Advances in Cardiovascular Imaging

Retinal Vascular Imaging
A New Tool in Microvascular Disease Research

Gerald Liew, MD, MMED; Jie Jin Wang, MMed, PhD; Paul Mitchell, MD, PhD; Tien Y. Wong, MD, PhD

Abstract—The microcirculation is relatively inaccessible to direct visualization and investigation. Recent methods have been developed which use advanced retinal photographic imaging techniques and computer-assisted image analysis to characterize, measure and quantify subtle variations and abnormalities in the retinal vasculature. These quantitative and qualitative assessments demonstrate a close association of retinal vascular signs to both clinical and subclinical cerebrovascular, cardiovascular and metabolic outcomes. Retinal vascular imaging may thus offer potential as a noninvasive research tool to probe the role and pathophysiology of the microvasculature, and as a cardiovascular risk prediction tool. Key areas where retinal vascular imaging has contributed to increased understanding of microvascular pathology and major areas of current and new research are discussed in this review. (Circ Cardiovasc Imaging. 2008;1:156-161.)

Key Words: arteriosclerosis ■ microcirculation ■ retinal vessels ■ cardiovascular disease ■ risk factors

The microcirculation represents the bulk of the circulatory system; yet, its role in the cardiovascular disease is less clear than the role of the macrocirculation (ie, large vessel disease). This is partly because current methods to investigate the microvasculature are invasive and can be performed only in highly specialized settings, which limits their utility as tools to understand the clinical consequences of microvascular disease.

The retina is a unique site where the in vivo microvasculature can be directly visualized and monitored repeatedly over time. Recent advances in retinal photographic imaging techniques have facilitated the development of computer-assisted methods to measure and quantify subtle variations and abnormalities in the retinal microvasculature. These quantitative assessments have been applied to large populations, of both adults and children, and in community and clinic settings. Existing data now convincingly show links between a range of retinal microvascular signs and both clinical and subclinical cerebrovascular, cardiovascular, and metabolic outcomes. Thus, retinal vascular imaging offers the potential to provide information that summarizes the cumulative microcirculatory effects of an individual’s lifetime exposures to lifestyle and environmental factors, and the body’s responses to these exposures, which may in turn be modified by genetic predisposition.

Retinal vascular imaging is currently used in 2 broad areas of cardiovascular research. First, retinal imaging is a novel, noninvasive research tool to probe the role and pathophysiology of the microvasculature, typically defined as vessels between 100 and 300 μm in size, in the development of clinical cardiovascular disease. Second, retinal vascular imaging is explored in clinical settings as a risk stratification tool to aid clinicians in identifying patients with microvascular signs who are at high risk of future clinical cardiovascular and cerebrovascular events. A third possibility is under investigation: retinal vascular imaging has potential as a surrogate measure of the microvascular benefits of new therapeutic agents in early phase II or even phase III studies.

Although this prospect has been raised, several critical issues must be addressed before retinal vascular imaging can be considered a valid surrogate for assessing outcomes in cardiovascular clinical trials. The unique perspective offered by retinal vascular image analysis is used by an increasing number of researchers and research groups to address scientific questions that are difficult to answer through other means. Some key questions this new technique has been applied to are described later, as are major issues that remain to be addressed.

Retinal Vascular Imaging Technologies
Several semiautomated programs to measure retinal vascular caliber have been developed and described. The most widely used program was developed and validated in the Atherosclerosis Risk in Communities Study to examine the retinal vascular signs of arteriosclerosis in 11 114 middle-aged...
adults and has since undergone further refinement and improvement. The program measures arteriolar and venular caliber from digital retinal photographs centered on the optic disc (or images digitized from photographic slides). Photographs of sufficient quality for grading can be obtained using nonmydriatic autofocus cameras, although higher quality images are taken if pharmacological pupil dilation is undertaken beforehand. With the assistance of a trained grader, the program identifies all retinal vessels greater than 25 μm in diameter that completely pass through the region between 1/2 and 1 disc diameter from the optic disc margin (zone B) and identifies their edges using a pixel density histogram (Figure 1). The cross-sectional diameter of retinal arterioles and venules is measured repeatedly and summarized using formulae to obtain values representing the average arteriolar and venular caliber of that particular eye. Reliability of this method is high, with intergrader reliability weighted kappa of 0.85 and 0.90, for arteriolar and venular caliber measurements, respectively, and intragraded reliability between 0.80 and 0.93 for both arteriolar and venular caliber measurements.

