Endothelial Function Testing as a Biomarker of Vascular Disease

Subodh Verma, MD, PhD; Michael R. Buchanan, PhD; Todd J. Anderson, MD

The endothelium is the monolayer of endothelial cells lining the lumen of all blood vessels. These cells function as a protective biocompatible barrier between all tissues and the circulating blood. Endothelial cells also function as a selective sieve to facilitate bidirectional passage of macromolecules and blood gases to and from tissues and blood. The strategic location of the endothelium allows it to “sense” changes in hemodynamic forces and blood-borne signals and “respond” by releasing a number of autocrine and paracrine substances. A balanced release of these bioactive factors facilitates vascular homeostasis. Endothelial cell dysfunction disrupts this balance, thereby predisposing the vessel wall to vasoconstriction, leukocyte adherence, platelet activation, mitogenesis, pro-oxidation, thrombosis, impaired coagulation, vascular inflammation, and atherosclerosis.1 Our understanding of these endothelial cell responses has led to the development of tests that are believed to reflect endothelial cell dysfunction or integrity in vivo. Given the central role of the endothelium in the development and clinical course of atherosclerosis, endothelial function testing may serve as a useful biomarker of atherosclerosis.

Nitric oxide (NO) is the key endothelium-derived relaxing factor that plays a pivotal role in the maintenance of vascular tone and reactivity.2 In addition to being the main determinant of basal vascular smooth muscle tone, NO acts to negate 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 to 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 that results in the formation of untoward oxidants such as superoxide and hydrogen peroxide (versus NO) with resultant impairment in endothelial function.3 Superoxide inactivates NO to peroxynitrite, which further decreases NO activity in this uncoupled state. Cardiac risk factors in general lead to an increase in oxidative stress, attenuating net NO bioactivity.

Assessment of Endothelial Function

Assessment of endothelial cell function refers to a measure of endothelial cell response to stimulation—for example, by vasoactive substances released by or those that interact with the vascular endothelium. Endothelium-dependent vasodilatation can be assessed in the coronary and peripheral circulations in humans. In addition, measures of platelet function and inflammation/leukocyte activation (such as C-reactive protein [CRP]) are other measures of endothelial health. Figure 1 shows methods of assessing endothelium-dependent vasomotion.

Coronary Circulation

Quantitative coronary angiography has been used to examine the changes in vascular diameter in response to an infusion of an endothelium-dependent vasodilator such as acetylcholine (Ach), bradykinin, substance P, or serotonin.4 In healthy...
vessels, Ach evokes a NO-mediated vasodilatory response; however, in patients with endothelial dysfunction, this effect is blunted or paradoxical vasoconstriction may occur.5 Endothelial function of the coronary microvasculature (resistance vessels) has been assessed by using intracoronary Doppler techniques to measure coronary blood flow in response to pharmacological or physiological stimuli.6 Although considered by many to be the best assessment of endothelial function, this technique is limited by its invasive nature, expense, and relative inaccessibility.

Peripheral Circulation
Brachial artery ultrasound is a widely used, noninvasive measure of endothelial cell function.7,8 The forearm blood flow is occluded for 5 minutes using a blood pressure cuff maintained at a standard pressure. When the pressure is released, reactive hyperemia occurs. This results in shear stress–induced NO release and subsequent vasodilatation (flow-mediated vasodilatation). This technique has the advantage of being noninvasive and can readily identify populations with attenuated endothelial function. The major limitations of this technique are the need for ultrasonographic expertise and a significant day-to-day variability (about 25%) due to biological circadian rhythms. Nonetheless, at present, this approach is widely used to assess vasomotion function. 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 intraarterial (brachial artery) administration of agonists. This technique is excellent for acute interventions with repeated measurements.9,10 The major drawbacks, again, are reproducibility and the technique’s more invasive nature compared with ultrasound. Noninvasive measures of arterial compliance and waveform morphology also provide a marker of vascular health that may in part be endothelium dependent.

