Comparison between invasive and noninvasive techniques of evaluation of microvascular structural alterations

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Background: The evaluation of the morphological characteristics of small resistance arteries in humans is challenging. The gold standard method is generally considered to be the measurement by wire or pressure micromyography of the media-to-lumen ratio of subcutaneous small vessels obtained by local biopsies. However, noninvasive techniques for the evaluation of retinal arterioles were recently proposed; in particular, two approaches, scanning laser Doppler flowmetry (SLDF) and adaptive optics, seem to provide useful information; both of them provide an estimation of the wall-to-lumen ratio (WLR) of retinal arterioles. Moreover, a noninvasive measurement of basal and total capillary density may be obtained by videomicroscopy/capillaroscopy. No direct comparison of these three noninvasive techniques in the same population was previously performed; in particular, adaptive optics was never validated against micromyography.

Methods: In the current study, we enrolled 41 controls and patients: 12 normotensive lean controls, 12 essential hypertensive lean patients, nine normotensive obese patients and eight hypertensive obese patients undergoing elective surgery. All patients underwent a biopsy of subcutaneous fat during surgery. Subcutaneous small resistance artery structure was assessed by wire micromyography and the media-to-lumen ratio was calculated. WLR of retinal arterioles was obtained by SLDF and adaptive optics. Functional (basal) and structural (total) microvascular density was evaluated by capillaroscopy before and after venous congestion.

Results and conclusion: Our data suggest that adaptive optics has a substantial advantage over SLDF in terms of evaluation of microvascular morphology, as WLR measured with adaptive optics is more closely correlated with the M/L of subcutaneous small arteries ($r = 0.84$, $P < 0.001$ vs. $r = 0.52$, $P < 0.05$, slopes of the relations: $P < 0.01$ adaptive optics vs. SLDF). In addition, the reproducibility of the evaluation of the WLR with adaptive optics is far better, as compared with SLDF, as intraobserver and interobserver variation coefficients are clearly smaller. This may be important in terms of clinical evaluation of microvascular morphology in a clinical setting, as micromyography has substantial limitations in its clinical application due to the local invasiveness of the procedure.

Keywords: adaptive optics, microcirculation, remodeling, retina, retinal arterioles, scanning laser Doppler flowmetry, small resistance arteries

Abbreviations: MLR, media-to-lumen ratio; ROC, receiver-operating characteristic; SLDF, scanning laser Doppler Flowmetry; WCSA, total wall cross-sectional area

INTRODUCTION

Essential hypertension is associated with structural alterations in the microvessels [1]; in particular, an increase in the media-thickness-to-internal-lumen ratio of subcutaneous small resistance arteries [media-to-lumen ratio (MLR)] [1,2] and a reduction in capillary density [2] were observed. An increased MLR, that is the consequence of a process defined as microvascular remodeling, is associated with a worse prognosis in hypertension and diabetes [3,4], as well as to functional consequences [5]; appropriate pharmacological intervention may induce a prevention or a regression of subcutaneous small resistance artery remodeling [6] and an improvement of prognosis [7]. For all these reasons, the evaluation of microvascular

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structural alterations might provide clinically relevant information, in terms of a better stratification of cardiovascular risk, before and also during treatment [4].

The wall–lumen ratio (WLR) or the MLR of small arteries can be measured by pressure or wire micromyography, usually in subcutaneous tissues obtained through surgical biopsies [8,9]; this methodological approach represents, currently, the gold standard for the evaluation of microvascular alterations [1,8,9]. However, also according to the 2013 European Society of Hypertension/European Society of Cardiology Guidelines for the management of arterial hypertension [10], 'the invasiveness of the method makes this approach unsuitable for general use'. This has stimulated the search for noninvasive, but clinically reliable methods for a noninvasive evaluation of small resistance artery structure. The retinal microcirculation represents perhaps the only microvascular district that may be directly and relatively easily observed; in addition, the vasculature of the eye and of the heart [11] as well as that of the brain [12] share several common characteristics. Therefore, the easily accessible vessels of the eye may be considered, to some extent, a window to the heart [11] and to the brain [4,13].

In 2007, Harazny *et al.* [14] proposed a new method of assessment of structural abnormalities in the retinal vascular district. A quantification of the WLR of retinal arterioles was obtained using scanning laser Doppler flowmetry (SLDF) [14]. We have validated this method, demonstrating a statistically significant correlation between a noninvasive evaluation of the WLR of retinal arterioles and a locally invasive evaluation of the MLR of subcutaneous small resistance arteries by wire micromyography ($r = 0.76$, $P < 0.001$) in 40 normotensive controls and hypertensive patients [15]. However, the reproducibility of the noninvasive measurement of the WLR with the concerned technical approach in real-life situations is probably suboptimal, because of possible problems in a correct estimation of the internal diameter with a Doppler approach [16]. In fact, while the outer arteriole diameter is measured in confocal reflection images, the lumen diameter is measured in Doppler perfusion images [14,16]. In addition, the Heidelberg retina flowmeter is no longer present in the market, thus limiting the scientific interest and the possible clinical development of this approach [16].

A couple of years ago, a novel and extremely promising approach was made commercially available: the direct measurement of WLR of retinal arterioles using an adaptive optics imaging system [17,18]. This is a considerably improved version of a traditional fundus camera based on an approach originally applied to correct for aberrations in astronomic optical systems [16,17]. A beam of light enters the eye, and a small amount is reflected back out of the eye and into the optical system [16,17]. Wavefront aberrations in the reflected image are sensed by a suitable image sensor in the system, and corrected for by a deformable mirror. The achieved image resolution is, with the current system version, of the order of 1 μm. The fundus illumination consists of incoherent, infrared flashes at 10 Hz frequency, and the final image provided by the instrument is the average of a sequence of such images. The system, thus, provides images of a quality and resolution never previously obtained [16,17]. Vessel walls are clearly visible in most circumstances provided that the eye fixation is correct and that the ocular media are clear.

Skin capillary density was demonstrated to be decreased in hypertension and may be evaluated noninvasively by videomicroscopy/capillaroscopy before and after venous congestion, in the nailfold, dorsum of the finger or in the forearm [18–21]. Although a reduction in the capillary density might have important consequences in terms of tissue perfusion [5], the prognostic meaning of microvascular rarefaction was never demonstrated [4].

At present, no direct comparison of the assessment of microvascular alterations with wire or pressure micromyography and with adaptive optics is available. Therefore, the aim of the current study was to investigate WLR of retinal arterioles, measured either by SLDF or adaptive optics, capillary density measured by videomicroscopy and MLR of subcutaneous small resistance arteries, measured by wire micromyography in the same controls and patients.

PATIENTS AND METHODS

Forty-one controls and patients admitted to the surgical department of our hospital for elective surgery were investigated. They were 12 normotensive lean controls, 12 essential hypertensive lean patients, nine normotensive severely obese patients and eight hypertensive severely obese patients.