Temporal Sequence of Microvascular Changes and Clinical Cardiovascular Disease

A key question in cardiovascular physiology is whether changes detected in the microvasculature are early markers of vascular disease secondary to the disease process or whether microvascular changes represent the primary causes that contribute etiologically and mechanistically to the development of vascular disease. For example, a distinctive feature of hypertension is increased peripheral vascular resistance, which is determined largely by narrower arterioles in the systemic microcirculation. However, one of the central unresolved issues in understanding the pathophysiology of hypertension is whether arteriolar narrowing is antecedent to and contributes to the development of hypertension, or whether it is consequential to and represents a secondary adaptation to elevated blood pressure, or whether both processes occur.

It has been hypothesized that relatively modest decreases in internal arteriole lumen caliber, particularly in the renal preglomerular vasculature, occur early in the genesis of essential hypertension. These caliber changes then act to “reset” blood pressure to a higher level by altering hemodynamics in a manner similar to renal artery stenosis. By providing noninvasive means to measure arteriolar caliber, retinal vascular imaging provided the first prospective clinical evidence, which showed that narrower arteriolar caliber preceded the development of clinical hypertension and was not purely a secondary response to established hypertension (Figure 2). Subsequent studies have since demonstrated that retinal arteriolar narrowing precedes by years the development of hypertension in initially normotensive individuals (Table), which supports the hypothesis that microvascular changes have a primary role in the development and evolution of hypertension. Such data probing the anatomic substrate underlying the development of hypertension have clear implications for its treatment and prevention.

Homology Between Retinal Microvasculature and Other Microvascular Beds

Retinal vascular imaging appears to have utility in the investigation of microcirculatory changes in cardiovascular disease, but there is an important caveat to insights obtained through this means. The extent to which the structure and function of the retinal microvasculature mirrors the cerebral microvasculature, and microvascular beds elsewhere, is not clear. Histopathologic studies suggest that retinal vascular signs are closely related to pathological microvascular changes in other organs (eg, hypertensive arteriolar changes in the brain and myocardium). Other studies demonstrate...
that persons with lacunar stroke have functional alterations in the retinal vasculature including reduced retinal arteriolar-venule passage time. 

Pathological changes in the retinal arteries parallel changes in the small cerebral arteries that cause white matter lesions and lacunes. The close relationship between retinal and cerebral vascular changes is unsurprising given the embryological, morphological, and functional similarities due to their common origin from the internal carotid artery. What is perhaps more surprising is that there are also strong indications that retinal vascular changes parallel pathology in the coronary micro and macrocirculations, with retinal arteriolar narrowing strongly associated with the presence and severity of angiographically defined coronary artery occlusion, and reduced myocardial perfusion measures on cardiac MRI, whereas retinopathy is correlated with coronary artery calcification on cardiac computed tomography scanning.

Although these lines of evidence support the hypothesis that retinal microvasculature changes provide insights into the vascular structure and function of the cerebral and coronary microcirculations, with retinal arteriolar narrowing strongly associated with the presence and severity of angiographically defined coronary artery occlusion, and reduced myocardial perfusion measures on cardiac MRI, whereas retinopathy is correlated with coronary artery calcification on cardiac computed tomography scanning.

