Arterial stiffness or endothelial dysfunction as a surrogate marker of vascular risk

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The understanding of the pathophysiology of atherosclerosis has advanced greatly in the past decade. Cardiovascular risk factors increase the likelihood of an adverse event by having a detrimental effect on the blood vessel wall. Abnormal interactions among cholesterol, inflammatory mediators, platelets and the vascular wall lead to atherogenesis and cardiac events. In an effort to better understand this process, develop surrogate end points for clinical trials and, ultimately, better risk stratify individuals, a variety of measures of arterial function have been studied. These include measures of endothelial health and arterial compliance. The current paper reviews the various techniques available for the study of vascular health. While not yet routinely used for clinical care, these measurements provide important insights into the pathophysiology and treatment of atherosclerosis.

Key Words: *Arterial stiffness; Atherosclerosis; Compliance; Endothelium*

La raideur artérielle ou la dysfonction endothéliale comme marqueur de substitution du risque vasculaire

La compréhension de la physiopathologie de l'athérosclérose a beaucoup progressé depuis dix ans. Les facteurs de risque cardiovasculaire augmentent la possibilité d'événement indésirable en raison de leur effet néfaste sur la paroi des vaisseaux sanguins. Des interactions anormales entre le cholestérol, les médiateurs inflammatoires, les plaquettes et la paroi vasculaire provoquent une athérogénèse et des troubles cardiaques. Afin de mieux comprendre ce processus, d'élaborer des paramètres ultimes de substitution en vue d'essais cliniques et, en bout de ligne, de mieux stratifier les individus selon leur risque, diverses mesures de la fonction artérielle ont été étudiées. Ces mesures incluent celles de la santé endothéliale et de la compliance artérielle. Le présent article permet d'analyser les diverses techniques disponibles pour étudier la santé vasculaire. Bien qu'elles ne soient pas encore utilisées systématiquement en soins cliniques, ces mesures donnent d'importants aperçus de la physiopathologie et du traitement de l'athérosclérose.

The understanding of the pathophysiology of atherosclerosis and its vascular complications has increased dramatically over the past decade. Numerous measurable biomarkers play a role in the atherosclerosis process, and are associated with clinically important vascular events. Not only has their evaluation advanced our knowledge of the atherosclerotic process but it has also accelerated a search for new diagnostic tools to aid in risk stratification. Putative factors include soluble biomarkers and imaging assessments of vascular structure and function. The endothelium plays a key role in vascular homeostasis through the release of a variety of paracrine factors that interact with platelets, inflammatory cells and the vessel wall. The endothelium's central location allows it to sense and respond to a variety of perturbations. Endothelial dysfunction is an early marker of disease. Risk factors can also affect mechanical properties such as arterial stiffness, which is now recognized as a major contributor to systolic hypertension in elderly people. Methods of assessing endothelial function and arterial stiffness are available for clinical research and recent evidence suggests that these measures may provide important prognostic information.

ATTRIBUTES OF A SURROGATE BIOMARKER

Epidemiological studies, such as Framingham, have allowed longitudinal assessment of cardiovascular events and the development of simple scoring tables for risk calculation (1,2). These factors involved include blood pressure, lipid profile, smoking status and age. More recently, many factors thought to be associated with the atherosclerotic process have been studied. Some of these have been shown to correlate with clinical outcomes and have been deemed biomarkers (3,4). However, association does not necessarily imply causation and, due to a variety of limitations, most of these markers will not be useful for the clinical evaluation of risk or drug therapy (5). To become clinically important, a biomarker must fulfill the criteria for a surrogate marker. A surrogate substitutes for clinically important end points with respect to how an individual feels, functions or survives. Classic examples include blood pressure and low density lipoprotein cholesterol. These are targets of treatment. A surrogate should meet the following criteria:

- Adds independent information above currently used criteria;
- Is reliably and reproducibly measured;
- Accounts for a significant proportion of disease risk;
- Provides good predictive value;
- Is reasonably associated with the underlying pathophysiology;
- If it is to be used in risk prevention, is present before the clinical appearance of the outcome; and
- Is available and practical for widespread application (5-8).