In the lean controls and patients, in 16 cases an election cholecystectomy was performed, in five indications for surgery was an incidentaloma of the adrenals (no evidence of hormonal secretion) and in three cases an elective intestinal resection for diverticulosis was performed. All obese patients were admitted to the surgical department of our hospital for indication to bariatric surgery (jejunoileal bypass or biliopancreatic derivation). Indication to bariatric surgery was made according to current clinical practice and ethics guidelines. Normal controls and patients were considered hypertensive or normotensive according to the European Society of Hypertension/European Society of Cardiology Guidelines [10]. The majority of the 20 hypertensive patients were on treatment with antihypertensive drugs. Blood pressure (BP) was recorded by standard sphygmomanometric approach (three measurements in lying position, 5 min apart) the day before surgical intervention. No wash-out of antihypertensive treatment was possible; therefore, BP values recorded were those on treatment in 18 out of 20 cases. Antihypertensive drugs used included diuretics, angiotensin-converting enzyme inhibitors, calcium channel blockers, AT1 blockers, beta-blockers and doxazosin. One normotensive individual had type 2 diabetes mellitus and was on treatment with oral antidiabetic drugs. None of them had additional signs of retinopathy (aneurisms, hemorrhages, exudates). Originally, 43 patients were included in the study, but in one case, due to poor collaboration, no proper evaluation of retinal arteriolar morphology could be performed, and in a second case, no suitable vessels were found in the subcutaneous fat biopsy.

Evaluation or retinal arteriolar morphology: scanning laser Doppler flowmetry

Twenty controls and patients underwent an evaluation of the retinal arteriolar morphology by SLDF, 3–4 days after

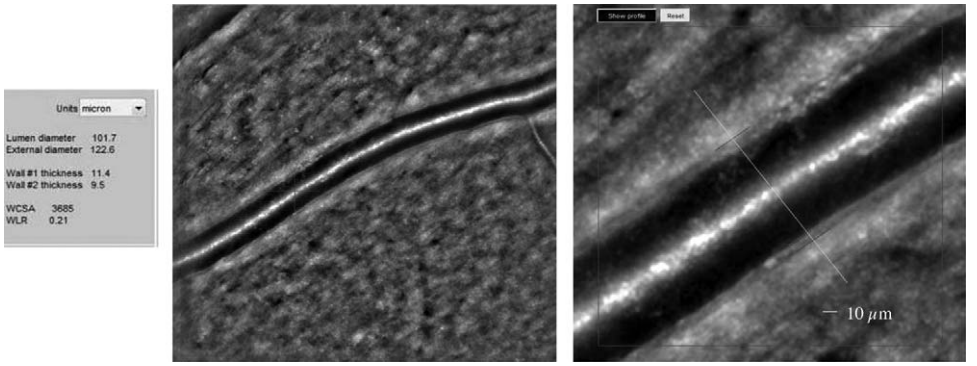

FIGURE 1 Example of images obtained with adaptive optics (adaptive optics camera, Rtx-1) (a) and measurement of morphological parameters with a dedicated software (b).

surgical intervention. WLR of retinal arterioles was assessed using SLDF at 670 nm (Heidelberg Retina Flowmeter; Heidelberg Engineering, Heidelberg, Germany) [14,15]. Details about the protocol adopted were previously described [15]. Briefly, an arteriole with a size between 80 and 140 μm of the superficial retinal layer in a retinal sample of $2.56 \times 0.64 \times 0.30$ mm was scanned within 2 s, at a resolution of 256 points × 64 lines × 128 lines. Measurements were performed in the juxtapapillary area of the right eye, 2–3 mm temporal superior to the optic nerve; the mean from three measurements was taken [14,22]. Analyses of diameters were performed offline with an automatic full-field perfusion imaging analysis program (Nirox Optoelectronics, Brescia, Italy). Outer arteriole diameter was measured in reflection images, and lumen diameter was measured in perfusion images [14,15,22]. WLR was calculated using the formula (outer diameter–lumen diameter)/lumen diameter [14,22].

The average values and SD were obtained by averaging 30 measurements.

Evaluation or retinal arteriolar morphology: adaptive optics

All normal controls and patients underwent an evaluation of the retinal arteriolar morphology by adaptive optics; 3–4 days after surgical intervention as mentioned, adaptive optics is a novel and accurate optoelectronic method which can provide noninvasively qualitative and quantitative microvascular morphometry of the different anatomical component of small vessels at a near-histological scale in the human retina [16–18]. Fundus images were obtained using a commercially available flood-illumination adaptive optics camera (Rtx-1; Imagine Eyes, Orsay, France). Briefly, the Rtx-1 camera measures and corrects wavefront aberrations with a 750-nm super luminescent diode source and an adaptive optics camera system operating in a closed loop [17,18]. A 4×4 fundus area (i.e. approximately 1.2×1.2 mm in emmetropic eyes) is illuminated at 840 nm by a temporally low coherence light emitting diode flashed flood source and a stack of 40 fundus images is acquired in 4 s by a camera. No pupil dilation was necessary. After a 5-min rest, the patient was installed on the chin rest. The live video image of the pupil allowed alignment with the incident light; the live display of adaptive optics camera-corrected fundus image allowed adjustment of brightness, contrast and focus. Gaze was oriented using a dedicated target to capture the region of interest which included a segment of the superotemporal artery of the right eye, devoid of bifurcations, with at least 250 μm long and an inner diameter of at least 50 μm. The site of interest was chosen to be free of the presence of focal arterial nicking or arteriovenous crossings. Additional information about the methods is reported in Koch *et al.* [17]. Image processing by appropriate software allowed measurements of the inner and outer diameters of the vessel [17] (Fig. 1). Wall thickness was defined as wall thickness = (outer diameter−inner diameter)/2, total wall cross-sectional area (WCSA) as $\text{WCSA} = \pi \times [(\text{outer diameter}/2)^2 - (\text{inner diameter}/2)^2]$ and the ratio of total parietal thickness over the lumen diameter averaged along 250-μm length defined the WLR [17,18].

The average values and SD were obtained by averaging 30 measurements.

Evaluation of capillary density

Skin capillary density was assessed by capillaroscopy before and after venous congestion, as described elsewhere [19–21]. Briefly, after a period of rest in sitting position in a quiet and temperature controlled room (21–22 °C), capillaries from nailfold and the dorsum of the fourth finger of the nondominant hand were visualized by using an epi-illuminated microscope containing a 100-W mercury vapor lamp light source, and pictures (final magnification of 200) were obtained by video-microscopy (Videocap 3.0 D1 200; DS Medica, Milano, Italy) in baseline conditions (basal capillary density) and after venous congestion (total capillary density), to visualize functionally excluded capillaries. Venous congestion was induced by inflating at to 60 mmHg for 2 min a miniature BP cuff applied to the base of the fourth finger of the nondominant hand [19–21]. Capillary density was defined as the number of capillaries per square millimeter of the microscopic field and was counted by hand. The first row of the nailfold capillaries was considered. Capillary density was determined by two independent operators and the findings were averaged.