The role of the venular circulation in cardiovascular disease involves new insights into vascular pathophysiology, particularly the pathophysiology of venules, an area that has previously received little attention. For example, recent studies have revealed that venular dilation, rather than arteriolar narrowing, is the stronger predictor of clinical stroke, a relationship hitherto unsuspected (Figure 3). This surprising result was confirmed in a subsequent work and suggests that venules are not merely passive conductance vessels but represent a dynamic component responsive to changes in the microcirculation. Such findings have already stimulated an increased interest in venular physiology, with new studies reporting that wider retinal venules are linked to cerebral hypoxia, endothelial dysfunction, hyperglycemia, and inflammation, in contrast to narrower retinal arterioles, which are consistently and strongly related to elevated blood pressure and endothelial dysfunction. The prospect that both these retinal microvascular changes result from underlying endothelial dysfunction, and could thus represent a directly visible microvascular phenotype of endothelial dysfunction, is enticing and offers scope for further potential applications, for example, as a surrogate marker of endothelial function in clinical trials of new antihypertensive or vasoprotective agents. Nonetheless, this possibility is remote at present as it is not clear whether the changes detected on retinal vascular imaging are directly related to the causal pathways of cardiovascular disease or are associated epiphenomena.

Role of the Venular Circulation in Cardiovascular Disease

Studies linking retinal microvascular signs to cardiovascular disease have discovered unexpected associations that give new insights into vascular pathophysiology, particularly the pathophysiology of venules, an area that has previously received little attention. For example, recent studies have revealed that venular dilation, rather than arteriolar narrowing, is the stronger predictor of clinical stroke, a relationship hitherto unsuspected (Figure 3). This surprising result was confirmed in a subsequent work and suggests that venules are not merely passive conductance vessels but represent a dynamic component responsive to changes in the microcirculation. Such findings have already stimulated an increased interest in venular physiology, with new studies reporting that wider retinal venules are linked to cerebral hypoxia, endothelial dysfunction, hyperglycemia, and inflammation, in contrast to narrower retinal arterioles, which are consistently and strongly related to elevated blood pressure and endothelial dysfunction. The prospect that both these retinal microvascular changes result from underlying endothelial dysfunction, and could thus represent a directly visible microvascular phenotype of endothelial dysfunction, is enticing and offers scope for further potential applications, for example, as a surrogate marker of endothelial function in clinical trials of new antihypertensive or vasoprotective agents. Nonetheless, this possibility is remote at present as it is not clear whether the changes detected on retinal vascular imaging are directly related to the causal pathways of cardiovascular disease or are associated epiphenomena. Future research in animal models and clinic settings to monitor these retinal vascular changes and their relationship to the onset, progression, and regression of cardiovascular disease is needed to validate this phenotype.

Table. Retinal Arteriolar Narrowing and Incident Hypertension

<table>
<thead>
<tr>
<th>Retinal arteriolar caliber</th>
<th>Atherosclerosis Risk in Communities</th>
<th>Blue Mountains</th>
<th>Beaver Dam</th>
<th>Rotterdam</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Affected</td>
<td>OR (95% CI)*</td>
<td>% Affected</td>
<td>OR (95% CI)*</td>
</tr>
<tr>
<td>1st quintile (narrowest)</td>
<td>22.3</td>
<td>1.6 (1.2–2.2)</td>
<td>54.2</td>
<td>1.8 (1.4–2.4)</td>
</tr>
<tr>
<td>2nd quintile</td>
<td>14.3</td>
<td>1.3 (1.0–1.8)</td>
<td>44.9</td>
<td>1.4 (1.1–1.8)</td>
</tr>
<tr>
<td>3rd quintile</td>
<td>13.7</td>
<td>1.3 (0.9–1.8)</td>
<td>43.4</td>
<td>1.3 (1.0–1.7)</td>
</tr>
<tr>
<td>4th quintile</td>
<td>12.3</td>
<td>1.2 (0.9–1.6)</td>
<td>38.3</td>
<td>1.1 (0.8–1.5)</td>
</tr>
<tr>
<td>5th quintile (widest)</td>
<td>8.9</td>
<td>1.0</td>
<td>34.7</td>
<td>1.0</td>
</tr>
</tbody>
</table>

OR indicates odds ratio; CI, confidence intervals.