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Surrogates must also stand the rigours of time and be well validated in multiple studies in a wide range of patient populations. The new biomarker that most closely meets these criteria is C-reactive protein (4,9-11).

ARTERIAL STIFFNESS

Blood pressure has long been known to be an important risk factor for coronary and cerebrovascular disease. While initial work concentrated on diastolic pressure, more recently, it has been recognized that systolic and pulse (difference between systolic and diastolic) pressures play a more important role, particularly with advancing age (12). Systolic pressure is influenced by arterial stiffness and increases continuously with age. It has long bothered physiologists that the shape of the waveforms in the proximal aorta differ markedly for pressure and flow. In the prevailing frequency-domain theory of blood pressure, pressure and flow waveforms are separated into mean and oscillatory components. This leads to the concept of reflected (backward-going) waves that can reinforce a forward-moving pressure wave (13-15). Recent elegant work by Wang et al (16) has suggested an alternative hypothesis. The arterial system can be thought of as a Windkessel type reservoir in which the level is controlled by peripheral resistance. A second component is very closely related to forward-travelling flow waves as a result of ventricular ejection (16). Aortic pressure is then the instantaneous summation of the reservoir pressure and the effects of the flow wave. In a situation in which velocity is increased within the system (increased arterial stiffness), a backward-travelling reflected wave could arrive back in the aorta in systole, further augmenting systolic pressure. This has a detrimental effect by increasing cardiac afterload and decreasing diastolic coronary filling. Alterations in the speed at which waves travel and the change in the arterial contour serve as the basis of evaluating structural properties of the arterial tree (17).

Arterial stiffness is determined in large part by the elastin to collagen ratio in their walls. The proximal large arteries (aorta and major branches) are quite elastic due to high elastin content. Aging leads to progressive arterial stiffness as a result of elastic fibre degeneration and atherosclerosis development. In addition, the elasticity of a given arterial segment is not constant. With increasing distending pressure, there is recruitment of collagen fibres leading to greater stiffness. Increases in heart rate can also change arterial characteristics and must be considered in these measurements as well (18,19).

Noninvasive methods of assessing arterial stiffness

Relating change in volume to distending pressure: There are many methods that have been used to measure arterial stiffness in vivo in humans (19). This discussion is restricted to those of a noninvasive nature. Arterial compliance is the relationship between change in vessel diameter (or area) for a given change in pressure. Bank et al (18) have described an ultrasound technique used to measure changes in brachial artery area while changing pressure over a wide range, with progressive occlusion with a blood pressure cuff. Radial artery waveforms are measured with applanation tonometry. This allowed the calculation of the following parameters:

- Compliance is the first derivative of area versus pressure curve
- Circumferential wall stress = pressure × midwall radius / intimal-medial thickness
- Circumferential strain = midwall radius / unstressed midwall radius (radius at 0 distending pressure)
- Young's incremental elastic modulus (E_{inc}) = 0.75 × stress / strain.
- Pulse wave velocity (PWV) = $[(E_{inc} \times \text{intimal-medial thickness}) / (2 \text{ blood density} \times \text{radius})]^{1/2}$ by the Moens-Korteweg equation.

In the study by Bank et al (18), nitroglycerin increased the compliance of the artery, decreased wall stress and decreased the elastic modulus (decreased stiffness). While this technique is reproducible and elegant, it is somewhat limited in that considerable expertise is required for the various measurements undertaken. In addition, it has not been extensively used in patients with risk factors to determine if differences can be detected in arterial compliance among different risk groups. Kinlay et al (20) used a modification of this technique and determined that nitric oxide (NO) is a regulator of arterial stiffness. This suggests an interplay between endothelial-derived factors that alter tone and structural properties of the arterial wall. Some investigators have assessed stiffness parameters by looking at diameter changes between systole and diastole (during a cardiac cycle). Ultrasound and tonometry would allow these measurements. While less physiological, these studies are easier to perform and the carotid artery can be studied directly (21). When applied at a population level, it has been clearly demonstrated that arterial stiffness increases with age (22,23). Magnetic resonance imaging has the potential to be useful in the measurement of aortic distensibility as well (24). A limitation of all of these approaches is that peripheral rather than central blood pressure was used in the calculation of stiffness. This can only be overcome by invasive approaches that use catheters within the aorta.

