End-organ dysfunction and cardiovascular outcomes: the role of the microcirculation

Christopher J. LOCKHART, Paul K. HAMILTON, Cathy E. QUINN and Gary E. McVEIGH
Department of Therapeutics and Pharmacology, Whitla Medical Building, School of Medicine, Queens University Belfast, Lisburn Road, Belfast BT9 7BL, U.K.

ABSTRACT

Risk factors for cardiovascular disease mediate their effects by altering the structure and function of wall and endothelial components of arterial blood vessels. A pathological change in the microcirculation plays a pivotal role in promoting end-organ dysfunction that not only predisposes to further organ damage, but also increases the risk for future macrovascular events. The microcirculation is recognized as the site where the earliest manifestations of cardiovascular disease, especially inflammatory responses, occur that may play a pivotal role in driving the atherosclerotic process in conduit vessels. Furthermore, the vast surface area of the endothelium compared with conduit vessels means that the vascular effects of endothelial dysfunction or activation will be most apparent in this section of the vasculature. Current techniques providing indices of vascular health focus on large arteries without providing insight into the structure and function of small vessels. Techniques capable of detecting microvascular damage and monitoring the response to therapeutic interventions, especially in vulnerable target organs of interest, may improve risk stratification and represent a valuable surrogate for future cardiovascular outcome.

INTRODUCTION

Target organ damage powerfully influences the occurrence of future cardiovascular events [1]. Screening for target organ damage has become a standard of care in diabetes clinics, with assessment of microalbuminuria and digital retinal screening now accepted as routine practice. Classification schemes recommend screening for target organ damage as part of the clinical assessment process as it influences the initiation and goals of therapy in order to prevent disease progression and delay or prevent future cardiovascular events [2,3]. The presence of target organ damage therefore defines a high-risk population who develop complications either because disease control is sub-optimal or have increased susceptibility to accelerated development and progression of the atherosclerotic process.

Although not traditionally considered a target organ, arterial blood vessels represent the site for the development of the atherosclerotic process that causes cardiovascular events [4]. By the time symptoms develop or clinical signs of atherosclerosis can be detected in conduit vessels, the disease process is already at an advanced stage. Although populations with overt disease are clearly at increased risk for future events, up to 50% of individuals present with a myocardial infarction or sudden cardiac death as the first manifestation of the atherosclerotic disease [5,6]. Thus screening for asymptomatic individuals with subclinical arterial vascular disease is now...
Figure 1  Mechanisms linking inflammatory signals initiated and amplified in the microvasculature with the development and progression of atheroma in large vessels

CRP, C-reactive protein; IFN-γ, interferon-γ; IL, interleukin; sCD40L, soluble CD40 ligand; sP-ser, soluble P-selectin; sVCAM-1, soluble vascular cell adhesion molecule-1; TNF-α, tumour necrosis factor-α. This Figure was reproduced with permission from Stokes, K. Y. and Granger, D. N., The microcirculation: a motor for the systemic inflammatory response and large vessel disease induced by hypercholesterolaemia? J. Physiol. 562, 647–53. © (2005) Wiley-Blackwell.
contention comes from studies showing that structural and functional changes in different microcirculatory beds can predate and predict the development of various cardiovascular disease states and event [22,26–28].

The vast surface area of endothelium in the microcirculation compared with large conduit vessels means the vascular effects of endothelial dysfunction or activation in altering structure and tone will be most apparent in this section of the circulation [29]. Altering the impedance properties of the microcirculation can limit organ flow reserve and detrimentally impact on ventricular–vascular coupling [30]. In addition, a consequence of stiffening of the aorta is an increased transmission of pulsatile pressure and flow into the microcirculation [30]. Transmission of pulsatile stress can damage microcirculatory beds, especially in susceptible target organs such as the brain, eye and kidney. It is known that microvascular dysfunction identified in these target organs not only predicts further organ dysfunction, but identifies an increased risk for future macrovascular events [31]. Organs such as the eye, brain and kidney therefore represent preferential targets susceptible to damage early in disease processes. The pathophysiological explanation may lie in the differential input impedance of the brain and kidney compared with other microvascular beds [32]. These end-organs represent low impedance networks that expose the microvasculature to large pressure and flow fluctuations.

Technical advances now permit the study of structure and function of susceptible target organs, such as the eye and kidney, or evaluation of the endothelium most often using the non-invasive technique of FMD (flow-mediated dilation). The common denominator of these investigations relates to the evaluation of the status of the microcirculation, a section of the vasculature where endothelial activation plays a pivotal role in the pathogenesis of arterial disease [33]. Although the FMD response to forearm ischaemia is estimated by measuring the percentage of dilation of the brachial artery, it essentially represents an indirect assessment of the status of the forearm microcirculation, as the magnitude of conduit artery dilation is known to be critically dependent on the response of the microcirculation to the stimulus (Figure 2).

As the structure and function of the microcirculation can be influenced within weeks of therapeutic interventions, unlike an atherosclerotic plaque or a degenerative change within large artery segments, assessment of this section of the vasculature may have particular value in monitoring the response to drug therapy and as a surrogate for future clinical outcome [34,35].

