Microvascular structure as a prognostically relevant endpoint

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Remodelling of subcutaneous small resistance arteries, as indicated by an increased media-to-lumen ratio, is frequently present in hypertensive, obese, or diabetic patients. The increased media-to-lumen ratio may impair organ flow reserve. This may be important in the maintenance and, probably, also in the progressive worsening of hypertensive disease. The presence of structural alterations represents a prognostically relevant factor, in terms of development of target organ damage or cardiovascular events, thus allowing us a prediction of complications in hypertension. In fact, media-to-lumen ratio of small arteries at baseline, and possibly their changes during treatment may have a strong prognostic significance. However, new, non-invasive techniques are needed before suggesting extensive application of the evaluation of remodelling of small arteries for the cardiovascular risk stratification in hypertensive patients. Some new techniques for the evaluation of microvascular morphology in the retina, currently under clinical investigation, seem to represent a promising and interesting future perspective. The evaluation of microvascular structure is progressively moving from bench to bedside, and it could represent, in the near future, an evaluation to be performed in all hypertensive patients, to obtain a better stratification of cardiovascular risk, and, possibly, it might be considered as an intermediate endpoint in the evaluation of the effects of antihypertensive therapy, provided that a demonstration of a prognostic value of non-invasive measures of microvascular structure is made available.

Keywords: microcirculation, peripheral circulation, remodelling, small resistance arteries, vascular biology

Abbreviations: ACE, angiotensin-converting enzyme; AVR, arteriolar-to-venular ratio; BP, blood pressure; TREG, T-regulatory lymphocytes

MICROVASCULAR STRUCTURAL ALTERATIONS IN HYPERTENSION, DIABETES MELLITUS, AND OBESITY

Alterations in the microcirculation are commonly associated with cardiovascular and metabolic diseases [1,2] and may involve small resistance arteries, arterioles, capillaries, and postcapillary venules [3]. In particular, the structure of subcutaneous [1–3] as well as of cerebral [4] small resistance arteries (lumen diameter 100–300 μm) in human may be altered in the presence of primary or secondary hypertension, diabetes mellitus, or morbid obesity [3].

Essential hypertension is associated with a narrowing of the internal lumen and with an increase of media wall thickness, with consequent increase in the media-to-lumen ratio [1]. The observed increase in the media-to-lumen ratio may be the consequence of an inward eutrophic remodelling (rearrangement of otherwise normal material around a narrowed lumen) or of an inward hypertrophic remodelling (vascular smooth muscle cell hypertrophy or hyperplasia) [5]. Eutrophic remodelling of subcutaneous small arteries is commonly seen in essential hypertension; on the contrary an hypertrophic remodelling, with evident smooth muscle cell growth, has been shown in patients with type II diabetes mellitus [6,7], obesity [8,9], and also with metabolic syndrome [10] [even regardless of the presence or absence of elevated or normal blood pressure (BP) levels], as well as in some forms of secondary hypertension, including renovascular hypertension [11], Cushing's syndrome [12], acromegaly [13] and, possibly, primary aldosteronism [11].

Structural remodelling of the microvascular networks includes also rarefaction in the most distal part, as suggested by the observation in hypertension, in diabetes, and in obesity, of a reduction of the density of microvessels and capillaries [3,4,14], with potential consequences for tissue perfusion and exchange or transport of nutrients [3,15,16]. A close correlation between media-to-lumen ratio of subcutaneous small arteries and capillary density in the skin has been previously observed [17], thus suggesting that vascular remodelling and rarefaction may occur in parallel.

Both a functional rarefaction (capillary perfused in basal conditions) and a structural rarefaction (capillary recruited during venous congestion,) were observed in

Journal of Hypertension 2017, 35:914–921

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Received 16 October 2016 **Revised** 24 November 2016 **Accepted** 15 December 2016

DOI:10.1097/HJH.0000000000001259

hypertension [3]. Therefore, capillary rarefaction observed in hypertension is very likely a permanent anatomical change rather than a functional one. Whether it is pathologically important in terms of worsening the disease or generating complications it is still a matter of debate, although it is probable that it might be associated with increased peripheral resistance and impaired tissue perfusion, thus consequently, to organ damage [16]. At present, however, we do not have convincing evidence of a prognostic value of a decreased capillary density in hypertension [3].