Micromyography

All normal controls were submitted to a biopsy of subcutaneous fat from the anterior abdominal region. The biopsy of the abdominal subcutaneous fat was taken during the surgical procedure. Small arteries (about 100–280 μm of

TABLE 1. Demographic data in the study population

	All controls, $n=41$	Lean normotensive controls, $n=12$	Lean hypertensive patients, $n=12$	Obese normotensive patients, $n=9$	Obese hypertensive patients, $n=8$
Age (years)	$53.0 \pm 13.9^{*}$	58.2 ± 12.9	62.9 ± 11.1	$38.4 \pm 10.2^{**,\#\#\#}$	$46.9 \pm 3.56^{*,\#\#\#}$
Sex (M/F)	19/22	10/2	7/5	0/9	2/6
BMI (kg/m^2)	$34.0 \pm 8.40^{**}$	26.7 ± 2.40	29.1 ± 3.31	$41.1 \pm 2.76^{***,\#\#\#}$	$43.5 \pm 8.24^{***,\#\#\#}$
Smokers	7 (17.07%)	6 (50%)	0	1 (11.11%)	0
SBP (mmHg)	124 ± 19.0	120 ± 21.7	122 ± 18.1	122 ± 13.5	136 ± 20.4
DBP (mmHg)	76.6 ± 11.7	74.8 ± 10.5	72.8 ± 11.0	75 ± 12.5	$86.9 \pm 8.83^{*,\#\#}$
Serum glucose (mg/dl)	106 ± 27.6	113 ± 35.2	110 ± 32.0	100 ± 20.0	98.8 ± 10.7
Serum creatinine (mg/dl)	0.90 ± 0.41	0.93 ± 0.32	1.12 ± 0.62	$0.7 \pm 0.07^{*}$	0.74 ± 0.12
Total cholesterol (mg/dl)	184 ± 37.2	185 ± 50.6	178 ± 35.2	183.7 ± 5.50	195 ± 14.1
Serum triglycerides (mg/dl)	139 ± 80.9	149 ± 100	116 ± 81.1	149 ± 29.1	155 ± 91.9
Serum sodium (mEq/l)	141 ± 1.60	141 ± 1.91	141 ± 1.56	141 ± 1.50	141 ± 1.51
Serum potassium (mEq/l)	3.91 ± 0.28	4.05 ± 0.23	3.92 ± 0.30	3.93 ± 0.28	$3.67 \pm 0.21^{**}$

$^{*}P<0.05$ vs. lean normotensive controls.
$^{**}P<0.01$ vs. lean normotensive controls.
$^{***}P<0.001$ vs. lean normotensive controls.
$^{\#}P<0.05$ vs. lean hypertensive patients.
$^{\#\#}P<0.01$ vs. lean hypertensive patients.
$^{\#\#\#}P<0.001$ vs. lean hypertensive patients.

average diameter in relaxed conditions, 2 mm long) were dissected from the subcutaneous fat of the biopsies and mounted as a ring preparation on an isometric myograph (410 A; Danish Myo Technology, Aarhus, Denmark), by threading onto two stainless steel wires (40-μm diameter). After equilibration, the micromyograph was transferred to the stage of a light microscope with immersion lens. The vessel was stretched slightly (wall tension about 0.1 N/m), and the structural characteristics of the vessels were evaluated. The following parameters were directly measured or calculated: wall thickness, media thickness, adventitia thickness, internal diameter, MLR, media cross-sectional area (MCSA), WCSA. Details about the micromyographic technique of evaluation of small artery morphology were previously reported [23–26]. The average values obtained from two vessels in each experiment were considered.

Calculation of intraobserver and interobserver reproducibility of scanning laser Doppler flowmetry and adaptive optics

We randomly selected 18 controls and patients (10 normotensives, eight hypertensive patients, 7/18 severely obese) that underwent both retinal investigations. From each of the controls and patients, a single image obtained with adaptive optics was selected. For each image, 30 locations were then selected. The average values and SD for the lumen diameter, the external diameter, the average of the two wall thicknesses, WCSA and the WLR were obtained by averaging 30 measurements. Similarly, we measured the same parameters in 18 single images obtained by SLDF in the same controls and patients. For each image, five locations (corresponding to a vascular segment comparable with that evaluated with adaptive optics) were selected and the results obtained averaged. The same operator evaluated the same acquired images in 2 different days, separated by 10 weeks (intraobserver variability) and two different operators evaluated the same images (after 1 week) (interobserver variability).

Both the retinal study and the micromyographic study were conducted blindly. The protocol of the study was approved by the ethics committee of our institution (Medical School, University of Brescia), and a written informed consent was obtained from each participant. The procedures followed were in accordance with the institutional guidelines.

Statistical analysis

All data are expressed as mean + SD, unless otherwise stated. Differences between groups were evaluated by Student's unpaired *t* test. Distribution of categorical variables between groups was evaluated by a χ^2 test. Relationships between variables were assessed by calculation of Pearson's correlation, and statistical comparisons of the slopes of the relations were performed (Student's *t* test based on the standard error of regression models). A calculation of the receiver-operating characteristic (ROC) curves of the two diagnostic tests (evaluation of WLR with SLDF or adaptive optics) was also performed. The cutoff value for normalcy of the MLR was set at 0.05, which is the value observed in normal controls in the majority of intervention studies [6].

All parameters were normally distributed. All analyses were carried out with SPSS software (version 13.0; SPSS Inc., Chicago, Illinois, USA) apart from comparisons of the ROC curves, performed with MedCalc v. 14.8.1.0 (MedCalc Software, Ostend, Belgium).

RESULTS

Demographic characteristics of controls and patients enrolled in the study are reported, for descriptive reasons, in Table 1. Differences between groups were observed for age, DBP values, BMI and serum creatinine and potassium (Table 1). Morphological characteristics of microvessels in the different districts explored both in the whole population and in the different subgroups of controls and patients are reported, again only for descriptive reasons, in Table 2.

