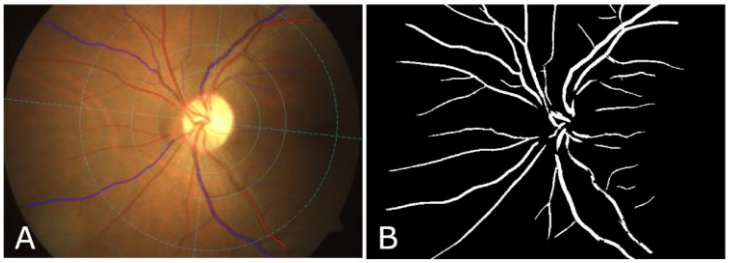

Table 1: **Reproducibility study from a series of 100 fundus images**

	ICC intra-operator 1	CI 95%	ICC intra-operator 2	CI 95%	ICC inter-operator	CI 95%
AVR	0.923	0.884-0.95	0.823	0.730-0.883	0.918	0.875-0.946
CRAE	0.941	0.910-0.961	0.914	0.869-0.943	0.944	0.915-0.963
CRVE	0.952	0.927- 0.968	0.968	0.952-0.979	0.979	0.968-0.986
Arterial tortuosity	0.969	0.952-0.979	0.958	0.937-0.972	0.972	0.957-0.981
Vein tortuosity	0.98	0.970-0.987	0.962	0.943-0.975	0.992	0.988-0.995

AVR: arteriole-to-venule ratio
CRAE: central retinal artery equivalent
CRVE: central retinal vein equivalent
ICC: intraclass correlation coefficient
CI: confidence interval
1: first operator
2: second operator

Table 2: **Vascular parameters of the population with an history of central retinal vein occlusion, compared to the control group.**

	Age (years)	Refraction (diopters)	CRAE (microns)	CRVE (microns)	AVR
Control eyes	62.6 ±16.1	−0.2 ±1.8	144.8±9.3	208.8±24.07	0.70 ±0.06

Contralateral eye of CRVO (n=34)	63.8 ±14.6	0.5 ±1.6	150 ±12.7	214.08 ±26.5	0.71 ±0.07

	Vein tortuosity	**Minimum vein tortuosity**	**Maximum vein tortuosity**
Control eyes	$9.86.10^{-5} \pm 2.5.10^{-4}$	$1.72.10^{-5} \pm 3.4.10^{-5}$	$4.96.10^{-4} \pm 9.7.10^{-4}$
Contralateral eye of CRVO	$1.1.10^{-4} \pm 1.2.10^{-4}$	$2.64.10^{-5} \pm 5.6.10^{-5}$	$4.28.10^{-4} \pm 3.7.10^{-4}$

	Arterial tortuosity (p = 0.01)	**Minimum arterial tortuosity** (p = 0.01)	**Maximum arterial tortuosity**
Control eyes	$6.71.10^{-5} \pm 8.4.10^{-5}$	$1.52.10^{-5} \pm 2.67.10^{-5}$	$5.9.10^{-4} \pm 1.6.10^{-3}$
Contralateral eye of CRVO	$1.69.10^{-4} \pm 2.1.10^{-4}$	$3.3.10^{-5} \pm 4.55.10^{-5}$	$6.17.10^{-4} \pm 9.10^{-4}$

AVR: arteriole-to-venule ratio
CRAE: central retinal artery equivalent
CRVE: central retinal vein equivalent

The results are expressed as mean ± SD.

***Table 3*: Fractal dimensions of the arterial vasculature of the population with an history of central retinal vein occlusion, compared to the control group.**
There was no statistically significant difference between the two groups for fractal dimension analysis. The results are expressed as mean ± SD.

	D0a	D1a	D2a	D0v	D1v	D2v
Control eyes	1.56 ±0.07	1.55±0.07	1.55±0.07	1.53±0.06	1.52±0.06	1.51±0.06
Contralateral eye of CRVO	1.57 ±0.08	1.56 ±0.08	1.56±0.08	1.54±0.08	1.53±0.08	1.53±0.08

D0: capacity dimension
D1: information dimension
D2: correlation dimension
A: arterial
V: veinule
Tot: total

***Table 4:* Vascular parameters of the population with an history of branch retinal vein occlusion and the control population.**
AVR: arteriole-to-venule ratio
CRAE: central retinal artery equivalent
CRVE: central retinal vein equivalent
BRVO : branch retinal vein occlusion
The results are expressed as mean ± SD.

	AGE (years)	**REFRACTION** (diopters)	**CRAE** (microns) (p=0.02)	**CRVE** (microns)	**AVR** (p=0.006)
Control eyes	61.87 ±13.3	−0.6 ±1.9	149.3 ±12.4	216.3 ±23.5	0.69 ±0.06
Contralateral eye of BRVO (n=15)	64.47 ±13.56	0.6 ±1.5	166.7 ±25.6	219.2 ±20.8	0.76 ±0.05

	Vein tortuosity	**Minimum vein tortuosity**	**Maximum vein tortuosity**
Control eyes	$8.08.10^{-5}$ ± $1.6.10^{-4}$	$1.22.10^{-5}$ ± $2.5.10^{-5}$	$1.02.10^{-3}$± $3.37.10^{-3}$

Contralateral eye of BRVO	$8.08.10^{-5}$ $\pm 8.77.10^{-5}$	$2.4.10^{-5} \pm 4.3.10^{-5}$	$1.58.10^{-3} \pm 4.9.10^{-3}$

	Arterial tortuosity	**Minimum arterial tortuosity** (p=0.05)	**Maximum arterial tortuosity**
Control eyes	$8.72.10^{-5} \pm 1.10^{-4}$	$8.59.10^{-6} \pm 1.12.10^{-5}$	$4.29.10^{-4} \pm 3.8.10^{-4}$
Contralateral eye of BRVO	$1.64.10^{-4} \pm 1.5.10^{-4}$	$2.74.10^{-5} \pm 3.33.10^{-5}$	$6.07.10^{-4} \pm 6.2.10^{-4}$

***Table 5*: Fractal dimensions of the arterial vasculature,**

	D0a (p=0.005)	**D1a** (p=0.008)	**D2a** (p=0.008)	**D0v**	**D1v**	**D2v**	**D0tot** (p=0.01)
Control eyes	1.55 ±0.06	1.55±0.06	1.55±0.06	1.52±0.06	1.51±0.06	1.51±0.06	1.71±0.05
Contralateral eye of BRVO	1.63±0.07	1.62±0.07	1.61±0.07	1.53±0.07	1.52±0.07	1.52±0.07	1.76±0.05

D0: capacity dimension
D1: information dimension
D2: correlation dimension
A: arterial
V: veinule
Tot: total