*OR are adjusted for factors such as age, gender, body mass index, smoking, total serum cholesterol, and blood pressure.

Figure 3. Retinal photograph of the right eye of a woman showing wide retinal venule (insert). She had a fatal stroke within 10 years of retinal photography.
Retinal Microvasculature Is Accessible and Information Rich

There is a wealth of geometric and structural data present in the visible retinal microvasculature, only parts of which have been investigated in detail. Theoretical and experimental work supports the concept that the vascular architecture develops in a way that is optimized for efficient flow and that deviations from this optimal state occur in disease processes. These deviations are reflected in abnormal, or suboptimal, geometric parameters of retinal microvascular architecture that may translate into an increased risk of cardiovascular disease. For example, geometric parameters of the retinal vascular network, such as vessel tortuosity, the angle, and number of bifurcations, and the length to diameter ratio of vessels appear to convey prognostic information on the risk of hypertension and cardiovascular disease, but to date, this has only been investigated in small case-control series. A mutation in the basement membrane collagen gene COL4A1 leads to both an increased retinal vessel tortuosity and an increased risk of hemorrhagic stroke. This genotype could underlie certain microvascular phenotypes that increase risk of cerebrovascular disease. Importantly, retinal imaging may provide a means to directly detect, measure, and quantify these phenotypes and thus evaluate risk.

One such recent analysis is the fractal dimension of the retinal vasculature, a novel means of measuring the complexity or density of the retinal vessel branching network (Figure 4). A less complex, less dense, and lower fractal dimension indicates rarefaction or loss of vessels, whereas a more complex, denser, and higher fractal dimension indicates a microvascular proliferation. This measure has been used in a limited number of studies to provide insights into the embryology and development of microvasculature and has shown promise as a sensitive indicator of early vascular changes in diabetic retinopathy, other retinal diseases, and systemic cardiovascular disease. The fractal dimension may combine contributions of the individual vessel parameters (eg, retinal vessel caliber, bifurcation angle, length to diameter ratio) with the vascular branching pattern into a single global value that summarizes the geometric complexity of the retinal vasculature. The variation and change in the fractal dimension may, thus, be a sensitive indicator of deviation from normal or optimized architecture and, thus, early microvascular disease. Measurement of fractal dimension may track the microcirculatory response to the progression of hypertension or, conversely, the microvascular response to antihypertensive treatments. Despite this promise, only very few, mostly clinic-based studies, have examined the role of retinal vessel fractal analysis in cardiovascular disease.

Functional Measures of the Retinal Microvasculature Complement Structural Retinal Vascular Imaging

Most studies to date have examined the prognostic information conveyed by static structural features of the microcirculation, largely because these are the most amenable to analysis from retinal images. In contrast, dynamic functional measures of the retinal microcirculation (eg, flowmetry and dynamic vessel assessments) and their relationship to cardiovascular risk have had relatively little attention. Such functional changes in the retinal microcirculation may well be more relevant to the prediction of cardiovascular risk. Techniques such as scanning laser doppler flowmetry and scanning laser ophthalmoscopy permit in vivo measurement of microcirculatory blood flow that is inaccessible in any other microcirculation. When coupled with imaging of the retinal vessels, estimates of retinal vessel wall thickness may be obtained, providing a unique means of determining actual arteriolar and venular wall thickness, which is clearly of value in assessing the health of the microcirculation. Some studies have shown that these measures may track the microcirculatory response to therapy for hypertension and may identify increased cerebrovascular risk. Stimulating the retina with flickering light increases retinal arterial and venous diameters, indicating a tight coupling among neural activity, blood flow, and vasoreactivity. Such flicker-dependent vasodilation may be an indicator of healthy endothelial function and is diminished in systemic diseases like hypertension and diabetes. However, whether these functional measures of retinal vascular hemodynamics will help to identify those at risk of cardiovascular diseases is unexplored.

