Noninvasive assessment of preclinical atherosclerosis

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Abstract: Initially considered as a semipermeable barrier separating lumen from vessel wall, the endothelium is now recognised as a complex endocrine organ responsible for a variety of physiological processes vital for vascular homeostasis. These include the regulation of vascular tone, luminal diameter, and blood flow; hemostasis and thrombolysis; platelet and leucocyte vessel-wall interactions; the regulation of vascular permeability; and tissue growth and remodelling. The endothelium modulates arterial stiffness, which precedes overt atherosclerosis and is an independent predictor of cardiovascular events. Unsurprisingly, dysfunction of the endothelium may be considered as an early and potentially reversible step in the process of atherogenesis and numerous methods have been developed to assess endothelial status and large artery stiffness. Methodology includes flow-mediated dilatation of the brachial artery, assessment of coronary flow reserve, carotid intimamedia thickness, pulse wave analysis, pulse wave velocity, and plethysmography. This review outlines the various modalities, indications, and limitations of available methods to assess arterial dysfunction and vascular risk.

Keywords: endothelial function, vascular risk, vascular stiffness

The vascular endothelium

The endothelium has an important role in maintaining vascular homeostasis. Although once considered simply as a semipermeable membrane, endothelial cells transduce a wide range of physiological stimuli, and in response, produce a variety of signalling molecules that exert autocrine and paracrine effects. The endothelium can therefore be considered as an important endocrine organ (Vanhoutte et al 1989; Vane et al 1990), and is responsible for maintaining vasomotor tone, hemostasis and thrombosis, inflammatory processes, platelet and leucocyte vessel-wall interactions, and controlling vascular permeability. The equilibrium between vasodilatation and vasoconstriction in regional vascular beds is largely controlled by the interaction between endothelium-derived vasoactive mediators and the vascular smooth muscle layer. Endothelial nitric oxide (NO), produced by constitutive activity of nitric oxide synthase (eNOS) (Schini-Kerth 1999), is a potent vasodilator and one of the most important regulators of vascular tone. In addition, NO is antiatherogenic, inhibiting platelet aggregation, smooth muscle proliferation, expression of adhesion molecules, and neutrophil aggregation (Vanhoutte et al 1989; Vane et al 1990). Arterial endothelial dysfunction is a key, early, and potentially reversible, event in the process of atherogenesis and is characterised by impaired NO bioavailability (Healy 1990; Ross 1993; Berliner et al 1995). Endothelial dysfunction causes impaired vasomotor responses to various neurohumoral stimuli which may contribute to transient myocardial ischemia, plaque rupture, thrombosis, and myocardial infarction (Maseri et al 1978). Endothelial dysfunction has so far been described in association with

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many established cardiovascular risk factors such as active and passive smoking, hypertension, hypercholesterolemia, obesity, and type II diabetes (McVeigh et al 1992; Anderson, Meredith, et al 1995; Treasure et al 1995; Goodfellow et al 1996; Williams et al 1996; Koller 2002; Williams et al 2002). The extent of endothelial dysfunction and vasomotor responsiveness correlates with the rate of progression of atherosclerosis and cardiovascular events (Schachinger et al 2000; Widlansky et al 2003). As such, endothelial function has importance, not only in determining predisposition to atherosclerotic disease, but also in determining prognosis in clinically affected patients.

Clinical assessment of endothelial function

Endothelial function can be measured in a variety of ways using invasive and noninvasive techniques in the coronary and peripheral circulation. The clinical examination of endothelial function involves assessing the ability of the endothelium to release NO in response to various exogenous and endogenous stimuli. The quantity of NO released from endothelial cells determines the degree of vasodilatation detected in clinical studies, and thus arterial "health". Ludmer and collegues (1986) initially tested this concept by infusing acetylcholine into coronary arteries at angiography and measuring subsequent changes in arterial diameter. In healthy arteries, infusion of acetylcholine caused vasodilation, whereas vasoconstriction occurred in the presence of a damaged endothelial lining. Further evidence that the observed vasodilation was secondary to NO release was derived from studies which blocked dilatation using inhibitors of the L-arginine-NO pathway (Hodgson and Marshall 1989; Lefroy et al 1993). Subsequent studies using vasoactive pharmacological and physiological agents have confirmed differences in dilatation and endothelial responsiveness between healthy vessels and atherosclerotic vessels (Gollino et al 1991; Yeung et al 1991).

