



Automatic image analyser to assess retinal vessel calibre (ALTAIR). A new tool to evaluate the thickness, area and length of the vessels of the retina

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ABSTRACT

Background and objectives: The examination of the fundus allows to evaluate retinal the microcirculation in vivo. We assess the reliability and validity of ALTAIR software, and to evaluate its clinical relevance by the association of thickness, area and length of the retinal vessels with other measures of vascular structure and function, target organ damage and cardiovascular risk.

Methods: Cross-sectional study involving a total of 250 subjects aged 62 ± 9 years, 51 % males. In a random subsample of 60 subjects (118 retinographies), we estimated the intraobserver, interobserver and interdevice intraclass correlation coefficients (ICC) of the measurements of retinal vascular thickness, area and length in 3 concentric circles. Concurrent validity was assessed with all 250 subjects (495 retinographies), analysing the relationship to age, blood pressure, target organ damage, vascular structure and function, and cardiovascular risk.

Results: Of the sample, 69 % were diagnosed with hypertension and 17 % with diabetes. Intraobserver ICC ranged from 0.640 for venous length to 0.906 for arterial area. Interobserver ICC ranged from 0.809 for arterial length to 0.916 for venous area, and interdevice ICC for arteriovenous ratio (AVR) was 0.887, thickness of arteries 0.590 and vein thickness 0.677. We found a moderate correlation between retinal vascular parameters and vascular structure and function, and target organ damage. In multiple linear regression analysis, the association with blood pressure, albumin/creatinine ratio, carotid intima-media thickness and cardiovascular risk is maintained.

Conclusion: The ALTAIR tool has been useful for analysing the thickness, area and length of retinal vessels, with adequate reliability and a concomitant association of retinal vessel measurements with other cardiovascular parameters and cardiovascular risk. Therefore, in addition to thickness, the area and length of retinal vessels could also play a role in the prediction of cardiovascular risk.

1. Introduction

The examination of the fundus is a harmless and non-invasive test

which allows retinal circulation to be evaluated in vivo [1] and microcirculation data to be obtained. Changes in geometry, vessel calibre, and microvascular circulation are early changes in the pathogenesis of

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different diseases such as arterial hypertension (HBP) [2,3], or the target-organ lesions of cardiovascular disease [4,5]. These facts, and the ease with which the retinal vessels can be accessed and evaluated, have enhanced the study of variations in retinal vessel calibre and the analysis of their relationship to cardiovascular morbidity and mortality [6]. Different studies have found an association between retinal vessel calibre and HBP [5], ictus [7], left ventricular hypertrophy [8], metabolic syndrome [9] and coronary artery disease in women [10]. However, results have not been consistent when evaluating the relationship to other pathologies such as arteriosclerosis [11] or lesions to target organs (cardiac, renal and vascular) [11,12]. The relationship of microvascular changes in the retina to the parameters which measure vascular structure and function [3,13] or cardiovascular risk (CVR) [14] is also unclear. Over the past two decades, multiple software systems have been developed to measure retinal vessel calibre from fundus photographs [15,16]. Retinal analysis [17] has been used in several studies such as the Atherosclerosis Risk in Communities study [18], Rotterdam study [19], Blue Mountains Eye Study, [20] and others [21,22]. Another system was the Integrative Vessel Analysis (IVAN) [23], used in the Wisconsin Epidemiologic Study of Diabetic Retinopathy [24] and Multi-Ethnic Study of Atherosclerosis [25], among others. Finally, the Singapore I Vessel Assessment (SIVA; version 3.0; National University of Singapore) [26] had more improved automation features and also provided a more global representation of the overall retinal vascular network, with measurements of additional geometry parameters such as branching angles, bifurcation, fractal dimension, and tortuosity and Witt NW et al. [27] have developed a novel measure to characterise optimality of diameter relationships at retinal vascular bifurcations. Our group has developed a valid and reliable semiautomatic tool to evaluate retinal vessel calibre, the AV index calculator [14]. However, an observer still needs to intervene to obtain the measurements, which may limit their reproducibility. While measurements have focused on the calibre of arteries and veins, the analysis of other vascular parameters such as area, length and branching patterns, and their links to cardiovascular parameters has been less frequently researched. The focus of the present study, the "Automatic image analyser to assess retinal vessel calibre" (ALTAIR) software platform, uses analytical methods and artificial intelligence (AI) algorithms to investigate retinal parameters such as calibre, area and length of vessels [28]. This additional information can help to better understand the ageing process of the retinal vessels. In addition, the intervention of the observer is less than most of the available tools, which implies a significant reduction of bias in the measure.

Our aim is to analyse the reliability and validity of the ALTAIR software, and to assess its clinical relevance by the association of thickness, area and length of the retinal vessels with other measures of vascular structure and function, target organ damage and cardiovascular risk.

2. Methods

2.1. Study design and setting

A descriptive cross-sectional tool validation study was carried out to assess the reliability and validity of the ALTAIR software platform. The study was implemented by the Research Unit of a primary healthcare centre.

2.2. Subjects

The study comprised 250 voluntary participants aged 35–74 with some cardiovascular risk factors according to the 2013 European Society of Hypertension/European Society of Cardiology Guidelines [29]. Participants were excluded due to the following criteria: psychic or cognitive disorders that interfere with the established requisites of the protocol; non-collaborative attitude; educational or comprehension

limitations; and severe comorbidities with a 12-month likelihood of life-threatening complications. In addition to these general criteria, those subjects who could not perform retinography due to cataracts or any ophthalmological disease that prevented the performance of this technique were excluded [28]. Of the 495 retinographies finally analysed, five were discarded for technical reasons. Intraobserver, interobserver and interdevice reliability was analysed and compared to the AV index calculator [14] using a random subsample of 60 subjects (118 retinographies) of the total studied. The 250 subjects included in the study allow us to treat as significant a Pearson correlation of $r = 0.175$ between the retinal parameters measured with ALTAIR (vessel thickness, area and length) and femoral-carotid pulse wave velocity (cfPWV), the gold standard of arterial stiffness measurement, with a power of 80 % and an alpha risk of 0.05.

