Retinal blood vessel calibre and vascular ageing in a general Spanish population: A EVA study

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Abstract

Introduction: The aim of this work was to analyse the association of the retinal arteriolar calibre and the arteriole/venule index (AV index) with vascular ageing in a general population without previous cardiovascular disease.

Materials and methods: Descriptive cross-sectional study. A total of 482 individuals without cardiovascular disease (mean age: 55.6 ± 14.2 years) were selected by random sampling, stratified by age and sex. The retinal arteriolar calibre was measured using digital fundus images of the back of the eye captured with a validated, semiautomated and computer-assisted software (Index calculator). Vascular ageing was defined using three criteria based on the values of: (1) Carotid-femoral Pulse Wave Velocity (cfPWV), (2) Brachial-ankle Pulse Wave Velocity (baPWV) and (3) Carotid Intima-Media Thickness.

Results: The AV index and arteriolar calibre show a negative correlation with age, arterial pressure, cardiovascular risk and parameters of vascular structure and function (p < 0.001 in all cases). We found lower mean values of the AV index and arteriolar calibre in the individuals with early vascular ageing...
INTRODUCTION

The development of new retinal imaging methods, such as digital photography, provides a unique advantage to carry out a more objective and reproducible in vivo evaluation of retinal vascularization in a non-invasive manner. This facilitates the identification of people with high risk of vascular disease, since changes in retinal microvascularity, especially in diabetics, can indicate changes in systemic vascularization. During the last decade, several prospective studies, based on general populations, have shown that retinal vascular calibres are associated with a wide range of subclinical and clinical cardiovascular diseases, corroborating the opinion that retinal arteriolar calibre can become a new marker of cardiovascular risk (CVR). Thus, several meta-analyses have associated retinal venular thickness with greater risk of stroke, greater rate of type 2 diabetes mellitus, especially in men, greater prevalence of metabolic syndrome and with markers of inflammation and endothelial dysfunction. Moreover, several studies carried out at the population level have shown that systemic inflammation, evaluated with ultrasensitive CRP or interleukin values, not only predicts future cardiovascular events, but that their evolution has a worse prognosis, all due to microvascular and endothelial involvement. Similarly, wider venules, narrower arterioles and lower arteriole-venule index (AV index) were associated with greater risk of coronary heart disease in women, arterial hypertension and higher body mass index.

Vascular ageing (VA) has gained considerable interest in the last decades, as it has been shown to be more strongly related to morbimortality due to cardiovascular diseases than biological ageing, with its alteration preceding the development of cardiovascular events. This imbalance between chronological age and life expectancy has led to search for biomarkers of VA, which include retinal blood vessel calibre alterations. VA depends mainly on arterial stiffness, arterial pressure and age. Several studies have analysed the relationship between retinal blood vessel calibre and arterial stiffness, measured with brachial-ankle pulse wave velocity (baPWV) and carotid-femoral pulse wave velocity (cfPWV) or arterial stiffness index, reporting that narrower arterioles and wider venules are associated with greater arterial stiffness. The study of Pathai et al associated narrower arteriolar calibres with higher chronological age.

However, the relationship between retinal blood vessel calibre and VA in the Spanish general population has not been studied to date, and in this work, we also analyse the association between retinal blood vessel calibre and AV index estimated with a software tool (AV index calculator). Therefore, the aim of this study was to analyse the association of retinal blood vessel calibre and AV index with VA in a Spanish general population without previous cardiovascular disease. This study contains results of secondary objectives of the EVA study (association between different risk factors and early vascular ageing).

DESIGN

2.1 Study design and population

This is a cross-sectional study with individuals recruited from the EVA study (association between different risk factors and early vascular ageing), registered in ClinicalTrials.gov (NCT02623894).
The study was conducted at the Salamanca Primary Healthcare Research Unit (APISAL). The reference population consisted of 43,946 people (35–75 years of age), who lived in Salamanca and were registered in 5 different urban health centres. A random sampling with replacement was carried out, stratified by age (35, 45, 55, 65 and 75 years) and sex, selecting 501 individuals, with 100 individuals (50 women and 50 men) in each group. The recruitment was performed between June 2016 and November 2017. Figure 1S shows the flowchart, included and excluded individuals, the causes of exclusion by age group and sex, and the reference population. Of the 501 individuals recruited from the EVA study, a total of 482 were analysed, since 19 were excluded for not having some of the main measurements recorded (10 retina, 3 cfPWV and 9 cIMT).