A close correlation was observed between MLR of subcutaneous small arteries and WLR of retinal arterioles evaluated by adaptive optics: $r=0.84$, $r^2=0.64$, P less than

TABLE 2. Morphological data in subcutaneous and retinal vessels as well as capillary density in the cutaneous tissue

	All controls, $n = 41$	Lean normotensive controls, $n = 12$	Lean hypertensive patients, $n = 12$	Obese normotensive patients, $n = 9$	Obese hypertensive patients, $n = 8$
Subcutaneous small resistance arteries					
Internal diameter (μm)	290 ± 65.6	297 ± 56.8	302 ± 71.3	271 ± 77.9	282 ± 48.2
External diameter (μm)	318 ± 68.1	318 ± 58.2	331 ± 79.2	306 ± 88.4	312 ± 42.0
Media thickness (μm)	13.4 ± 5.44	10.0 ± 3.83	13.4 ± 4.13*	16.9 ± 5.44**	14.6 ± 6.87*
Wall thickness (μm)	28.2 ± 10.4	21.6 ± 7.21	28.1 ± 7.63*	35.6 ± 11.6**	29.7 ± 11.7*
Media cross-sectional area (μm^2)	13 056 ± 6803	9800 ± 5507	13 610 ± 5873	16 553 ± 9120*	13 175 ± 5751
Wall cross-sectional area (μm^2)	28 713 ± 14 289	21 860 ± 10 767	29 591 ± 11 888	36 866 ± 20 408*	28 505 ± 10 914
Media-to-lumen ratio – MLR	0.0530 ± 0.0260	0.0326 ± 0.0118	0.0394 ± 0.0101	0.0720 ± 0.0188***,###	0.0867 ± 0.0148***,###
Retinal arterioles SLDF					
Outer diameter (μm)	102 ± 17.6	76.5 ± 21–3	108 ± 19.3	96.2 ± 8.01	97.8 ± 15.8
Inner diameter (μm)	93.3 ± 20.8	69.7 ± 22.8	84.7 ± 21.4	73.5 ± 9.58	63.2 ± 24.1
Wall thickness (μm)	12.8 ± 5.46	11.8 ± 1.61	11.7 ± 4.16	11.4 ± 2.66	17.3 ± 9.81
Wall-to-lumen ratio – WLR	0.398 ± 0.340	0.377 ± 0.172	0.294 ± 0.184	0.347 ± 0.057	0.710 ± 0.665#
Wall cross-sectional area (μm^2)	3481 ± 1406	2984 ± 662	3490 ± 1502	3015 ± 702	4184 ± 2000
Retinal arterioles AO					
Outer diameter (μm)	113 ± 21.9	112 ± 15.5	112 ± 23.9	121 ± 21.9	107 ± 28.0
Inner diameter (μm)	88.3 ± 18.6	89.6 ± 11.9	88.5 ± 20.9	92.9 ± 19.2	80.8 ± 23.9
Wall thickness (μm)	12.4 ± 2.48	11.4 ± 2.26	11.4 ± 2.29	14.2 ± 2.14**,##	13.1 ± 2.27
Wall-to-lumen ratio – WLR	0292 ± 0062	0.253 ± 0.034	0.259 ± 0.037	0.315 ± 0.056**,#	0.373 ± 0.042***,###
Wall cross-sectional area (μm^2)	4024 ± 1388	3687 ± 1162	3723 ± 1298	4874 ± 1423*,#	4024 ± 1628
Capillary density					
Basal capillary density nailfold (number of capillaries/mm^2)	7.29 ± 1.46	6.44 ± 0.88	6.83 ± 1.60	9.01 ± 1.41	8.00 ± 0.71
Total capillary density nailfold (number of capillaries/mm^2)	7.46 ± 1.41	6.67 ± 0.87	7.00 ± 1.67	9.22 ± 1.44	8.20 ± 0.45
Basal capillary density dorsum of the finger (number of capillaries/mm^2)	72.9 ± 13.2	73.2 ± 17.5	72.1 ± 12.8	70.3 ± 10.3	76.6 ± 10.4
Total capillary density dorsum of the finger (number of capillaries/mm^2)	76.6 ± 14.6	77.9 ± 17.1	74.4 ± 12.0	71.3 ± 11.8	83.5 ± 14.8
Capillary density					
Basal capillary density forearm (number of capillaries/mm^2)	54.6 ± 11.3	54.6 ± 12.1	51.8 ± 10.0	56.9 ± 12.7	55.7 ± 11.9
Total capillary density forearm (number of capillaries/mm^2)	61.5 ± 10.7	60.4 ± 8.62	60.2 ± 10.0	65.9 ± 14.7	60.0 ± 10.2

SLDF, scanning laser Doppler flowmetry.
*$P < 0.05$ vs. lean normotensive controls.
**$P < 0.01$ vs. lean normotensive controls.
***$P < 0.001$ vs. lean normotensive controls.
#$P < 0.05$ vs. lean hypertensive patients.
##$P < 0.01$ vs. lean hypertensive patients.
###$P < 0.001$ vs. lean hypertensive patients.

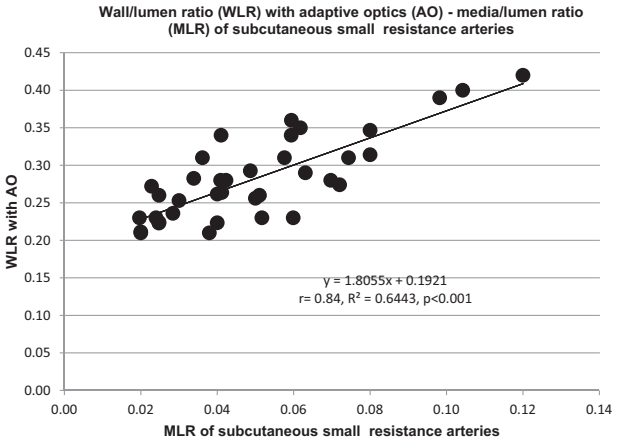

FIGURE 2 Correlation between wall-to-lumen ratio of retinal arterioles evaluated by adaptive optics and media-to-lumen ratio of subcutaneous small arteries.

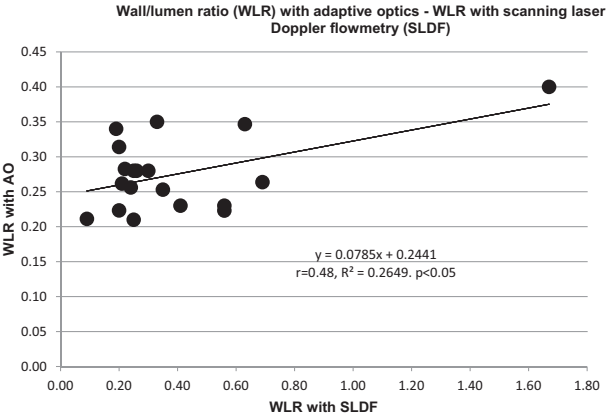

FIGURE 4 Correlation between wall-to-lumen ratio of retinal arterioles evaluated by adaptive optics and by scanning laser Doppler flowmetry.