Retinal Vascular Imaging May Provide Insights Into the Genetics of Microvascular Disease

Considerable research is currently focused on the role of genetic factors in the development of vascular disease processes and risk of cardiovascular disease. However, most major genetic studies, such as the Wellcome Trust Case Control Consortium, have focused on the genetic associations of large vessel atherosclerosis. Data on the genetic
determinants of microvascular disease are sparse. Much of the variability in arteriolar and venular calibers is believed to be related to genetic factors,\textsuperscript{44,45} and although a number of candidate genes have been postulated,\textsuperscript{44} to date, no strong evidence implicates specific genes. In twin studies,\textsuperscript{45} heritability estimates for retinal arteriolar caliber are higher than those for mean arterial blood pressure, suggesting that genetic influences may potentially be direct and potent for the retinal arteriolar phenotype and its responses to environmental insults. A recent genome-wide linkage analysis of retinal vascular caliber demonstrated a number of linkage signals for the arteriolar caliber trait,\textsuperscript{44} which deserve further scrutiny.

**Potential for Retinal Vascular Imaging as a Clinical Tool for Cardiovascular Risk Prediction.**

The intimate association of retinal vascular signs and cardiovascular risk factors has been shown repeatedly in numerous clinical and epidemiological studies. These are reviewed elsewhere.\textsuperscript{1–3} In brief, the pattern of association of arteriolar and venular caliber appears fairly distinct, with narrower arteriolar caliber related mainly to current and past elevated blood pressure and greater waist-to-hip ratio, whereas wider venular caliber is related to metabolic abnormalities such as hyperglycemia, hypertriglyceridemia, inflammatory markers, obesity, and renal disease.\textsuperscript{1–3} Many studies have demonstrated prospective associations of retinal microvascular signs with incident cardiovascular outcomes. Narrower retinal arteriolar caliber predicts coronary heart disease events as well as mortality, with some evidence of stronger associations in women; wider venular caliber appears to predict both coronary heart disease and stroke events, including mortality from these causes.\textsuperscript{1–3}

Despite these strong and consistent associations, much more work remains before retinal vascular imaging can be translated into clinical practice. To be clinically useful, retinal microvascular signs must demonstrate an additional prognostic information for cardiovascular risk prediction, over contributions from traditional risk factors. Previous studies on novel cardiovascular risk markers did not add substantially to risk prediction over traditional risk factors,\textsuperscript{46} partly because traditional risk factors have already moderately high predictive ability.\textsuperscript{47} In this regard, there remains skepticism that a retinal vascular assessment will offer sufficient prognostic potential beyond traditional cardiovascular assessment strategies.\textsuperscript{48} Recent work in populations without diabetes suggest that retinal vascular caliber measurements provide slightly superior coronary heart disease risk prediction to the use of traditional risk factors alone, but the magnitude of this improvement was considered unlikely to be relevant in clinical practice.\textsuperscript{49}

There are certain population subgroups, however, where retinal microvascular imaging may be more strongly predictive of risk. These are subgroups in which the microvascular contribution to cardiovascular disease is more prominent, for example, in women\textsuperscript{50} and in persons with diabetes, suggesting that the targeted application of retinal vascular imaging may be more productive. The cost-effectiveness of retinal vascular imaging must also be considered, especially as the measurement of traditional risk factors (eg, blood pressure, serum cholesterol) is relatively inexpensive and is widely available. Such issues need to be resolved before retinal microvascular imaging could be considered for clinical risk prediction.

**Conclusions**

Progress in retinal vascular imaging technologies in the last 2 decades has provided clinicians and researchers with a noninvasive means to measure and quantify subtle variations and abnormalities in the retinal microvasculature. These new technologies have linked retinal microvascular signs with a greater risk of a range of important clinical and subclinical cardiovascular outcomes. Data from these studies are providing unique insights into the microvascular contribution to hypertension and cardiovascular disease. However, further work is needed to determine whether retinal vascular imaging will live up to its promise and attain translation as a valuable new clinical modality of assessing cardiovascular disease.

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**Disclosures**

None.

**References**

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