Coronary angiography

Methods of assessment

Quantitative coronary artery angiography of the left anterior descending artery and intracoronary doppler techniques have been applied to measure coronary artery vaso-responsiveness in response to endothelium-dependent agonists such as bradykinin and mechanical stimuli such as increased flow. Quantitative angiography can assess coronary arterial luminal diameter following cardiac catheterization, whilst doppler probes evaluate blood flow velocity in response to infusion of vasoactive agents (Groves et al 1995).

The integrity of coronary arteries has recently been evaluated angiographically via stimulation of the sympathetic nervous system using exercise and cold pressor tests. Sympathetic neuronal stimulation using cold pressor testing induces vasodilatation in healthy vasculature and vasoconstriction in proatherogenic disease states and atherosclerotic coronary arteries (Zeiher et al 1991; Antony et al 1994; Nitenberg et al 1998).

Although the coronary angiography technique is undoubtedly a useful tool for assessing vascular risk, widespread use is not practical. Coronary angiography is invasive and unsuitable for studying early preclinical atherosclerosis in asymptomatic subjects, or for the serial evaluation of vascular physiology in response to potential antiatherogenic strategies. Furthermore, its use is limited as a consequence of serious adverse reactions reported following the intracoronary infusion of acetylcholine at angiography (Tio et al 2002).

Association with coronary artery disease and atherosclerotic risk factors

Impaired vascular reactivity in the coronary artery circulation is associated with traditional coronary risk factors such as type 2 diabetes, insulin resistance, hypertension, and dyslipidemia (Nitenberg et al 1998; Dagres et al 2004; Mokelke et al 2005), even in the absence of clinically overt atherosclerotic lesions. Impaired vascular reactivity may also serve as an index integrating the overall stress imposed by coronary risk factors (Vogel and Corretti 1998). Coronary endothelial vasodilator dysfunction persists after angiographically significant coronary atherosclerotic plaque is evident and has been shown to predict long-term disease progression and cardiovascular event rates in patients at risk of coronary disease (Schachinger et al 2000). However, disease modifying agents which reduce cholesterol and exhibit antioxidant qualities improve coronary artery endothelium-dependent dilatation (Anderson, Meredith, et al 1995; Treasure et al 1995) and may reflect the cardioprotective qualities of these agents.

Flow-mediated dilatation

Method of assessment

A noninvasive technique using high-resolution ultrasound to overcome the practical constraints of invasive coronary artery testing has been developed to assess endothelial function in the peripheral circulation (Celermajer et al 1992).

Using this technique, changes in brachial artery diameter are measured by following the endothelium-dependent stimulus of increased blood flow and may be compared with changes in vessel diameter following the oral administration of endothelium-independent agonists such as glyceryl trinitrate (GTN) (Celemajer et al 1992). Since endothelial dysfunction is a generalized systemic process, it occurs in both the coronary and systemic circulation (Anderson, Gerhard, et al 1995). Indeed, a close relationship has been demonstrated between vasodilator responses in the brachial artery and those in the coronary circulation (Anderson, Uehata, et al 1995; Matsuo et al 2004). The sensitivity of the original technique developed by Celermajer et al (1992) has been improved by using a validated computerized vessel wall tracking system (Vadirec Medical Systems® [Ramsey et al 1995]) to follow changes in brachial artery diameter throughout the cardiac cycle.

