Brief Review: Current Perspective

Clinical Utility of Endothelial Function Testing
Ready for Prime Time?

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"...Arteriosclerosis...is the expression of the natural wear and tear to which the tubes are subjected...the onset of arteriosclerosis depends on the quality of arterial tissue (vital rubber)...in the make up of the machine, bad material was used for the tubing..."1

William Osler,
The Principles and Practice of Medicine, 1892

The arterial endothelium comprises cells resting on a basement membrane that exert autocrine, paracrine, and endocrine functions. This monolayer of endothelial cells plays a crucial role in the regulation of vascular tone in part by the release of vasoactive substances, notably NO, endothelin, prostacyclin, and angiotensinogen.2–4 In addition, endothelial cells are involved in the modulation of platelet activation, leukocyte adhesion, and thrombosis. The endothelium, therefore, delicately balances the counterregulatory pathways that control vasomotion, cell proliferation, thrombosis, inflammation, and oxidation.

Endothelial function becomes impaired early in the atherogenic process, and this diminishes the normal vasodilator response. Impaired endothelium-dependent vasorelaxation can be detected by measuring the response to pharmacological and physiological stressors before the development of angiographically significant atherosclerotic plaque in the coronary5,6 and peripheral vasculature.7 Well-known cardiac risk factors, including age, gender, hypertension, hyperlipidemia, diabetes mellitus, and smoking, as well as novel risk factors, such as inflammation and hyperhomocystinemia, have been associated with abnormal vasorelaxation. Pharmacological therapies and lifestyle changes aimed at improving cardiovascular risk, in many instances, may also improve vasomotor function.8

Techniques for Assessing Endothelial Function
Because atherosclerosis is a diffuse disease process, endothelial function can be assessed in either the coronary or the peripheral circulation. Coronary artery endothelial function is most commonly assessed by intracoronary infusion of acetylcholine, which, acting via muscarinic receptors on endothelial cells, causes release of NO and coronary artery dilation. In patients with risk factors for atherosclerosis and in those with overt coronary artery disease, infusion of acetylcholine results in a diminished vasodilatory response or paradoxical vasoconstriction.5 Coronary artery endothelium-dependent vasomotion can also be assessed by other modalities, including the cold pressor test, or by distal administration of direct vascular smooth muscle cell–relaxing agents, such as papaverine or adenosine, which increase coronary blood flow. Although coronary artery endothelial function testing evaluates the vessels most likely to cause cardiovascular events, this methodology is limited primarily by the risk of the invasive procedure as well as its expense. Therefore, there has been interest in developing less invasive methods of evaluating endothelial function.

To date, the most developed methods that have emerged as alternatives to invasive testing of coronary endothelial function involve evaluation of peripheral arterial endothelial function. Testing peripheral endothelial function with noninvasive techniques provides an opportunity to evaluate large patient populations. Brachial artery imaging with high-resolution ultrasound during reactive hyperemia is now a widely used method of determining peripheral vascular function.9,10 Typically, forearm or hand ischemia is induced by interrupting arterial blood supply with a cuff inflated to suprasystolic pressure. Release of the tourniquet induces reactive hyperemia caused by dilation of the distal microvasculature. Shear stress and alterations in hydrostatic pressure during reactive hyperemia result in the local release of NO. The magnitude of the change in vessel diameter from the baseline period to the peak observed during reactive hyperemia is indicative of the degree of endothelial function. Although the brachial artery circulation is most commonly interrogated to determine changes in blood vessel diameter during reactive hyperemia, other peripheral arteries may be evaluated, including the carotid, superficial femoral, and radial arteries.11–14 Despite widespread use in clinical research, however, the methods being used for the noninvasive assessment of peripheral endothelial function are insufficiently standardized.15 For example, in brachial artery reactivity studies, the occluding cuff is placed above the elbow by some, whereas others place it below the elbow. The time after cuff deflation when measurements are taken also varies between laboratories. Finally, there is considerable operator dependence in several aspects of how the test is performed. Therefore, this tool will not be clinically useful until standard
methodology is developed that will provide more consistency for longitudinal follow-up and allow comparisons of data from various laboratories.