2.3. Variables and measurement instruments

General variables such as age, gender, occupation, smoking, alcohol consumption, personal history and drug use were recorded. A detailed description of the measurements and other variables analysed has been published [28].

2.4. Retinal vascular evaluation

Nasal and temporal images centred in the papilla, taken in a sitting position, were obtained by a trained nurse using a non-mydratic retinograph, TOPCON TRC NW 200 (Topcon Europe BC, Capelle aan den IJssel, The Netherlands). Using the ALTAIR software specifically developed by our group, vessel thickness, area and length of the retina were then calculated semiautomatically (Fig. 1) [28].

2.5. Development of the ALTAIR platform

The "Automatic image analyser to assess retinal vessel calibre" platform, ALTAIR, uses AI techniques and analytical algorithms to measure the characteristics of retinal veins and arteries and calculate the parameters of interest [30,31]. Methodology and measurements have been described previously, and are briefly described below [28] (see complete information on supplementary material I). The methodology is separated into two phases:

2.5.1. Digitisation of the retina

To perform this phase, the following steps are necessary:

- 1 **Load image and eye detection:** The platform automatically tries to determine which eye (left or right) is the image, based on the detection of the macula. In this step, if the automatic detection is wrong, the supervisor can modify this value by simply clicking on the correct eye.
- 2 **Processing:** In this step, noise is reduced, contrast is improved, blurriness corrected, and edges sharpened. Some of these actions can be carried out at the hardware level, which is to say, with the features included with the camera.
- 3 **Detection limits:** In this step, the platform is capable of locating the disk and identifying the centre and edges of the retina (Fig. 1). The platform builds a data structure that identifies each part of the retina based on the matrices of colours representing the images obtained. In this step, image processing techniques [32,33] were used to detect intensity based on the boundaries of the structures.
- 4 **Segmentation:** Segmentation is the process that divides an image into regions or objects of which the pixels have similar attributes. [34–36] This step can be considered the heart of the methodology proposed and used in the platform and performs the following actions: a) Identification of vessels; b) Structure of vessel; c) Cataloguing of veins and arteries.

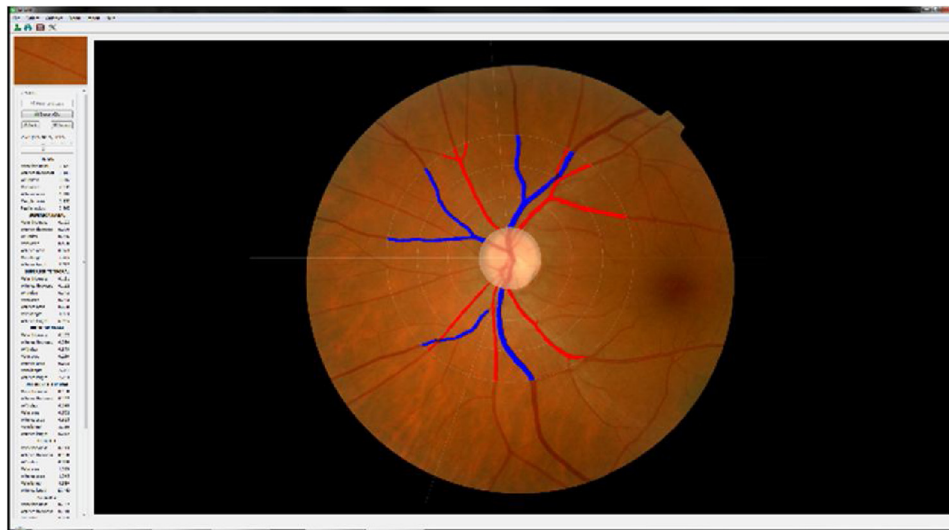


Fig. 1. Detection and identification of vessels. Cataloguing of veins and arteries.

2.5.2. Measurements

The following measures can be displayed by the platform: venous thickness in μm , arterial thickness in μm , arteriovenous ratio (AVR) (ratio between retinal artery and vein thickness), vascularised area in square μm , arterial area in square μm , area of all vessels in square μm and papilla radius in millimeters. The ALTAIR platform also produces internally combined parameters using circles and quadrants. For this purpose, it delimits three circles concentric to the papilla, of a width equivalent to the radius of the papilla and selects the retinal vessels and their branches in each one of them.

2.6. Retinal software validation

After training in imaging appreciation, the evaluators completed the following steps to validate the retinal software platform:

Evaluation of reliability or precision:

- 1 Intraobserver variability: For an assessment of measurement repeatability, an operator twice evaluated a total of 118 images of a random subsample of 60 patients with a one-week gap between measurements. On both days, the operator and the images analysed were the same, and the previous measurement information was not available.
- 2 Interobserver variability: To assess the measurement system's reproducibility, an operator different to the one carrying out the measurement in phase 1 evaluated the same 118 images previously measured. The information regarding the results from the previous phase was not available to this operator, and both operators were equally experienced in the subject matter and the use of the software. Both operators had also taken the same preparatory training.

2.6.1. Assessing validity (accuracy)

- 1 To assess the degree of agreement between ALTAIR and the AV Index calculator software [14] the same 118 retinographies were also analysed with the latter device and the results were compared with those obtained in circle 2 of the ALTAIR, which is where the measurements are made with the AV Index calculator.
- 2 2-Measurement validity was analysed in a total sample of 250 participants and 495 retinographies evaluating the relationship of the retinal vascular measures to age, blood pressure, Carotid intima-media thickness (IMT) as a measurement of vascular structure, carotid femoral pulse wave velocity (cfPWV), the gold standard for measuring arterial stiffness, Cardio Ankle Vascular index (CAVI),

kidney function, electrocardiographic parameters and estimated cardiovascular risk. The values of the retinal vessel parameters used in this process were the average of the measurements of the left and right eye of each subject, except for five subjects from whom only one retinography was obtained.

2.7. Clinic blood pressure

To measure clinical blood pressure, three samples of systolic blood pressure (SBP) and diastolic blood pressure (DBP) were taken with a validated OMRON model M10-IT sphygmomanometer (Omron Health Care, Kyoto, Japan). The average of the last two samples was used, following the recommendations of the European Hypertension Society [37].