Many wall tracking systems have been developed to determine flow-mediated dilatation (FMD). One such system comprises an adapted duplex colour flow echo machine, giving high axial resolution (Ramsey et al 1995). With this technique, the brachial artery is identified using an ultrasound transducer with a stand-off device containing ultrasound coupling gel placed between the transducer and the arm to prevent compression of the anterior wall of the artery. Vessel wall movements are tracked using the stored radio frequency signals to produce displacement waveforms of the anterior and posterior walls together with the distension waveform (diameter change as a function of time [Hoeks et al 1990]). The distension waveform allows measurement of end-diastolic diameter for each beat, providing a theoretical resolution of 3 m. Forearm blood flow is measured throughout the study using a continuous wave doppler probe positioned over the brachial artery (Smith et al 2002). Once baseline measurements of brachial artery diameter are established, a cuff placed at the wrist is inflated to suprasystolic pressure, causing relative hand ischemia. Release of the occluding cuff results in reactive hand hyperemia and an associated increase in blood flow through the brachial artery, which induces shear stress on the arterial wall and provides a stimulus for endothelium-dependent dilatation. Similar measurements can also be made using a NO donor, eg, glyceryl trinitrate (GTN) for an assessment of endothelial-independent vasodilatation (Celermajer et al 1992).

A degree of investigator expertise is required to determine brachial artery vasodilation using ultrasonography and no consensus exists regarding the degree of vasodilation which should be expected in individuals with healthy endothelial function (Faulx et al 2003). Significant changes in brachial artery reactivity have been reported within healthy subjects throughout the course of a day when measured by the same operators, suggesting that variability occurs between morning and evening measurements, in addition to variability between subjects examined on different days (De Roos et al 2003). However, this is disputed by others who have carefully controlled for confounding factors (ter Avest et al 2005). Despite this, FMD will continue to be an extensively used technique for assessing endothelial function. Improvements in available equipment and operator expertise will reduce variability in results.

Association with coronary artery disease and atherosclerotic risk factors

The FMD technique is now one of the most widely used noninvasive methods of assessing endothelial function and closely correlates with cardiovascular risk (Kuvin and Karas 2003). Impaired FMD is described in insulin resistant states and type 2 diabetes, dyslipidemia, hypertension, end stage renal disease, and smoking (Yildiz et al 2003; Esen et al 2004; Holay et al 2004; Thomas et al 2004). Subsequently, FMD has been used extensively to assess the potential antiatherogenic qualities of treatment options, and continues to be indispensable in determining endothelial integrity.

Carotid intimamedial thickness

Method of assessment

Another noninvasive method of assessing subclinical atherosclerosis involves measurements of carotid intima-media thickness (IMT) with high resolution B-mode ultrasonography. This is a well established technique which has been extensively used to estimate coronary artery events and the extent of established atherosclerosis in central and peripheral vasculature (Bots et al 1993), with early increases in IMT possibly reflecting adaptation to elevated intravascular shear stress (Bots et al 1997). As a noninvasive imaging technique, quantitative carotid IMT is versatile for use in large populations with minimal risk to subjects (Barth 2004). However, accurate measurements, particularly of the near wall, require a high level of technical expertise. Consequently, some authors suggest the administration of contrast media during the examination period (Macioch et al 2004; Martin and Lekaris 2004).

A variety of techniques have been used in the determination of carotid IMT. Measurements of the common

carotid, internal carotid, and carotid bifurcation are all technically acceptable, including combination measurements of 12 carotid arterial sites, eg, using Meijer's Arch (Bots et al 2003). In view of the diversity and lack of uniformity in determining carotid IMT, meta-analysis suggests that circumferential scanning of the carotid artery and calculation of the mean maximum carotid IMT provides a more accurate measurement of carotid atherosclerosis (Bots et al 2003). However, all sites of carotid IMT measurement appear to have equivalent value in predicting future coronary artery events (Iglesias del Sol et al 2002).