Venous occlusion plethysmography, another method for evaluating peripheral vasomotor function, involves measuring volume changes in the forearm by mercury strain gauges during hyperemia. In addition to changes induced by an ischemic challenge, infusion of vasoactive agents allows for an in vivo evaluation of the mechanisms that mediate specific responses. For example, infusion of inhibitors of endothelial NO synthase can be an effective tool for defining the role of NO in contributing to enhanced vasodilation after medical interventions.6,17 These approaches, however, require brachial artery cannulation.

Abnormalities in peripheral endothelial function, detected by methods such as brachial artery ultrasound and venous occlusion plethysmography, correlate with the presence of coronary vasomotor dysfunction.18 Furthermore, as with the coronary circulation, peripheral vasomotor function is diminished in subjects at risk for atherosclerosis,19 and medical interventions and lifestyle changes that reduce atherosclerotic risk are also associated with improved peripheral vascular function.20–22 The correlation in endothelial function in both the coronary and the peripheral vasculature suggests that a common pathway contributes to endothelial dysfunction in both vascular beds. On the basis of the literature, which demonstrates an association between endothelial dysfunction and the presence of either risk factors for atherosclerosis or atherosclerosis itself, endothelial function testing has rapidly grown into a widely used research tool. However, the lack of data demonstrating that vascular function testing contributes specific and independent prognostic value continues to limit its clinical utility.

In addition to vascular imaging techniques, a number of even more novel techniques are being explored to assess the integrity of the endothelium. For example, cellular adhesion molecules, which play a key role in leukocyte adherence and transmigration, are expressed on the surface of damaged endothelial cells. Cellular adhesion molecules are shed into the circulation, and elevated levels of these circulating proteins have been observed in patients with atherosclerosis. Preliminary data suggest that these types of measurements may be markers of increased cardiovascular risk.23,24 Similarly, elevated levels of C-reactive protein, a systemic marker of inflammation, have also recently been shown to predict the presence of endothelial dysfunction, atherosclerosis, and the risk of future cardiovascular events.25 Metabolites of NO are excreted in the urine, thereby suggesting that measurements of levels of these compounds may also be reflective of endothelial function or dysfunction. Although urinary excretion of NO metabolites may be reduced in patients with atherosclerosis, this type of evaluation is complicated by the fact that many stimuli, such as exercise, regulate their release.26 Finally, in patients with coronary artery disease, vascular extracellular superoxide dismutase, an important antioxidant enzyme system, is substantially reduced, and thus this compound too is being evaluated as a marker of endothelial dysfunction.27

Endothelium-independent vasodilation can be evaluated in the coronary or peripheral circulation after administration of agents that directly relax smooth muscle cells, such as nitroglycerin or sodium nitroprusside. This technique allows one to assess the ability of the artery to maximally dilate. The role of vascular smooth muscle cells in vasodilation has important implications for endothelial function testing. For example, it is unclear whether factors that result in endothelial dysfunction also lead to abnormalities in vascular smooth muscle cell–mediated relaxation. In addition, it is controversial whether or not abnormalities in endothelium-independent vasodilation are predictive of increased cardiovascular risk. Finally, because vascular smooth muscle cells are the final common pathway mediating vasorelaxation, it is important to realize that individuals with decreased endothelium-independent vasodilation responses, by definition, will have decreased endothelium-dependent vasodilation. Thus, further work is needed to dissect the importance and relevance of this complex cellular interaction.