In the present review article, we provide a systematic overview relating end-organ microcirculatory dysfunction, with particular emphasis on the retinal, forearm, coronary, renal and cerebral microcirculations, to cardiovascular risk assessment and outcome. Recent results indicate that comprehensive assessment of the signatures of wave reflection from target organs of interest represents a particularly sensitive metric of end-organ microvascular status that could prove useful in refining cardiovascular risk for individual patients [36].

RETINAL MICROCIRCULATION

Retinal microcirculation and the development of cardiovascular risk factors

Cardiovascular risk assessment has focused on traditional risk factors such as measurement of blood cholesterol levels, blood pressure and blood glucose levels. These
are major risk factors that exhibit strong, positive and continuous relationships with the risk of cardiovascular disease, even within defined normal ranges. However, the development and progression of arterial vascular disease is a multifactorial process and isolated abnormalities of such risk factors are poor predictors of risk [37,38]. In current clinical practice, available techniques employed for the diagnosis of cardiovascular disease tend to be utilized in response to the development of symptoms and, thus, detect disease at a relatively late stage. Structural and functional changes in the retinal microcirculation may reflect early inflammation and endothelial dysfunction, and alteration in vascular structure and endothelial function acts as the substrate for accelerated disease development [39]. Thus a method of evaluating individuals at a pre-clinical stage, even before the development of the risk factor itself, holds profound potential for disease prevention and early intervention and treatment.

Generalized arteriolar narrowing is a recognized finding with systemic hypertension; however, it is also known that a lower AVR (arteriolar-to-venular ratio) predicts the risk of developing hypertension in the future [22]. Traditionally, the AVR has been accepted as a measure that reflects arteriolar narrowing [40–42]; however, previous studies have confirmed that retinal venular dilation can occur and play an independent role in predicting cardiovascular disorders [43].

Impaired microvascular haemodynamics have been implicated in the pathogenesis of diabetes [44]. Microvascular dysfunction has been shown not only in patients with diabetes, but also in subjects at high risk of developing diabetes, such as those with impaired glucose tolerance or first-degree relatives of those with diabetes [45]. In tandem with subjects at risk of developing hypertension [46], it appears the association between a smaller retinal AVR and incident diabetes may also be due to arteriolar narrowing, venular dilation, or both [47,48]. Ikram et al. [48] have explored whether the AVR was explained by retinal arteriolar narrowing or venular dilation. The results suggested that the relationship between AVR and impaired fasting glucose and diabetes was explained by venular dilation rather than arteriolar narrowing.

Retinal microcirculatory dysfunction and CHD (coronary heart disease)
The retinal vasculature permits a unique visualization of long-term cumulative exposure to elevated blood pressure and other cardiovascular risk factors [49]. The presence of retinopathy signifies an increased CHD risk, independent of known risk factors [50], with results supporting the role of microvascular disease in the pathogenesis of CHD in diabetes. In addition, retinopathy signs are independently associated with the presence of CAC (coronary artery calcium) [51], supporting the concept that common pathophysiological processes may underlie both micro- and macro-vascular disease. Furthermore, microvascular disease has also been hypothesized to influence LV (left ventricular) remodelling [52,53], with studies linking coronary microvascular dysfunction to abnormal LV remodelling and subsequent risk of heart failure [54,55]. Most recently, Cheung et al. [52] have shown that narrower retinal arteriolar calibre is associated with LV concentric remodelling independent of traditional risk factors and coronary atherosclerotic burden, adding further support to the hypothesis that microvascular disease may contribute to the cardiac remodelling process.

Coronary microcirculatory dysfunction
There is a clear well-established link between myocardial morbid events and obstructive coronary atherosclerosis. Coronary angiography and subsequent intervention by means of balloon angioplasty and stent placement is common place in today’s medical practice. However, there is increasing evidence that abnormalities in the structure and function of the coronary microcirculatory network exist in a number of clinical conditions and such abnormalities may contribute to the pathogenesis of myocardial ischaemia [56].

Assessment of coronary endothelial function requires selective catheterization and Doppler flow measurement, which can be supplemented by angiography to identify the concomitant increase in coronary artery calibre [57]. Microvascular function of the coronary system can be assessed by quantifying the flow increase induced by local intra-arterial infusion of endothelium-dependent vasodilator substances, such as acetylcholine, bradykinin, substance P or serotonin [58]. Functional assessment of the coronary microvascular system is limited by its invasive nature and lack of accessibility, apart from specialized centres.

Several studies have now shown that the degree of coronary microcirculatory and endothelial dysfunction is related independently to the risk of future cardiovascular events [14]. Coronary vasoreactivity has been assessed in 147 patients with minimal disease after single vessel angioplasty [59], and cardiovascular events served as outcome variables over a median follow-up period of 7.7 years. Patients suffering from cardiovascular events during follow-up had significantly increased vasoconstrictor responses to acetylcholine infusion and cold pressor testing, as well as significantly blunted vasodilator responses to increased blood flow and the intracoronary injection of nitroglycerine. The authors [59] concluded that coronary endothelial vasodilator dysfunction predicts long-term atherosclerotic disease progression and cardiovascular event rates. Suwaidi et al. [60] followed 157 patients with mild coronary disease who had undergone evaluation of coronary vascular reactivity by graded administration of intracoronary acetylcholine, adenosine and nitroglycerine, and intracoronary ultrasound at the time of diagnostic study. They demonstrated severe

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endothelial dysfunction in the absence of obstructive coronary artery disease. Atherosclerotic burden, assessed by intravascular ultrasound, bore no relationship to either endothelial function or outcomes, indicating the function of the coronary circulation had greater prognostic value.