2.8. Central augmentation index (CAIx) and carotid femoral pulse wave velocity (cfPWV)

These parameters were measured using the SphygmoCor System (AtCor Medical Pty Ltd, West Ryde, Australia). With the patient in a sitting position and the arm supported on a rigid surface, a pulse wave analysis was performed by means of a sensor placed in the radial artery and the application of a mathematical transformation to estimate the aortic pulse wave and the CAIx. The value was automatically adjusted to a heart rate of 75 bpm by the SphygmoCor System [38]. With the patient in the supine position, the pulse waves of the carotid and femoral arteries were recorded using the SphygmoCor system (pulse wave velocity V_x) to estimate the delay with respect to the ECG wave and to calculate the cfPWV. Distances from the suprasternal recess to the location of the sensor in the carotid and femoral arteries were measured with a tape measure, and the values multiplied by 0.8. Vascular target organ damage was considered when cfPWV was > 10 m/s [29].

2.9. Carotid intima-media thickness (IMT)

The carotid intima-media thickness was analysed by ultrasonography. In order to optimise reproducibility, automatic measurements of the carotid IMT were made with a Sonosite Micromax ultrasound system (FUJIFILM SonoSite Europe, Amsterdam, Netherlands) together with a 5–10 MHz multifrequency high-resolution linear transducer with Sonocal software. Measurements of the common carotid were made after examining a 10 mm longitudinal section at a distance of 1 cm from the bifurcation, with measurements taken on the proximal and distal wall in the lateral (90°), anterior (45°) and posterior

planes (135°) following an axis perpendicular to the artery to separate two lines, one for the intima-blood interface and the other for the media-adventitia interface. A total of six measurements of the right carotid and six of the left carotid were obtained, using mean values (average mean IMT) calculated automatically by the software [39]. The measurements were obtained with the subject in decubitus, with the head extended and slightly turned in the direction opposite to the carotid artery examined. Average mean IMT was considered abnormal if > 0.90 mm, or if there were atherosclerotic plaques with a diameter of 1.5 mm or a focal increase of 0.5 mm or 50 % of the adjacent IMT [29].

2.10. Cardio ankle vascular index and ankle-brachial index

The CAVI and the ankle-brachial index (ABI) were measured using a VaSera VS-1500 device (Fukuda Denshi, Tokyo, Japan). CAVI is a measure of vascular rigidity and independent of blood pressure [40]. Their values were calculated automatically, with the stiffness parameter β estimated by means of the following equation: $\beta = 2\rho \times l / (Ps-Pd) \times \ln(Ps / Pd) \times PWV^2$, where ρ was blood density, Ps and Pd were SBP and DBP in mmHg, and PWV was measured between the aortic valve and the ankle. CAVI and ABI were measured at rest. For the study, the lowest ABI values and the highest CAVI values obtained from each extremity were considered.

2.11. Renal assessment

Renal damage was assessed by measuring the glomerular filtration rate using the CKD-EPI formula (chronic kidney disease epidemiology collaboration) [41], and proteinuria as measured by the albumin-creatinine ratio (ACR), following the criteria of the European Society of Hypertension/European Society of Cardiology Guidelines [29].

2.12. Cardiac assessment

The electrocardiographic examination was performed using a General Electric MAC 3500 ECG System Niskayuna, New York, USA, which automatically measures the voltage and duration of the waves and estimates the Cornell voltage-duration product criteria Cornell VDP [42]. Electrocardiographic left ventricular hypertrophy was defined as a Sokolow-Lyon index > 3.5 mV or Cornell VDP > 2440 mm \times ms.

2.13. Cardiovascular risk assessment

Cardiovascular risk was estimated using the risk equation (D'Agostino scale) based on the Framingham study [43], with an Anglo-Saxon population and the SCORE low risk chart [44], based on the MONICA study with a European population.

2.14. Anthropometric measurements

Body weight and height were measured twice using an electronic scale and an approved stadiometer (Seca 770, Medical Scale and Measurement Systems, Birmingham, United Kingdom), with the patient wearing light clothing and no shoes. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). Obesity was defined as BMI > 30 kg/ m^2 . Waist circumference was measured on the standing patient wearing no clothes using a flexible graduated measuring tape.

2.15. Analytical measurements

Venous blood samples were collected between 8:00 and 9:00 following a 12-h fast. The concentrations of plasma glucose, HbA1C, creatinine, total cholesterol in serum and high-density lipoprotein (HDL-cholesterol) in plasma and albumin and creatinine in urine were

measured. Blood and urine samples were analysed at Salamanca University hospital, approved by the external quality assurance programmes of the Spanish Society of Clinical Chemistry and Molecular Pathology.

2.16. Statistical analysis

The continuous variables were expressed as the mean \pm standard deviation and frequency distribution for categorical data. The comparison of means, in the case of two groups, was performed using the Student's *t*-test for independent samples. Pearson's correlation coefficients and intraclass correlation coefficients (ICC) were calculated to evaluate the relationships between continuous variables. Using the Bland-Altman method, the mean difference and 95 % limits of agreement between a) the measurements of the two observers and b) the two measurements of the same observer were evaluated. Multiple linear regression analysis was performed to analyse the relationship of the retinal parameters generated by ALTAIR to the vascular structure and function parameters, the presence of target organ injury, and the cardiovascular risk score. The dependent variables used were the AVR, thickness, area and length of the arteries and veins. The independent variables for vascular structure and function were carotid IMT, cfPWV and CAVI. We also used clinical SBP and DBP and renal function calculated with the CKDEPI and ACR formulas, the last two parameters being divided by 100 to facilitate interpretation of the coefficients. Age and gender were included in the model as adjustment variables. Data were analysed using SPSS version 23.0 (IBM Corp, Armonk, NY, USA) A value of $P < 0.05$ was considered to be statistically significant.

2.17. Ethical and legal issues

The study was approved by the clinical research ethics committee (CEIC) of the healthcare area of Salamanca (CEIC of Area de Salud de Salamanca, January 29, 2014). Participants were required to sign an informed consent form prior to inclusion in the study, in accordance with the Declaration of Helsinki [45] (28).