Prognostic Value of Endothelial Function Testing

Until recently, only correlative relationships have been drawn between endothelial dysfunction and atherosclerosis progression. This is now beginning to change. The first step toward supporting the clinical utility of endothelial function testing has come from recent studies demonstrating that it can provide prognostic value independent of that provided by assessment of the traditional cardiovascular risk factors. Suwaidi et al28 examined 157 patients with mild coronary artery disease and showed that there were significantly more cardiovascular events over a 2.3-year follow-up period in subjects with impaired coronary artery endothelial function than in those with normal endothelial function or mild dysfunction. Schachinger et al29 noted similar findings over a 7.7-year follow-up period in 147 patients. In this study, coronary endothelial function was assessed using acetylcholine infusion, cold pressor testing, and vasodilator responses to increased blood flow. A greater number of cardiovascular events, primarily revascularization procedures, occurred during the follow-up period in patients with poor coronary endothelial function, regardless of which technique was used. In addition, the endothelium-independent response to nitroglycerin also predicted adverse prognosis, suggesting a role for functional abnormalities in the smooth muscle vasodilator capacity of coronary arteries. Most recently, Halcox et al30 reported similar findings over a 46-month period while measuring changes in coronary vascular resistance and epicardial artery diameter in response to intracoronary acetylcholine. As in previous studies, this study showed that endothelial dysfunction predicted cardiovascular events in patients with coronary artery disease. Importantly, endothelial function also predicted an increase in cardiovascular events in patients with angiographically normal coronary arteries. Other studies have noted that coronary artery endothelial function testing may also have predictive value for the development of cardiac allograft vasculopathy and cardiac death in heart transplant recipients.31 Importantly, however, despite the provocative findings linking endothelial function
and risk of future cardiovascular events, larger prospective studies with varied patient populations are needed.

Extending the observations from studies on coronary artery endothelial function, recent trials assessing peripheral arterial endothelial function also indicate an ability to predict future cardiovascular events. For example, we recently showed that the presence of well-preserved brachial artery vasoreactivity predicts the absence of coronary artery disease and good exercise tolerance.32 Neunteuff et al33 studied patients undergoing evaluation for chest pain with coronary angiography over a 5-year period. They found that patients with decreased peripheral endothelial function had an increase in cardiovascular events and the need for revascularization procedures. These retrospective data, however, are based on a small, highly selected patient population. Impaired brachial artery flow-mediated dilation has also been shown to independently predict postoperative outcomes in high-risk patients, even after correcting for cardiovascular risk factors. Goke et al34 examined brachial artery vasomotion in patients undergoing peripheral vascular surgery and monitored these patients for 30 days after surgery. Flow-mediated dilation was significantly lower in the patients who had a postoperative event than in those who did not. In multivariate analyses, impaired brachial artery vasodilation was an independent predictor of short-term cardiovascular risk in this population. Heitzer et al35 demonstrated an increase in cardiovascular events over a 4.5-year follow-up period in coronary artery disease patients with blunted peripheral vascular responses to brachial artery infusion of acetylcholine. Interestingly, patients experiencing cardiovascular events showed a greater vasodilatory effect after coadministration of vitamin C, thereby suggesting that increased oxidative stress may contribute to worse outcomes in patients with endothelial dysfunction. In addition, assessment of forearm endothelial function has been shown to be a marker of long-term cardiovascular events in patients with hypertension.36

The next major conceptual step toward supporting a role for endothelial function testing in clinical practice is the observation that enhanced endothelial function in response to an intervention identifies a group of patients who have an improved cardiovascular prognosis. Modena et al37 studied 400 postmenopausal women with hypertension and impaired brachial artery vasomotion. After 6 months of treatment with optimal medical therapy for blood pressure control, brachial artery vasoreactivity remained abnormal in 150 patients, whereas it improved (flow-mediated dilation increased to >10%) in the remaining 250 patients. After an average of 67 months of therapy, patients whose flow-mediated dilation remained impaired had significantly more cardiovascular events than did those patients whose endothelial function improved with treatment. To our knowledge, this is the first study to suggest that sequential testing of endothelial function may be useful in determining long-term response to a specific medical intervention. The intriguing findings in this study suggest the hypothesis that endothelial function testing may identify individuals who benefit from a given intervention. Conversely, by highlighting patients who have a poor vasodilator response, it may be possible to determine who might benefit from more aggressive or combination therapies. This hypothesis will need to be tested in large-scale randomized, controlled trials.