0.001 (Fig. 2), whereas the correlation observed between MLR of subcutaneous small arteries and WLR of retinal arterioles evaluated by SLDF was less close: $r = 0.52$, $r^2 = 0.31$, P less than 0.05 (Fig. 3); a difference between the slopes of the two linear relations (adaptive optics vs. SLDF) was observed ($P < 0.01$). A statistically significant correlation was observed between WLR, of retinal arterioles evaluated by adaptive optics and SLDF ($r = 0.48$, $r^2 = 0.26$, $P < 0.05$) (Fig. 4).

No statistically significant correlation was observed between the internal or external diameter of subcutaneous small arteries and the internal and external diameter of retinal arterioles (evaluated by either adaptive optics or SLDF). A statistically significant correlation was observed between wall thickness or WCSA of subcutaneous small resistance arteries and the same morphological parameters of retinal arterioles evaluated with adaptive optics ($r = 0.45$, $P < 0.01$ and $r = 0.43$, $P < 0.01$, respectively), whereas no significant correlations were observed with wall thickness or WCSA of retinal arterioles evaluated by SLDF. No statistically significant correlations were observed between WCSA, wall thickness, inner or outer diameter evaluated by adaptive optics or the same parameters evaluated by SLDF.

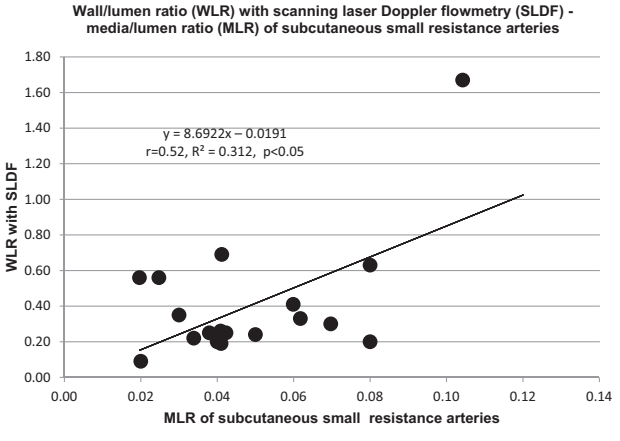

FIGURE 3 Correlation between wall-to-lumen ratio of retinal arterioles evaluated by scanning laser Doppler flowmetry and media-to-lumen ratio of subcutaneous small arteries.

Intraobserver and interobserver coefficient of variation for morphological parameter obtained by adaptive optics or SLDF are reported in Table 3. The reproducibility of the evaluation of the WLR and of the other indices of microvascular morphology with adaptive optics is far better, as compared with SLDF, as intraobserver and interobserver variation coefficients are clearly smaller.

We have also evaluated intraobserver and interobserver coefficient of variation in the subgroup of 10 normotensive controls: data are similar to those obtained in the whole population (WLR: adaptive optics 3.74 ± 1.47 and 6.59 ± 4.35, SLDF 30 ± 18 and 39 ± 20; internal diameter: adaptive optics 0.66 ± 0.40 and 0.87 ± 0.88, SLDF 16.2 ± 12.3 and 19.1 ± 4.2; wall thickness: adaptive optics 0.77 ± 0.37 and 1.59 ± 1.33, SLDF 25.6 ± 15.7 and 27.1 ± 11.8, respectively, for intraobserver and interobserver coefficient of variation; P value at least <0.01 between SLDF and adaptive optics in any comparison).

A calculation of ROC curves for WLR evaluated with either adaptive optics or SLDF was performed (Fig. 5). Sensitivity and specificity were evaluated in terms of ability of WLR measurements to discriminate normal controls or patients with a MLR above 0.05 ($n = 10$) or below 0.05 ($n = 10$). There was a significant difference between ROC curves in term of sensitivity and specificity in favor of adaptive optics ($P < 0.05$).

Finally, correlations between MLR of subcutaneous small resistance arteries and basal or total capillary density in the nailfold dorsum of the finger and forearm. Data are reported in Table 4. Although a statistically significant direct correlation was observed in the nailfold, no significant correlation was observed for the dorsum of the finger and the forearm (Table 4).

DISCUSSION

The current study for the first time has compared three noninvasive techniques of evaluation of microvascular morphology against the gold-standard, locally invasive micromyographic approach. The evaluation of the WLR

TABLE 3. Reproducibility of wall–lumen ratio measured by adaptive optics or scanning laser Doppler flowmetry

	Intraobserver AO	Interobserver AO	Intraobserver SLDF	Interobserver SLDF
WLR of retinal arterioles	3.25 ± 1.40	7.05 ± 4.59	38 ± 22*	43 ± 19*
Internal diameter of retinal arterioles	0.69 ± 0.35	0.87 ± 0.78	17.8 ± 14.0*	20.5 ± 5.3*
External diameter of retinal arterioles	0.74 ± 0.32	1.45 ± 1.37	11.7 ± 8.38*	13.1 ± 10.4**
Wall thickness of retinal arterioles	2.94 ± 1.43	7.02 ± 4.59	28.5 ± 17.0*	28.1 ± 12.1*
Wall cross-sectional area of retinal arterioles	3.09 ± 1.42	6.81 ± 5.51	28.8 ± 19.9*	24.6 ± 18.0**

AO, adaptive optics; SLDF, scanning laser Doppler flowmetry; WLR, wall–lumen ratio.
*$P < 0.001$ vs. AO.
**$P < 0.01$ vs. AO.

of retinal arterioles by adaptive optics has a clear advantage over SLDF, due to a better reproducibility and a closer correlation with the MLR of subcutaneous small resistance arteries, an index of vascular remodeling with demonstrated prognostic and clinical significance in hypertension and diabetes mellitus [3,4,27].

In a previous study [28], the reproducibility of the WLR or retinal arterioles evaluated by SLDF was better than in the current study, with values of intraobserver coefficient of variation of 7.7–8.9% and of interobserver coefficient of variation of 4–15% depending on the clinical conditions examined. Our protocol was similar to that adopted by Harazny *et al.* [28], however, we averaged a number of measurements (five for each image for SLDF, 30 for adaptive optics). In our experience, the main problem related to the evaluation of WLR of retinal arterioles with SLDF is the relatively high variability of the estimation of the internal diameter, due to the indirect nature of the measurement (image of Doppler flow), whereas the estimation of the morphological parameters of retinal arterioles by adaptive optics is quite stable throughout a length of the vascular segment of more than 500 μm [17]. Variation coefficients measured in our study for WLR evaluated by adaptive optics are in agreement with previously published data [17,29].

There is hardly any doubt that the retinal vascular district represents an easily accessible and pathophysiologically relevant target, in terms of stratification of cardiovascular risk [11,30,31].

