Detection of presymptomatic atherosclerosis: a current perspective

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ABSTRACT
Atherosclerosis, the pathological process underlying myocardial infarction, stroke and other occlusive vascular disease, is the major cause of death in the Western world. The development of techniques to accurately and reproducibly detect and measure the early changes of atherosclerosis and/or to identify subjects at highest cardiovascular risk may aid in the development of prevention strategies and facilitate a decrease in morbidity and mortality from atherosclerosis. Increasing understanding of the pathophysiology of early atherosclerosis has allowed the development of a number of potential methods for the assessment of the early stages of atherosclerosis in humans. These include techniques for assessing early structural changes in the coronary arteries with electron-beam computed tomography and magnetic resonance imaging. External vascular ultrasound has also been used to image other circulations as a surrogate marker for coronary atherosclerosis, e.g. the measurement of carotid artery intima-media thickness. Early functional changes of atherosclerosis have also been described many years before the development of structural changes. A number of techniques have been developed to measure endothelial dysfunction, one of the earliest changes of atherosclerosis, including non-invasive measurement of endothelial function using external vascular ultrasound. A variety of serum markers have also been described, and may be useful markers of atherosclerosis. We discuss some of the more promising techniques for the detection of early, presymptomatic atherosclerosis.

INTRODUCTION
Atherosclerosis, the pathological process underlying myocardial infarction, stroke and other occlusive vascular disease, is the major cause of death in the Western world [1]. Although death rates from coronary artery disease have declined in the United States of America and many other industrialized countries during the last two or three decades, cardiovascular causes of death still account for 40–50% of deaths in these countries [2]. Importantly, if causes of premature death (under 70 years) are examined, coronary artery disease is by far the largest single cause of death. In addition, since the prevalence of atherosclerosis appears to be increasing in developing countries [1], it may become the single largest health problem worldwide in the near future.

Although there have been some recent improvements in the treatment of the manifestations of atherosclerosis, most of the improvements in mortality seem to be due to lifestyle changes in the community, such as smoking cessation and better eating habits, and to improvement in risk factor management [3]. Coronary atherosclerosis is a disease that has a long ‘lag phase’ between the early phases of the disease and the development of clinical syndromes [4]. Therefore interventions aimed at improving death rates from coronary artery disease may be more effective if the early stages of atherosclerosis are targeted, rather than secondary prevention and treatment of the complications of coronary artery disease. Macroscopic changes, such as fatty streaks and atheroma, are frequently present in the coronary arteries of teenagers and young adults [5], with up to half of children

Key words: atherosclerosis, endothelium, nitric oxide, ultrasound.
Abbreviations: ICAM, intercellular adhesion molecule; LDL, low-density lipoprotein.
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of 10–14 years of age having fatty streaks in their coronary arteries [6]. It has been demonstrated that endothelial dysfunction, a key early event in atherogenesis, may be present even in children as young as 6 years of age [7,8]. The endothelial dysfunction present in childhood and teenage years relates to the presence of traditional risk factors, including hypercholesterolaemia, a family history of premature coronary artery disease, hypertension and cigarette smoking. Intervention at such an early stage, before the establishment of advanced atherosclerotic plaques, might be expected to give the maximum possible benefit in reducing the late complications of atherosclerosis.

Older subjects also manifest endothelial dysfunction, as well as structural changes in the vessel wall, such as intimal thickening and overt atherosclerotic plaques. Both of these factors, and their interaction, are intimately involved in the development of clinical ischaemic syndromes [9]. Although there may be a continuum between the early stages of functional abnormalities and those subjects with advanced atherosclerosis, different strategies may be required in managing these groups. In subjects with established atherosclerosis, it may be more important to measure the structural changes of atherosclerosis to aid in guiding more aggressive therapeutic interventions.

The development of techniques to accurately and reproducibly detect and measure the early changes of atherosclerosis and/or to identify subjects at highest cardiovascular risk would allow the evaluation of therapeutic strategies, and may allow identification of those individual subjects likely to benefit from intervention. Increased understanding of the pathophysiology of early atherosclerosis has allowed the development of a number of potential methods for the assessment of the early stages of atherosclerosis in humans.