Association with coronary artery disease and atherosclerotic risk factors

Carotid IMT correlates with cardiovascular risk factors such as the 'metabolic syndrome' (McNeill et al 2004), insulin resistance in type 1 and 2 diabetics (Fujiwara et al 2003; Singh 2003), microalbuminuria (Jadhav and Kadam 2002), hypercholesterolemia (Wendelhag et al 1992), and atherogenesis (Salonen and Salonen 1993).

Carotid IMT and the progression of IMT correlates well with cardiovascular and cerebrovascular end-points (Bots et al 1997; Hodis et al 1998; O'Leary et al 1999). However, although extensive data supports the use of carotid IMT as a predictor of cardiovascular risk, endothelial dysfunction manifesting as impaired brachial artery reactivity may be an earlier predictor of coronary artery disease, with increased carotid IMT being evident at a later stage in the process of atherogenesis (Furumoto et al 2002).

Cardiovascular risk and vascular stiffness

Large artery stiffness

Arteriosclerosis is an integral part of the aging process (Pearson et al 1994) and is now also recognised as an important and independent risk factor for cardiovascular disease (Arnett et al 1994). As a result of vascular stiffening, the diastolic blood pressure decreases and pulse pressure widens, as occurs with advancing age (Franklin et al 1997). Consequently, brachial artery pulse pressure is a surrogate marker of vascular stiffness and is used to determine cardiovascular risk in normotensive and hypertensive subjects (Benetos et al 1997, 1998; Franklin et al 1999), having a higher predictive value of cardiovascular risk than mean arterial blood pressure (Domanski et al 1999; Miller et al 1999). Isolated systolic hypertension and elevated pulse pressure have also been identified as a major cardiovascular risk factor in the Systolic Hypertension in the Elderly Programme and the Systolic Hypertension in Europe trial (Frishman 2000).

Confirming the association between pulse pressure and cardiovascular risk, Philippe et al (2002) demonstrated a direct correlation between aortic pulse pressure, measured with intra-aortic balloons at coronary angiography, and the extent of atherosclerotic disease. Wave reflection within the vascular tree leads to a higher pulse pressure in central vessels than in the periphery (Pauca et al 1992; Nichols and O'Rourke 1998, 2005). The resulting increase in left ventricular after-load increases myocardial oxygen consumption and promotes left ventricular hypertrophy. In addition, increasing systolic pressure elevates arterial wall circumferential stress and predisposes to atherosclerotic plaque generation. Left ventricular hypertrophy that arises from increased aortic systolic pressure (Lakatta 1991) predisposes to coronary artery disease (MacMahon et al 1990), cerebrovascular events (Kannel et al 1981), and is an independent predictor of cardiovascular mortality (Levy et al 1990). Furthermore, increased arterial stiffening has also been demonstrated in subjects with increased cardiovascular risk including diabetes mellitus (Wahlqvist et al 1988, Salomaa et al 1995), hypertension (McVeigh et al 1991, Armentano et al 1991), hypercholesterolemia (Dart et al 1991, Giannattasio et al 1996), and end stage renal disease (Blacher et al 2002).

Regulation of large artery stiffness

Elastin and collagen are the major determinants of large artery stiffness, with smooth muscle originally thought to play a minor role (Wilkinson and McEniery 2004). Advancing age causes gradual destruction in arterial wall elasticity, with increasing demands placed on the collagenous element (O'Rourke 1976; Avolio et al 1983). This redistribution of the heterogeneous element in the vascular wall can be triggered by endothelial dysfunction (Nili et al 2002). However, recent work has highlighted the importance of smooth muscle in determining vessel stiffening. Vasoconstrictors such as noradrenaline can increase vascular stiffness and dilators such as hydrallazine and sodium nitroprusside have the opposite effect (Nichols and O'Rourke 2005). Such vasoactive pharmacological agents can alter vessel diameter by up to 30%, independent of changes in peripheral resistance or blood pressure (Latson et al 1988), demonstrating the important dynamic contribution of vascular smooth muscle to large vessel stiffness.