This sample size is enough to detect a mean difference of AV index between individuals with healthy vascular ageing (HVA) (40) and individuals with early vascular ageing (101) equal to or greater than 0.035, accepting an alpha risk of 0.05 and a beta risk of 0.20 with two-tailed contrast, assuming a standard deviation of 0.068. Following the recommendations of the Helsinki Declaration,20 all participants were informed about the study and signed informed consent before being included in the project. The study was approved on 4/5/2015 by the Salamanca ethics committee on research with medicines.

The study report conforms to the EQUATOR general guidelines.21

2.2 | Variables and measurement instruments

A detailed description of the procedures followed in this study and the inclusion and exclusion criteria have been previously published.19,22 A physician and a nurse collected blood samples examined the participants and administered the questionnaires used in this study. Both of them were trained following a standardized protocol.

2.2.1 | Evaluation of retinal blood vessels

The retinal images were captured using a Topcon NW 200 non-mydriatic retinograph (Topcon Europe B.C., Capelle a/d Ijssel), obtaining nasal and temporal images centred on the optic disc. These images were analysed using the AV Index Calculator software developed by our group (registration number 00/2011/589).18 This software automatically recognizes and draws two outer circles concentric to the optic discs, delimiting an area A (0–0.5 cm in diameter) and an area B (0.5–1 cm in diameter). The software identifies the limits of the different blood vessels, automatically recognizes the arteries and veins of the retina, and then performs multiple measurements of the diameter of the segment of the blood vessels located in area B. Lastly, it estimates the mean calibre of veins and arteries in µm, and these measurements are summarized as arteriole thickness, venule thickness and arteriole/venule index (AV index). An AV index of 1.0 suggests that the diameter of the arterioles is, on average, equal to that of the venules in that eye, whereas values below 1 indicate that the arterioles are narrower than the venules. With the aim of improving the reliability of the process and increasing its efficacy, the main blood vessels in the upper and lower temporal quadrants were measured, rejecting the rest of the blood vessels. The measures were calculated separately for each quadrant and then in combination to estimate the mean measure in each eye. Figure 2S shows the described process.18

2.2.2 | Carotid-femoral pulse wave velocity (cfPWV)

This variable was measured using a SphygmoCor device (AtCor Medical Pty Ltd, Head Office). The pulse wave was measured with the participant sitting and leaning his/her dominant arm on a rigid surface. Central and peripheral blood pressure were obtained using a sensor located in the radial artery, estimating the morphology of the pulse wave in the aorta. Carotid and femoral pulse waves were analysed, with the patient in the supine position, estimating the delay time with respect to that of the wave of the ECG and calculating the pulse wave velocity. The distances were measured with a measuring tape from the sternal notch to the point where the sensor was placed over the carotid and femoral arteries.23

2.2.3 | Brachial-ankle pulse wave velocity (baPWV)

This variable was measured using a VaSera VS-1500 device (FukudaDenshi), following the manufacturer instructions. The cuffs were adapted to the circumference of the arms and ankles. The electrodes were connected to the right and left arms and ankles, and a heart sound microphone was fixed with double-sided tape to the chest, over the sternum, in the second intercostal space. BaPWV was estimated using the following equation: baPWV = (0.5934 × height (cm) +14.4724)/TBA (TBA is the time interval between the arm and ankle waves).24
2.2.4 | Carotid intima-media thickness

This variable was measured using a Sonosite Micromax ultrasound device (Sonosite, Inc.), with a 5–10 MHz multi-frequency high-resolution linear transducer and with Sonocal software, which automatically measures the cIMT. The cIMT measurements were performed following the protocol published by our research group.25

2.2.5 | Vascular ageing index (VAI)

VAI was estimated with cfPWV and cIMT, using the formula developed by Nilsson et al.26: VAI = (log (1.09) × 10 cIMT + log (1.14) cfPWV) × 39.1 + 4.76. This index integrates the carotid artery’s cIMT and cfPWV, which are two of the most individually used methods to estimate VA, providing information about arterial stiffness and subclinical atherosclerosis.

