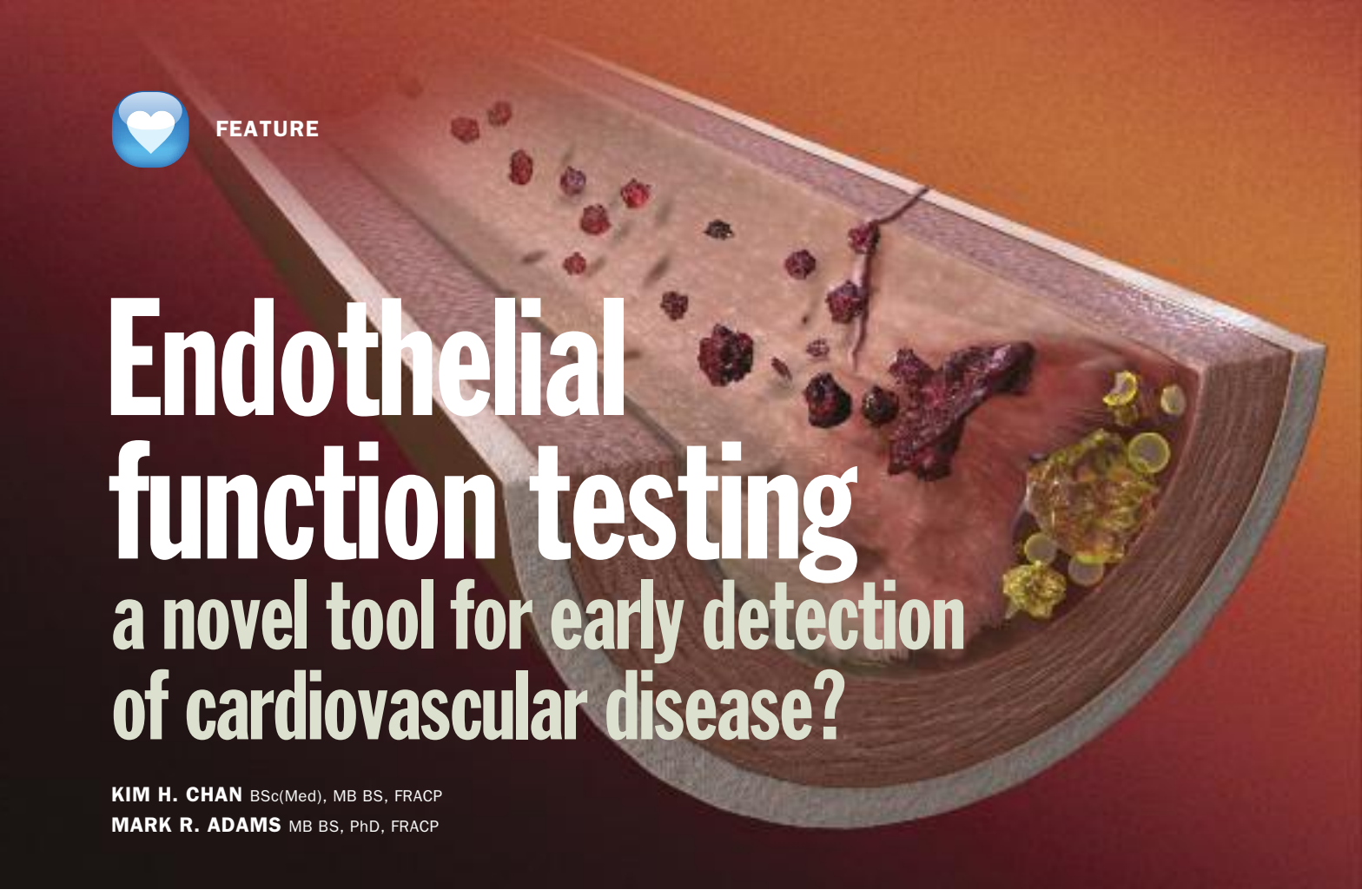

FEATURE

Endothelial function testing

a novel tool for early detection of cardiovascular disease?