PWV: While PWV can be measured by the above approach, it can also be directly measured by simultaneous assessment of arterial waveforms in two locations. Velocity is then calculated as distance/time. Both applanation tonometry and Doppler ultrasound can be used to obtain this measure and can be applied to population-based studies. It has been widely studied and is a reproducible measure (25,26). The carotid and femoral arteries are often used. PWV increases directly with increasing arterial stiffness. Increases in distending pressure increase PWV and, therefore, levels of blood pressure need to be taken into account when comparing groups. The effect of heart rate is less clear but may be a confounding factor as well (17).

Systolic contour analysis: One of the predictable effects of an increase in PWV is augmentation of systolic pressure due to reflected pressure waves. A late systolic wave can be detected by contour analysis of a peripheral or carotid artery. Applanation tonometry with software algorithms has allowed widespread study of systolic contour. An augmentation index (AIx) is the ratio of the pulse pressure of the reflected wave divided by the overall pulse pressure. Karamanoglu et al (27) developed a method of studying peripheral arteries, using a generalized transfer function, a derived aortic waveform is generated. The SphygmoCor device (Atcor Medical, Australia) derives an AIx and can also calculate ejection duration (19). While the general transfer function has been validated (28), there is much controversy over the reliability of this measurement for the determination of arterial stiffness (29). The AIx is increased with increasing blood pressure and decreased heart

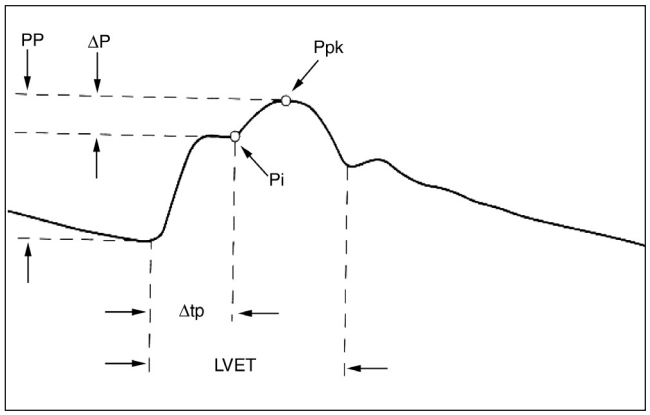

Figure 1) *Carotid arterial contour. A reflected wave due to increased arterial stiffness generates a systolic wave (Ppk). The difference between this wave and the initial forward-going systolic wave (Pi) is then divided by the pulse pressure (systolic – diastolic pressure [ΔP]) to generate the augmentation index (AIx). AIx = ΔP/pulse pressure (PP). LVET Left ventricular ejection time; Δtp Time to Pi*

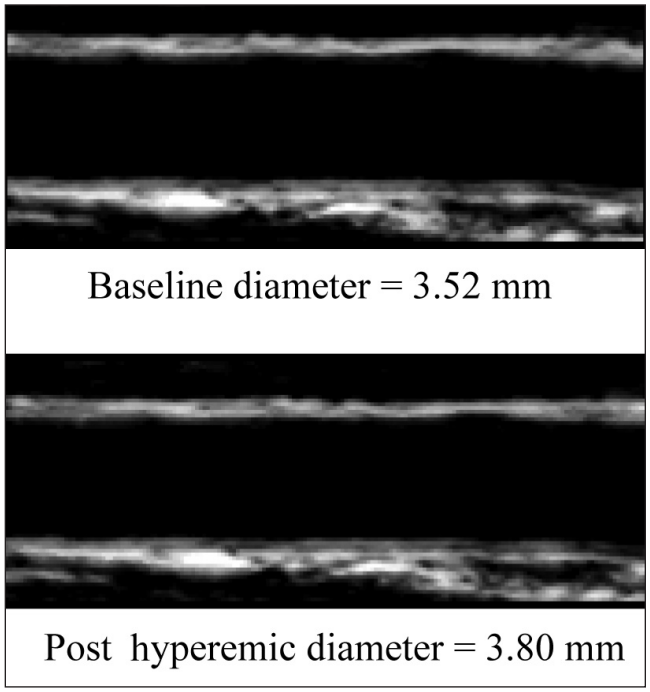

Figure 2) *Ultrasound image of the brachial artery. At rest, the diameter is 3.52 mm, and between 60 s and 90 s postocclusion, the hyperemic diameter is 3.80 mm. Flow-mediated dilation = 3.80–3.52/3.52 × 100 = 8.0%*