Halcox et al. [61] measured the change in coronary vascular resistance and epicardial diameter in response to intracoronary acetylcholine in 132 patients with CAD (coronary artery disease) and 176 without evidence of CAD. The patients were followed-up for a mean of 46 ± 3 months. Multivariate analysis that included CAD and conventional risk factors for atherosclerosis demonstrated that coronary vascular resistance and epicardial constriction with acetylcholine, together with increasing age, CAD and BMI (body mass index), were independent predictors of adverse events. Endothelium-independent responses were not predictive of outcome. Epicardial and microvascular coronary endothelial dysfunction, however, were found to independently predict acute cardiovascular events in patients without and with overt CAD.

**Retinal microcirculatory dysfunction and cerebrovascular disease**

The retina and brain share a similar pattern of vascularization during embryological development, and the retinal and cerebral circulations have been found to have many anatomical and physiological similarities [62,63]. Pathological studies have shown that moderate or severe changes in the retinal vessels, especially around the optic disc, are associated with increased risk of cerebral haemorrhage or infarction, and retinal and cerebral arterioles share similar histopathology in stroke decedents [64]. The retinal microcirculation may therefore be a useful surrogate to identify cerebral microvascular disease as it can be easily visualized and photographed in vivo [65].

Retinal microvascular abnormalities reflect cumulative small vessel damage from elevated blood pressure and may even reflect subclinical cerebral microvascular changes [66]. In a population-based cross-sectional study of 1684 subjects 55–74 years of age without a history of clinical stroke, retinal photographs were recorded and graded for the presence of retinal microvascular abnormalities, including arteriovenous nicking, focal arteriolar narrowing, retinal haemorrhages, soft exudates and microaneurysms [66]. Retinal vessel diameters were measured and summarized as the AVR and changes were correlated with cerebral MRI (magnetic resonance imaging) findings graded for the presence of cerebral infarcts. Smaller AVRs and focal arteriolar narrowing were associated with MRI-defined subclinical cerebral infarcts, although these associations were demonstrated only in subjects with hypertension. Another group has also recently shown that evidence of SVD (small vessel disease) in the retina increases the likelihood of finding it in the brain [67]. This suggests that retinal photography may be useful for studying subclinical cerebrovascular disease in population-based studies.

WMLs (white matter lesions) reflect ischaemic SVD, although the underlying pathophysiological determinants are not well understood [68,69]. They are associated with cardiovascular risk factors such as hypertension [70] and diabetes [71], and also with increased risk of future ischaemic stroke [72]. Lacunar infarcts occur when there is occlusion of a small end-artery by microatheroma [73]. WMLs have been found to be present on MRI scans with increased frequency in patients with retinal microvascular abnormalities. Furthermore, there appears to be a multiplicative interaction between retinal microvascular abnormalities and WMLs on the risk of future stroke. In a subset of the ARIC (Atherosclerosis Risk In Communities) study, subjects with both WMLs and retinopathy were 18 times more likely to develop stroke than those without either finding (relative risk, 18.1 [95% CI (confidence interval), 5.9–55.4]) [74]. Another study examining the relationship between retinal artery disease and cerebral SVD found that pathological changes in the retinal arteries paralleled changes in the small cerebral arteries that cause WMLs and lacunae [75]. All types of retinal vascular pathology occurred more frequently in patients with cerebral SVD, but only retinal arterial narrowing and sclerosis significantly correlated with MRI signs of cerebral SVD.

Studies relating retinal microcirculatory changes with the incidence of cardiovascular disease have produced conflicting results. In the middle-aged (51–72 years) cohort from the ARIC study, a smaller AVR (representing either smaller arteriolar calibre or larger venular calibre) was associated with incident CHD [76] and stroke [77]. However, a further analysis of the ARIC study showed that, after adjusting for present and previous blood pressure and medication, the AVR was not associated with any clinical (cardiovascular disease or stroke) or subclinical (carotid or popliteal artery thickness or lower limb obstructive disease) indicators of atherosclerosis, except carotid plaques [78]. Generalized arteriolar narrowing was associated with risk factors for vascular disease such as smoking, inflammatory markers (white blood cell count, fibrinogen and reduced albumin), greater BMI, higher plasma triacylglycerols (triglycerides), low HDL (high-density lipoprotein)-cholesterol and high fibrinogen levels. This suggests that these arteriolar changes are pathologically distinct from atherosclerosis. In the Beaver Dam Eye Study, a smaller AVR was associated with cardiovascular mortality only in younger (43–74 years of age) but not older (75–84 years of age) subjects [79]. Pooled data analysis of another older population from the Beaver Dam Eye Study and Blue Mountains Eye Study (43–97 years) demonstrated that both smaller arterioles and larger venules predicted stroke mortality among
subjects 43–69 years of age, but not those over 70 years of age [80].