Not only microvascular structure may be altered in cardiovascular and/or metabolic diseases, but also functional characteristics of small vessels may be impaired, in particular those mediated by a normal-functioning endothelium. In fact, an impairment of the endothelial function, as evaluated by the vasodilator response to acetylcholine, has been detected in human small arteries in essential hypertension [6,11] as well as in type 2 diabetes mellitus [6,7] and obesity [8,9].

As mentioned, alterations in the microcirculation represent a common finding in patients with hypertension [1] and also in those with diabetes mellitus [6]; remodelling of small resistance arteries might represent an important structural vascular damage involved in the development of mechanism of disease and, ultimately, of clinical events. The media-to-lumen ratio of subcutaneous small resistance arteries in human hypertension was demonstrated to be related to left ventricular geometry [18] and to the intima–media thickness of the carotid artery [19].

An important consequence of the presence of increased media-to-lumen ratio may be an impairment of vasodilator reserve. In fact, remodelling of small resistance arteries is characterized by a narrowing of the lumen, which leads to an increase of flow resistance even at full dilatation, that is, in the absence of vascular tone. A close correlation was observed, in hypertensive patients, between media-to-lumen ratio of subcutaneous small resistance arteries and coronary flow reserve [20,21] or minimum vascular resistance in the forearm, measured from the maximum post-ischaemic flow [22], suggesting that structural alterations in small resistance arteries may be present at the same time in different vascular districts, including the coronary circulation. Alterations in the microcirculation may therefore play an important role in the development of organ damage in hypertension.

The extent of structural alterations in subcutaneous small resistance arteries is particularly pronounced in hypertensive patients with type 2 diabetes mellitus [6] or obesity [8–10]. It seems, therefore, that the association of several cardiovascular risk factors may have a synergistic, deleterious effect on the microcirculation.

It is not clear whether an increase in BP values precedes or follows the onset of microvascular alterations. Some data in animal models of hypertension, in particular in the spontaneously hypertensive rat suggest that an alteration in microvascular structure, namely an increase in the media-to-lumen ratio of mesenteric small resistance arteries might be present in a prehypertensive phase [23], when BP values are, substantially, similar to those of normotensive control animals. Data in humans are obviously scarce and relatively difficult to obtain, as there is no certainty about what could be considered as a true prehypertensive condition, and no longitudinal data are presently available. An increase in forearm minimal vascular resistance (an indirect index of microvascular structure) in young study participants with positive family history of hypertension was observed [24]; however, when resistance vessels from offspring of essential hypertensive patients were investigated with direct approaches (micromyography), no morphological alterations were observed, compared with controls [25]. According to Park and Schiffrin [26], small artery remodelling is the most prevalent and probably the earliest form of target organ damage in human mild essential hypertension. In a review, Schiffrin [2] addressed the topic of the time course of changes in morphologic and mechanical aspects of resistance arteries as hypertension evolves with time. He suggested that an increase in the media-to-lumen ratio in small resistance arteries might be present very early, but its severity parallels the increase in BP values [2].

MECHANISMS INVOLVED IN THE DEVELOPMENT OF MICROVASCULAR REMODELLING

Several mechanisms have been proposed to explain the development of vascular remodelling. For eutrophic remodelling, an alteration of the integrins pattern might be involved [27,28]. In addition, extensive work of Bakker *et al.* [29,30] have demonstrated a crucial role of tissue transglutaminase in the development of eutrophic remodelling. On the contrary, hypertrophic remodelling might be the consenquence of long-standing, severe hypertension, and/or of an impaired myogenic response to increased BP [7]. The wall myogenic tone [7,31] is the ability of an artery to contract in response to an increase in intraluminal pressure. This autoregulatory function is vital to ensure stabilization of distal capillary pressures and so prevent (or limit) organ damage [31]. When myogenic autoregulation is damaged, wall stress is increased and target organ damage ensues [31]. In particular, when myogenic autoregulation is impaired in the context of hypertension, eutrophic remodelling is replaced by hypertrophic remodelling [31]. Two particular integrins, $\alpha 5\beta 1$ and $\alpha\nu\beta 3$, have been found to be necessary for both normal myogenic autoregulation and eutrophic remodelling [31], and their altered expression might be involved in the development of vascular remodelling.