STRUCTURAL AND FUNCTIONAL CHANGES IN EARLY ATHEROSCLEROSIS

The process of atherogenesis is complex, and involves interactions between many cell types, the development of endothelial injury, vessel wall thickening and, ultimately, the formation of plaque [4]. In recent years there have been many advances in the understanding of early functional changes, and as these events generally occur before the development of structural changes in the vessel wall, they have been of particular interest to researchers. Such functional changes include decreased local availability of endothelial-derived nitric oxide, enhanced surface expression of cellular adhesion molecules, changes in markers of inflammation, production of cytokines, and oxidative modification of lipoproteins, among others [10–12]. Among the many functional changes in the early stages of atherosclerosis, the measurement of endothelial nitric oxide has been of particular interest, and a variety of both invasive and non-invasive techniques have been developed to study this in vivo [13]. Similarly, a variety of methods have been developed for studying some of the other functional changes, as manifest in peripheral blood samples [13], and these will be discussed below.

Structural alterations in the vessel wall occur at a later stage than functional changes; however, subtle changes, such as increased vessel wall thickness and calcification, may develop many years prior to the onset of symptomatic atherosclerosis. Investigators have studied the peripheral circulation with ultrasonography, measuring intima-media thickness and presymptomatic atherosclerotic plaque, either as a marker of disease in peripheral circulations or as a surrogate for the coronary circulation [14]. In addition, early changes in the coronary circulation have been documented with invasive techniques such as coronary angiography and intravascular ultrasound, and more recently with non-invasive techniques such as electron-beam computed tomography and magnetic resonance imaging [15–17].

Stress testing is used by some clinicians to screen individuals for presymptomatic coronary artery disease. Regardless of whether electrocardiographic, echocardiographic or nuclear imaging end-points are used, the technique relies on the presence of obstructive coronary artery lesions. As a result, there is a high incidence of false-positive stress tests in low-risk populations; in addition, considering that the majority of acute cardiac events occur at the site of non-obstructive plaques, the technique has a poor positive predictive value for future clinical events in asymptomatic subjects [18,19].

In detecting presymptomatic atherosclerosis, it is important that the technique used is reliable and reproducible. When utilized in clinical trials, it should be accurate and reflect the clinical outcome, and when used for screening individuals it needs to have good sensitivity and specificity. A number of measures have proven to be predictive of coronary artery disease in population studies, but not when applied to individual subjects [20,21]. In either case the test should be safe and free from unpleasant side effects, and ideally the technique should be non-invasive. At present no ideal test for the detection and measurement of early atherosclerosis exists; however, there have been many recent advances in this area, particularly with non-invasive imaging and the study of peripheral blood samples [13].

ARTERIAL ENDOTHELIAL FUNCTION TESTING

Endothelial dysfunction is an early event in the development of atherosclerosis and plays a central role in
the pathogenesis of atherosclerosis, as well as in the progression to clinical syndromes of ischaemia [4]. As a result, the measurement of endothelial dysfunction has been of interest to investigators studying both the early stages of atherogenesis and its later consequences. A method for studying endothelial function in vivo was first described by Ludmer et al. in 1986, involving the assessment of the conduit vessels of the coronary circulation [22]. This technique is invasive, requiring cardiac catheterization, and involves the measurement of changes in coronary artery diameter in response to intra-arterial infusions of various pharmacological agents. Although this technique has proven to be a valuable research tool, giving many insights into coronary artery physiology [23–27], it has the disadvantage of being invasive. The risk of complications from cardiac instrumentation has made serial studies using this method unattractive, and studies of truly early atherosclerosis, which may be present in asymptomatic children and young adults [6], practically impossible.