However, other studies would suggest that SVD may also contribute to the risk of cardiovascular disease specifically in older subjects. This has been demonstrated in a recent sub-study of the CHS (Cardiovascular Health Study), a prospective population-based cohort study composed of 1992 men and women 69–97 years of age, in which retinal arteriolar and venular calibres were measured from retinal photographs, using a computer-assisted method, and related to incident CHD and stroke events [81]. After 5 years of follow-up, there were 115 incident CHD events and 113 incident stroke events. Participants with a larger retinal venular calibre had a higher incidence of CHD and stroke. At multivariable analysis, controlling for age, gender, race, arteriolar calibre, systolic and diastolic blood pressure, diabetes, glucose concentration, cigarette smoking, pack-years of smoking and HDL- and LDL (low-density lipoprotein)-cholesterol levels, larger retinal venular calibre remained independently associated with incident CHD and incident stroke. The Rotterdam study also showed that a larger venular diameter was associated with an increased risk of stroke, specifically cerebral infarction [82]. Retinal venular dilation was also related to progression of cerebral SVD [69]. No similar associations with arteriolar diameter were observed. This again highlights the importance of a complete interrogation of the microcirculation in terms of both arteriolar and venular diameters.

**FUNCTIONAL FOREARM MICROCIRCULATORY DYSFUNCTION AND CARDIOVASCULAR OUTCOMES**

Non-invasive assessment of vascular endothelial function can be assessed by ultrasound or MRI techniques to measure changes in brachial artery diameter in response to an increase in flow velocity (FMD). The most commonly used stimulus is temporary arterial occlusion, which is followed by reactive hyperaemia and release of NO and other vasodilator mediators. Although not usually viewed as an end-organ, impaired FMD has traditionally been recognized as an indirect marker of NO bioactivity [84]. However, the exact mechanisms responsible for producing the FMD response in the forearm are poorly understood; some suggesting that FMD directly reflects NO bioavailability [85], others that it is simply a reflection of baseline brachial artery diameter [86] or that it is a measure of microvascular dysfunction, reflecting the degree of post-ischaemic vasodilation [87]. Endothelium-dependent FMD has been shown to be a homoeostatic response to short-term increases in local shear stress, which is known to be critically dependent on the magnitude of the imposed stimulus [88,89] and on the distal forearm microcirculation [90,91].

Ultrasound can be used to track the calibre of the brachial artery, which normally dilates in response to the flow increase. NO is an important contributor to this dilation since the effect is at least partially blocked by NOS (NO synthase) inhibition. Guidelines for the use of this technique have been published [92]. This method, although non-invasive, is technician-dependent. Ultrasound assessment of artery calibre requires technical skill and interpretive precision. Temporal variability, alterations in baseline calibre and variations in the magnitude of the flow-induced stimulus all may influence the results [92–94]. Although more suitable for screening than invasive methods, the meticulousness and variability of the method reduce its applicability in multi-centre studies.

A number of studies have demonstrated that brachial artery reactivity as assessed by ultrasound improves with risk factor modification and treatment with drugs known to reduce cardiovascular risk. Gokce et al. [96] pre-operatively examined brachial artery vasodilation using ultrasound in 187 patients undergoing vascular surgery. Patients were prospectively followed for 30 days after surgery for post-operative events, including cardiac death, myocardial infarction, unstable angina/ischaemic ventricular fibrillation, stroke or elevated troponin I, reflecting myocardial necrosis. Pre-operative endothelium-dependent FMD was significantly lower in patients with an event than in those without an event, whereas endothelium-independent vasodilation to nitroglycerine was similar in both groups.

In a study of 73 patients suffering from angina, those with impaired FMD had greater rates of revascularization procedures, including bypass surgery and angioplasty, than those with preserved FMD during a 5 year follow-up period [97]. This finding remained significant after a multivariate adjustment. Katz et al. [98] investigated the association between endothelial function and subsequent mortality risk in chronic heart failure. They assessed FMD in the brachial artery and exhaled NO production during submaximal exercise as biomarkers of endothelial function in the systemic arterial circulation in 259 subjects with NHYA (New York Heart Association) class II–III CHF (chronic heart failure). Both decreased FMD and decreased exhaled NO production were associated with increased risk of death or urgent transplantation.

We have shown previously [99] that an impaired FMD response observed in patients with SLE (systemic lupus erythmatosus) is closely related to an impairment of downstream microcirculatory haemodynamic function, as assessed by DSS (diastolic shear stress), not simply a relative lack of NO. This highlights the dependence of FMD results on the function of the distal microcirculatory network. This relationship between FMD and the degree of evoked DSS is important. Previously, Mitchell et al. [88] have shown that higher baseline diastolic flow and DSS were associated with larger baseline diameter and higher FMD% (percentage FMD). Further hyperaemic...
flow and DSS were strongly related to FMD%. Other recent studies have also shown an association between larger baseline diameter and higher FMD% [86], but this was in a much older cohort of patients. Our findings [99] show that higher mean diastolic velocity and DSS were again associated with higher FMD%, but there was no association with baseline diameter.