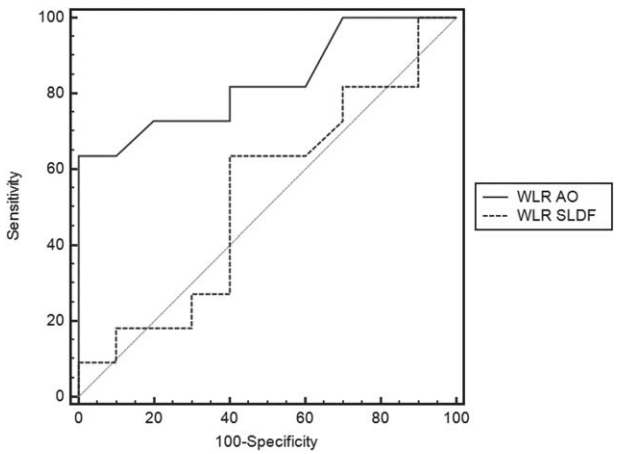

FIGURE 5 Receiver-operating characteristic curves for the two estimation of the wall-to-lumen ratio of retinal arterioles considered as diagnostic test for discrimination of controls and patients with a media-to-lumen ratio of subcutaneous small resistance arteries above or below the median value of the population examined. $P = 0.04$ between curves (Hanley & McNeil test).

Different parameters measured in the retinal vasculature have been proposed as clinically relevant, including fractal dimension, arteriolar-to-venular ratio, number of terminal branching of retinal vessels [32]. Although for some of them some prognostic relevance was demonstrated [33,34], it seems that at least 'retinal arteriolar and venular narrowing, similarly to capillary rarefaction in other vascular beds may be an early structural abnormality of hypertension, but its additive value to identify patients at risk for other types of organ damage needs to be better defined' [10]. Advantages and limitations of different indices of retinal microvascular morphology have been addressed by Cheung *et al.* [32]. The demonstration provided in the current study that WLR of retinal arterioles, especially when evaluated by adaptive optics, is closely correlated with MLR of subcutaneous small arteries, the most potent predictor of cardiovascular and cerebrovascular events also in multivariate analyses [3,4], strongly supports the clinical interest in a noninvasive evaluation of microvascular structure, and the possibility to transfer such measurements to the bedside [35], considering indices of microvascular morphology as intermediate endpoint in the evaluation of antihypertensive patients [36] and ready for prognostic translation [37]. As mentioned, cerebral and retinal circulation share anatomic, physiological and embryological features [12], and this renders the evaluation of microvascular structure in the retina a very good candidate for noninvasive assessment of hypertension-induced changes in the microvessels. However, a direct demonstration of the prognostic relevance of the easement of WLR in retinal arterioles with either SLDF or adaptive optics is still lacking. Data available with adaptive optics are limited, at present, to the observation of correlations of WLR with age [38] and BP [17] or of parallelisms between changes in WLR during treatment and extent of BP

TABLE 4. Correlation coefficients for capillary density in different locations and media-to-lumen ratio of subcutaneous small resistance arteries

Correlation coefficients, $n = 41$	r	r^2	P
Basal capillary density in the nailfold/MLR	0.46	0.22	<0.001
Total capillary density in the nailfold/MLR	0.50	0.25	<0.001
Basal capillary density in the dorsum of the finger/MLR	0.09	0.007	NS
Total capillary density in the dorsum of the finger/MLR	0.08	0.006	NS
Basal capillary density in the forearm/MLR	−0.05	0.003	NS
Total capillary density in the forearm/MLR	−0.07	0.004	NS

MLR, media-to-lumen ratio.

reduction [18]. Different drugs or drug combinations seem to differently affect WLR evaluated with adaptive optics or SLDF [39], as previously observed with micromyography [6].

A reduction in capillary density might be associated with an impairment of tissue perfusion [40], thus possibly being involved in the worsening of organ damage in hypertension, and being also a possible target for treatment [41,42]. In a previous study, we have demonstrated that a correlations exists between MLR of subcutaneous small resistance arteries and microvascular density in the derma as evaluate by immunohistochemistry of fixed tissues, suggesting that different alterations in different locations of the microvasculature may be present simultaneously and may be interrelated [43]. Also changes during treatment seem to be similar as far as WLR of retinal arterioles and capillary density in the dorsum of the finger are concerned [39]. However, in the current study, correlations between MLR of subcutaneous small resistance arteries and capillary density in the dorsum of the finger or in the forearm were not statistically significant, whereas that with capillary density in the nailfold was statistically significant, but it was a direct one, suggesting, unexpectedly, that more pronounced alterations in subcutaneous small resistance arteries is associated with a greater capillary density in the nailfold, instead of a rarefaction. This finding is not easy to be explained; however, previous data demonstrating a reduction of capillary density in hypertension or obesity using videomicroscopy/capillaroscopy are restricted to the dorsum of the finger and to the forearm [19–21]; thus, it is possible that capillaries in the nailfold might behave differently. In any case, it is clear from these data that a noninvasive estimation of capillary density in the cutaneous tissue is probably less relevant, from a clinical point of view, with respect to an evaluation of retinal arteriolar morphology.

Limitation of the study

Being a methodological study, no selection of patients was made, and a large proportion of controls and patients admitted to the surgical department were considered eligible. Hypertensive patients were previously treated, and no therapeutic wash-out was possible. Classification of controls and patients into the four groups was made retrospectively, and only for descriptive purposes. The study was not powered enough to evaluate differences between groups. For all these reasons, no significant difference was observed in some morphological parameters between hypertensive patients and normotensives. In this study, we did not specifically evaluate intraobserver and interobserver reproducibility of the measurement of the MLR of subcutaneous small arteries by wire micromyography, because this was not our main aim, and data in this regard were previously published [9]. In our laboratory, the intra-assay coefficient of variation of the MCSA calculation (the morphological parameter with the highest variability) was 10.4% (six vessels, 10 measurements in each vessel in a single session), and the interassay coefficient of variation was 11.2% (six vessels, 10 measurements in each vessel performed in two sessions by two different observers) [26]. Although we observed a significant correlation between three different morphological parameters measured in retinal arterioles with adaptive optics vs. those measured in subcutaneous small resistance arteries (WLR/MLR, wall thickness/wall thickness, WCSA/WCSA), it is generally accepted that the most relevant and useful parameter obtained with micromyographic approaches is represented by the MLR, as it appears to be independent form the vessel dimension, whereas this is not the case for media thickness and vessel diameters, which are obviously dependent on the dimensions of vascular segments [8,9]. Also for the WLR evaluated with adaptive optics, some independency from the vascular diameter was demonstrated [17].

An important issue is how to standardize measurements of WLR when BP differ between different groups. When micromyiography is used isolated vessels are completely relaxed and then stretched to an equivalent of 100 mmHg at which all measurements are made. Lumen size may change depending on levels of vasoconstrictor tone in microvessels and this could, to some extent, affect WLRs. The measurements of retinal vessels obtained with SLDF or adaptive optics have both structural and functional components, as the vessels are not relaxed or normalized for a standardized wall tension, thus reflecting in any case an in-vivo rather than an in-vitro situation [4].