Some inroads have been made in the non-invasive measurement of endothelial function in the coronary microcirculation, as manifest by coronary flow reserve. Using transthoracic doppler/echocardiography, it has recently become feasible to assess flow in the left anterior descending artery, and thus to assess coronary flow reserve in this vascular territory [28]. Although this technique is not suitable in all subjects and is in the early stages of development, it may become more useful as ultrasound technology improves. Similarly, cardiac magnetic resonance imaging with breath-holding and phase-contrast velocity mapping has allowed the non-invasive assessment of coronary flow reserve [29]. This technique is also at an early stage of development, and further investigation is required to determine its clinical utility. Positron emission tomography also allows measurement of myocardial blood flow, utilizing isotopes such as ¹⁸O-labelled water [30]. This technique has been used to demonstrate reduced coronary flow reserve in subjects without overt coronary artery disease but with coronary risk factors such as hypercholesterolaemia [31,32]. Its widespread use, however, may be limited by a lack of access to such technology, its expense, and the problem of radiation exposure.

**Peripheral large vessel function**

At present, invasive methods remain the only well-established means of assessing both large and small vessel coronary endothelial function in vivo. For this reason, measurement of endothelial function in peripheral arterial beds as a surrogate for the coronary circulation has been assessed. In vivo measurement of endothelium-dependent dilatation in peripheral arteries using ultrasound was first described in 1992 [7]. This technique uses high-resolution external vascular ultrasound to measure changes in arterial diameter (in most cases in the brachial or femoral artery) in response to reactive hyperaemia. This hyperaemic response is induced by inflating a pneumatic cuff distal to the imaging site to suprasystolic pressure for approximately 4 min, resulting in transient ischaemia distally. This results in increased flow within the study artery; in normal arteries, this is known to cause an increase in the activity of endothelial nitric oxide synthase, via the activation of shear force receptors on the cell surface. Following this, the artery is assessed using an endothelium-independent stimulus such as the administration of sublingual nitroglycerin, thus assessing the vessel’s intrinsic ability to dilate. It has been shown that this flow-mediated dilatation is dependent mainly on the endothelial release of nitric oxide, as it can be almost completely eliminated by the intra-arterial infusion of the nitric oxide synthase inhibitor N⁶-monomethyl-L-arginine [33]. In addition, the peripheral response in the brachial artery has been shown to correlate closely with endothelial function measured invasively in the coronary arteries [34], and with the severity of coronary artery disease [35].

It has been possible using this technique to demonstrate that endothelial dysfunction is present in children as young as 6 years of age who have risk factors for coronary artery disease, such as familial hypercholesterolaemia or passive smoking [7,8,36]. Investigators have shown that healthy adults with risk factors such as hypercholesterolaemia, older age, cigarette smoking, diabetes and male gender may have abnormal endothelial function [37–40], and that these risk factors may interact in producing impairment of endothelial...
function [38] (Figure 1). The technique is non-invasive, well tolerated and reproducible [41], factors that have facilitated its use as an end-point in clinical trials of interventions aimed at improving endothelial function. A number of clinical trials have utilized the measurement of brachial artery flow-mediated dilatation to evaluate such interventions in both acute and longer-term studies [42–45]. For example, Clarkson and colleagues [43] found that 1 month of dietary supplementation with oral L-arginine in healthy hypercholesterolaemic young adults resulted in an improvement of flow-mediated dilatation from 1.7% to 3.6%. Similarly, Kugiyama et al. [44] recently found that 1 month of supplementation with α-tocopherol in older subjects produced a 3.3% improvement in flow-mediated dilatation.

Although this technique has now been used extensively in placebo-controlled trials and cross-sectional population studies, its utility in assessing the prognosis of individual subjects requires further evaluation. In this respect, it may be limited by a number of factors. The technique is difficult to perform, being dependent on an experienced operator. In addition, results are influenced by a number of factors, such as vessel size and fasting state, and there is diurnal variation [45,46]. For these reasons, the use of brachial artery flow-mediated dilatation measurement cannot be recommended in routine clinical practice, although this may change as improved scanning methods and more data become available.