Whilst NO profoundly alters basal arterial tone, its effect on arterial stiffness remains unclear. However, the acute administration of a NO donor such as GTN produces changes in the peripheral pressure pulse that are consistent with a reduction in arterial stiffness (Cockcroft et al 1997). β-adrenergic drugs, in particular albuterol, act via the L-arginine-NO pathway to stimulate NO release and cause vasodilatation in resistance arteries (Cardillo et al 1997; Dawes et al 1997) via their action on β_2-adrenoreceptors. β_2-agonists have therefore been used to evaluate endothelial integrity in healthy controls (Hayward et al 2002).

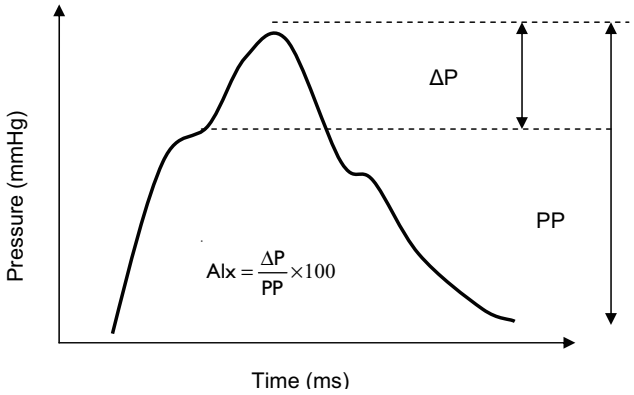

Figure 1 Representation of central arterial waveform.
Abbreviations: AIx; Augmentation Index; PP, pulse pressure.

Clinical assessment of arterial stiffness

Pulse wave analysis and pulse wave velocity

Method of assessment

The peripheral pressure waveform can be useful in determining cardiovascular risk. However, an adequate assessment of central arterial pressure waveforms cannot be determined from peripheral pulse wave analysis (PWA) because changes in vessel stiffening throughout the vascular tree causes location-dependent changes in the pressure waveform. In addition, central arterial waveforms will be influenced by the reflective wave phenomenon as described by Nichols and O'Rourke (2005). The systolic waveform leaves the aortic root and travels to the periphery, where smaller arterioles provide multiple points of reflection. A resulting 'reflective' wave is generated and returns to central arteries (O'Rourke and Kelly 1993). In individuals with healthy compliant arteries, the reflective wave will return to the central vasculature in diastole and augment diastolic coronary arterial blood flow. The speed of the advancing wave is termed pulse wave velocity (PWV) (Lehmann et al 1997). With age, a combination of increased reflective capacity at peripheral sites and faster PWV within stiffened vessels causes premature augmentation of the systolic waveform, forming a 'late systolic peak' on waveform analysis (Figure 1). This explains the differences between the brachial and aortic pressure waveforms, which may be as high as 20 mmHg (Pauca et al 1992). The central pressure waveform is important in view of determining left ventricular workload, which is relatively independent of the brachial pressure.

In view of the above observations, a technique has been derived by O'Rourke and Gallagher (1996), which is able to noninvasively record the peripheral pulse pressure wave and generate a corresponding central arterial waveform. The technique involves the use of an applanation tonometer to record the radial pulse wave. Applanation tonometry causes partial flattening of the arterial wall and equilibration of intra-arterial circumferential pressure. The accuracy of arterial tonometry in recording peripheral waveforms has been described by previous investigators who evaluated waveforms derived from noninvasive tonometry and direct arterial puncture (Cohn et al 1995). The central arterial waveform can subsequently be derived from the peripheral waveform using a validated generalized transfer factor (Karamanoglu et al 1993; Chen et al 1997; Takazawa et al 1998; Fetics et al 1999). This is then expressed in terms of an Augmentation Index (AIx) which can be used to assess vascular stiffness and cardiovascular risk between study groups.