The relationship between impaired FMD% and the presence of cardiovascular risk factors, being in part determined by an abnormal distal microcirculatory response to DSS and hyperaemic flow, highlights the implications for many studies employing FMD as a surrogate marker of NO bioavailability; namely that it is essential to consider the hyperaemic flow and shear stress response when interpreting endothelium-dependent vasodilation. It is often suggested that FMD specifically reflects NO-mediated endothelial function; however, we believe that this is too simplistic a statement given that FMD% is affected by the nature of the shear-stress stimulus, and also given that shear stress itself acts as a stimulus for endothelial vasoconstictors [100]. To this end, analysis of Doppler flow velocity waveforms is helpful, as changes in the evoked waveform reflect alterations in the distal microcirculatory network. We assert that this provides a clear basis for the future development of methods to non-invasively assess cardiovascular health in patients at risk of future complications, thus providing a base to tailor preventive drug therapy and intervene if necessary at an earlier stage than would otherwise be possible.

**EARLY KIDNEY DISEASE AND CARDIOVASCULAR OUTCOMES**

It has been known for many years that most patients with chronic kidney disease do not die of kidney failure, but rather of cardiovascular disease [101]. In the HOPE (Heart Outcomes Prevention Evaluation) study, patients with renal insufficiency had a higher incidence of cardiovascular death, myocardial infarction or stroke than those without (22 compared with 15 %) [102]. The early detection of altered kidney function, therefore, offers an opportunity to identify individuals at increased risk of cardiovascular disease at a preclinical stage. This section will highlight methods that can be used to identify kidney disease before it becomes clinically apparent. The evidence linking early kidney dysfunction to cardiovascular outcome will also be discussed.

**Serum markers of kidney function**

Most evidence relating to the adverse outcomes in kidney disease relates to individuals with a GFR (glomerular filtration rate) < 60 ml·min$^{-1}$·1.73 m$^{-2}$, with the suggestion in older literature of a biological threshold effect above which no harm is likely [103]. Recent work has shown that minor degrees of renal dysfunction can be associated with adverse cardiovascular outcomes. Mild renal impairment was found to be an independent risk factor for cardiovascular mortality within 10 years in an apparently healthy unselected population [104]. The results indicated that the risk began with a GFR in the region of 90 ml·min$^{-1}$·1.73 m$^{-2}$ and remained an independent determinant after correction for other established risk factors.

Cystatin C is a protein that is a member of a family of competitive inhibitors of lysosomal cysteine proteinases. It is expressed constantly, is filtered freely at the glomerulus, undergoes complete reabsorption and catabolism by the proximal tubules with no reabsorption into the bloodstream and no renal tubular secretion [105], and is therefore an ideal marker of GFR. Measurement of cystatin C allows a more precise estimation of GFR when compared with measurement of creatinine, as its levels are independent of muscle mass, age and gender [106]. Shlipak et al. [107] investigated the value of cystatin C as a measure of kidney function and defined preclinical kidney disease as the presence of an estimated GFR > 60 ml·min$^{-1}$·1.73 m$^{-2}$, with a cystatin C concentration > 1 mg/l [107]. They convincingly demonstrated that, in an elderly cohort of more than 4500 individuals, early renal dysfunction was strongly associated with death, cardiovascular death, stroke, myocardial infarction and incident heart failure.

**Urine markers of kidney function**

The glomerulus is composed of a network of specialized capillaries. Excretion of albumin in the urine is indicative of the degree of glomerular permeability, with increasing levels of albumin in the urine indicating increasing levels of kidney damage [108]. Arbitrary cut-off limits for albumin excretion have been defined, with normal excretion being < 30 mg/day and albuminuria < 300 mg/day. The intermediate zone (30–300 mg/day) is described by the term microalbuminuria. The finding of microalbuminuria in an individual therefore allows the detection of endothelial dysfunction in the glomerular microvasculature. Microalbuminuria can also be defined in terms of the urinary albumin-to-creatinine ratio. A ratio > 30 in the first voided sample in the morning is considered abnormal [109]. Two elevated albumin/creatinine ratios separated by 3–6 months are generally required to define microalbuminuria [110]. The presence of microalbuminuria is known to predict the development of proteinuria and progressive renal failure in patients with Type 1 diabetes [111]. More recent work has shown that it can also predict cardiovascular events in various groups of patients, as highlighted below.

**Normal populations**

Ljungman et al. [112] have shown that a baseline measure of urinary albumin excretion was an efficient and
independent predictor of cardiovascular disease in middle-aged normotensive men [112]. This idea that microalbuminuria can be present in otherwise healthy individuals and that it can predict disease is supported by a study by Corona et al. [113]. These authors studied a group of unselected men and non-pregnant women between 35 and 64 years of age. Subjects were classified as being either without or with microalbuminuria. The probability for myocardial infarction bordered on significance, being 1.90 (95% CI, 0.97–3.72) times higher in those with microalbuminuria. In a further population study involving 20911 individuals between 40 and 79 years of age, an increased all-cause mortality (particularly from cardiovascular disease) was noted in subjects with microalbuminuria [114].

Type 1 diabetes

Traditional thinking suggested that the relationship between microalbuminuria and cardiovascular morbidity and mortality was mainly attributable to the later development of overt nephropathy [115]. However, in adults with Type 1 diabetes, microalbuminuria alone is associated with excess cardiovascular mortality. Viberti et al. [116] measured the overnight urinary albumin excretion rate in 87 patients with Type 1 diabetes and followed the cohort for 14 years. Clinical proteinuria developed in two subjects out of 55 with an albumin excretion rate <30 μg/min, but in seven out of eight subjects excreting between 30 and 140 μg/min. The risk of clinical diabetic nephropathy in the latter group was 24 times higher than for the former group. In addition, 9.1% of patients with albumin excretion rates <30 μg/min had died compared with 37.5% with the higher rate.