3. Results

General, anthropometric and clinical characteristics of the 250 study participants were included, and the results from the subsample of 60 are presented in Table 1. Mean age was 62.0 ± 9.0 years, and 51 % of the subjects were male. A total of 172 (69 %) were diagnosed with hypertension, 43 (17 %) with diabetes, 80 (32 %) with obesity and 101 (40 %) were on treatment with lipid lowering drugs. Average cardiovascular risk on the D'Agostino scale was 18.6 ± 13.3 .

The mean AVR values, thickness, area and length of the vessels by gender can be seen in Table 2. Significant differences were observed in gender, with greater vein thickness and lower AVR and venous length in males ($p < 0.01$). The intraobserver ICC in the evaluation of the whole retina ranged from 0.963 (95 % CI 0.947 to 0.974) ($p < 0.01$) for the vascular area to 0.697 (95 % CI 0.564 to 0.790) ($p < 0.01$) for the AVR, with the exception of vein length (0.640, 95 % CI 0.482 to 0.750) (Table 3) Similarly, interobserver ICC values for the evaluation of the parameters of the retinal arterioles and venules in the total area of the retina ranged from 0.936 (95 % CI 0.907 to 0.955) in vascular area to 0.770 (95 % CI 0.669 to 0.841) in AVR ($p < 0.01$ in all) (Table 3) The interdevice ICC, comparing the measurements obtained with the AV Index calculator and the results of ALTAIR in circle 2, was 0.887 (95 % CI 0.838 to 0.992) for the AVR, 0.590 (95 % CI 0.396 to 0.721) with arteries and 0.677 (95 % CI 0.525 to 0.781) with veins (Table 3).

The separate analysis of the three concentric circles into which the retina is divided showed similar results, both for intraobserver ICC (Table S1), interobserver ICC (Table S2), and interdevice ICC (Table S3). The Bland-Altman plots for intraobserver and interobserver ICC are

Table 1
Demographic and clinical characteristics of the study population.

Variables	Full sample: N = 250		Subsample: N = 60		P
	Mean/n ^o	SD/ (%)	Mean/n ^o	SD/(%)	
Age, years	62	9	61	7.8	0.340
Gender, male	128	(51)	37	(62)	0.145
Smokers	36	(14)	6	(10)	0.371
Inadequate alcohol consumption	40	(16)	6	(10)	0.240
Waist perimeter, cm	97	10	96	10	0.607
Body mass index	29	4	28	4	0.717
Systolic blood pressure, mmHg	134	18	131	15	0.260
Diastolic blood pressure, mmHg	79	10	80	9	0.567
Heart rate, bpm	69	11	69	11	0.854
Glycaemia, mg/dl	96	30	95	22	0.705
HbA1c,%	5.7	0.7	5.8	0.7	0.668
Total Cholesterol, mg/dl	192	33	195	37	0.498
HDL Cholesterol, mg/dl	54	14	53	14	0.632
Albumin/creatinine index, mg/g	13.9	54.2	10	34	0.600
Glomerular filtration (CKDEPI), ml/min	85	16	86	15	0.733
Obesity	80	(32)	16	(27)	0.422
Hypertension	172	(69)	43	(72)	0.665
Diabetes	43	(17)	11	(18)	0.835
Antihypertensive drugs	146	(58)			
Antidiabetic drugs	38	(15)			
Lipid lowering drugs	101	(40)			
High CAVI	8.7	1.3			
Low ABI	1.1	0.1			
Central Alx75,%	31.3	10.3			
Pulse wave velocity, m/sc	7.5	2.1			
IMT Medio, mm	0.74	0.10			
Cardio vascular risk (D'Agostino),%	18.6	13.3			
Cardio vascular risk (SCORE),%	3.6	3.7			
Pulse wave velocity increased	21	(8)			
Carotid IMT thickening	78	(31)			
ABI pathologic	9	(4)			
Microalbuminuria	11	(4)			
Left ventricular hypertrophy	29	(12)			

Subsample: Random subsample to analysed intra observer, inter observer and inter device reliability. SD: Standard deviation, HbA1c: glycosylated haemoglobin. HDL: high density lipoprotein, CKDEPI: chronic kidney disease epidemiology collaboration, CAVI: Cardio Ankle Vascular index, ABI: ankle-brachial index, Alx75: Augmentation index adjusted 75 bpm, IMT : intima media thickness. Pulse wave velocity increased: > 10 m/sc, Carotid IMT engrossed: > 0.90 mm, o presence of a plaque identified by an IMT > 1.5 mm or by a focal increase in thickness of 0.5 mm or 50 % of the surrounding carotid IMT value. ABI pathologic < 0.9. Microalbuminuria > 30 mg/g. Left ventricular hypertrophy: Cornell PDV > 2240 mm/ms.

shown in Figs. 2 and 3, while Figure S1 shows the Bland-Altman plots for interdevice ICC and Table S4 the limits of agreement.

Table 4 shows the correlations of the retinal vascular parameters analysed by vascular structure and function and target organ damage. We found that age was negatively correlated with the parameters measured with ALTAIR: vessel thickness (artery $r = -0.209$, vein $r = -0.286$, $p < 0.01$), area ($r = -0.546$, $p < 0.01$) and length ($r = -0.513$; $p < 0.01$). There is also a negative correlation with systolic (area and length) and diastolic (thickness) blood pressure. While no correlation was observed with Cornell VDP, for renal function there was a positive correlation of CKDEPI with thickness, ($r = 0.161$, $p < 0.05$), area ($r = 0.274$, $p < 0.01$) and length ($r = 0.247$; $p < 0.01$), and a negative correlation of the ACR with thickness ($r = -0.163$, $p < 0.01$).

Regarding arterial function, CAVI and cfPWV show negative correlations with thickness, area and length of arteries and veins. IMT correlated negatively with vascular area and length, but not thickness, with a correlation between $r = -0.196$ for venous and $r = -0.423$ for arterial length. Estimated CV risk also showed a negative correlation with the area and length of arteries and veins. The global correlations are also found in the analysis of the three circles (Table S5).