Peripheral microcirculation

Techniques have also been developed to evaluate microvascular endothelial function in peripheral systemic circulations. One such method is that described by Panza et al. [47] for measuring endothelium-dependent vasodilation in the forearm circulation with plethysmography, in response to intra-arterial infusions of endothelium-dependent vasodilators. This technique has demonstrated abnormal microvascular endothelial function in the presence of coronary risk factors [48,49], and has shown that these abnormalities can be acutely reversed with certain interventions [50,51]. Although this method is less invasive than coronary angiography, it still requires the placement of an intra-arterial catheter. Other less invasive techniques of measuring small vessel function have been described, e.g. measurement of laser doppler fluximetry responses to acetylcholine and sodium nitroprusside delivered by iontophoresis [52]. It is not known, however, whether the changes in the microvascular circulation are related to the development of atherosclerosis in larger conduit vessels, or whether forearm microvascular responses correlate with coronary physiology.

Despite the important information gained from studies of endothelial function, there are only limited data to link endothelial dysfunction with later clinical outcomes [53]. There is evidence to suggest that the degree of endothelial dysfunction may correlate with the severity of coronary artery disease [54]; however, the prognostic value of finding endothelial dysfunction in individual subjects is unknown. Large prospective studies, with clinical outcome measures, are required to assess whether asymptomatic, healthy subjects with endothelial dysfunction are at increased risk of later cardiac events.

MEASUREMENT OF BIOCHEMICAL, MOLECULAR AND CELLULAR CHANGES

Although a multitude of factors have been measured in the blood of subjects with established atherosclerosis, as yet no blood test exists that can reliably and accurately predict the presence and severity of atherosclerosis. Most attention has focused on the measurement of major serum risk factors, such as total cholesterol, low-density lipoprotein (LDL)-cholesterol and lipoprotein (a), which correlate with the extent of coronary artery disease with r-values in the range 0.2–0.3 [55–57]. Serum and lipoprotein levels of antioxidant vitamins, and the level of oxidation of LDL, have been disappointing predictors of coronary artery disease [58]. Although the susceptibility of LDL to oxidation has been correlated with the presence of some coronary risk factors [59], there is little evidence to suggest that this will be a useful measure of atherosclerosis in vivo in human subjects. Products of lipid peroxidation, such as F2-isoprostane, have been found in relation to risk factors such as smoking and in the presence of coronary artery disease [60,61]; these, as well as novel products of lipid oxidation and lipoprotein-bound oxysterols such as 7-oxocholesterol, may prove to be useful markers of early atherosclerosis in the future [62].

In response to various insults promoting endothelial damage, endothelial cells and products of such cells have been detected in peripheral blood samples. Some of these have been investigated as potential markers of atherosclerosis. Levels of von Willebrand factor, a glycoprotein involved in haemostasis and produced by endothelial cells, are elevated in the setting of endothelial damage [63]. Furthermore, levels of von Willebrand factor are elevated in hypercholesterolaemia, and may be reduced after lowering of cholesterol levels [64]. Tissue plasminogen activator and plasminogen activator inhibitor-1 are produced by endothelial cells, and the physiological balance of these two factors is involved in controlling fibrinolysis. Levels of both may be increased in response to endothelial cell insults, such as those mediated by hypercholesterolaemia [65]. These levels also correlate with increased carotid intima-media thickness, as well as with the subsequent risk of vascular events [66,67]. Adhesion molecules produced by endothelial cells may be released into peripheral blood due to endothelial cell
changes within the coronary arteries, from the detection of very early intimal thickening to the measurement of plaque volume in advanced atherosclerosis [79–81]. Utilizing this technique, early presymptomatic changes have been documented in high-risk subjects, such as cardiac transplant recipients [79]. While the information derived from an intravascular ultrasound examination is potentially of great interest, its use as a research tool is limited by the expense of the procedure, and more importantly by its invasive nature [82]. As a result, much interest has focused on the use of non-invasive external vascular ultrasound to examine other vascular beds as surrogate markers of coronary artery disease.