2.3 | Definition of healthy vascular ageing (HVA), normal vascular ageing (NVA) and early vascular ageing (EVA)

Vascular ageing (VA) was defined as follows: the participants that presented vascular lesion in carotid arteries or peripheral artery disease were classified as early vascular ageing (EVA). Considering the percentiles of baPWV and VAI by age group and sex, the different types of VA were classified as follows: more than P90 was considered EVA, between P10 and P90 was considered normal vascular ageing (NVA) and less than P10 was considered healthy vascular ageing (HVA). The individuals diagnosed with hypertension or type 2 diabetes mellitus were excluded from the HVA group. The distribution of participants with the three criteria in each of the groups is shown in Figure 1.

Prior to performing all the measurements, the participants could not smoke or consume caffeine 1 h before the examination, used comfortable clothes and remained at rest and quiet for at least 10 min before the measuring.

2.3.1 | Measurement of the cardiovascular risk factors

Clinical arterial pressure was measured using a validated M10-IT OMRON sphygmomanometer (Omron Health Care). The measurements were performed following the guidelines of the European Association of Hypertension.27 Weight and height were measured twice using a certified electronic scale (Seca 770) and a height rod (Seca 222), and the average of the two measurements was recorded for both parameters. The blood samples, collected in the health centres between 8 a.m. and 9 a.m. from the participants, who fasted overnight and had neither smoked nor consumed alcohol or caffeine within the previous 12 h, were used to determine plasma glucose, total cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides, through automatized standard enzyme methods. Low-density lipoprotein (LDL) cholesterol was determined through Friedewald’s formula. CVR was estimated using Framingham’s equation (D’Agostino version).28

It was considered that a person had hypertension if he/she was under anti-hypertensive drug treatment or showed blood pressure values ≥140/90 mmHg. Diabetes mellitus was considered for those who were under anti-hyperglycaemic drug treatment or showed blood glucose values ≥126 mg/dl or HbA1c ≥6.5%. Dyslipidaemia was considered for the participants who were under lipid-lowering drug treatment or showed fasting total cholesterol ≥240 mg/dl, LDL cholesterol ≥160 mg/dl, HDL cholesterol ≤40 mg/dl in men and ≤50 mg/dl in women, or triglycerides ≥200 mg/dl. Obesity was considered if the body mass index was ≥30. The participants were considered smokers if they smoked during the testing or had stopped smoking within 12 months prior to the study.

2.4 | Statistical analysis

The data of the continuous variables are presented as mean ± standard deviation, and those of the categorical variables are presented as number and percentage. To represent the values of the parameters used to estimate vascular ageing, the 10th and 90th percentiles were calculated. The comparison of means between two independent groups and between more than two groups was carried out with Student’s t test and one-way analysis of variance (ANOVA), respectively, using LSD correction in post hoc comparisons. To analyse the association between categorical variables, the χ² test was applied. The correlation between quantitative variables was evaluated with Pearson’s correlation coefficient.

To analyse the association between retinal blood vessel calibre and VA, several models of multiple regression and logistic regression were performed. Three multiple regression models were conducted using vascular ageing evaluated with cfPWV, baPWV and VAI as dependent variables. AV index and retinal arteriole and venule diameter were used as independent variables, and age, sex and hypotensive, hyperglycaemic and lipid-lowering drugs were used as adjustment variables in all models. In the logistic regression models, the presence of HVA or NVA vs EVA
was used as dependent variable in all three models (HVA or NVA = 0, EVA = 1). The independent and adjustment variables used in the logistic regression models were those used in the multiple regression models. All analyses were conducted using SPSS for Windows v25.0 software (IBM Corp). For the hypothesis testing, an alpha risk of 0.05 was established as the limit of statistical significance.