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Endothelial dysfunction is a key early event in atherosclerotic disease. Testing of endothelial function may therefore allow early detection of those individuals at risk of atherosclerotic disease.

The endothelium plays an important role in vascular homeostasis and health. Rather than being just a single layer of cells lining blood vessels, it is closely involved in a wide range of functions, including regulation of vascular tone, thrombosis and inflammation. The disruption of any of these regulatory processes will result in endothelial dysfunction, which is an early event in the development of atherosclerosis. The progression of atherosclerosis over time may result in plaque instability, rupture and subsequent thrombosis. Indeed, several studies have reported a link between endothelial dysfunction and risk of cardiovascular events.[1]

Given that endothelial dysfunction may be potentially reversible, early detection of this disorder may have prognostic implications. This has led to the development of several clinical tools for assessing endothelial function. This article provides an overview of the techniques used to assess endothelial function, and discusses the link between endothelial dysfunction and cardiovascular risk factors.

Key points

- **Endothelial function testing is an emerging tool for early detection of vascular disease, especially in young asymptomatic patients, and has the potential to improve cardiovascular outcomes. These patients often first present to their GP for a routine medical 'check-up'.**
- **Endothelial dysfunction is associated with a range of traditional cardiovascular risk factors and worse outcomes.**
- **Endothelial function testing that is noninvasive, easy to use, accurate and reproducible is now available.**
- **Studies demonstrating early detection of vascular disease with endothelial function testing leading to improved clinical outcomes are currently lacking; therefore further studies are needed before the widespread and routine clinical use of this testing can be recommended.**

CARDIOLOGY TODAY 2011; 1(2): 12–16

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The clinical importance of endothelial dysfunction

It is intuitive from endothelial dysfunction being a key early event in atherosclerosis that impaired endothelial function will lead to increased risk of future cardiovascular events. A study reported that endothelial dysfunction diagnosed on coronary angiography predicted progression of atherosclerosis and cardiovascular events over a median follow up of 7.7 years; the results remained significant even after adjusting for traditional cardiovascular risk factors.[2] Another study reported that 14% of patients with severe endothelial dysfunction had cardiac events (myocardial infarction, coronary revascularisation and/or cardiac death) during follow up of more than two years, while those with normal function or mild dysfunction had no cardiac events.[3] The results from these studies therefore suggest that endothelial function testing may be useful in assessing risk of future cardiac events.

Endothelial function testing

The identification of the endothelium-derived vasodilatory factor nitric oxide led to a flurry of research findings on vascular tone and function. The importance of the discovery of nitric oxide's biological functions is indicated by nitric oxide being proclaimed 'Molecule of the Year' in 1992.

As loss of endothelium-dependent vasodilation occurs in the earliest stages of atherosclerosis, this discovery subsequently led to the development of endothelial function testing as a potential tool in clinical practice for the early detection of atherosclerotic vascular disease, a leading cause of morbidity and mortality worldwide. The initial techniques used for assessment of endothelial function were invasive and required arterial instrumentation, thereby potentially posing complications. However, with improvements in technology, noninvasive techniques have since been developed to allow for more widespread clinical use. The methods are described below and summarised in the Table.

Invasive assessment of endothelial function

The assessment of endothelial function in the human coronary circulation was first performed during coronary angiography and involved infusion of a variety of vasodilatory agents.[4] The change in arterial diameter to these agents was determined and compared with the baseline measurement, giving a measure of endothelial function. Interestingly, paradoxical vasoconstriction can be observed after administration of vasoactive agents in the presence of endothelial dysfunction.[4] Similarly, endothelial function of peripheral arteries (e.g. brachial artery) can also be measured.