Although several therapies that have been shown to improve endothelial function also reduce cardiovascular risk, the paradigm is not necessarily consistent throughout. For example, various estrogen38 and antioxidant39 preparations are linked to enhanced endothelial function; however, large clinical trials have not demonstrated improved clinical outcomes with their use.40,41 L-Arginine, the substrate for NO synthase, has also been shown to improve endothelial function in patients with atherosclerosis.32 However, rapid administration does not alter exercise tolerance or ECG abnormalities in patients with coronary artery disease despite improving endothelial function, and there are no data to suggest that L-arginine improves long-term cardiovascular prognosis.33 Furthermore, although the beneficial effects of lipid-lowering therapy with HMG-CoA reductase inhibitors are clearly evident,44 the effect of this class of medications on endothelial function remains mixed.45 For example, the Coronary Artery Reactivity After Treatment with Simvastatin (CARATS) study showed no benefit of simvastatin on coronary artery endothelial function in patients with mild to moderate coronary artery disease and average cholesterol levels.46 Thus, the link between improved endothelial function and clinical cardiovascular outcomes remains complex.

Implications for the Future

As physiological testing of endothelial function evolves toward becoming a helpful tool for the evaluation and management of patients, it is important to ask, what are the implications of these data regarding potential future applications of endothelial function testing? Perhaps endothelial function testing will be useful to identify subgroups of patients who are at higher risk for cardiovascular events, yet who do not qualify for medical therapy according to present guidelines. For example, it is possible that endothelial function testing in patients with angiographically normal epicardial coronary arteries, by identifying those patients with decreased endothelial function, might define a subgroup of patients who would benefit from aggressive medical or lifestyle interventions. Endothelial function testing might also be useful in evaluating patients with abnormal myocardial perfusion as detected by nuclear imaging or who have wall motion abnormalities detected by stress echocardiography, yet no coronary artery lesions identifiable by angiography. Although recent primary prevention studies have demonstrated a clinical benefit of statins, the number “needed to treat” to prevent cardiovascular events in these lower-risk populations is relatively high. One can therefore also envision that endothelial function testing might be used in the primary prevention setting in an effort to identify patients most likely to benefit from aggressive therapy. Finally, repeat testing of the endothelium might prove valuable in monitoring the response to various forms of therapies, aid in decisions regarding titration of medications, and assess the need for further therapy. Importantly, these potential applications of endothelial function testing will clearly require further study before they are applicable to clinical practice.
Conclusions

Coronary and peripheral endothelial function represents a gauge of vascular health, and impaired vasomotion is important to the pathogenesis and prognosis of cardiovascular disease. Although other new noninvasive modalities of assessing cardiovascular risk, including quantification of coronary calcification by electron-beam CT\(^\text{47}\) and measurement of carotid artery intima-media thickness,\(^\text{48}\) have prognostic implications, physiological vascular testing of the endothelium is evolving into an important adjunctive tool for assessing cardiovascular risk and response to treatment. The present-day methods of assessing endothelial function vary significantly in terms of cost, invasiveness of the procedure, and standardization of the technique (Table). Newer, simpler methods of accurately assessing endothelial function will be necessary to bring endothelial function testing into the clinical arena.

The Executive Summary from Prevention Conference V in 2000, which focused on high-risk patients for primary prevention, states that, although “assessment of endothelial function, as measured most typically by flow-mediated brachial artery vasodilation, is a promising technique that may reflect an independent measure of cardiovascular disease (CVD) risk, additional prospective research is needed to demonstrate that this technique can truly add to standard CVD risk prediction” (Smith et al,\(^\text{49}\) p 116). Indeed, the new information supporting the prognostic value of endothelial function testing continues to add support for the concept that endothelial function testing can provide useful information independent of other cardiac risk factors in selected groups of patients. However, before the curtain rises to welcome endothelial function testing onto the clinical stage, further research is needed, including prospective, randomized studies focusing specifically on the clinical utility of endothelial function testing as a means of assessing cardiovascular risk or adequacy of therapy.

References


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## Table: Endothelial Function Testing

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<th>Coronary Artery Endothelial Testing</th>
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+++ indicates strongly supported; +, supported; and –, not well supported.