Type 2 diabetes

Mogensen [111] followed a cohort of 76 subjects with Type 2 diabetes for 9 years and showed that those with microalbuminuria were more likely to have clinically detectable proteinuria than those with lower concentrations, and also that mortality was 148% higher than in normal control subjects. A subsequent meta-analysis confirmed that, in subjects with Type 2 diabetes, the presence of microalbuminuria doubled the risk of having a cardiovascular event, and that the risk was similar to or even higher than that conferred by other established atherosclerotic risk factors [117]. Using a combined pool of 2138 patients, followed up for a mean of 6.4 years, the odds ratio for death was 2.4 (95% CI, 1.8–3.1), and that for cardiovascular morbidity/mortality was 2.0 (95% CI, 1.4–2.7). It is important to note that microalbuminuria has not yet been clearly demonstrated to predict overt nephropathy in Type 2 diabetes, therefore the main reason for the high frequency of cardiovascular disease in this population is not clearly related to diabetic nephropathy.

Hyperinsulinaemia

Individuals with insulin resistance and hyperinsulinaemia have an increased incidence of microalbuminuria [118]. The combination of hyperinsulinaemia and microalbuminuria in elderly individuals has been shown to be a strong predictor of CHD death as well as all CHD events [119].

Hypertension

It was first shown in 1974 that microalbuminuria occurs in patients with hypertension who do not have diabetes [120]. It has also been shown that the magnitude of protein excretion correlates with the severity of hypertension, especially systolic blood pressure and pulse pressure [121,122]. Abnormalities in arterial mechanical properties have been inferred in patients with hypertension and microalbuminuria. For example, Tsiofis et al. [123] showed that such patients exhibited an earlier systolic augmentation of the arterial pressure than normal, reflecting a more impaired arterial elasticity [123]. In addition, patients with hypertension and microalbuminuria have been shown to have an increased incidence of dynamic electrocardiographic changes during exercise [124]. The authors claim that this demonstrates an increased prevalence of silent coronary ischaemia in such patients.

Microalbuminuria has been linked with many cardiovascular disease outcomes and death in patients with hypertension. Ljungman et al. [112] showed that a baseline measure of urinary albumin excretion was an efficient and independent predictor of cardiovascular disease in middle-aged men with hypertension. A value of urinary albumin-to-creatinine ratio more than the median has been associated with a composite end point of cardiovascular death, non-fatal stroke or non-fatal myocardial infarction in patients with hypertension and LV hypertrophy [125]. Agrawal et al. [126] linked the finding of microalbuminuria to a significantly higher incidence of coronary artery disease, LV hypertrophy, previous stroke and peripheral vascular disease. In those subjects with microalbuminuria and cardiovascular disease, the amount of albumin in the urine was significantly higher than in those who did not present with cardiovascular disease. A prospective trial of non-diabetic patients with hypertension demonstrated a relative risk of 3.5 (95% CI, 1–12.1) when adjusted for all other atherosclerosis risk factors [127].

Individuals known to have cardiovascular disease

The HOPE study was carried out to assess the effect of therapy in patients over 55 years of age deemed to be at high risk of cardiovascular events. An analysis of the characteristics of the participants in the study showed that age, waist-to-hip ratio, diabetes, smoking, hypertension, vascular disease and LV hypertrophy were independent determinants of microalbuminuria in all participants [128]. In participants with diabetes, the odds of
microalbuminuria increased 16% for every 10.4 years of diabetes duration, and increased 8% for every 0.9% increase in HbA1c (glycated haemoglobin). Post-myocardial infarction, microalbuminuria has been shown to be a strong independent predictor of an adverse cardiac event in the next 3 years [129].

Evidence that lowering albumin excretion improves outcome
It has been shown that in subjects with hypertension, the presence of proteinuria increases the incidence of cardiovascular events, but that normalization of protein excretion with an ACE (angiotensin-converting enzyme) inhibitor correlated with a reduction in such events [130]. Furthermore, lowering blood pressure using an agent known to reduce microalbuminuria may provide greater cardiovascular risk reduction than an agent that lowers blood pressure without affecting this [131]. Treatment of patients with diabetes with a thiazolidinedione can also improve albumin excretion, and this fact may account for some of the efficacy that has been reported with these agents [132].

Blood flow waveform analysis
Doppler ultrasound has been used to study blood flow in renal blood vessels. The most commonly measured parameters are the RI (resistive index) and PI (pulsatility index), which attempt to characterize velocity waveforms using a small number of discrete points.

The RI and PI in intrarenal arteries increases with increasingly severe nephropathy [133]; however, these authors report no advantage in using such measures over more routine parameters such as serum creatinine, blood pressure and age.

Frauchiger et al. [134], administering GTN (glyceryl trinitrate) to healthy volunteers and patients with diabetic nephropathy (13 with proteinuria and seven with microalbuminuria), showed a significant difference in interlobar artery RI between young healthy volunteers and those with nephropathy. The reduction in RI after administration of GTN was significantly diminished in patients compared with controls. In addition, the reduction in RI correlated with age in the healthy subjects and with duration of diabetes in the subjects with diabetes. The authors [134] postulate that these results demonstrate fixed arteriolar damage in diabetes.