This could be a limitation, but also a clinical advantage, as the concerned measurements reflect truly a 'real life' situation. In any case, there is no feasible method to get rid of a functional component of these measurements, although the flicker-light technique has been used in an attempt to reduce vascular tone [4,44].

In summary, our data suggest that adaptive optics has a substantial advantage over SLDF in terms of evaluation of microvascular morphology, as it is more closely correlated with the MLR of subcutaneous small arteries. In addition, the reproducibility of the evaluation of the WLR with adaptive optics is far better, as compared with SLDF, as intraobserver and interobserver variation coefficients are clearly lower. This may be important in terms of clinical evaluation of microvascular morphology in a clinical setting, as micromyography is generally considered a gold-standard approach for this purpose, but it is limited in its clinical application by the local invasiveness of the procedure. Therefore, a possible future perspective related to our results is represented by the wider use of noninvasive approaches for the stratification of cardiovascular risk in the majority of hypertensive patients, as microvascular structure may also represent an intermediate endpoint in the evaluation of the effects of antihypertensive treatment. It is probable that a noninvasive evaluation of WLR of retinal arterioles might meet the criteria requested by the European Guidelines on Hypertension management [10], although it would also be clinically important to determine whether the therapeutic regression of cardiac, renal and vascular target organ damage seen in hypertension has the same temporal pattern of regression as retinal vascular damage.

In conclusion, a noninvasive and easily repeatable procedure such as the evaluation of the arterioles in the fundus oculi by adaptive optics may provide similar information regarding microvascular morphology compared with an invasive, accurate and prognostically relevant micromyographic measurement of the MLR of subcutaneous small arteries. Therefore, there is hardly any doubt that new technologies, currently under clinical evaluation, may help

us in the future to noninvasively assess microvascular structural alterations and to better stratify cardiovascular risk of our patients with consequent optimization of treatment [45].

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Mulvany MJ, Aalkjaer C. Structure and function of small arteries. *Physiol Rev* 1990; 70:921–971.
2. Agabiti Rosei E, Rizzoni D. The effects of hypertension on the structure of human resistance arteries. In: Lip GYH, Hall JE, editors. *Comprehensive hypertension*. Philadelphia, PA: Mosby Elsevier; 2007. pp. 579–590; 207, chapter 47.
3. Rizzoni D, Porteri E, Boari GEM, De Ciuceis C, Sleiman I, Muiesan ML, *et al*. Prognostic significance of small artery structure in hypertension. *Circulation* 2003; 108:2230–2235.
4. Agabiti-Rosei E, Rizzoni D. Microvascular structure as a prognostically relevant endpoint. *J Hypertens* 2017; 35:914–921.
5. Rizzoni D, Agabiti-Rosei C, Agabiti-Rosei E. Hemodynamic consequences of changes in microvascular structure. *Am J Hypertens* 2017; 30:939–946.
6. Agabiti-Rosei E, Heagerty AM, Rizzoni D. Effects of antihypertensive treatment on small artery remodelling. *J Hypertens* 2009; 27:1107–1114.
7. Buus NH, Mathiassen ON, Fenger-Grøn M, Præstholm MN, Sihm I, Thybo NK, *et al*. Small artery structure during antihypertensive therapy is an independent predictor of cardiovascular events in essential hypertension. *J Hypertens* 2013; 31:791–797.
8. Schiffrin EL, Hayoz D. How to assess vascular remodelling in small and medium-sized muscular arteries in humans. *J Hypertens* 1997; 15:571–584.
9. Virdis A, Savoia C, Grassi G, Lembo G, Vecchione C, Seravalle G, *et al*. Evaluation of microvascular structure in humans: a 'state-of-the-art' document of the Working Group on Macrovascular and Microvascular Alterations of the Italian Society of Arterial Hypertension. *J Hypertens* 2014; 32:2120–2129.
10. Mancia G, Fagard R, Narkiewicz K, Redón J, Zanchetti A, Böhm M, *et al*., Task Force Members. 2013 ESH/ESC Guidelines for the management of arterial hypertension. The Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *J Hypertens* 2013; 31:1281–1357.
11. Flammer J, Konieczka K, Bruno RM, Virdis A, Flammer AJ, Taddei S. The eye and the heart. *Eur Heart J* 2013; 34:1270–1278.
12. Wong TY, Mitchell P. Hypertensive retinopathy. *N Engl J Med* 2004; 351:2310–2317.
13. Rizzoni D, De Ciuceis C, Porteri E, Paiardi S, Boari GE, Mortini P, *et al*. Altered structure of small cerebral arteries in patients with essential hypertension. *J Hypertens* 2009; 27:838–845.
14. Harazny JM, Ritt M, Baleanu D, Ott C, Heckmann J, Schlaich MP, *et al*. Increased wall:lumen ratio of retinal arterioles in male patients with a history of a cerebrovascular event. *Hypertension* 2007; 50:623–829.
15. Rizzoni D, Porteri E, Duse S, De Ciuceis C, Agabiti Rosei C, La Boria E, *et al*. Relationship between media-to-lumen ratio of subcutaneous small arteries and wall-to-lumen ratio of retinal arterioles evaluated noninvasively by scanning laser Doppler flowmetry. *J Hypertens* 2012; 30:1169–1175.
16. Rizzoni D, Docchio F. Assessment of retinal arteriolar morphology by noninvasive methods: the philosopher's stone? *J Hypertens* 2016; 34:1044–1046.
17. Koch E, Rosenbaum D, Brolly A, Sahel JA, 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; 32:890–898.
18. Rosenbaum D, Mattina A, Koch E, Rossant F, Gallo A, Kachenoura N, *et al*. Effects of age, blood pressure and antihypertensive treatments on retinal arterioles remodeling assessed by adaptive optics. *J Hypertens* 2016; 34:1115–1122.
19. Antonios TF, Singer DR, Markandu ND, Mortimer PS, MacGregor GA. Rarefaction of skin capillaries in borderline essential hypertension suggests an early structural abnormality. *Hypertension* 1999; 34 (4 Pt 1):655–658.
20. Antonios TF, Singer DR, Markandu ND, Mortimer PS, MacGregor GA. Structural skin capillary rarefaction in essential hypertension. *Hypertension* 1999; 33:998–1001.
21. De Ciuceis C, Rossini C, Porteri E, La Boria E, Corbellini C, Mittempergher F, *et al*. Circulating endothelial progenitor cells, microvascular density and fibrosis in obesity before and after bariatric surgery. *Blood Press* 2013; 22:165–172.
22. Ritt M, Harazny JM, Ott C, Schlaich MP, Schneider MP, Michelson G, Schmieder RE. Analysis of retinal arteriolar structure in never-treated patients with essential hypertension. *J Hypertens* 2008; 26:1427–1434.
23. Aalkjaer C, Heagerty AM, Petersen KK, Swales JD, Mulvany MJ. Evidence for increased media thickness, increased neural amine uptake, and depressed excitation-contraction coupling in isolated resistance vessels from essential hypertensives. *Circ Res* 1987; 61:181–186.
24. Mulvany MJ, Hansen PK, Aalkjaer C. Direct evidence that the greater contractility of resistance vessels in spontaneously hypertensive rats is associated with a narrowed lumen, a thickened media, and an increased number of smooth muscle cell layers. *Circ Res* 1978; 43:854–864.
25. Rizzoni D, Porteri E, Guelfi D, Muiesan ML, Valentini U, Cimino A, *et al*. Structural alterations in subcutaneous small arteries of normotensive and hypertensive patients with non insulin dependent diabetes mellitus. *Circulation* 2001; 103:1238–1244.
26. Rizzoni D, Porteri E, Castellano M, Bettoni G, Muiesan ML, Muiesan P, *et al*. Vascular hypertrophy and remodeling in secondary hypertension. *Hypertension* 1996; 28:785–790.
27. Heagerty AM. Predicting hypertension complications from small artery structure. *J Hypertens* 2007; 25:939–940.
28. Harazny JM, Raff U, Welzenbach J, Ott C, Ritt M, Lehmann M, *et al*. New software analyses increase the reliability of measurements of retinal arterioles morphology by scanning laser Doppler flowmetry in humans. *J Hypertens* 2011; 29:777–782.
29. Rosenbaum D, Koch E, Girerd X, Rossant F, Pâques M. Imaging of retinal arteries with adaptative optics, feasibility and reproducibility. *Ann Cardiol Angeiol (Paris)* 2013; 62:184–188.
30. Muiesan ML, Grassi G. Assessment of retinal vascular changes in hypertension: new perspectives. *J Hypertens* 2006; 24:813–814.
31. Ritt M, Schmieder RE. Wall-to-lumen ratio of retinal arterioles as a tool to assess vascular changes. *Hypertension* 2009; 54:384–387.
32. Cheung CY, Ikram MK, Sabanayagam C, Wong TY. Retinal microvasculature as a model to study the manifestations of hypertension. *Hypertension* 2012; 60:1094–1103.
33. Liew G, Mitchell P, Rochtchina E, Wong TY, Hsu W, Lee ML, *et al*. Fractal analysis of retinal microvasculature and coronary heart disease mortality. *Eur Heart J* 2011; 32:422–429.
34. Ding J, Wai KL, McGeechan K, Ikram MK, Kawasaki R, Xie J, *et al*., Meta-Eye Study Group. Retinal vascular caliber and the development of hypertension: a meta-analysis of individual participant data. *J Hypertens* 2014; 32:207–215.
35. Schiffrin EL, Touyz RM. From bedside to bench to bedside: role of renin-angiotensin-aldosterone system in remodeling of resistance arteries in hypertension. *Am J Physiol Heart Circ Physiol* 2004; 287:H435–H446.
36. Mulvany MJ. Small artery structure: time to take note? *Am J Hypertens* 2007; 20:853–854.
37. Heagerty AM. Changes in small artery structure in hypertension: ready for prognostic translation? *J Hypertens* 2017; 35:945–946.
38. Meixner E, Michelson G. Measurement of retinal wall-to-lumen ratio by adaptive optics retinal camera: a clinical research. *Graefes Arch Clin Exp Ophthalmol* 2015; 253:1985–1995.
39. De Ciuceis C, Salvetti M, Paini A, Rossini C, Muiesan ML, Duse S, *et al*. Comparison of lercanidipine plus hydrochlorothiazide vs. lercanidipine plus enalapril on micro and macrocirculation in patients with mild essential hypertension. *Intern Emerg Med* 2017; 12:963–974.
40. Struijker Boudier HAJ. Microcirculation in hypertension. *Eur Heart J* 1999; 1 (Suppl L):L32–L37.
41. Levy BI, Ambrosio G, Pries AR, Struijker-Boudier HAJ. Microcirculation in hypertension: a new target for treatment? *Circulation* 2001; 104: 735–740.
42. Tsioufis C, Dimitriadis K, Katsiki N, Tousoulis D. Microcirculation in hypertension: an update on clinical significance and therapy. *Curr Vasc Pharmacol* 2015; 13:413–417.