Measurement of vessel wall thickness is feasible in superficial arteries, such as the carotid and femoral arteries, using external vascular ultrasound. Carotid artery intima-media thickness in particular has been proposed as an early manifestation of atherosclerosis, and its measurement has been used as an end-point in clinical trials of disease regression [83–85]. Carotid intima-media thickness, measured with B-mode ultrasound, correlates well with the distance between the vessel lumen and the periadventitia–adventitia interface [86]. This measurement can be made reliably and reproducibly in the common carotid and bifurcation, and to a lesser extent in the internal carotid [87]. Ultrasound has not proven to be as useful in the reliable measurement of actual plaque size or volume; however, it may prove to be useful in assessing the morphology of plaques, such as in demonstrating intraplaque haemorrhage [88,89].

Carotid intima-media thickness has been shown to increase with age and to correlate with atherosclerosis in other vascular beds [90], as well as with the presence of vascular risk factors such as older age, cigarette smoking, increased LDL-cholesterol, hypertension and diabetes [83,85,91]. Increased carotid intima-media thickness may pre-date the development of symptomatic atherosclerosis by many years, and it has been demonstrated in children at risk of atherosclerosis as young as age 7 years [92]. Furthermore, several studies have linked increased carotid intima-media thickness with an increased risk of subsequent cardiovascular events [83,93] (Figure 2). These factors, as well as the simple, non-invasive and rapid nature of carotid ultrasound, have made it an excellent technique for serial studies of subjects in clinical trials.

A number of investigators have therefore used such measurements as an end-point in trials of atherosclerosis regression [84,85]. For example, the CLAS trial demonstrated a reduction in carotid intima-media thickness of 0.05 mm over 3 years in subjects receiving cholesterol-lowering therapy, compared with a progression of 0.07 mm in subjects with placebo therapy [85]. Although measurement of intima-media thickness in the carotid artery has been useful in such clinical trials, the small magnitude of the changes seen has limited its serial use to trials studying large numbers of subjects. Whether carotid
ultrasound will prove to be a useful tool in guiding treatment of high-risk subjects and following their progress requires further evaluation; however, its use as a surrogate measure in predicting the presence of clinically relevant coronary artery disease may be limited by a lack of precise correlation [20].

Arterial wall thickness in other vascular beds has been less extensively studied than in the carotid circulation; however, there is some evidence that femoral artery intima-media thickness correlates with coronary artery atherosclerosis [88], and that descending aortic plaque, as assessed by trans-oesophageal echocardiography, may relate to the presence of coronary artery disease [94]. A variety of other simple functional measures have been described that have been used in large-scale epidemiological studies as markers of pre-symptomatic atherosclerosis. For example, the ankle/brachial pressure indices are often used in studies of atherosclerosis and risk factors [95], although they are probably more useful in the assessment of asymptomatic, but established, disease. Also, their predictive power for coronary artery disease makes them less useful in individual subjects. These techniques require further evaluation.

**ELECTRON-BEAM COMPUTED TOMOGRAPHY**

Ultrafast computed tomography is a new imaging technique using electron-beam technology that allows image acquisition at a very rapid rate. The speed of its scanning rate makes it useful in cardiac imaging, where movement during the cardiac and respiratory cycle has limited conventional computed tomography [96]. Calcium makes up approximately 20% of plaque volume, and the association of coronary artery calcium with atherosclerotic plaque has been well known for many years [97]. In addition, the deposition and turnover of calcium within atherosclerotic plaques is an active process, with cells within plaques elaborating osteocalcin and osteopontin, among other products [98]. Despite this, however, the deposition of calcium within plaques is not uniform. Smaller plaques, which may be active, as well as some high-grade stenoses may completely lack detectable levels of calcium [99]. Until the development of ultrafast computed tomography, however, accurate imaging of this calcium in vivo was not possible. This detection of coronary calcium seems to correlate well with histological changes [15,100], and several studies have documented sensitivity for detecting obstructive coronary artery disease in the range 80–100% [98]. Although the specificity is less impressive, some of the studies have found a relationship between the degree of coronary calcification and the risk of subsequent coronary events [101].