Ishimura et al. [135] studied patients with Type 2 diabetes and divided subjects into four groups depending on urinary albumin and creatinine. They showed that the RI and PI were higher in the most severely affected group compared with the other groups. RI values were significantly influenced by creatinine clearance, age and duration of diabetes. The correlation of ultrasound markers of vascular function and microalbuminuria has been investigated. It has been shown that the RI in the interlobar artery correlates with urinary albumin-to-creatinine ratio [136]. Patients with a higher RI had a significantly higher prevalence of microalbuminuria.

In patients with hypertension, those with the highest renal RI have increased LV mass indexes, higher carotid intima-medial thickness and a higher prevalence of LV hypertrophy, carotid plaques and microalbuminuria [137].

Microvascular disease in the kidney predicts disease elsewhere
There is a link between microvascular dysfunction in the diabetic eye and kidney. In a study of 220 patients with Type 1 diabetes followed up for 15 years, 74% of those with nephropathy developed proliferative retinopathy compared with 14% in patients free of proteinuria [138]. Thus microvascular disease in one organ is associated with microvascular disease in another. The combination of retinopathy and nephropathy confers a particularly unfavourable cardiovascular risk on the patient. The presence of both retinopathy and albuminuria conferred a relative risk of death, cardiovascular or renal events of 6.83 compared with a risk of 1.61 for retinopathy alone and 4.34 for albuminuria alone [139]. In addition, in a study linking retinopathy and cardiovascular outcome, urinary albumin excretion was found to explain a considerable part of the association found [140]. In a case-control study involving 102 patients with Type 2 diabetes, macular ischaemia was shown to be independently associated with nephropathy, irrespective of the severity of retinopathy [141].

NOVEL METHODS OF ASSESSING MICROCIRCULATORY FUNCTION
Microvascular dysfunction that can precede and contribute to the development of disease and end-organ damage play a pivotal role in influencing pressure and flow patterns in the circulation [25,27,29]. Quantitative analysis of pressure and flow velocity waveforms can identify and track changes in haemodynamics associated with risk factors for cardiovascular disease and in response to physical and pharmacological interventions [142]. Drug therapy has little direct effect on the aorta and the hemodynamic actions predominantly influence small vessels to alter the pattern of wave reflection and waveform morphology [143–146]. Previous studies have confirmed the sensitivity and utility of tracking changes in pulse waveform morphology as a non-invasive monitoring tool to identify pharmacological modulation of smooth muscle tone [147–149]. Changes in waveform morphology in response to therapeutic interventions can be related to improvement in organ function and therefore holds potential to refine cardiovascular risk stratification.

Ultrasound and the Doppler effect have long been used to measure blood velocity and its temporal and spatial
variation within the vascular tree in order to diagnose and monitor vascular disease [25]. Cyclical changes induced in the pulsatile circulation produce systolic-diastolic variations in velocity ranges responsible for the various shapes of the Doppler time-velocity spectral waveforms [142]. Change in the linear flow velocity spectral envelope is not representative of any single vessel, but is determined by changes in the properties and total cross-sectional area of the downstream vascular network.

Quantitative analysis of Doppler time-velocity waveforms has traditionally relied on estimates of arterial pulsatility in the feeding vessel supplying microvascular beds to derive an RI [143,144]. Although this index can mirror changes in downstream vascular resistance, changes in resistance and flow pulsatility are not closely correlated in all circumstances [150–152]. The relationship is confused by changes in the pulsatile function or compliance characteristics, in addition to resistance to flow, of downstream vessels and microvasculature distal to the site of measurement [151,153]. Traditionally viewed predominantly as a feature of elastic conduit arteries, it is recognized that the compliance of microvascular beds contributes significantly to altering the impedance, or total opposition to flow, presented by a microcirculatory network [30,154]. Remodelling processes that involve changes in the amount, organization and constituents of vessel walls could alter the compliance characteristics of the microvasculature without necessarily altering the resistance to blood flow [155–157]. Furthermore, given that compliance depends not only on the wall properties of blood vessels but also on diameter, it can be appreciated that compliance will be altered significantly in a section of the vasculature capable of dilating by orders of magnitude more than conduit vessels [158].

The microcirculation is the most important site for wave reflection and the dynamic nature of this section of vasculature in modulating structure and tone suggests haemodynamic change can be monitored by pulse wave analysis. RI assessment cannot capture or quantify the entire signature of wave reflection that alters waveform morphology. This index, similar to the augmentation index derived from pressure pulse recordings, is determined from single inflection points on the waveform that fail to account for the bulk of wave reflection that becomes embedded in, and distorts the morphology of, the incident wave over the duration of the cardiac cycle [19]. We [99,159] and others [35a] have shown that comprehensive analysis of the flow velocity waveform is more sensitive in detecting and monitoring dynamic change in microvascular tone than limited approaches that focus on isolated points on the waveform.