In the multiple linear regression analysis adjusted for age and gender (Table 5), the AVR showed a negative relationship with DBP ($\beta = -0.001$; $p = 0.041$) and with ACR ($\beta = -0.020$; $p = 0.026$). Arterial thickness was also negatively associated with ACR ($\beta = -2.122$; $p = 0.024$). When arterial area was the dependent variable, SBP was significant ($\beta = -0.010$; $p < 0.001$), as was mean IMT ($\beta = -1.081$; $p = 0.008$). Arterial length was associated with systolic ($\beta = -0.114$; $p < 0.01$) and diastolic blood pressure ($\beta = 0.105$; $p = 0.031$) and with mean IMT ($\beta = -14.278$; $p = 0.01$). Cardiovascular risk was associated with venous thickness and area and with arterial length (Table S6).

4. Discussion

To the best of our knowledge, ALTAIR is the first tool capable of measuring the thickness, area and length of the retinal vessels semi-automatically. The software has shown good reliability, with a high intraobserver and interobserver ICC in the measurement of arterial and venous area, length and thickness. The results were similar in the three circles analysed around the papilla. In the comparison with the AV index calculator, which estimates the thickness of the vessels mainly in circle 2, the interdevice ICC was slightly lower in veins and arteries, but the AVR attained an ICC of 0.887. On the other hand, although the intra-observer and inter-observer Bland Altman analysis shows a good limit of agreement for measuring the thickness of the arteries, some bias values are shown in the veins. Conversely, in the inter-device analysis, the limit of agreement of the AVR and veins was better than that of the arteries. Regarding concomitant clinical validity, we found that the retinal parameters analysed correlated negatively with age, blood pressure, vascular structure and function (IMT, CAVI and cfPWV), cardiovascular risk and ACR, and positively with glomerular filtration, while no association was found with electrocardiographic parameters. These results remain unchanged after adjustment except for CAVI, cfPWV and CKDEPI.

4.1. Comparison with other studies

4.1.1. Reliability

The reliability found in this study was slightly lower than our group's results with the AV Index calculator, which yielded interobserver ICCs of between 0.96 and 0.98 for veins, arteries and AVR, with intraobserver ICCs ranging from 0.97 to 0.99 [14]. This difference is possibly due to the fact that with ALTAIR, the entire vascular tree of the retina is analysed over a very wide area, while the AV Index only covers the temporal vessels in a more limited area of the retina. Nevertheless, the reliability of our ALTAIR measurements was similar to that reported by Pose-Reino et al. [46], who used a different methodology for measuring retinal vessel calibre and found interobserver correlations ranging from 0.83 to 0.98 in 30 images. The Atherosclerosis Risk in Communities (ARIC) study [47] reported intraobserver and interobserver correlation coefficients of 0.69 and 0.74 for artery calibre, 0.89 and 0.77 for vein calibre, and 0.84 and 0.79 for AVR respectively, slightly lower than our study. Sherry et al. [48] used a method based on the ARIC study, which improved both interobserver (ranging from 0.78 to 0.90) and intraobserver correlation coefficients (ranging from 0.79 to 0.92). In sum, both the intraobserver and interobserver reliability of the instrument used in this study are equal to or greater than those of other previously used methods. Finally, Yip et al. [15] compared three software applications developed to evaluate

Table 2
Mean values of the retinal parameters evaluated by gender.

	All		Female		Male		P-value
	Mean	SD	Mean	SD	Mean	SD	
AVR	0.74	0.08	0.76	0.08	0.73	0.08	0.001
Arteriolar thickness, μm	86.90	8.21	87.01	6.99	86.81	9.19	0.850
Venular thickness, μm	117.27	12.34	114.02	11.30	120.31	12.55	< 0.001
Arteriolar area, μm^2	1.66	0.62	1.67	0.60	1.65	0.64	0.774
Venular area, μm^2	2.07	0.47	2.09	0.53	2.05	0.41	0.538
Vascular area, μm^2	3.65	1.03	3.64	1.14	3.66	0.92	0.873
Arteriolar length, mm	19.01	6.75	19.23	6.77	18.81	6.74	0.635
Venular length, mm	17.77	4.15	18.38	4.71	17.19	3.46	0.023
Vascular length, mm	35.93	10.04	36.27	11.38	35.62	8.64	0.611

AVR: Ratio between the thickness of the arteries and veins. SD: Standard deviation.

Table 3
Intraclass Correlation Coefficient intraobservers, interobservers and inter-devices.

Intraobservers (global)	ICC	95 % Confidence Interval	P-value
Arteriolar thickness, μm	0.902	0.858 0.932	< 0.001
Venular thickness, μm	0.780	0.683 0.847	< 0.001
AVR	0.697	0.564 0.790	< 0.001
Arteriolar area, μm^2	0.906	0.865 0.935	< 0.001
Venular area, μm^2	0.835	0.763 0.886	< 0.001
Vascular area, μm^2	0.963	0.947 0.974	< 0.001
Arteriolar length, mm	0.869	0.812 0.909	< 0.001
Venular length, mm	0.640	0.482 0.750	< 0.001
Vascular length, mm	0.925	0.893 0.948	< 0.001
Interobservers (global)			
Arteriolar thickness, μm	0.815	0.733 0.872	< 0.001
Venular thickness, μm	0.884	0.833 0.920	< 0.001
AVR	0.770	0.669 0.841	< 0.001
Arteriolar area, μm^2	0.871	0.813 0.910	< 0.001
Venular area, μm^2	0.916	0.878 0.941	< 0.001
Vascular area, μm^2	0.936	0.907 0.955	< 0.001
Arteriolar length, mm	0.809	0.725 0.868	< 0.001
Venular length, mm	0.816	0.735 0.873	< 0.001
Vascular length, mm	0.867	0.808 0.907	< 0.001
Interdevices (circle 2)			
AVR	0.887	0.838 0.922	< 0.001
Arteriolar thickness, μm	0.590	0.396 0.721	< 0.001
Venular thickness, μm	0.677	0.525 0.781	< 0.001

ICC: Intraclass Correlation Coefficient. AVR: Ratio between the thickness of the arteries and veins. Interdevice: compare ALTAIR with AV index calculator.

retinal vessels with different methodologies. Although the limit of agreement between them was broad, the measurements of the three correlated with each other, as well as with other clinical parameters; this is similar to our findings in terms of the limit of agreement, both intra and interobserver correlations, while some of the measures have shown greater discrepancies than expected.

