Wall-to-Lumen Ratio of Retinal Arterioles as a Tool to Assess Vascular Changes

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The retina offers a beautiful and unique opportunity to visualize and examine the body’s microvasculature safely, repeatedly, quickly, and noninvasively in vivo. Retinal arterioles appear to undergo similar changes as cerebral and peripheral arterioles in hypertension, indicating that retinal arteriolar abnormalities mirror structural and functional microvascular changes elsewhere in end-organ tissues.1-4 Since the pioneering work by Keith et al5 in 1939, several studies have confirmed the prognostic significance of retinal vascular abnormalities on mortality attributed to a cardiovascular cause.5-7

However, although there is solid evidence for the prognostic significance of advanced retinopathy, the evidence of a prognostic impact of early retinal vascular abnormalities on cardiovascular risk stratification is less well established.1,8 It was suggested that methodological issues might be the cause for the lack of a solid evidence that early retinal vascular abnormalities are closely linked to cardiovascular risk.1,9 Therefore, much research effort over the last decade has focused on the development of new methodological approaches to enable more precise and reliable detection and evaluation of early retinal vascular abnormalities in hypertensive patients.

A new approach focuses on retinal arteriolar structural parameters by using scanning laser Doppler flowmetry (SLDF) with automatic full-field perfusion imaging analyses (AFFPIAs).2,10 This approach allows the assessment of both the outer diameter (OD) and inner diameter (ID) of retinal arterioles in vivo and, thus, analyzes vascular remodeling of retinal arterioles by calculating wall:lumen ratio, wall thickness, and wall cross-sectional area (volume of wall per unit of length) of retinal arterioles. These methods do not need to determine diameter of retinal venules, which are also subject to changes in cardiovascular disease. This review introduces and describes this new methodology, explains the improved power of measuring retinal vascular changes, and discusses our recent findings using this tool.

Remodeling of Arterioles and Small Arteries and the Prognostic Significance

The idea of assessing the wall:lumen ratio of retinal arterioles as an in vivo parameter of vascular damage goes back to the finding that remodeling of arterioles and small arteries, indicated by the increase in the media:lumen ratio of the peripheral arterial resistance vessel, represents an early step, possibly the earliest step, in hypertension-associated vascular damage and target organ disease.11 Such remodeling processes have been evidenced in isolated arterioles and small arteries obtained from biopsies of subcutaneous tissue.11-14 In hypertension, elevated blood pressure either directly or indirectly via vasoactive peptides (angiotensin II and endothelin 1 among others) leads to vasoconstriction of peripheral arterioles and small arteries, growth and apoptosis of smooth muscle cells of the arteriolar and small artery vascular wall, and vascular fibrosis (ie, accumulation of collagen, fibronec-tin, and other extracellular matrix proteins in the vessel wall), resulting in eutrophic and/or hypertrophic remodeling.4,15,16 Isolated subcutaneous arterioles and small arteries from subjects with essential hypertension stages 1 and 2 were found to have a reduction in ID and rearrangement of the smooth muscle cells around a smaller lumen without a marked growth response, ie, the media cross-sectional area (volume of media per unit of length) is unchanged, reflecting eutrophic remodeling.15,16 In contrast, a marked growth response with an increase in media cross-sectional area of isolated subcutaneous arterioles and small arteries was found in subjects with severe, long-standing, and some forms of secondary hypertension, reflecting hypertrophic remodeling (vascular smooth muscle cell hypertrophy or hyperplasia).15,16 Both types of remodeling result in an increase in the media:lumen ratio of the arteriole and small artery.15,16 Blood pressure reduction by pharmacological intervention was found to be able to decrease the media:lumen ratio of isolated subcutaneous arterioles and small arteries.16 Other than blood pressure, metabolic factors were found to remodel arterioles and small arteries. Subjects with diabetes mellitus were found to reveal hypertrophic remodeling of isolated subcutaneous arterioles and small arteries.17

Of clinical importance, an increased media:lumen ratio of isolated subcutaneous arterioles and small arteries was found to be of prognostic significance with respect to cardiovascular events in patients with hypertension, with adverse prognosis in those patients with greater media:lumen ratio.12-14 Arteriolar narrowing and remodeling are, likewise, described as

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initial steps of retinal and peripheral changes in hypertension.\textsuperscript{1–4} Thus, retinal arterioles may undergo remodeling similar to that seen in arterioles of the subcutaneous tissue.