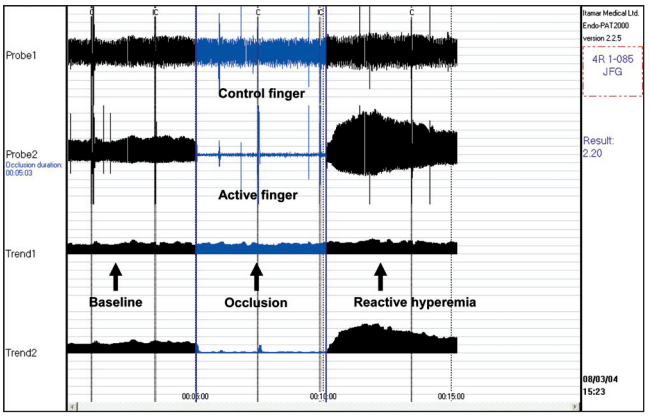

Figure 3) *Determination of endothelial function by peripheral artery tonometry. This finger tip plethysmograph measures pulse volume amplitude (PVA) in the index finger of both hands at rest, during a 5 min arm occlusion and then postocclusion. The PVA index is the relative increase in PVA postocclusion in the active finger compared with the control finger. Values greater than approximately 1.4 are normal*

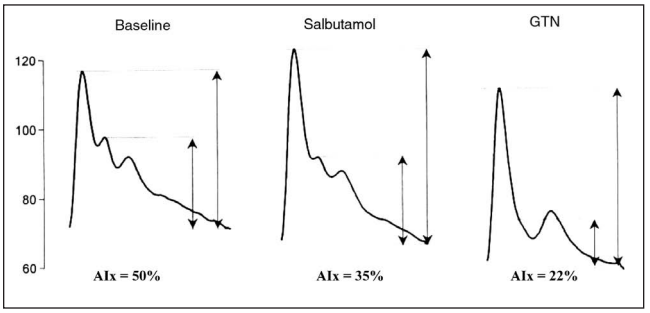

Figure 4) *Use of systolic contour to assess endothelial function. The augmentation index (AIx) is measured from a peripheral artery without a generalized transfer function. Measurements are made at rest, in response to the endothelium-dependent stimulus (inhalation of a beta$_2$-agonist) and in response to nitroglycerin (GTN). A fall in AIx with salbutamol is related to nitric oxide release and a decrease in arterial stiffness. Data from reference 118*

rate and by vasoactive drugs (30). Wilkinson et al (31,32) were able to demonstrate in healthy subjects that NO inhibition increased the AIx, again suggesting functional regulation of large artery stiffness by the endothelium. Some of the limitations of the generalized transfer function can be overcome by measuring contour at the carotid artery (26,33).

Diastolic contour analysis: Based on the Windkessel model of blood pressure, the decay of the reservoir pressure in part relates to peripheral resistance. An exponential decay curve represents capacitance (large) artery compliance and is referred to as C_1. The C_2 component provides a measure of small artery compliance (34,35). Again, peripheral arterial tonometry can be used to obtain the waveform for further calculations. Of the methods described, there is probably the most uncertainty in the reliability of this methodology.

Relationship of arterial stiffness to risk factors and their treatment

Since systolic pressure and pulse pressure increase with increasing age, it would be surprising if there were not a very important effect of age on measures of arterial stiffness (36). Indeed, this has been found in almost all reported studies. In a recent study of healthy Framingham participants, Mitchell et al (26) assessed PWV and carotid contour. Central arterial stiffness increased with increasing age to a greater degree than peripheral arterial stiffness (which is usually higher than aortic stiffness). Interestingly, reflected wave amplitude (AIx) was only weakly related to age. This suggests that central aortic stiffness and forward wave amplitude, as opposed to reflected waves, are the primary mechanisms for the increased central systolic and pulse pressure of aging. This increase in forward-going waves from the central aorta, not dampened by a concomitant increase in reflected waves to buffer the effect, could result in damaging

levels of pressure in a variety of microvascular beds including the brain and kidney (37). Studies previous to this have shown a stronger relationship between the AIx and age (38).