4.1.2. Validity

4.1.2.1. Blood pressure. In a recent systematic review, Chew et al. [49] found that arterial narrowing is not only linked to chronic exposure to hypertension, but may precede its development. In line with the findings of a study by Wong TY et al. [50], our study showed an inverse relationship of the area and length of the retinal vessels to systolic blood pressure and of the thickness of the artery to diastolic blood pressure.

4.1.2.2. Renal function. We found a positive association between glomerular filtration and retinal vascular length and the area of both arteries and veins, although this disappears after adjustments in the regression analysis, and a negative association between ACR and arterial thickness. This could indicate that arteriole narrowing of the retina reflects coexisting damage in renal microcirculation, which leads

to a subsequent deterioration of kidney function. However, the association between retinal vessel abnormalities and chronic kidney disease varies according to the series. Our group found a relationship between AVR and venular caliber with ACR [14], and Yau et al. [51] observed an association between retinal microvascular calibre and the incidence of chronic kidney disease in white adults. However, Sabanayagam et al. [52] found no association between the diameters of arterioles or venules and the risk of developing reduced glomerular filtration within 15 years. These discrepancies are probably due to the different characteristics of the samples analysed, with different evolution periods and different pathologies.

4.1.2.3. Arterial stiffness. Using a semiautomated computer application developed by the Institute of Computer Science, Foundation for Research and Technology–Hellas, Triantafyllou et al. [3] found a relationship between the narrowing of retinal arterioles and arterial stiffness measured with PWV and AIx. Similarly, Katsvi et al. [53] found a progressive stiffening of the aorta in parallel with the progression of retinal changes in hypertensive subjects. Our software records the measurements of the papilla’s three concentric circles and analyses thickness, area and length in each circle, finding a correlation with structure and vascular function, not only of the width of the vessels, but also of area and length in the three circles analysed; as far as we are aware, this is the first study to describe this association. This opens up new line of research into the relationship of retinal vascularization, i.e. microcirculation, to macrocirculation.

4.1.2.4. Cardiovascular risk. The negative association found in the current study between vascular risk and the thickness, area and length of the retinal vessels is in line with the results of previous studies [28] and supports the usefulness of incorporating retinal vessel measurement into the assessment of vascular risk. Numerous authors have associated the presence of retinopathy, whether linked to diabetes [54], renal disease [55] or other target organ damage [13] or not, with increased cardiovascular risk. Given the predictive value of both retinal arteriolar narrowing and arterial stiffness in terms of cardiovascular mortality and morbidity, the identification of combined micro and macrovascular damage could be useful in cardiovascular risk stratification in hypertensive patients and the assessment of other cardiovascular risk factors [3].

To complete a satisfactory validation of the tool, it is necessary to conduct a longitudinal study to assess the capacity of the retinal vessel measurements obtained by the ALTAIR tool to predict the appearance of lesions of cardiovascular target organs, as well as cardiovascular morbi-mortality.

4.1.2.5. Limitations. This study has some limitations. First, since it is not a fully automatic tool, some decisions in its administration need to be made by the observer; despite the process being standardized as far as possible, there may still be some type of variability which could