Furthermore, alteration in the pattern of wave reflection, marking the presence of early microvascular disease in target organs, may not be identified by pulse wave analysis techniques that employ a global assessment of wave reflection [14]. This is because the magnitude of wave reflection from low impedance beds will be small and overwhelmed by contributions from high impedance circulations and not detectable in recordings obtained from proximal conduit arteries [160]. In addition, the small amount of reflected energy emanating from the ocular or renal microcirculations will undergo a degree of attenuation and dissipation as it travels towards the major conduit arteries [150,160]. The versatility of ultrasound in permitting the study of target organs of interest provides a significant advantage over tonometric techniques in this regard.

The prognostic importance of measures of microvascular structure and function are limited. It has been shown that microvascular function measured by reactive hyperaemic velocity and shear stress relates to cardiovascular risk factors more closely than FMD and also to outcome. Reactive hyperaemic velocity is taken to indicate a decrease in the stimulus for FMD change. It provides a measure of dilation of the downstream microvasculature that will result in increased flow and shear stress in the feeding conduit vessel. We have shown previously [99] that, despite the lack of difference in peak reactive hyperaemic velocity, a downstream microvascular abnormality can be identified by arterial waveform analysis. Microvascular dilation will influence not only the steady-state phenomena or resistance to flow, but also the pulsatile component of flow that depends on the compliance characteristics of the microvascular bed. Traditionally viewed predominately as a feature of elastic conduit arteries, it is recognized that the compliance of microvascular beds contribute significantly to altering the impedance presented by a microcirculatory network. Given that compliance depends not only on the wall properties of blood vessels but also on diameter, it can be appreciated this parameter will be altered significantly in a vasculature capable of dilating by as much as 80%.

The input into the microvascular bed is certainly pulsatile and a change in the downstream impedance, which incorporates both steady-state and pulsatile components of flow, will influence wave reflection that can be assessed by quantitative arterial waveform analysis. Measuring changes in hyperaemic flow may not fully characterize changes in downstream microvascular function. Our observations would support previous findings [142,161,162] indicating that vascular tree stiffening, which influences the pattern of wave reflection, represents an early and subtle marker of changes in vasomotor tone beyond that indicated by measures of microvascular dilation.

It is recognized that quantitative analysis of pressure pulse and flow waveforms recorded from arterial blood vessels provide valuable information about the status of the arterial circulation. Indices derived from analysis of pulse waveforms recorded from large arteries have been employed to infer changes in the mechanical properties of the arterial system. The pulse contour is determined...
by the interaction between LV output and physical properties of blood vessels with the microcirculation representing the most important site for pulse wave reflection. Changes in the structure or function of microcirculatory beds influence the amplitude and timing of reflected waves that become embedded in the incident or forward pulse to alter the arterial waveshape. The signature of wave reflection is known to distort the morphology of the incident wave over a considerable portion of the cardiac cycle. Current techniques that focus on isolated points or sections of the waveform cannot fully capture the effects of wave reflection in altering waveform morphology. A more comprehensive approach that enables capture and quantification of all the components of the wave reflection signature would add value in marking the presence of abnormalities in the arterial microvasculature.

We have shown in recently presented, but as yet unpublished, work that wavelet spectra of normalized time-averaged signals at baseline revealed significant variations ($P < 0.05$) between a group of patients at increased cardiovascular risk, namely Type 1 diabetes, and healthy volunteers (C. J. Lockhart, A. McCann, C. E. Agnew, P. K. Hamilton, C. E. Quinn, M. Cross, R. D. Plumb, R. C. McGivern, M. T. Harbinson and G. E. McVeigh, unpublished work). This indicates that more information can be extracted from the differences in morphology between groups. Wavelets are small short signals which look at the time/frequency domain simultaneously; they function to decompose a signal into factors of scale and position. They provide information about the frequency content and, crucially, the time at which these frequencies occur. In contrast with Fourier-based approaches, frequency and time information is known but not at the expense of the other [163–165]. In addition, wavelets do not require that the signal is stationary and so are useful in the interrogation of real-life signals, such as post-ischaemic flow waveforms. Because the derived indices capture the whole of the waveform, unique information is provided concerning the downstream microcirculation on a beat-to-beat basis (see Figure 3 for examples of Doppler waveforms and subsequent comprehensive analysis via wavelet-based techniques).

Therefore, in order to assess the effects of disease and to monitor the actions of drug interventions that alter pulsatile and steady-state haemodynamics, a sensitive assessment tool capable of identifying and tracking changes in waveform morphology and the pattern of wave reflection is required. The altered wave reflection signature becomes embedded in the incident waveform and can be employed to detect and track changes in microvascular haemodynamics.

CONCLUSIONS

The importance of early management in the preclinical phase of atherosclerosis has been emphasized recently. Endothelial activation that contributes to microvascular...
dysfunction may drive inflammation which plays a key role in CAD and other manifestations of atherosclerosis. Microvascular dysfunction is recognized as one of the earliest manifestations associated with cardiovascular risk factors, is at least in part reversible and holds prognostic significance. To assess the effects of disease and monitor the action of drug interventions requires a sensitive assessment tool to identify and track changes in the pattern of wave reflection resulting from altered structure and tone in the microcirculation. Ultrasound-based techniques that enable recording of waveforms in the immediate proximity of target organs of interest coupled with comprehensive waveform analysis approaches represents a significant advance in this regard.

FUNDING
This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

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Received 4 March 2008/3 April 2008; accepted 18 April 2008
Published on the Internet 8 January 2009, doi:10.1042/CS20080069