Serial changes in coronary calcium content have been documented in clinical trials of lipid lowering [101], although it is not certain that these changes reflect changes in plaque volume. Ultrafast computed tomography may prove to be a useful measure in evaluating the progression/regression of coronary atherosclerosis. Unfortunately, the technique has a low specificity for clinically relevant obstructive coronary artery disease [102], and as some plaques lack calcium altogether its positive predictive value is not sufficient to replace coronary angiography. In addition, coronary angiography remains relatively safe [103], allows imaging of the complete coronary circulation, and may serve to guide therapeutic interventions such as coronary angioplasty. Although electron-beam computed tomography has proven useful in a variety of large epidemiological studies, more prospective data are required to determine its utility in clinical decision-making situations. Initial data, however, suggest that the technique may not accurately predict clinical events in individuals. Recently Detrano and colleagues [21] studied 1196 high-risk individuals and found that the presence of calcium in coronary arteries did not accurately predict future cardiac events. For these reasons, as well as those of cost and radiation exposure, the technique cannot yet be recommended as a routine screening test in high-risk subjects [104]. Some of these problems may be overcome in the future with the use of contrast enhancement and the development of scanners with improved resolution.

**MAGNETIC RESONANCE IMAGING**

Magnetic resonance imaging is a potentially useful tool for assessing early atherosclerosis, as not only is it non-
invasive and safe, but it yields information on flow as well as tissue characterization [29,105]. Magnetic resonance imaging is used routinely in clinical practice to assess arteries in the peripheral circulation, in particular the cerebral arteries and the aorta [106]; however, the application of magnetic resonance imaging to the coronary circulation has been limited by the relatively small size of these arteries and the rapid movements they undergo during the cardiac and respiratory cycles. Application of magnetic resonance imaging to the study of the coronary circulation is particularly attractive, as it would allow simultaneous acquisition of information on the anatomy and ultrastructure of coronary arteries, as well as cardiac perfusion, metabolism and function. The use of ultrafast magnetic resonance imaging angiography with breath holding and electrocardiographic gating still provides only a relatively poor image of the coronary lumen, resulting in poor sensitivity for the detection of coronary atherosclerosis [17]. Magnetic resonance imaging has been used effectively to measure plaque and to visualize plaque ultrastructure in arteries ex vivo, as well as in experimental animal studies and some human superficial arteries in vivo [107]. These studies have yielded important information regarding plaque architecture and behaviour under various conditions. Although the use of magnetic resonance imaging is limited by cost, the ability to apply this technique to the coronary circulation would represent an exciting development in the study of presymptomatic atherosclerosis.

NUCLEAR MEDICINE

The majority of nuclear medicine techniques have proven disappointing in the imaging of preclinical atherosclerosis, or even clinically relevant lesions. Techniques such as the use of radiolabelled lipoproteins and immunoglobulins have been limited by poor target-to-background ratios in the coronary and carotid circulations [108]. Peptides and monoclonal antibody fragments directed at different components of the atherosclerotic plaque and labelled with Technitium-99m have shown some promise in early studies, and radiolabelled peptides directed against the glycoprotein IIb/IIIa receptors on activated platelets may have some use in imaging intracoronary thrombus [30].

CONCLUSIONS

Increased understanding of the pathophysiology involved in the early stages of atherosclerosis has led to a proliferation of methods to detect the early stages of the disease in high-risk subjects. Techniques have been developed to assess early functional changes, such as the loss of endothelial nitric oxide production, and to assess early structural changes, such as increased intima-media thickness and coronary calcium. Although these tests have proven useful in defining risk in large population studies and in clinical trials, no one test currently exists that is suitable for screening individuals in clinical practice. Without a reliable test to identify high-risk individuals, the task of targeting those who may benefit from early intervention is difficult. At this stage novel blood tests, ultrasound imaging of arteries and coronary artery computed tomography appear to be the most promising avenues for future research. The increasing incidence of atherosclerosis worldwide, and increasing pressure on health resources, make the development of such a screening test a vital aim of cardiovascular research at the start of the new millennium.

REFERENCES


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