In previous studies, direct relationships were observed between media-to-lumen ratio of the subcutaneous small arteries and BMI, waist circumference, plasma insulin levels, and homeostasis model assessment (HOMA) index [9] or insulin/insulin-like growth factor 1 [6,13], thus suggesting an important role also of humoral factors such as insulin/insulin resistance in the development of vascular structural alterations. Finally, it is also possible that oxidative stress/inflammation might be involved in the development (and possibly in the regression) of both types of vascular remodelling [32,33].

Recently, the interest of researchers has been focused on a possible role of the immune system in the development/maintenance of high BP values and vascular remodelling. As previously mentioned, low-grade inflammation is a

process that might have an important role to play as a mechanism in the progression of cardiovascular disease [33,34]. Circulating and tissue leukocytes and macrophages are components of the mechanisms leading to inflammatory responses. Inflammation participates in many processes that contribute to the development of elevated BP. In the vasculature, for example, inflammation can increase the proliferation of smooth muscle cells and may participate in vascular remodelling [35]. Infiltration of immune cells in various organs such as blood vessels, kidney, and perivascular adipose tissue is an important component of the inflammatory mechanisms leading to cardiovascular damage and hypertension [36].

In summary, it seems that most of the structural changes are generated by the need to offset the increased wall stress observed when BP rises or, in the case of diabetes, when blood flow increases. Other processes probably follow, namely inflammation and the upregulation of some integrins.

VASCULAR STRUCTURAL ALTERATIONS AND PROGNOSIS

An increase in the media-to-lumen ratio of subcutaneous small arteries was proved to be a powerful predictor of cardiovascular events in hypertension [37], and a hypertrophic remodelling of small vessels seems to be associated to an even higher incidence of events, compared with eutrophic remodelling [38]. Structural alterations in the subcutaneous vascular district might reflect similar alterations in the vasculature of target organs, such as the heart [20] or the brain [4]. Therefore, structural alterations of small arteries are associated with an increased cardiovascular risk in hypertensive and diabetic patients [38,39], perhaps as a consequence of an impaired organ flow reserve in several relevant vascular districts.

More than a decade ago, a direct demonstration of a prognostic role of microvascular structural alterations, independently of BP values, was made available [37]. In the concerned study, a Cox multivariate regression analysis was performed, and the conventional cardiovascular risk factors were considered. Only the media-to-lumen ratio of subcutaneous small resistance arteries and the pulse pressure remained in the model, thus suggesting that the majority of the prognostic information provided by classical cardiovascular risk factor was present in these two indices of microvascular and macrovascular alterations [37] (Table 1).

The prognostic meaning of structural alterations of small resistance arteries was then confirmed in a larger population (more than 300 study participants and patients) at lower global risk, also taking into account only major cardiovascular events [40]. Similar data were also obtained by Mathiassen *et al.* [41] in a population of essential hypertensive patients. Hypertrophic remodelling, such as that observed in diabetic or obese patients, seems to be associated with an even worse prognosis [38,39], compared with eutrophic remodelling.

In the study by De Ciuceis *et al.* [40] 65 normotensive study participants, 111 patients with essential hypertension (37 of them with type 2 diabetes mellitus), 109 patients with secondary forms of hypertension, and 18 normotensive diabetic patients, for a total number of 303 study participants and patients were included. Study participants were re-evaluated after an average follow-up time of 6.9 years to assess the occurrence of cardio–cerebrovascular events. In total, 11 patients died of a fatal cardio–cerebrovascular event, 14 had a major, nonfatal cardiovascular event (stroke or myocardial infarction), 23 had a minor cardiovascular event, and 255 had no cardiovascular event.

TABLE 1. Variables significantly associated with the occurrence of cardiovascular events according to a Cox proportional hazard model

Study participants and patients	*P* value
Whole population ($n = 151$)	
Pulse pressure	$P = 0.009$
Media-to-lumen ratio of subcutaneous small arteries	$P < 0.0001$
Patients in whom data about left ventricular mass were available ($n = 86$)	
Media-to-lumen ratio of subcutaneous small arteries	$P = 0.002$

Prognostic factors considered: age, sex, clinic SBP, DBP, pulse pressure, dyslipidaemia, presence of diabetes, smoking status, baseline diagnosis (essential hypertension, pheochromocytoma, primary aldosteronism, renovascular hypertension, normotensive diabetic patients), and the media-to-lumen ratio of subcutaneous small arteries. Only pulse pressure and the media-to-lumen ratio of subcutaneous small arteries of subcutaneous small arteries entered the model in the whole population. Adapted with permission from [37].