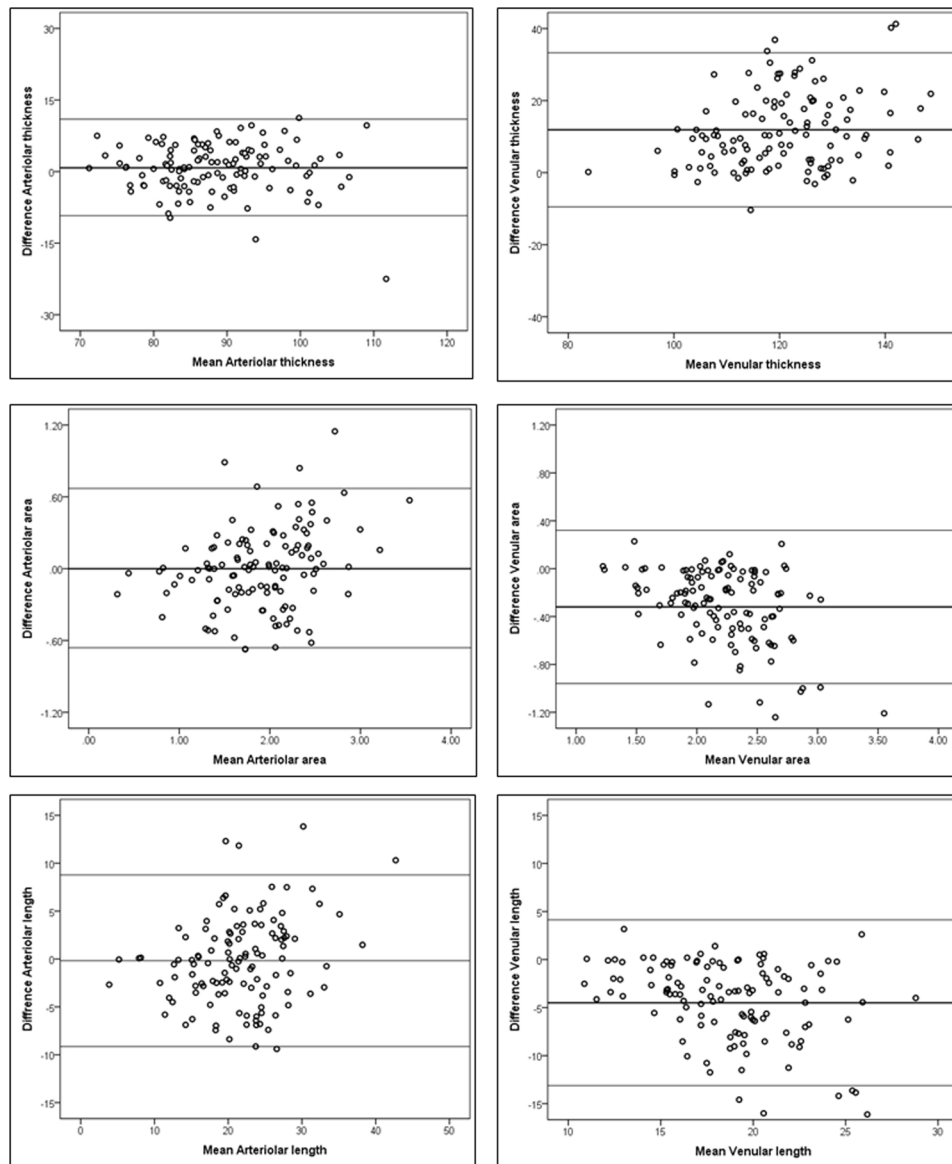


Fig. 2. Bland-Altman intraobserver. Thickness of veins and arteries. Area of veins and arteries. Length of veins and arteries. Limits of agreement in Table S4.

generate bias. Second, the phase of the cardiac cycle in which the photograph of the retina was taken was not considered, which may result in increased variability in the thickness and area of the vessels [56]. Third, although the number of retinas not analysed for technical reasons was low, we must take this fact into account and the influence that using the mean values of the left and right retinas may have had on the calculation of results.

5. Conclusions

The ALTAIR tool has been shown to be useful for analysing the thickness, area and length of retinal vessels, with acceptable intraobserver, interobserver and interdevice ICCs, but with wide interobserver limits of agreement. This tool has shown a concomitant association of the retinal vessel measurements with other cardiovascular parameters and cardiovascular risk. Therefore, in addition to thickness, retinal vascular area and length could play a role in the prediction of cardiovascular risk. Greater automation of the tool is necessary to avoid observer influence and facilitate transfer to clinical practice.

Summary Points
What is known

- 1 There are different software that mainly analyze the thickness of the retinal vessels in which the observer's intervention is very important.
- 2 The thickness of the retinal vessels is related to the state of the micro and macrocirculation.
- 3 The association of the retina vessels with hypertension, myocardial infarction, stroke and other cardiovascular diseases is already known

What this study has added

- 1 ALTAIR analyzes, in addition to thickness of the vessels, the length of these and the surface of vascularization.
- 2 The Altair has shown adequate reliability and validity for the analysis of the retina vessels with low influence of the observer.
- 3 The length of the vessels and the surface of vascularization is related to the state of the micro and macro systemic circulation.

;1;

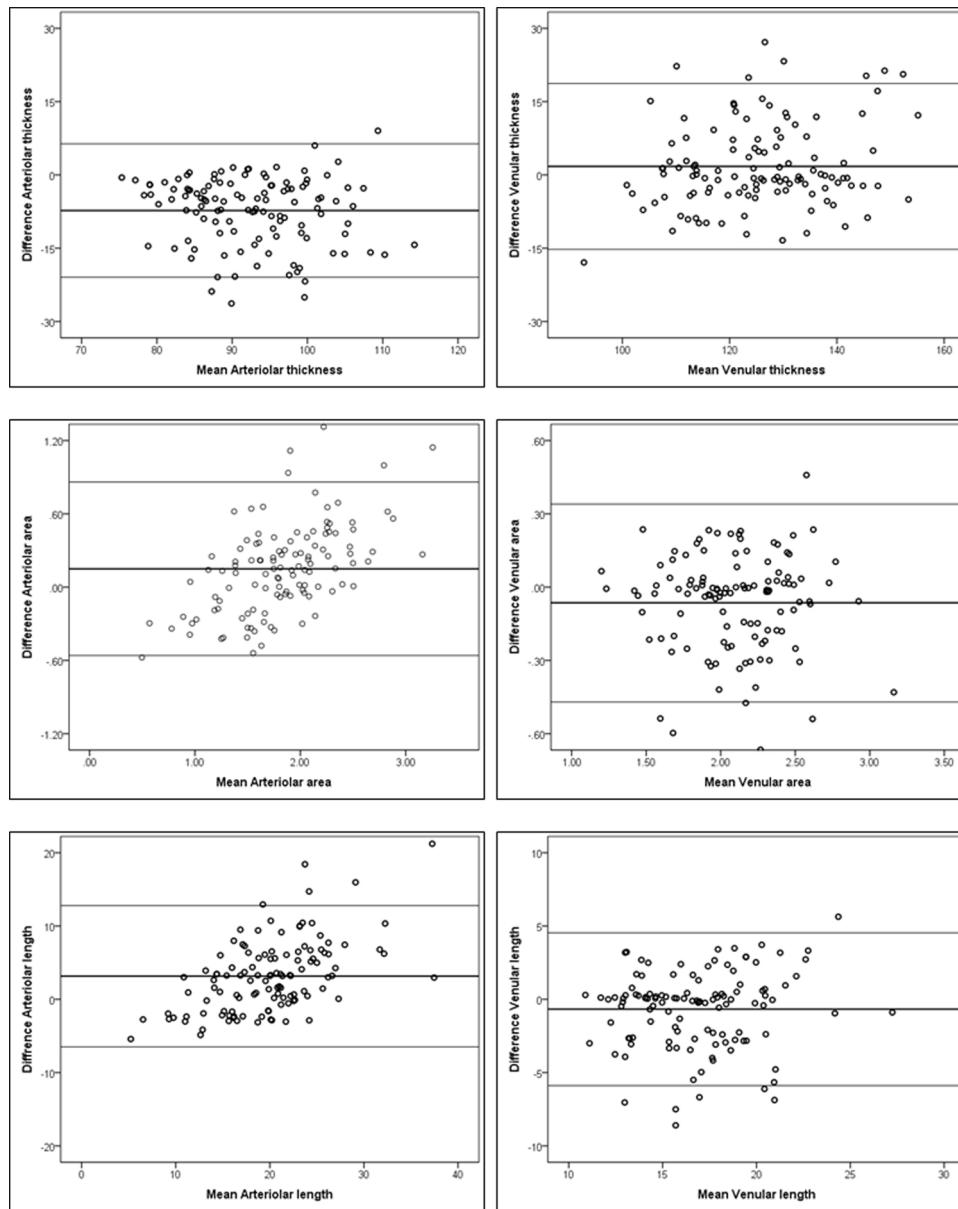


Fig. 3. Bland-Altman interobserver. Thickness of veins and arteries. Area of veins and arteries. Length of veins and arteries. Limits of agreement in Table S4.