The relationship between blood pressure and arterial stiffness is more difficult to ascertain because blood pressure is a major covariate in the various measurements of stiffness. That being said, PWV and the AIx have been shown to be predictors of cardiovascular events in hypertensive subjects (25). In the previously mentioned study by Mitchell et al (26), mean arterial pressure was a strong predictor of PWV and the AIx. Other factors associated with increased aortic stiffness include diabetes (39), hypercholesterolemia (40) and end stage renal disease (41). Recent studies have also demonstrated an association between PWV and C-reactive protein, independent of age and mean pressure (42).

The effect of cardiovascular drugs on measures of arterial stiffness has been extensively studied and reviewed (17,36). Many of the agents tested also lower blood pressure, and this effect must be differentiated from any effects on structural remodelling or improvement in endothelial function that would favourably affect arterial stiffness. Agents that decrease arterial stiffness include nitroglycerin (18,20), angiotensin-converting enzyme inhibitors and angiotensin receptor blockers (43), and calcium channel blockers (44). The data for beta-blockers are not nearly as clear. Drugs that have favourable effects on outcomes and endothelial function, such as statins, have been shown to decrease arterial stiffness, but further studies are needed in this area (45,46).

Prognostic implications of alterations in arterial stiffness

A biomarker can only truly elevate itself to the status of a surrogate marker when it has been shown to be an independent predictor of cardiovascular events. While measures of arterial stiffness are pertinent research end points, their use as surrogate markers require further study. That said, several longitudinal studies by research groups in France have suggested an independent prognostic importance of measures of arterial stiffness. In a cohort of 242 subjects with end stage renal disease, Blacher et al (47) measured PWV and the AIx (carotid artery). Both measures were shown to be independent predictors of mortality in this cohort. Age was the other major contributor to outcomes (33,41,47). The same group also followed a large cohort of hypertensive individuals (n=710). PWV was higher in a group with atherosclerosis, and at a particular age, PWV was the best predictor of cardiovascular mortality (25). In a second large hypertensive cohort, Boutouyrie et al (48) and Laurent et al (49,50) were able to show that PWV independent of age and pulse pressure was a predictor of coronary events, stroke and all-cause mortality. The RRs were between 1.4 and 2.1. In a recent cross-sectional study, Weber et al (51) demonstrated a relationship between the AIx and angiographically proven coronary disease. Finally, a study of elderly men (23) established a relationship between carotid plaque burden and all cause mortality. However, carotid arterial stiffness (Young's modulus) added very little to risk predictions in this cohort. The data to date, while intriguing, certainly do not firmly establish an important prognostic role for measures of arterial stiffness. Ongoing observations from large randomized studies will be awaited with much interest.

ENDOTHELIAL DYSFUNCTION

The endothelium is a single-cell lining covering the internal surface of blood vessels. The strategic location of the endothelium allows it to sense changes in hemodynamic signals and respond by releasing a number of substances. A balance between endothelium-derived relaxing and contracting factors is critical in maintaining vascular homeostasis. When this balance is disrupted, it predisposes the vasculature to vasoconstriction, leukocyte adherence, platelet activation, mitogenesis, pro-oxidation, thrombosis, impaired coagulation, vascular inflammation and atherosclerosis (52).

NO is the key endothelium-derived relaxing factor, which plays a pivotal role in the maintenance of vascular tone and reactivity (53). In addition to being the main determinant of basal vascular smooth muscle tone, NO opposes the actions of potent endothelium-derived contracting factors such as angiotensin-II and endothelin-1. In addition, NO serves to inhibit platelet and white cell activation and maintain the vascular smooth muscle in a nonproliferative state. NO is synthesized from L-arginine under the influence of the enzyme NO synthase (NOS). NOS requires a critical cofactor, tetrahydrobiopterin, to facilitate NO production. Tetrahydrobiopterin deficiency leads to an 'uncoupling of NOS' with the resultant production of potent oxidants such as superoxide and hydrogen peroxide instead of NO. Superoxide inactivates NO to peroxynitrite, further decreasing NO activity in this uncoupled state. Cardiac risk factors in general lead to an increase in oxidative stress, attenuating net NO bioactivity and leading to endothelial dysfunction (54).