A significant difference was observed in event-free survival between study participants and patients with a media-to-lumen ratio above or below two different cutpoints: 0.11 (two SDs above the mean of normal control study participants) and 0.098 (mean and median value of the entire population; Mantel–Cox test between survival curves: $P = 0.0001$ for the cutpoint of 0.098).

Similar results were obtained by restricting the analysis to patients with essential hypertension.

Similarly, Mathiassen *et al.* [41] have investigated 159 essential hypertensive patients. In total, 30 patients suffered from cardiovascular events during a follow-up period of more than 10 years. The authors tested two different cutpoints of media-to-lumen ratio: 0.083 (mean value of the hypertensive cohort) and 0.098 (two SDs above the mean of normal control study participants). Also in this study, event-free survival was significantly different between patients with low or high media-to-lumen ratio of subcutaneous small resistance arteries (Mantel–Cox test between cumulative survival curves: $P = 0.010$ for the cutpoint of 0.098, $P = 0.022$ for the cutpoint of 0.083) [41].

We have pooled together the two studies [40,41], using Comprehensive Meta-Analysis Software (Biostat Inc, Englewood, NY, USA). The results are reported in Fig. 1. A total of 270 patients with essential hypertension were included [40,41]. The cutpoint of 0.098 for media-to-lumen ratio of subcutaneous small resistance arteries was used, as it was the same in the two studies. The results clearly indicate a worse prognosis for those hypertensive patients with a media-to-lumen ratio above 0.098 (favours B), with a *P* value more than 0.0001.

Moreover, we have performed an additional analysis in our database of study participants and patients in whom an evaluation of small resistance artery structure was performed (part of them were included in the previously mentioned study) [40], restricting the analysis to 119

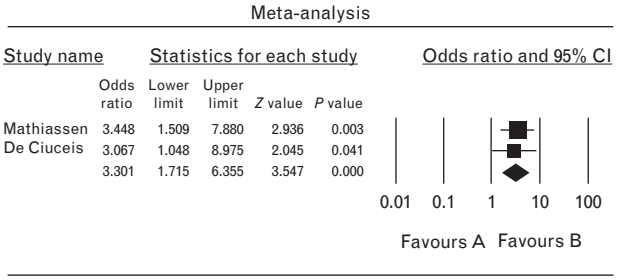

FIGURE 1 Meta-analysis of two studies [49,50]: only patients with essential hypertension are included ($n = 270$). Odds ratio for cardiovascular events in favour of patients with a media-to-lumen ratio of subcutaneous small arteries below 0.098 ($P < 0.0001$). CI, confidence interval.

patients with diabetes mellitus, using the two cutpoints (0.098 and 0.11) previously identified [37,40]. The results are reported in Figs. 2 and 3. A significant difference was observed for cumulative survival, in favour of those with a lower media-to-lumen ratio ($P < 0.05$ at least).

Recently, Buus *et al.* [42] demonstrated a prognostic role of changes in microvascular structure, as evaluated by the media-to-lumen ratio of subcutaneous small resistance arteries during antihypertensive treatment. This demonstration of the prognostic role of changes in microvascular structure during treatment, independently of the extent of BP reduction, could substantially support the idea to consider microvascular structure as an intermediate endpoint in the evaluation of the benefits of antihypertensive treatment.

As mentioned, it is not currently known whether capillary rarefaction may possess a prognostic significance. As a preliminary study suggests that microvascular rarefaction might be correlated with media-to-lumen ratio of small arteries [17], it is also possible that vascular changes at a more distal level might contribute to the higher incidence of cardiovascular events observed in hypertensive and/or diabetic patients.

Recently, interest has been focused on the possible interrelationships between alterations in the microcirculation and macrocirculation (in particular changes in the