3 | RESULTS

3.1 | Characteristics of the study population

The general characteristics of the participants, the retinal blood vessel thickness and the parameters of vascular structure and function, globally and according to sex, are shown in Table 1. The mean age was 55.59 ± 14.22 years. The mean value of AV index was 0.793 (0.780 in men and 0.805 in women; p < 0.001). The mean value of arteriolar and venular calibre was 112.00 µm and 142.70 µm, respectively, with no differences between men and women. The male participants presented higher values of cIMT (0.696 vs. 0.665), cFPWV (6.824 vs. 6.222) and VAI (63.122 vs. 59.041) than the female participants. The characteristics of the 19 individuals who were excluded from the study for not having some of the main measurements recorded are shown in Table 1S.

Figure 2 shows the mean values of AV index and arteriolar and venular calibre by age group and sex. In the age group of 65 years, the women showed higher AV index values than the men.
### 3.2 Correlation analysis

Table 2 shows the correlation of retinal blood vessel thickness with age, blood pressure, CVR and the parameters of vascular structure and function. AV index and arteriolar calibre were negatively correlated with age, blood pressure, CVR and the parameters of vascular structure and function (p < 0.001 in all cases). Venular calibre was correlated positively only with age and CVR (p < 0.001). Table 2S shows the correlation in subjects diagnosed with hypertension and type 2 diabetes mellitus.

#### TABLE 1 General characteristics of the subjects included in the study, global and by sex

| Risk factors                  | Global (482) | Men (236) | Women (246) | p value |
|------------------------------|-------------|-----------|-------------|---------|
| Age (years)                  | 55.59 ± 14.22 | 55.40 ± 14.23 | 55.77 ± 14.25 | 0.775 |
| Smoking, n (%)               | 83 (17%) | 45 (19%) | 38 (15%) | 0.335 |
| Systolic blood pressure (mmHg) | 119.88 ± 19.22 | 126.21 ± 19.42 | 113.82 ± 16.96 | <0.001 |
| Diastolic blood pressure (mmHg) | 75.51 ± 10.13 | 77.34 ± 9.34 | 73.76 ± 10.56 | <0.001 |
| Pulse pressure (mmHg)        | 45.17 ± 19.80 | 49.06 ± 16.68 | 41.31 ± 21.83 | <0.001 |
| Hypertensive, n (%)          | 141 (29%) | 78 (33%) | 63 (26%) | 0.045 |
| Anti-hypertensive drugs, n (%) | 92 (19%) | 47 (20%) | 45 (18%) | 0.650 |
| Total cholesterol (mg/dl)    | 194.97 ± 32.52 | 192.77 ± 32.17 | 197.08 ± 32.78 | 0.146 |
| LDL cholesterol (mg/dl)      | 115.57 ± 29.46 | 117.43 ± 30.22 | 113.79 ± 28.66 | 0.178 |
| HDL cholesterol (mg/dl)      | 58.94 ± 16.22 | 53.39 ± 14.15 | 64.25 ± 16.32 | <0.001 |
| Triglycerides (mg/dl)        | 102.82 ± 53.73 | 112.13 ± 55.21 | 93.90 ± 50.80 | <0.001 |
| Dyslipidaemia, n (%)          | 181 (38%) | 85 (36%) | 96 (40%) | 0.278 |
| Lipid-lowering drugs, n (%)  | 96 (20%) | 43 (18%) | 53 (22%) | 0.212 |
| Fasting plasma glucose (mg/dl) | 87.86 ± 16.93 | 89.54 ± 17.84 | 86.24 ± 15.88 | 0.033 |
| HbA1c, (%)                   | 5.48 ± 0.53 | 5.52 ± 0.58 | 5.44 ± 0.47 | 0.100 |
| Diabetes mellitus, n (%)     | 34 (7%) | 23 (10%) | 11 (4%) | 0.032 |
| Hypoglycaemic drugs, n (%)   | 31 (6%) | 20 (8%) | 11 (4%) | 0.054 |
| Body mass index (kg/m²)      | 26.47 ± 4.18 | 26.82 ± 3.37 | 26.14 ± 4.82 | 0.071 |
| Waist circumference (cm)     | 93.13 ± 11.90 | 98.52 ± 9.41 | 87.94 ± 11.75 | <0.001 |
| Obesity, n (%)               | 90 (19%) | 39 (17%) | 51 (21%) | 0.245 |
| Abdominal obesity, n (%)     | 184 (38.3%) | 71 (30%) | 113 (46%) | <0.001 |
| CVR D’Agostino               | 11.30 ± 12.54 | 16.60 ± 15.06 | 6.25 ± 6.24 | <0.001 |