Endothelial dysfunction detected using the above technique has been shown to correlate with early atherosclerotic disease, as well as vascular risk factors including hypertension, diabetes, hypercholesterolaemia, smoking and age.[5] However, this technique requires arterial puncture and performance of coronary angiography, and is therefore limited by its invasive nature. Although the risk of angiography is small, the potential complications from cardiac instrumentation make serial studies and studies of very early atherosclerosis (as may occur in children or asymptomatic young adults) unattractive. Therefore, less invasive techniques for measuring endothelial function have been developed to overcome these issues.

Noninvasive assessment of endothelial function

Brachial artery flow-mediated dilatation

In 1992, an Australian researcher, David Celermajer, first described a noninvasive technique of measuring arterial endothelial function. The technique used high resolution external vascular ultrasound to measure changes in brachial artery diameter in response to reactive hyperaemia.[1] It is based on the concept that the increased shear stress (provided by the reactive hyperaemia) imposed upon the endothelium leads to a vasodilatory response and thus increased arterial diameter, otherwise known as flow-mediated dilatation. Briefly, the baseline brachial artery diameter is measured at rest using vascular ultrasound.

Table. Methods for assessing endothelial function

Technique	Measurement involved	Invasiveness	Advantages	Disadvantages
Coronary angiography	Changes in coronary artery diameter or blood flow	Invasive	Accurate and reproducible; predicts clinical outcomes	Invasive; hence not suitable for serial assessments
Ultrasound flow-mediated dilatation	Changes in brachial artery blood flow	Noninvasive	Noninvasive; accurate and reproducible in experienced hands; predicts clinical outcomes	Measurement influenced by external factors; requires operator expertise; lack of standardisation
Pulse amplitude tonometry	Changes in digital pulse volume amplitude	Noninvasive	Noninvasive; accurate and reproducible	Lack of data on clinical outcomes
Circulating endothelial progenitor cell levels	Endothelial progenitor cell levels measured on a blood test	Minimally invasive	Simple blood test; minimally invasive; correlates with cardiac risk factors	Minimal data on clinical outcomes

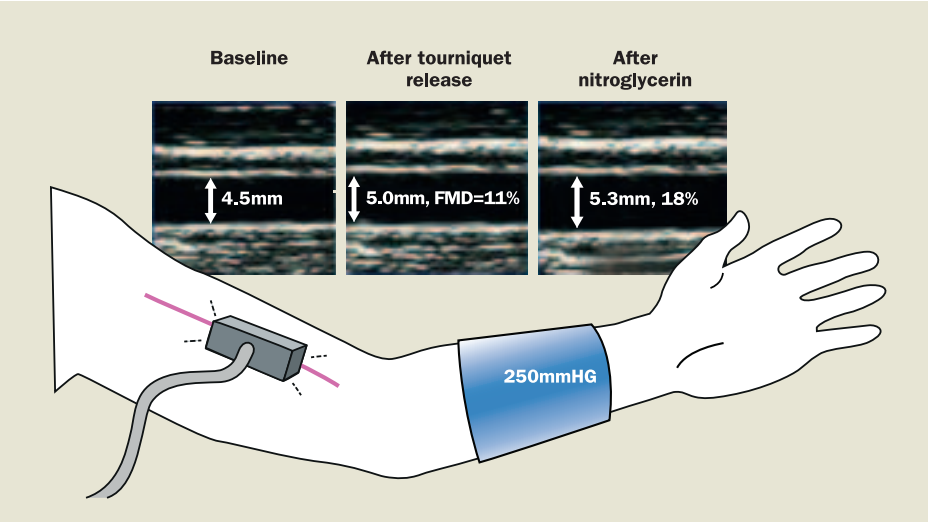

Figure 1. Brachial artery flow-mediated dilatation (FMD) using vascular ultrasound. The brachial artery diameter is increased compared with the baseline measurement in reactive hyperaemia (after release of a tourniquet distal to the imaging site inflated to above systolic pressure, i.e. to about 250 mmHg) and after administration of glyceryl trinitrate, giving a measure of endothelial function. (Illustrations courtesy of Professor David Celermajer.)