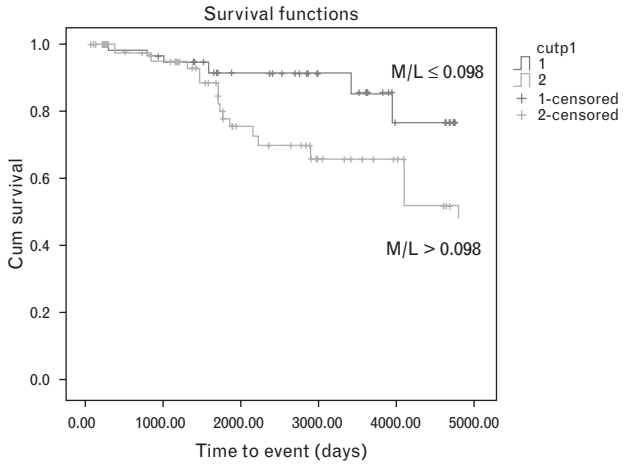

FIGURE 2 Kaplan–Meier cum survival curves between normotensive or hypertensive diabetic patients with an M/L of subcutaneous small arteries below or above 0.098 (cutp1): log rank (Mantel–Cox test $P = 0.049$, Breslow–Wilcoxon test: $P = 0.081$, Tarone–Ware test: $P = 0.058$. (Re-analysis of data, part from [40]). Cum, cumulative; M/L, media-to-lumen ratio.

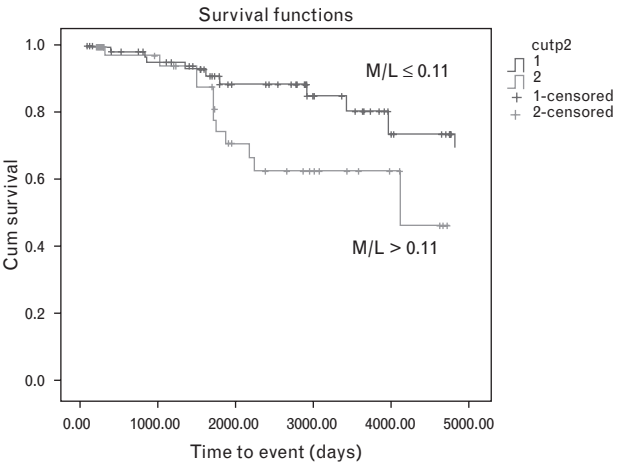

FIGURE 3 Kaplan–Meier cum survival curves between normotensive or hypertensive diabetic patients with an M/L of subcutaneous small arteries below or above 0.11 (cutp2): log rank (Mantel–Cox test $P = 0.034$, Breslow–Wilcoxon test: $P = 0.057$, Tarone–Ware test: $P = 0.041$. re-analysis of data, part from [40]). Cum, cumulative; M/L, media-to-lumen ratio.

mechanical properties). The media-to-lumen ratio of subcutaneous small resistance arteries was demonstrated to be related to indices of large artery stiffness (pulse wave velocity, augmentation index), as well as with central SBP and pulse pressure [43]. Thus, the crosstalk between the small and large arteries exaggerates arterial damage, following a vicious circle [44,45]; a relevant role is probably played by an enhancement of pulse wave reflection from distal reflection sites [44,45]. This hypothesis is in agreement to what was found in the previously mentioned multiple regression analysis, in which indices of alterations in large and small arteries were the only predictors of cardiovascular events remaining in the model [37].

TREATMENT

Effect of treatment on small artery structure

Some intervention studies with specific drugs have demonstrated an improvement or even an almost complete normalization of the structure of subcutaneous small resistance arteries with angiotensin-converting enzyme (ACE) inhibitors (cilazapril, perindopril, lisinopril), calcium channel blockers (nifedipine, amlodipine, irsradipine), and angiotensin II receptor blockers (losartan, irbesartan, candesartan, olmesartan, and valsartan) [2,3,46]. On the contrary, the β-blocker atenolol and the diuretic hydrochlorothiazide had limited effects on resistance vessels, despite a similar BP reduction [2,3,46]. More than 300 patients were investigated in these intervention studies, using a reliable and precise micromyographic approach [47–49]. ACE inhibitors proved to be significantly more effective than the β-blocker atenolol in terms of changes in media-to-lumen ratio [46]. The same result was obtained comparing dihydropyridinic calcium channel blockers and atenolol, or angiotensin receptor blockers and atenolol [46].