Methods of assessing endothelial function

Endothelial function refers to a physiological observation that is the result of stimulation of vasoactive substances released by or that interact with the vascular endothelium. Endothelium-dependent vasodilation can be assessed in the coronary and peripheral circulations in humans. In addition, measures of platelet function and inflammation or leukocyte activation are indirect measures of endothelial health. Most studies concentrate on vasomotor responses as a marker of endothelial function. There is no agreed on gold standard for the measurement of endothelial function.

TABLE 1
Methods to determine arterial stiffness and endothelial function

Artierial stiffness	Endothelial function
Compliance measures (diameter-pressure relationship)	Coronary vasomotion with QCA to Ach
Stress, strain, elastic modulus measurements from diameter-pressure relationship	Coronary blood flow with Doppler wire to Ach
Pulse wave velocity	Ultrasound determination of flow-mediated vasodilation
Peripheral contour analysis with generalized transfer function – AIx	Impedance plethysmography with brachial artery infusion of Ach
Central systolic contour analysis with tonometry to calculate AIx	Peripheral arterial tonometry with hyperemia
Diastolic contour analysis to determine C_1 and C_2 compliance	Soluble biomarkers of inflammation
Use of beta$_2$-agonists to measure change in AIx as measure of endothelial function	Studies of venous distensibility

Ach Acetylcholine; AIx Augmentation index; C_1 Large artery compliance; C_2 Small artery compliance; QCA Quantitative coronary angiography

TABLE 2
Prognostic value of arterial stiffness measurements

City, author, year (reference)	Patient status (n), measurement	Duration of study	Event	Conclusion
Paris, Blacher et al, 1999 (47)	241 ESRD patients, PWV	72 months	73 deaths	Age and aortic PWV were predictors
Paris, London et al, 2001 (33)	Same 180 ESRD patients, PWV and AIx	52 months	70 deaths	Both PWV and AIx were predictors
Paris, Blacher et al, 2003 (41)	Same 242 ESRD patients, PWV	78 months	91 deaths, 58 CV deaths	Used PWV index – advanced age was predictor
Paris, Blacher et al, 1999 (25)	710 hypertension patients, PWV	Less than two years	Not available	PWV related to FRS and CV mortality
Paris, Laurent et al, 2001 (49)	1980 HT patients, PWV	112 months	107 deaths, 46 CV deaths	PWV was independent predictor
Paris, Boutouyrie et al, 2002 (48)	1045 HT patients, PWV	5.7 years	97 CV events	PWV predicts Coronary events
Paris, Laurent et al, 2003 (50)	1715 HT patients	7.9 years	25 fatal CVAs	Age and aortic PWV predicts stroke
Rotterdam, Stork et al, 2004 (23)	367 men IMT, wall tracking United States	Four years	70 deaths	Stiffness not independent of FRS
Paris, Meaume et al, 2001 (123)	141 patients >70 years of age, PWV	Four years	56 deaths; 27 CV deaths	OR 1.16 for PWV
Sydney, Weber et al, 2004 (51)	465 men undergoing catheterization SphygmoCor (Altcor Medical, Australia)	Cross-sectional	Not applicable	AIx correlated with angiographic CAD

AIx Augmentation index; CAD Coronary artery disease; CV Cardiovascular; CVA Cerebrovascular accident; ESRD End stage renal disease; FRS Framingham risk score; HT Hypertension; IMT Intima media thickening; PWV Pulse wave velocity