Hyperaemia is then induced by inflating a tourniquet to above systolic pressure around the forearm, distal to the imaging site. The tourniquet is then released, resulting in reactive hyperaemia, and the change in brachial artery diameter in response to this is measured, giving a measure of endothelial function (Figure 1). Likewise, the subject can be administered glyceryl trinitrate, and the measurement of change in brachial artery diameter obtained.

It has been reported that endothelial dysfunction assessed using flow-mediated dilatation is associated with cardiovascular risk factors including hypercholesterolaemia, both active and passive smoking, and older age.[6] Furthermore, these brachial artery responses have been shown to correlate with coronary artery endothelial function and extent of coronary disease, as well as risk of future cardiovascular events.[5]

The greatest advantage of flow-mediated dilatation is its non-invasive nature, thereby allowing serial assessments of endothelial function. In addition, the test usually takes only 30 to 40 minutes to perform, is well tolerated and is relatively accurate and reproducible in experienced hands. It can be technically challenging, however, and it requires specific training. Other disadvantages are that it can be influenced by intercurrent viral illness, it can be transiently impaired after a meal and it varies with ambient temperature. To overcome these issues, different laboratories use variations of the technique. Unfortunately this has resulted in a lack of standardisation, making interpretation of flow-mediated dilatation data across different laboratories difficult. Given these issues, endothelial function assessment with brachial artery flow-mediated dilatation is currently not in widespread use for cardiovascular risk stratification.

Digital pulse amplitude tonometry

Given the potential issues with flow-mediated dilatation, endothelial function assessment using digital pulse amplitude tonometry (PAT) has been developed.[7] This technique assesses endothelial function in the peripheral finger circulation by measuring changes in digital pulse volume during reactive hyperaemia. Briefly, the reactive hyperaemia pulse amplitude tonometry (RH-PAT) technique involves a blood pressure cuff being placed on one arm (the study arm), while the other arm serves as a control arm. PAT probes are placed on one finger of each hand to obtain a continuous baseline recording of the PAT signal. The blood pressure cuff is inflated to above systolic pressure for a brief duration and then deflated, and the hyperaemic PAT signal is recorded. The ratio between hyperaemic and baseline PAT signals is obtained from the study arm, and this ratio is then normalised to the ratio obtained from the control arm, giving a measure of endothelial function (Figure 2).

Studies using the PAT probe have demonstrated impaired endothelial function in children with type 1 diabetes mellitus, and have shown that impaired RH-PAT responses are present in patients with endothelial dysfunction measured during coronary angiography.[8] More recently, endothelial dysfunction as assessed by RH-PAT has been shown to be associated with male gender, body mass index, ratio of total to HDL cholesterol, diabetes mellitus and smoking.[7] Better endothelial function was seen with lipid-lowering treatment.[7] However, some findings in this study were counterintuitive, such as the lack of a significant association between hypertension and endothelial dysfunction, and a small but positive association between older age and better endothelial function. In addition, there are as yet no data on RH-PAT and risk of future cardiovascular events. Thus, further studies are needed to clarify the role of RH-PAT in cardiovascular risk stratification.

Arterial elasticity assessment

Age-related reduction in arterial elasticity is associated with endothelial dysfunction, and may precede significant atherosclerosis and cardiovascular events. Therefore, another potential technique for the detection of endothelial dysfunction is measurement of arterial elasticity using pulse waveform analysis.[9] This technique is being developed, but it is currently unknown whether endothelial dysfunction detected this way correlates with cardiovascular risk factors