Several reasons may be advocated to explain the disparate effects of different drug classes on small artery structure. It is possible that ACE inhibitors and angiotensin receptor blockers possess growth-inhibiting and antioxidant properties that may be responsible for their beneficial

effect on the microcirculation [2]. Additional possible reasons are the lack of vasodilator properties of atenolol [50] or a parallel improvement of alterations in the macrovasculature observed with other drugs [51,52]. In fact, drugs that improve microvascular structure are also particularly effective in reducing central BP, thus providing probably an additional benefit [44,45], probably by a reduction of the reflection of waves from the periphery [44,45,51,52]. Also in patients with type 2 diabetes mellitus, drugs that block the renin–angiotensin–aldosterone system seem to be more effective than atenolol in terms of effects on microvascular structure [15,53–55]. However, in patients with more severe hypertension (i.e. patients with left ventricular hypertrophy or concomitant diabetes mellitus), a reduction, but not a full normalization of the media-to-lumen ratio of subcutaneous small resistance arteries could be obtained by effective antihypertensive treatment [3,46]. In fact, media-to-lumen ratio remained significantly higher in respect to what observed in normotensive controls.

Few data are currently available about patients with type 1 diabetes mellitus. A study from Greenstein *et al.* [56] suggests that, with poor metabolic control, small arteries from patients with type 1 diabetes mellitus show hypertrophic growth in response to elevated BP, similar to that seen in type 2 diabetes mellitus. However, metabolic improvements enable eutrophic remodelling to occur in response to an increase in BP [56].

After surgical correction of obesity and consistent weight loss, a significant improvement of microvascular structure and of some oxidative stress/inflammation markers were observed [8]. Therefore, consistent weight loss obtained by bariatric surgery may substantially improve microvascular structure [8]. It should also be noted that, during antihypertensive treatment, the regression of microvascular structural alterations in the subcutaneous small arteries of hypertensive patients is paralled by an improvement of coronary flow reserve [57].

In addition, it was also demonstrated that the severity of structural alterations in subcutaneous small resistance arteries might predict the outcome after adrenalectomy in patients with primary aldosteronism, as the presence of vascular remodelling implies lower chances of BP normalization at long-term follow-up postadrenalectomy [58].

In general, there is evidence that the pathophysiological consequences of the regression of small artery remodelling might be the following:

1. A better control of BP with reduced vascular reactivity [58];
2. An increased organ flow reserve, especially in the heart [57];
3. A reduction of central BP [44,45,51,52].

NEW TECHNIQUES OF EVALUATION OF MICROCIRCULATION

Although the prognostic value of structural alterations in small subcutaneous arteries has been confirmed by two independent studies [37,41], according to the guidelines for the management of arterial hypertension of the European Society of Hypertension and of the European Society of Cardiology 'the invasiveness of the method makes this approach unsuitable for general use' [59].

Hence, the development of new, non-invasive approaches for the evaluation of microvascular damage is needed. The interest of researchers was focused, in the last decade, on the retinal vascular district, as it represents the only microvascular bed that may be directly viewed with relatively simple approaches, such as an ophthalmoscope or a slit lamp [60]. In addition, it is of interest that cerebral and retinal circulation share anatomic, physiological, and embryological features [61]. In fact, we observed that the same kind of structural alterations previously observed in subcutaneous small resistance arteries are also present in cerebral small arteries of hypertensive patients [4].

One of the first attempts to precisely quantify structural alterations of retinal microcirculation was made by Wong *et al.* [62]. By means of an automated computerized method, the authors have calculated the ratio between the arteriolar and venular external diameters (arteriolar to venular ratio: AVR) in circular segments of the retina. AVR resulted lower in hypertensive patients compared with normotensive controls [62]. Recently, a meta-analysis has confirmed that retinal arteriolar narrowing and venular widening are independently associated with an increased risk of hypertension [63]. However, its prognostic meaning is still controversial, as a correlation between AVR and incidence of cardiovascular events was detected only in women [62]. Further studies have substantially challenged the ability of this parameter to correctly stratify hypertensive patients according to the extent of target organ damage. Indeed, no relationship between quartiles of AVR and left ventricular mass, carotid artery intima–media thickness, or urinary albumin excretion was observed [64]. The relationship of retinal vessel calibre to future stroke events has been analysed in a systematic review and individual participant meta-analysis: wider retinal venular calibre predicted stroke, whereas the calibre of retinal arterioles was not associated with stroke [59]. Advantages and limitation of this methodological approach were discussed elsewhere [65].