TABLE 3
Prognostic significance of flow-mediated dilation

Source (reference)	Patient status	Duration of study	Patient end points	Conclusion
University of Vienna, Austria (114)	73 patients undergoing angiography	Five years	27 CV events	Not related to events
Boston University, USA (112)	187 elective vascular surgery patients	30 days	45 (25 TnI elevations) CV events	ED was independently predictive
University of Federico, Italy (109)	131 PVD patients	23 months	39 CV events	ED and ABI were predictive
University of Modena, Italy (113)	400 PM women with hypertension	67 months	32 CV events, change in ED to meds	No change in ED with BP medications was predictive
University of British Columbia British Columbia (110)	152 CAD patients in cardiac rehabilitation	34 months	22 CV events	ED and carotid plaque were predictive
University of Queensland, Australia (111)	444 at-risk patients	24 months	70 CV events	ED was not predictive

ABI Ankle-brachial index; BP Blood pressure; CAD Coronary artery disease; CV Cardiovascular; ED Endothelial dysfunction; PM Postmenopausal; PVD Peripheral vascular disease; TnI Troponin I

Coronary circulation: Quantitative coronary angiography can be used to examine the change in diameter to intracoronary infusions of the endothelium-dependent vasodilators such as acetylcholine. In healthy vessels, acetylcholine evokes an NO-mediated vasodilatory response; in patients with endothelial dysfunction, this effect is blunted, or paradoxical vasoconstriction may occur (55). Endothelial function of the coronary microvasculature (resistance vessels) can be assessed by employing intracoronary Doppler techniques and assessing coronary blood flow in response to pharmacological or physiological stimuli (56). Although considered by many to be the best assessment of endothelial function, this technique is limited by its invasive nature, expense and inaccessibility.

Peripheral circulation: Celermajer et al (57) were the first to describe a noninvasive assessment of flow-mediated vasodilation (FMD) in the brachial or femoral artery. Brachial artery occlusion for 5 min resulted in reactive hyperemia after the cuff was released (blood flow increased five- to sevenfold), and this increase in shear stress resulted in FMD, a NO-dependent process. Normal arteries dilate 10% to 15% depending on the laboratory, position of the cuff and equipment used. The variability is acceptable (approximately 2% absolute on repeated measures, with a coefficient of variation of 20%) (58). While differences exist in the performance of the test among research laboratories, recent guidelines have suggested uniform methodology to reduce variability (59,60). Brachial artery FMD has been shown to correlate with measures of coronary endothelial function (61). This technique has been extensively used with very good reproducibility and low observer variability.

Resistance vessel function in the forearm is assessed by strain-gauge venous impedance plethysmography. This methodology examines the change in forearm blood flow in response to direct intra-arterial (brachial artery) administration of agonists (62). This technique is excellent for acute interventions with repeated measurements. The major drawbacks again are reproducibility and its more invasive nature than ultrasound. A new finger plethysmographic technique offers some promise as a reproducible noninvasive tool (63,64).

Relationship of endothelial function to risk factors and their treatment

Endothelial injury with resulting dysfunction appears to be the initiating event in atherosclerosis (65-67) and plays an important role in the ischemic manifestations of coronary disease

(68). Ludmer et al (55) demonstrated that whereas acetylcholine induces epicardial coronary vasodilation in patients without atherosclerosis, paradoxical vasoconstriction occurs in patients with atherosclerosis. Atherosclerosis also impairs coronary resistance vessel function, despite the fact that resistance vessels are rarely affected by atherosclerosis (56,69). In addition, coronary atherosclerosis is characterized by peripheral vessel endothelial dysfunction (61).

Oxidative stress appears to play a pivotal role in the alteration of endothelial function associated with risk factors. Every traditional risk factor associated with accelerated atherosclerotic vascular disease has been associated with endothelial dysfunction in both animal and human models (70). Celermajer et al (57) demonstrated impaired endothelial function in children with familial hypercholesterolemia. This study was among the first in humans to demonstrate the important role of endothelial dysfunction early in the course of atherosclerosis. Similarly, abnormal vasomotion occurs in subjects with uncomplicated hypertension (71), diabetes (72-75), cigarette smoking (76,77) and low levels of high density lipoprotein (78,79). Oxidized low density lipoprotein plays an important role in abnormal endothelial vasorelaxation, leukocyte adhesion and endothelin production (80-83). In addition, oxygen free radicals impair endothelial function through direct inactivation of NO (84). While all traditional risk factors have been associated with peripheral endothelial dysfunction, a close quantitative relationship does not exist (85-88). This suggests that endothelial function measurements may add unique information not captured by Framingham risk scores. The potential effect of emerging risk factors on endothelial function needs to be fully explored. We have recently demonstrated no relationship between brachial FMD and C-reactive protein, for example (89).