Pulse wave analysis has more recently been used as a noninvasive tool to assess endothelial function (Wilkinson, Hall, et al 2002). Administration of β-agonist therapy induces repeatable reductions in the AIx, which are inhibited by infusion of L-N^G-monomethyl arginine (L-NMMA), suggesting that observed differences are endothelial- and NO-dependent (Wilkinson, Hall, et al 2002). Nitroglycerin administration induces further reductions in the AIx which are unaffected by L-NMMA and thus endothelial-independent (Wilkinson, Hall, et al 2002). Furthermore, the technique has been shown to correlate with FMD in healthy and type 2 diabetic subjects (Wilson et al 2004).

The analysis of PWV uses a similar system that calculates pulse wave propagation velocity between two sites, commonly the carotid and femoral pulses, or carotid and radial (Oliver and Webb 2003), although brachial-ankle PWV has been assessed by some (Katayama et al 2004; Igarashi et al 2005). Pulse wave velocity is inversely

proportional to vessel stiffness and distensibility (Nichols and O'Rourke 2005). Waveform data is recorded from two sites using noninvasive tonometry and stored electronically. Following documentation of the distance between the two recording sites, determination of the pulse transit time allows calculation of PWV. In order to assess pulse transit time a correlation point is identified within the pressure waveform, which may be the foot of the pressure wave (using SphygmoCor system) or the point of maximal upstroke (using Complior system) (Millasseau et al 2005). If the two pressure waveforms are not recorded simultaneously, an R wave on the electrocardiograph can be used to calculate wave transit time. Elevation of PWV leads to augmentation of the ascending aortic systolic waveform as previously outlined, resulting in higher left ventricular afterload and amplification of pulse pressure (Nichols 2005).

Pulse wave analysis and PWV are both noninvasive simple techniques that can be used to assess vascular stiffness in research and clinical settings (O'Rourke and Gallagher 1996; Hayward et al 2002; Sutton-Tyrrell et al 2005). Both techniques are influenced by factors that may confound data. For example, elevation in pulse rate will lower the AIx as a result of a reduction in reflective wave amplitude, and does not represent a change in vascular stiffness (Wilkinson, Mohammed, et al 2002). Consequently, a correction factor has been suggested to standardize for variation in heart rate (Wilkinson et al 2000). In addition, an inverse relationship between AIx, PWV, and height has been described, which may result from shorter reflective wave propagation time in individuals with short stature (McGrath et al 2001). While some investigators consider this a confounding variable in data analysis, it may explain the elevation in cardiovascular risk observed in short individuals (Kannam et al 1994). Despite the lack of data to confirm correlation between noninvasive and invasive measurements of PWV (Chiu et al 1991; Davies and Struthers 2003), the evidence that tonometer-derived PWV is an important determinant of cardiovascular risk is difficult to dispute.

Association with coronary artery disease and atherosclerotic risk factors

Pulse wave analysis has revealed accelerated large artery stiffening and endothelial dysfunction in association with several well established cardiovascular risk factors such as obesity (Suh et al 2005), end-stage renal failure (Covic et al 2003), and hypercholesterolemia (Wilkinson, Prasad, et al 2002). Similarly, PWV is increased in microalbuminuria (Smith et al 2005), renal dysfunction (Haydar et al 2004), type 2 diabetes (Tsuchiya et al 2004), and insulin resistance (Sengstock et al 2005).

Using PWA and PWV, vascular stiffness has been assessed and identified as an independent risk marker for cardiovascular mortality (Laurent et al 2001; Meaume et al 2001) and cerebrovascular events (Laurent and Boutouyrie 2005), and has a prognostic value equivalent to currently available biomarkers.