| Retinal vessels              |             |           |             |         |
|------------------------------|-------------|-----------|-------------|---------|
| AV index                     | 0.793 ± 0.073 | 0.780 ± 0.076 | 0.805 ± 0.067 | <0.001 |
| Arteriolar calibre (µm)      | 112.00 ± 13.20 | 110.89 ± 13.74 | 113.06 ± 16.20 | 0.071 |
| Venular calibre (µm)         | 142.70 ± 16.38 | 143.40 ± 16.66 | 142.03 ± 16.13 | 0.360 |

| Vascular structure and function |             |           |             |         |
|--------------------------------|-------------|-----------|-------------|---------|
| cIMT (mm)                      | 0.680 ± 0.109 | 0.696 ± 0.116 | 0.665 ± 0.100 | 0.002 |
| baPWV (m/s)                    | 12.910 ± 2.679 | 13.102 ± 2.447 | 12.725 ± 2.879 | 0.123 |
| cfPWV (m/s)                    | 8.151 ± 2.490 | 8.542 ± 2.680 | 7.712 ± 2.233 | 0.001 |
| VAI                            | 61.035 ± 12.768 | 63.122 ± 13.657 | 59.041 ± 11.536 | <0.001 |

Note: Values are means ± standard deviations for continuous data and number and proportions for categorical data.

Abbreviations: AV index, Retinal arteriolar-venular index; baPWV, Brachial-ankle pulse wave velocity; cfPWV, Carotid to femoral aortic pulse wave velocity; cIMT, Carotid intima-media thickness; CVR, Cardiovascular risk; HbA1c, Glycosylated haemoglobin; HDL, High-density lipoprotein; LDL, Low-density lipoprotein; VAI, Vascular Ageing Index.

p value: differences between men and women.
3.3 | Relationship of AV index and arteriolar and venular calibre with vascular ageing

Table 3 show the mean values for AV index and arteriolar and venular calibre in the individuals classified as HVA, NVA and EVA with the three criteria used to define them. The mean values of AV Index and arteriolar calibre were lower in the individuals with EVA than in those with HVA (Table 4).

3.4 | Association of vascular ageing with AV index and vascular calibre

The multiple regression analysis is shown in Table 5. AV index was negatively associated with cfPWV ($\beta = -2.89$)
and baPWV ($\beta = -3.18$), and VAI ($\beta = -1.74$) was negatively associated with arteriolar calibre. Arteriolar calibre was also negatively associated with baPWV ($\beta = -0.13$).

The results of the logistic regression models are shown in Figure 3. Higher values of AV index (OR = 0.04, OR = 0.03 and OR = 0.09) were more weakly correlated with developing EVA, defined with cfPWV, baPWV and VAI, respectively. Similarly, higher values of arteriolar calibre (OR = 0.71) were more weakly correlated with developing EVA, defined with VAI.

### 4 | DISCUSSION

This is the first study to analyse the association between retinal blood vessel calibre and AV index, estimated with Index Calculator, a software tool developed by our research group and previously validated\(^1\) for VA, defined with three criteria. The main finding of this study is that the participants who showed lower values of AV index and lower arteriolar calibre presented higher values of arterial stiffness with the three parameters used. These
results are confirmed in both the multiple and logistic regression analyses, with AV index and arteriolar calibre showing a negative correlation with the three criteria used to evaluate VA.