Table 4
Pearson correlations of retinal parameters with clinical variables.

	Age	SBP	DBP	Cornell VDP	CKDEPI	ACR	CAVI	PWV	IMT	CVR
AVR	0.021	-0.094	-0.174**	-0.012	0.068	-0.152*	0.047	0.004	0.030	-0.119
Arteriolar thickness, μm	-0.209**	-0.116	-0.038	-0.014	0.161*	-0.163*	-0.156*	-0.169**	-0.053	-0.124
Venular thickness, μm	-0.286**	-0.029	0.181**	0.026	0.121	0.007	-0.212**	-0.186**	-0.095	0.015
Arteriolar area, μm^2	-0.504**	-0.312**	0.051	0.110	0.202**	-0.062	-0.283**	-0.352**	-0.403**	-0.338**
Venular area, μm^2	-0.426**	-0.134*	0.116	0.000	0.259**	-0.054	-0.266**	-0.225**	-0.227**	-0.238**
Vascular area, μm^2	-0.546**	-0.234**	0.123	0.050	0.274**	-0.055	-0.312**	-0.324**	-0.347**	-0.306**
Arteriolar length, mm	-0.486**	-0.306**	0.078	-0.104	0.181**	-0.048	-0.272**	-0.339**	-0.423**	-0.354**
Venular length, mm	-0.309**	-0.130*	0.042	-0.006	0.205**	-0.062	-0.174**	-0.157*	-0.196**	-0.239**
Vascular length, mm	-0.513**	-0.241**	0.116	-0.055	0.247**	-0.050	-0.280**	-0.304**	-0.364**	-0.329**

*: $P < 0.05$; **: $P < 0.01$. AVR: Ratio between the thickness of the arteries and veins, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, Cornell VDP: Cornell voltage-duration product, CKDEPI: chronic kidney disease epidemiology collaboration ACR: Albumina/creatinine ratio, CAVI: Cardio Ankle Vascular index, PWV: Pulse wave velocity, IMT: intima media thickness, CVR : Cardiovascular risk.

Table 5
Multiple regression of measurements of the vessels of the retina with vascular parameters.

Dependent variables	Independent var.	B	95 % CI		P-value
AVR	SBP	0.000	-0.001	0.001	0.632
	DBP	-0.001	-0.003	0.000	0.041
	CKDEPI	0.035	-0.036	0.106	0.335
	ACR	-0.020	-0.037	-0.002	0.026
	CAVI	0.008	-0.003	0.018	0.143
	PWV	-0.001	-0.007	0.005	0.725
	IMT	0.077	-0.039	0.192	0.192
Arteriolar thickness, μm	SBP	-0.010	-0.086	0.066	0.794
	DBP	-0.063	-0.197	0.071	0.352
	CKDEPI	3.234	-4.190	10.658	0.392
	ACR	-2.122	-3.955	-0.288	0.024
	CAVI	-0.161	-1.224	0.902	0.766
	PWV	-0.363	-0.945	0.220	0.221
	IMT	8.211	-3.978	20.401	0.186
Venular thickness, μm	SBP	-0.068	-0.174	0.039	0.210
	DBP	0.147	-0.040	0.335	0.124
	CKDEPI	-3.022	-13.725	7.682	0.579
	ACR	0.176	-2.468	2.819	0.896
	CAVI	-1.164	-2.653	0.325	0.125
	PWV	-0.318	-1.133	0.498	0.444
	IMT	-0.901	-17.972	16.170	0.917
Arteriolar area, μm^2	SBP	-0.010	-0.014	-0.005	< 0.001
	DBP	0.006	-0.002	0.015	0.152
	CKDEPI	-0.246	-0.756	0.263	0.342
	ACR	-0.015	-0.141	0.111	0.817
	CAVI	0.040	-0.029	0.110	0.255
	PWV	-0.033	-0.071	0.005	0.091
	IMT	-1.081	-1.881	-0.280	0.008
Venular area, μm^2	SBP	-0.002	-0.006	0.001	0.214
	DBP	0.002	-0.005	0.008	0.571
	CKDEPI	0.058	-0.311	0.428	0.756
	ACR	-0.022	-0.113	0.070	0.642
	CAVI	0.008	-0.044	0.060	0.767
	PWV	0.000	-0.028	0.029	0.990
	IMT	-0.161	-0.758	0.436	0.596
Arteriolar length, mm	SBP	-0.114	-0.168	-0.061	< 0.001
	DBP	0.105	0.009	0.200	0.031
	CKDEPI	-3.426	-8.991	2.139	0.226
	ACR	0.010	-1.364	1.384	0.989
	CAVI	0.462	-0.298	1.223	0.232
	PWV	-0.314	-0.731	0.102	0.139
	IMT	-14.278	-22.995	-5.560	0.001
Venular length, mm	SBP	-0.015	-0.049	0.020	0.413
	DBP	-0.001	-0.063	0.060	0.970
	CKDEPI	0.674	-2.799	4.147	0.703
	ACR	-0.260	-1.118	0.598	0.551
	CAVI	0.282	-0.207	0.771	0.257
	PWV	0.028	-0.240	0.296	0.840
	IMT	-1.779	-7.388	3.831	0.533

Multiple regression adjusted by age and gender. AVR: Ratio between the thickness of the arteries and veins, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, CKDEPI: chronic kidney disease epidemiology collaboration ACR: Albumin/creatinin ratio, CAVI: Cardio Ankle Vascular index, PWV: Pulse wave velocity, IMT: intima media thickness.

ALTAIR tool

If interested in the Altair tool for evaluation purposes, information can be requested from: apisal2020@gmail.com.

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Declaration of Competing Interest

There are no conflicts of interest

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at [doi:https://doi.org/10.1016/j.ijmedinf.2020.104090](https://doi.org/10.1016/j.ijmedinf.2020.104090).

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