Elevations of central systolic pressure and consequent proatherosclerotic effects can be modified by vasodilatory compounds which reduce PWV in peripheral vessels without altering brachial blood pressure. Such reductions in augmentation of central pressure may underlie the observed benefits of cardiovascular drugs which are not currently attributed to blood pressure modification (Nichols 2005). Vascular stiffness has consequently gained increasing importance as a therapeutic target.

Photophlesymographic assessment of pulse wave reflection

Method of assessment

Photophlethysmography involves measuring the digital volume pulse via infrared light transmission through the finger. Previous investigators have demonstrated that the digital volume pulse resembles the carotid pressure wave and alters in a similar way to vasoactive mediators (Takazawa et al 1998). Reflected peripheral waveforms cause a second peak in the digital volume pulse (DVP) in a similar fashion to that seen in the peripheral pressure waveform, as measured using PWA (Chowienczyk et al 1999). With this technique, the point at which the reflective wave meets the systolic waveform is termed the inflection point (IP). When suprasystolic pressure is applied to both lower limbs at thigh level, the reflective wave returns sooner and causes an expected elevation of the IP (Chowienczyk et al 1999). In a similar way to the peripheral pressure wave, the DVP undergoes changes in response to exogenous NO donors such as GTN (Morikawa 1967; Lund 1986), which is independent of changes in heart rate (Chowienczyk et al 1999). Using both techniques, the major change seen is a reduction in the diastolic component of the waveform and the preceding IP (Chowienczyk et al 1999). This has been demonstrated recently in vivo, where acetylcholine-induced endothelial-derived NO release resulted in lowering of the IP in the photophlethysmographic waveform recorded in

cholesterol-fed rabbits. This response is diminished in cholesterol-fed rabbits when compared with healthy rabbits and antagonised by NOS inhibitors (Klemsdal et al 1994). As with PWA, pharmacological preparations which induce NO release, such as β-adrenergic drugs, will also cause a reduction in the IP of the pulse volume waveform (Chowienczyk et al 1999). Such effects are blunted by L-NMMA administration which suggests that this effect involves the L-arginine-NO pathway (Chowienczyk et al 1999).

Association with coronary artery disease and atherosclerotic risk factors

With evidence supporting the use of this technique to assess both endothelial-dependent and endothelial-independent vasodilatation, investigators have studied patient groups who are known to demonstrate marked endothelial dysfunction. Photophlethysmographic examination of type 2 diabetics has shown impairment of albuterol-induced responses with preservation of the endothelial-independent vasodilatation seen with GTN (Chowienczyk et al 1999). Vascular stiffness using this technique has also been described in cases of impaired glucose tolerance (Ohshita et al 2004) and hypertension in aging individuals, although some authors believe the technique to be inferior to PWV (Bortolotto et al 2000). However, this noninvasive technique provides a useful method of assessing vascular stiffening, in which both endothelial-dependent and independent responses can be determined (Millasseau et al 2002).

Biomarkers of endothelial function

As outlined previously, the antiatherogenic functions of the endothelium are complex. Several biochemical markers have been identified that correlate with coronary artery disease and conventional cardiovascular risk factors (Szmitko et al 2003). Further investigation of the inflammatory and thrombotic processes involved in atherogenesis will allow the assessment of potential biomarkers which may be incorporated into current cardiovascular risk stratification models. Such biomarkers include, oxidized low-density lipoprotein (oxLDL), high sensitivity C-reactive protein (CRP), endothelial progenitor cells (EPC), prothrombotic factors such as von Willebrand factor (VWF), and inflammatory markers including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and intracellular adhesion molecule-1 (ICAM-1) (Ridker et al 2001; Szmitko et al 2003). The clinical use of many of the biomarkers is restricted due to lack of end-point data (Tsimikas and Witztum 2001), with the exception of CRP which may correlate to a greater extent than traditional risk factors such as LDL-cholesterol (Ridker et al 2005). In consequence, CRP has already been incorporated into some risk stratification models.