### Table 4 Differences between participants with healthy vascular ageing and participants with early vascular ageing in AV index and vascular calibre

|          | Difference  | 95% CI      | p value |
|----------|-------------|-------------|---------|
| cfPWV    |             |             |         |
| AV index | 0.045       | 0.017 - 0.074 | 0.002 |
| Arteriolar calibre | 0.805   | 0.359 - 1.251 | <0.001 |
| Venular calibre | 0.156  | -0.430 - 0.742 | 0.580 |
| baPWV    |             |             |         |
| AV index | 0.054       | 0.032 - 0.076 | <0.001 |
| Arteriolar calibre | 0.797   | 0.421 - 1.157 | <0.001 |
| Venular calibre | 0.067  | -0.449 - 0.583 | 0.799 |
| VAI      |             |             |         |
| AV index | 0.047       | 0.026 - 0.069 | <0.001 |
| Arteriolar calibre | 0.685   | 0.309 - 1.061 | <0.001 |
| Venular calibre | -0.007 | -0.482 - 0.468 | 0.977 |

**Abbreviations:** AV index, Retinal arteriolar-venular index; baPWV, Brachial-ankle pulse wave velocity; cfPWV, Carotid to femoral aortic pulse wave velocity; CI, Confidence interval; VAI, Vascular Ageing Index.

**p value:** differences between healthy vascular ageing and early vascular ageing.

### Table 5 Association of vascular ageing parameters with the calibre of the retinal vessels. Multiple regression analysis

|          | β        | 95% CI      | p value |
|----------|----------|-------------|---------|
| cfPWV    |          |             |         |
| AV index | -2.887   | -4.691 - -1.083 | 0.002 |
| Arteriolar calibre | -0.078   | -0.178 - 0.021 | 0.123 |
| Venular calibre | 0.044  | -0.036 - 0.123 | 0.278 |
| baPWV    |          |             |         |
| AV index | -3.178   | -5.433 - -0.924 | 0.006 |
| Arteriolar calibre | -0.127   | -0.251 - -0.003 | 0.044 |
| Venular calibre | 0.018  | -0.081 - 0.117 | 0.725 |
| VAI      |          |             |         |
| AV index | -1.740   | -2.744 - -0.736 | 0.001 |
| Arteriolar calibre | -0.532   | -1.087 - 0.024 | 0.061 |
| Venular calibre | 0.231  | -0.212 - 0.674 | 0.306 |

**Note:** Multiple regression analysis using as dependent variables cfPWV, baPWV and VAI, as independent variables, AV index, arteriolar calibre and venular calibre and as adjustment variables age, sex and hypotensive, hypoglycaemic and hypolipidaemic drugs.

**Abbreviations:** AV index, retinal arteriolar-venular index; baPWV, brachial-ankle pulse wave velocity; cfPWV, carotid to femoral aortic pulse wave velocity; CI, confidence interval; VAI, Vascular Ageing Index; β: regression coefficient.

### 4.1 Association of AV index and retinal blood vessel calibre with cardiovascular risk and the parameters of vascular structure and function

In line with the results of previous studies, both arteriolar calibre and AV index showed a negative correlation with age, blood pressure and CVR. However, in contrast with the findings of other studies, there was no correlation between blood pressure and retinal venular calibre.

Arteriolar calibre and AV index showed a negative correlation with all the parameters of vascular structure and function analysed, which was not the case for venular calibre. Several studies have shown this association with cfPWV in hypertensive adults, with baPWV and with the presence of atherosclerosis plaque in the carotid artery. However, not all studies have found this correlation. Thus, in a recent study, Meyer et al only obtained a correlation with venular calibre, attributing the lack of association with arterial calibre to the old age of the participants. The different associations of retinal arteriolar and venular calibre with arterial stiffness suggest the presence of a different underlying pathogenesis.