Assessment of Wall:Lumen Ratio of Retinal Arterioles and Other Parameters of Retinal Arteriolar Structure and Remodeling Using SLDF With AFFPIA

For the assessment of retinal arteriolar structural parameters, the SLDF at 670 nm (Heidelberg Retina Flowmeter, Heidelberg Engineering) is used. The Figure shows the screen shot of the retinal arteriolar structure analysis software program of AFFPIA (SLDF version 4.0 by Welzenbach). Briefly, an arteriole with a size between 80 and 140 \( \mu \text{m} \) of the superficial retinal layer in the juxtapapillary area of the right eye, 2 to 3 mm temporal superior to the optic nerve, in a retinal sample of 2.56\( \times \)0.64\( \times \)0.30 mm, is scanned within 2 seconds at a resolution of 256 points\( \times \)64 lines\( \times \)128 lines. A specific length of the arteriole reflecting arteriolar structure during 1 heart beat (1 systole and 1 diastole) is used for analyses, and OD and ID are assessed every 10 \( \mu \text{m} \) of this specific length of the arteriole. If the vessel segment does not run exactly in a straight line, the software automatically adjusts the cross-sections perpendicular to this line against each other, referring to the cross-section with the lowest \( y \) coordinate. The mean of measured diameters is finally calculated, and the average from 3 singular measurements is completed for further analyses. Analyses of diameters are performed offline with AFFPIA (SLDF version 4.0 by Welzenbach). OD is measured in reflection images, and ID is measured in perfusion images. The reflection image is created from the direct current of the Doppler signal and approximates the amount of reflected laser light of the nonmoving tissue. At the outer vessel wall border is the weakest reflection because of the angle between light direction and vessel wall border. The turning points with maximum slope (triangles in the top left panel of the bottom of Figure 1) are considered for the definition of outer vessel wall border with respect to the largest difference of reflectivity between 2 points lying side by side. The perfusion image is generated by the Doppler effect caused by moving blood corpuscles. Because blood flow velocity is greatest in the center of the blood vessel and decreases toward the ID of the blood vessel, a Poisson velocity distribution of the bloodstream within the vessel
lumen can be adapted. The crossing points of the parabolic curves (reflecting the velocity distribution of blood flow within the blood vessel) and straight lines (reflecting baseline reflectivity) define the inner vessel wall borders (triangles in the bottom left panel of the bottom part of the Figure). On the basis of such measurements of OD and ID of the retinal arteriole wall:lumen ratio, wall thickness and wall cross-sectional area of the retinal arteriole are finally calculated using the formulas \((OD−ID)/ID\), \((OD−ID)/2\), and \((\pi/4)\times(OD^2−ID^2)\).

The examination is performed in a sitting position after 20 minutes of rest, at room temperature and daylight conditions between 8 AM and 2 PM but before lunch. There is no need to dilate the pupil, which is of great advantage. The readers of retinal scans have to be blinded to the clinical data of the subjects. It is strongly recommended that only specially trained and certified readers perform these analyses, and quality control has to be performed by independent, experienced personnel. We have demonstrated previously that the assessment of wall:lumen ratio of retinal arterioles is reliable, with a coefficient of variation for the wall:lumen ratio of \(\approx 10\%\). The new software has decreased the variability of the wall:lumen ratio substantially (J. Harazny, PhD, 2009, unpublished data). Nevertheless, this approach of measuring vascular retinal changes is, at the moment, a research tool that needs to be further developed for general use.

**Limitations of the Assessment of Wall:Lumen Ratio of Retinal Arterioles Using SLDF With AFFPIA**

Our approach is an in vivo measurement, and, therefore, it is not possible to distinguish functional (vasoconstriction because of vascular smooth muscle activity) from structural changes. In contrast, the assessment of media:lumen ratio of isolated subcutaneous arterioles and small arteries is undertaken with the vessels in a fully relaxed state and, hence, measures the structural condition of the vessels without the influence of active tone because of smooth muscle contraction.

With the SLDF at 670 nm, we measure the OD, ie, total wall thickness. Thus, in addition to changes of media thickness, the methodology is sensitive to adventitial changes that may also occur in hypertension. Thickening of the basal lamina was reported in retinal arterioles of stroke-prone spontaneously hypertensive rats. An increased adventitial thickness and reduction in the number of endothelial cells of the basilar artery were found in \(N^2\)-nitro-L-arginine methyl ester–treated Wistar-Kyoto rats.