43. Paiardi S, Rodella LF, De Ciuceis C, Porteri E, Boari GE, Rezzani R, *et al*. Immunohistochemical evaluation of microvascular rarefaction in hypertensive humans and in spontaneously hypertensive rats. *Clin Hemorheol Microcirc* 2009; 42:259–268.
44. Ritt M, Harazny JM, Ott C, Raff U, Bauernschubert P, Lehmann M, Michelson G. Schmieder RE impaired increase of retinal capillary blood flow to flicker light exposure in arterial hypertension. *Hypertension* 2012; 60:871–876.
45. Bosetti F, Galis ZS, Bynoe MS, Charette M, Cipolla MJ, Del Zoppo GJ, *et al*., 'Small Blood Vessels: Big Health Problems' Workshop Participants. 'Small Blood Vessels: Big Health Problems?': scientific recommendations of the National Institutes of Health Workshop. *J Am Heart Assoc* 2016; 5; e004389.

Reviewers' Summary Evaluations

Reviewer 1

Microvessel structural changes are associated with poor prognosis in hypertension and diabetes.

Current approaches that assess microvessel structure are either invasive (wire micromyiography of subcutaneous microvessels) or no longer available (Doppler flowmetry of retinal arterioles). Here the authors examine whether a noninvasive adaptive optics imaging system (AO) might be useful for assessing retinal vessel structure. The authors provide convincing data indicating that AO is reproducible and better suited for clinical use than wire micromyiography as it is noninvasive and easier to perform. A limitation of AO may be an inability to standardise the resultant wall/lumen ratio measurements to blood pressure.

Reviewer 2

This methodological study compares a novel technique employing adaptive optics (AO) for measurement of wall to lumen ratio (WLR) of retinal arterioles with the established scanning laser doppler flowmetry (SLDF). The AO device has impressively high resolution with excellent vessel boundary definition compared to SLDF, allowing accurate measurement of vessel calibre and wall thickness with reduced variability. WLR measured by AO shows a strong correlation with media to lumen ratio of subcutaneous microvessels, suggesting a potential clinical noninvasive replacement for the invasive method of micromyography. The sensitivity of the technique in various disease states is yet to be established.

Reviewer 3

This methodological study allows to conclude that the non invasive adaptive optic technique shows a good correlation with invasive gold-standard technique used to investigate arteriolar structural alterations. Thus, this technique could be safety used in clinical setting and for stratification of cardiovascular risk.