Oxidized LDL

Oxidation of LDL occurs within the subendothelial cells of the vascular tissue which promotes the binding and transformation of monocytes to foam cells (Parhami et al 1993; Watson et al 1997). Mechanisms include enhancing chemotaxis and monocyte adhesion, upregulation of inflammatory genes and growth factors, causing endothelial cell dysfunction and apoptosis, enhancing platelet aggregation with thrombus formation, and inducing plaque destabilisation (Berliner et al 1995; Aikawa et al 1998; Norata et al 2002). Consequently, oxLDL levels are increased in cases of acute coronary syndrome and myocardial infarction (Tsimikas et al 2003).

Prothrombotic factors

The prerequisites for thrombogenesis in atheromatous plaques include activation of the coagulation cascade and platelet activation (Smith et al 2003). As such, prothrombotic factors are often evaluated in studies aimed at assessing cardiovascular risk. von Willebrand factor is a multimeric glycoprotein that is synthesised in endothelial cells and released following endothelial damage (Mannucci 1998). As such, levels can be indicative of the degree of endothelial injury and subsequent atherosclerotic potential (Mannucci 1998). Prospective trials suggest that elevated VWF may predict future cardiovascular events in patients with established coronary atherosclerosis (Jansson et al 1991; Thompson et al 1995), which may be a reflection of the role of VWF in initiating platelet aggregation and thrombus formation (Mannucci 1998). von Willebrand factor also has a role in stabilising factor VIII and the latter, together with fibrinogen, have been incorporated into cardiovascular risk profiles in numerous studies (Wilhelmsen et al 1984; Folsom et al 1999; Chambless et al 2003; Chaves et al 2004).

Endothelial progenitor cells

Endothelial progenitor cells have also gained importance as a potential surrogate marker of endothelial health (Schmidt-Lucke et al 2005). These are essentially stem cells

which are recruited to sites of endothelial injury in order to perform a therapeutic function by differentiating into mature endothelial cells (Szmitko et al 2003). Depletion of circulating EPCs with impaired adhesion to vasculature may reflect repeated and enhanced demand for EPC mobilization from bone marrow, and indicate a state of endothelial dysfunction predisposing to enhanced cardiovascular risk (Hill et al 2003). In support of this, reduced EPC levels with impaired activity have been demonstrated in subjects known to have impaired endothelial function, such as hypertension and ischemic heart disease (Vasa et al 2001) and their use for therapeutic intervention of vascular dysfunction continues to be evaluated (Silva et al 2005).

Inflammatory markers

Several inflammatory markers have been described in association with cardiovascular risk, including TNF-α, IL-6, ICAM-1, and CRP, of which CRP has greatest prognostic value (Ridker et al 2001; Ridker 2002). CRP is not only a marker of cardiovascular risk, but may itself function as a proatherogenic molecule (Szmitko et al 2003). The acute phase reactant has been demonstrated to enhance proinflammatory cytokines, such as IL-6, TNF-α, and monocyte chemoattractant protein-1 (MCP-1), promoting chemotaxis and lipid accumulation (Verma, Li, et al 2002; Li and Fang 2004). In addition, CRP may interfere with NO synthesis, inhibit angiogenesis, and influence vascular remodelling (Verma, Wang, et al 2002; Wang et al 2003). Although CRP correlates well with atherogenesis, it is feasible that elevation reflects the impact of traditional risk factors on inflammatory processes, as opposed to the direct influence of CRP on endothelial function (Vita et al 2004; Verma et al 2004).

Summary

As the number of individuals suffering from the 'metabolic syndrome' escalates, the cardiovascular morbidity and mortality rates of future generations may continue to rise despite advances in pharmaceutical interventions. Disruption of endothelial function is multifactorial and complex, and precedes clinically apparent coronary and cerebrovascular disease.

Therefore, the evaluation of reproducible, noninvasive techniques for assessing endothelial function should enable screening of large populations and may guide interventions designed specifically to reduce the individual's vascular risk.

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