In general, in agreement with the literature, retinal venular calibre was correlated with blood pressure, blood glucose and body mass index, whereas the main variables related to arteriolar diameter were blood pressure and age. The differences among studies are due to the characteristics of the population, age, racial-ethnic differences, the prevalence of CVR factors and the consumption of drugs to treat hypertension.
4.2 Association of AV index and retinal blood vessel calibre with vascular ageing

The ageing process has been associated with the decrease of retinal blood vessel density, inner retinal layer thickness and retinal blood flow velocity. Therefore, it is suggested that there is a variety of vascular characteristics of the eye, which can be measured objectively through images, with the latter showing different physiological parameters of ageing and providing information about biological age, ageing trajectories and a variety of chronic systemic diseases. Thus, retinal vascular calibre is considered a structural marker of vascular pathology that reflects the interaction of systemic, environmental and genetic factors since the association between the increase of age and the narrowing of retinal blood vessels has been demonstrated in several populations.33

The results of this study are novel, as we show, in a Spanish general population without previous cardiovascular disease that arteriolar calibre and AV index are correlated with VA defined with three criteria. Our study suggests that the analysis of retinal blood vessels through images can be a useful and valid method to evaluate VA. In this line, Tapp et al16 developed an ‘ocular ageing index’ using ocular parameters to predict not only visual morbidity (vision impairment/blindness), but also systemic morbidity and mortality. Moreover, Poplin et al.34 showed that retinal imaging could predict CVR as accurately as the CVR equations that use the classic CVR factors.

To sum up, our findings suggest that the evaluation of retinal vascular calibre can provide information about VA and highlight the relevance of microcirculation in both systemic and ocular diseases. Furthermore, we believe that this measurement is simple to perform in clinical practice and provides information on microvascular circulation. Health professionals could be aided by this measurement tool to stratify cardiovascular risk more precisely.

4.3 Limitations and strengths

The main limitations of this study are as follows: (1) the transversal analysis does not allow inferring causality; (2) the results are from an urban population of a Spanish city that does not include people under 35 or over 75 years of age; (3) there are no data about the ocular factors that can affect vascular calibre, such as intraocular pressure; and (4) the prevalence of CVR factors in this study was lower with respect to that of other studies conducted in Caucasian populations. The main strength of this study is the fact that the participants were recruited through
population-based random sampling. However, further longitudinal studies are required to determine whether the measures of retinal blood vessel calibre can be used as a non-invasive evaluation of VA.

5 | CONCLUSIONS

Low values of AV index and arteriolar calibre are associated with vascular ageing in the general population without previous cardiovascular disease. Therefore, measuring the retinal blood vessel calibre could be useful in the clinical practice to evaluate vascular health in the general population.

ACKNOWLEDGEMENT

Researchers of the EVA group: Manuel A Gómez-Marcos, Luis García-Ortiz, José I Recio-Rodríguez, Carlos Martínez-Salgado, Jesús M Hernández-Rivas, Rogelio González-Sarmiento, Pedro L Sánchez-Fernández, Emiliano Rodríguez-Sánchez, María C Patino-Alonso, José A Maderuelo-Fernández, Leticia Gómez-Sánchez, Jesús González-Sánchez, Rosa Alonso-Dominguez, Carmela Rodríguez-Martín, Marta Gómez-Sánchez, Ángela de Cabo-Laso, Benigna Sánchez-Salgado, Natalia Sánchez Aguadero, Sara Mora-Simón, Olaya Tamayo-Morales, Cristina Agudo Conde, Cristina Lugones Sánchez, Susana González Sánchez, José Ramón González-Porras, José María Bastida-Bermejo and Isabel Fuentes-Calvo.

CONFLICTS OF INTERESTS

None of the authors does have any conflicts of interests.

AUTHOR CONTRIBUTIONS

LGS, MGS and MAGM contributed to the conception or design of the work. LGS, MGS, CPA, JIRR, JGS, CLS, CAC, JAM, ERS, LGO and MAGM contributed to the acquisition, analysis or interpretation of data for the work. LGS and MGS drafted the manuscript. All authors critically revised the manuscript and gave final approval and agree to be accountable for all aspects of the work ensuring integrity and accuracy.

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