We were among the first to demonstrate that low density lipoprotein reduction with an 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor improves coronary endothelium-dependent vasodilation (90). Numerous studies of lipid lowering have subsequently confirmed these results in a variety of patient populations (91-93). The subject of pharmacological treatment of endothelial dysfunction has been reviewed by myself and others recently (52,94,95). Suffice it to say, interventions associated with favourable cardiovascular outcomes have been associated with attenuation of endothelial dysfunction for the most part (96-101). The notable exception to this is hormone replacement therapy, which has been associated with improvement in endothelium-dependent vasomotion, but not cardiovascular outcome.

Prognostic implications of endothelial dysfunction

In the past four years, several retrospective studies have demonstrated an association between endothelial dysfunction and cardiovascular events (102). These studies have used coronary endothelial function testing (103-107), brachial impedance plethysmography (83,108) and brachial flow-mediated dilation (109-114). These studies in general are of small sample size (all less than 500 patients), retrospective in nature and included only individuals with documented vascular disease or hypertension. All of these recent studies showed an association between a measure of endothelial function and outcome, except for a recently reported Australian study of 444 patients that demonstrated that endothelial dysfunction was not predictive of mortality (111). Larger prospective studies are underway to more definitively address this question. These include a cohort from the Framingham study group (115) and a Canadian cohort of healthy middle-aged firefighters (Firefighters And Their Endothelium [FATE] Study) (116). These studies are required to clarify the prognostic significance of endothelial function testing.

INTERRELATIONSHIP BETWEEN ARTERIAL STIFFNESS AND ENDOTHELIAL DYSFUNCTION

Arterial stiffness and endothelial dysfunction represent different aspects of vascular disease. However, there is certainly some crosstalk between these two pathophysiological processes. NO is continuously released and has been shown to contribute to arterial compliance (20). Pharmacological agents that improve endothelial function, such as statins and angiotensin-converting enzyme inhibitors, also decrease arterial stiffness. This is related to favourable effects on endothelial-derived paracrine factors, structural remodelling and direct effects on blood pressure (117). Several authors have also described the use of pulse wave analysis to assess endothelial function. In these studies, the AIx was measured from a peripheral artery at baseline and then in response to an inhaled $beta_2$-agonist (endothelium-dependent). A reduction in the AIx was noted and this was blocked by NOS inhibition. In addition, the response was blunted in subjects with coronary artery disease or hypercholesterolemia (118,119). The original description of this approach used photoplethysmography to assess digital volume pulse (120). Arterial stiffness has also been demonstrated to be related to brachial artery FMD vasodilation in a recent small study (121). And finally, a recently published study by Mitchell et al (122) has challenged our thinking about the effect of risk factors on brachial endothelial function. In a large cohort from Framingham, it was demonstrated that hyperemic-induced shear stress was an important predictor of brachial FMD. In addition, risk factors diminished the magnitude of shear stress, suggesting that a reduced stimulus explains the diminution in endothelial function in subjects with cardiovascular risk factors (122).

CONCLUSIONS

The study of vascular biomarkers has greatly enhanced our understanding of the underlying pathophysiology of atherosclerosis. This includes soluble biomarkers and physiological parameters such as measures of arterial stiffness and endothelial function. It is unlikely that any new marker will be as important a surrogate marker as blood pressure or cholesterol. Certainly, endothelial function and arterial compliance in theory could be important markers, but difficulty in defining the best measure, modest reproducibility and lack of convincing prognostic studies to date have limited their clinical utility thus far. Ongoing prospective studies will determine whether surrogate status will be obtained in the next decade for either of these markers. Until that time, they will remain important research tools to further our understanding of vascular biology and to assess the impact of new risk factors and their treatment on the vessel wall.

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