Even more recently, Harazny *et al.* [66] proposed a further, interesting and promising approach. The method is based on the association between a confocal measurement of the external diameter of retinal arteriole and an evaluation of the internal diameter with a laser Doppler technique (Heidelberg Retinal Flowmeter, Heidelberg Engineering, Heidelberg, Germany), with calculation of the ratio between wall thickness and internal lumen [66]. The same authors, using this approach, could observe that wall thickness and internal lumen ratio is increased in untreated essential hypertensive patients compared with normotensive controls [67], and that an even more marked increase is present in hypertensive patients with a history of cerebrovascular events [66]. Finally, a close relationship was observed between wall thickness and internal lumen ratio and urinary albumin excretion, expression of the microvascular damage at the kidney level [68]. When wall thickness/internal lumen ratio and AVR of retinal vessels were evaluated in the same patients, only the first parameter

increased progressively from normotensives to treated hypertensives and to hypertensives with a history of a cerebrovascular event, and these differences closely paralleled those observed for carotid artery intima–media thickness [69]. A recent study compared in the same study participants and patients, wall thickness and internal lumen ratio of retinal arterioles evaluated with scanning laser Doppler flowmetry and media/lumen ratio of subcutaneous small resistance arteries evaluated by wire micromyography, that is commonly considered the reference approach for the measurement of structural alterations in the small vessels, because of accuracy and well demonstrated prognostic value. A rather good agreement between the two techniques, with a Pearson's correlation index of 0.76 was observed [70].

A couple of years ago a novel and extremely promising approach was made commercially available: the direct measurement of wall thickness and internal lumen ratio of retinal arterioles using an adaptive optics imaging system. This is a markedly improved version of a traditional fundus camera based on the approach originally applied to correct aberrations in astronomic optical systems [71]. The system provides images of a quality and resolution never previously obtained. Vessel walls are clearly visible in most circumstances, provided that the eye fixation is correct and that the ocular media are clear. Rosenbaum *et al.* [72] observed that BP and age both independently increased wall thickness and internal lumen ratio by thickening arterial wall. A short-term reduction in BP obtained by antihypertensive treatment induced a wall thickness and internal lumen ratio decrease because of lumen dilatation rather than wall thickness changes. By contrast, no modifications were observed in study participants with no reduction in BP [72].

It should be mentioned that the measurements of retinal vessels made with the above-mentioned techniques 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. 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 [73].

In our view, the results of the few available studies support the possible clinical relevance of the measurement of retinal arteriolar structure in clinical practice. Possible advantages related to the evaluation of wall thickness and internal lumen ratio of retinal artery with adaptive optics are clear, in terms of non-invasiveness and consequent possibility to obtain prognostic data about the presence and the regression of retinal microvascular alterations by antihypertensive treatment, which are currently lacking. These experiments have to be conducted.

In conclusion, in hypertension, the increase in peripheral resistance occurs at the microvascular level. It was clearly demonstrated that wall thickness is increased in relation to internal lumen, and that this alteration contributes to peripheral resistance. The increased media/lumen ratio may impair organ flow reserve [20]. This may be important in the maintenance and, probably, also in the progressive worsening of hypertensive disease. The presence of structural alterations represents a prognostically relevant factor, in terms of development of target organ damage or cardiovascular events, thus allowing a prediction of hypertension complications [38,39].

However, new, non-invasive techniques are needed before suggesting extensive application of the evaluation of microvascular morphology for the cardiovascular risk stratification in hypertensive patients. Some new techniques for evaluation of microvascular morphology in the retina, currently under clinical investigation, seem to represent a promising and interesting future perspective.

Currently, we may safely state that the evaluation of microvascular structure is progressively moving from bench to bedside [74] and it could represent, in the immediate future, an evaluation to be performed in all hypertensive patients, to obtain a better stratification of cardiovascular risk, and perhaps, it might be considered as an intermediate endpoint in the evaluation of the effects of antihypertensive therapy [75], provided that a demonstration of a prognostic value of non-invasive measures of microvascular structure is made available.

ACKNOWLEDGEMENTS

Conflicts of interest

There are no conflicts of interest.

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Reviewers' Summary Evaluations

Referee 1

This is a useful review showing the current evidence that abnormalities of the resistance vasculature provide prognostic information about later cardiovascular complications. The evidence is based largely on data requiring minor invasive surgery, and that methodology is thus not suitable for general screening. New noninvasive methods include examination of retinal vessels, and the review points to the need for prospective studies to determine if results based on such investigations also have prognostic validity.

Referee 2

Strengths: This is an internationally recognized group actively researching in the area of small arteries in cardiovascular disease. They are well placed to distil all the evidence that has been published and to produce a very timely and up to date review. Weaknesses: They have addressed their weaknesses.