Moreover, the validity of the estimate of wall thickness using SLDF with AFFPIA is not yet confirmed. There are many similarities between the approach of measuring media:lumen ratio in isolated subcutaneous arterioles and small arteries and the approach of measuring wall:lumen ratio in retinal arterioles, but it is possible that the 2 indices are not always coincident.

**Wall:Lumen Ratio of Retinal Arterioles in Hypertension**

In our first study, we demonstrated that treated hypertensive patients with poor blood pressure control have a greater wall:lumen ratio of retinal arterioles than those with good blood pressure control. Interestingly, in this cohort we did not find a significant relation between blood pressure and the wall:lumen ratio of retinal arterioles using correlation analysis. We have concluded that this lack of a relation of blood pressure and wall:lumen ratio of retinal arterioles might well be the result of the effects of some antihypertensive drugs to beneficially influence vascular structure. Subsequently, we analyzed the wall:lumen ratio of retinal arterioles in a cohort of never-treated patients with essential hypertension and normotensive controls. In this cohort, both systolic blood pressure and diastolic blood pressure were significantly related to wall:lumen ratio of retinal arterioles independent of traditional cardiovascular risk factors and other confounders, eg, subclinical inflammation, endothelial dysfunction, and dietary salt intake. Moreover, in this cohort, the wall:lumen ratio of retinal arterioles was greater in hypertensive than in the normotensive subjects, whereas wall cross-sectional area did not differ between the 2 groups, indicating a predominant role of eutrophic remodeling (and not growth) of retinal arterioles in stage 1 and stage 2 essential hypertension. Similar findings have been observed previously in studies examining arteriolar and small artery structures of subcutaneous tissue.

**Wall:Lumen Ratio of Retinal Arterioles and Hypertensive Cerebrovascular Damage**

In hypertensive patients with a history of a cerebrovascular event, we found a greater wall:lumen ratio of retinal arterioles than in patients with treated hypertension and normotensive controls. In another study comparing the wall:lumen ratio of retinal arterioles and the arteriole:venule ratio of retinal vessels (the arteriole:venule ratio of retinal vessels is widely used and describes the ratio of arteriolar and venular blood column diameters assessed by digitized retinal photography according to a method described previously by Hubbard et al), we found that the wall:lumen ratio of retinal arterioles was highest in patients with a history of transient ischemic attack or lacunar cerebral infarction compared with treated hypertensive subjects and normotensive controls, whereas the arteriole:venule ratio of retinal vessels did not differ among the study groups.

**Wall:Lumen Ratio of Retinal Arterioles and Changes in Other Vascular Beds**

In line we observed that the wall:lumen ratio of retinal arterioles and the intima-media thickness of the carotid artery are both greater in hypertensive patients with cerebrovascular damage than in treated hypertensive and normotensive controls, indicating that microvascular changes occurred in parallel to macrovascular changes in hypertensive patients with cerebrovascular damage. In addition, in a recent study (M.R., 2009, unpublished data) with obese prehypertensive and hypertensive subjects, we found a relation between the wall:lumen ratio of retinal arterioles and urinary albumin excretion. This relation points to parallel changes in the microcirculation of the retinal and renal vascular beds.
Conclusions
The assessment of retinal arteriolar structure and remodeling using SLDF with AFFPIA is a reliable tool. It requires strict standardization and trained, certified readers. Blood pressure is a major determinant of retinal arteriolar structure and remodeling. In never-treated hypertensive patients, wall:lumen ratio of retinal arterioles was increased but was noted to be normal if blood pressure was controlled. An increased wall:lumen ratio of retinal arterioles is associated with cerebrovascular and other hypertensive organ damage. The association between a greater wall:lumen ratio of retinal arterioles and urinary albumin excretion further supports the concept that wall:lumen ratio of retinal arterioles is a valid parameter of hypertension-related vascular damage.

Perspectives
Future studies assessing both the wall:lumen ratio of retinal arterioles and media:lumen ratio of isolated subcutaneous arterioles and small arteries in subjects with hypertension should be performed. In addition, the validity of the estimate of wall thickness using SLDF with AFFPIA needs to be further examined. Moreover, future carefully designed, prospective, multicenter studies are needed to elucidate the prognostic role of the wall:lumen ratio of retinal arterioles on cardiovascular outcome in hypertensive subjects independent of other established cardiovascular parameters.

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