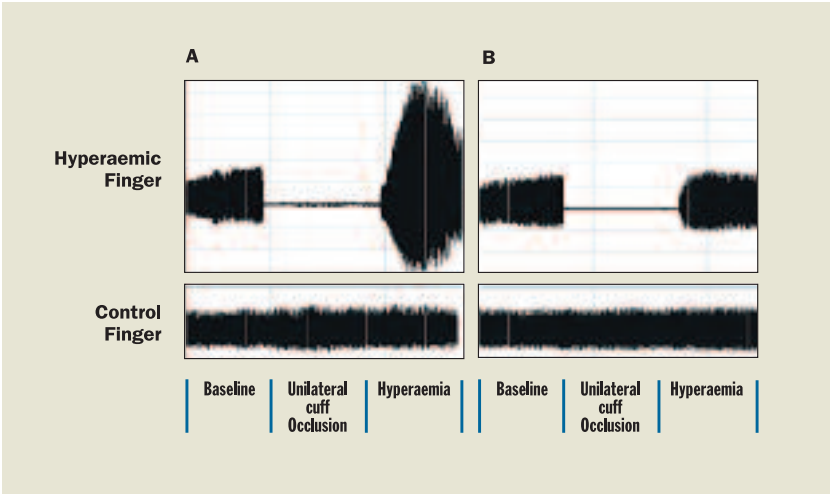

Figure 2. Reactive hyperaemia pulse amplitude tonometry. The traces in A show marked increases in blood flow in response to reactive hyperaemia, which is normal; the traces in B show impaired hyperaemic response, indicating endothelial dysfunction. (Reproduced with permission from: Hamburg NM, Keyes MJ, Larson MG, et al. Cross-sectional relations of digital vascular function to cardiovascular risk factors in the Framingham Heart Study. Circulation 2008; 117: 2467-2474.)

and events. Further studies are therefore needed to clarify the role of arterial elasticity measurement in endothelial function testing.

Blood tests and circulating endothelial progenitor cell levels

A simple blood test has long been the 'holy grail' of endothelial function testing, and such a test would be particularly attractive for widespread use in clinical practice. A previous study reported that elevated levels of serum C-reactive protein levels, a systemic marker of inflammation, were independently associated with endothelial dysfunction.[10] More recently, levels of circulating endothelial progenitor cells have been shown to correlate with better endothelial function.[11] Furthermore, levels of circulating endothelial progenitor cells were a better predictor of vascular reactivity than was the presence or absence of conventional cardiovascular risk factors.[11]

Hence, a simple blood test measuring endothelial progenitor cell levels may form the basis of endothelial function assessment in the future.

Cardiovascular interventions leading to improvement in endothelial function

Endothelial dysfunction is potentially reversible, and future cardiovascular events might be prevented when it is detected early. Indeed, standard cardiovascular therapies such as statins and antihypertensive medications, as well as lifestyle changes such as exercise and smoking cessation, have all been shown to improve endothelial function.[5]

Although cardiovascular treatment can improve endothelial function, it remains to be established whether earlier detection and treatment of vascular disease as a result of endothelial function testing will lead to improved clinical outcomes. Further studies are therefore needed before such measurements can be suggested for widespread and routine use in clinical practice. It is envisaged that such studies will involve randomising patients to either endothelial function testing or conventional cardiovascular risk factor assessment, followed by implementation of early preventative therapies as appropriate (depending on the results of the initial cardiovascular assessment). The cardiovascular event rates will then be compared between the two different assessment strategies, therefore indicating whether endothelial function testing is now ready for 'prime time' clinical use.

Conclusion

Endothelial dysfunction occurs early in cardiovascular disease, and correlates with adverse clinical outcomes. Early detection and treatment may reverse this disorder and have prognostic implications. This has led to the development of several techniques to assess endothelial function. For endothelial function testing to have widespread clinical applicability, it must be simple to use, noninvasive, reproducible and accurate, and early detection of endothelial dysfunction should also lead to improved clinical outcomes. Currently, none of the available tests fulfill all these requirements. Hence, much work remains to be done before widespread and routine clinical use of endothelial function testing can be incorporated into pre-existing cardiovascular management guidelines. **CT**

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COMPETING INTERESTS: None.