Inflammatory Markers
Over the past few years, we have witnessed a paradigm shift in our understanding of the underlying principles of atherosclerosis. This new view supports the concept that vascular inflammation is a central orchestrator of atherosclerotic lesion formation, progression, and eventual rupture.11
Chronic inflammation results in endothelial dysfunction and facilitates the interactions among modified lipoproteins, monocyte-derived macrophages, T cells, and normal cellular elements of the arterial wall, thus inciting early and late atherosclerotic processes. This paradigm has fueled interest in evaluating inflammatory markers of atherosclerosis, of which high-sensitivity CRP has emerged as one of the most important. As such, CRP is a powerful independent predictor of myocardial infarction, stroke, and vascular death in a variety of settings and appears to be a better prognosticator of cardiovascular events than LDL cholesterol. Over the past year, much interest has been generated into unraveling the mechanistic basis of the CRP – atherosclerosis connection (Figure 2). Indeed, recent studies, including work from our laboratory, suggest that CRP is not only a predictor but also a mediator of lesion formation. CRP, at concentrations known to predict vascular disease, has a direct effect to stimulate diverse early atherosclerotic processes, including the expression of endothelial cell adhesion molecules, the production of chemoattractant chemokines, and macrophage LDL uptake. In addition, CRP directly modulates the production of endothelium-derived vasoactive factors, including downregulating endothelial NOS (eNOS) –derived NO while augmenting production of the potent endothelium-derived vasoconstrictor endothelin-1. Additionally, CRP facilitates endothelial cell apoptosis and attenuates angiogenesis, which is an important compensatory mechanism in ischemia. More recently, CRP has also been demonstrated to promote the release of plasminogen activator inhibitor-1 from endothelial cells, up-regulate angiotensin- mediated neointimal formation, and alter endothelial progenitor cell survival and differentiation. CRP therefore seems to function as an important circulating marker of endothelial dysfunction. ICAM indicates intercellular adhesion molecule; VCAM, vascular cell adhesion molecule; and MCP, monocyte chemoattractant protein.

Figure 2. The mechanistic basis of the predictive value of CRP may be its ability to incite endothelial dysfunction. CRP can decrease eNOS mRNA, augment endothelin-1 (ET-1), and upregulate diverse adhesion molecules and chemoattractant chemokines, uncovering a proinflammatory and proatherosclerotic phenotype. Preliminary observations also suggest that CRP upregulates the nuclear factor-κB signaling in endothelial cells while attenuating endothelial progenitor cell survival and differentiation (Verma et al, unpublished observations, 2003). Also, the proatherogenic effects of CRP are augmented in the hyperglycemic milieu. Also, CRP has been demonstrated to potently upregulate angiotensin type 1 (AT₁) receptor in vascular smooth muscle cells in vivo and in vitro, augmenting vascular smooth muscle (VSM) proliferation and migration, reactive oxygen species (ROS) production, and restenosis. CRP therefore seems to function as an important circulating marker of endothelial dysfunction. ICAM indicates intercellular adhesion molecule; VCAM, vascular cell adhesion molecule; and MCP, monocyte chemoattractant protein.

Endothelial Function as a Biomarker of Atherosclerosis
Dysfunction of endothelial cells is probably the earliest event in the process of lesion formation—hence, the concept that
assessments of endothelial function exist; and (6) endothelial cell perturbations often are reflected in peripheral vasodilator abnormalities, thereby allowing the assessment of peripheral endothelial function as a measure of coronary vasomotion.27 Recent studies also suggest that there is a correlation between endothelium-dependent vasodilation and CRP levels.26 Thus, endothelial dysfunction may be reflected systematically, thereby allowing for a less invasive approach to the assessment of overall endothelial cell biocompatibility.

The rationale for why endothelial function testing, either directly or indirectly, may serve as an indicator of vascular damage or disease is as follows: (1) The healthy endothelium is nonthrombogenic; (2) endothelial dysfunction occurs in response to vascular risk factors and is an early event in atherosclerosis; (3) endothelial dysfunction precedes structural atherosclerosis; (4) interventions that improve endothelial function also decrease cardiovascular events in patients with stable coronary disease; (5) reproducible, noninvasive assessments of endothelial function exist; and (6) endothelial function testing fulfills the criteria for an acceptable biomarker. Because risk factors, even those not yet identified, target the endothelium, it is a logical “window” of future atherosclerotic outcomes.

Prognostic Relevance of Endothelial Function

The clinical manifestations of coronary artery disease depend on a multitude of interrelated pathophysiological processes, of which endothelial dysfunction is only one.

Studies of Vasomotion

We demonstrated that endothelial dysfunction was associated with the development of transplantation atherosclerosis as assessed by intravascular ultrasound.28 Those subjects with normal vasodilator responses to Ach immediately after transplantation developed atherosclerosis at a rate one third that of those with endothelial dysfunction during the first year of follow-up. Three retrospective trials have assessed the relationship between Ach-mediated coronary endothelial function and clinical events (Table). Subjects with vasoconstrictor responses to Ach were more likely to develop adverse cardiovascular events during follow-up of 5 to 10 years despite having minimal coronary disease at baseline. Although important, these observations are diminished by the relative low frequency of end point events.29,30 A recently published trial of 308 subjects who underwent coronary endothelial function testing revealed improved survival in those subjects with better conduit and resistance vessel responses to Ach. Endothelial function was a predictor of outcome in a multivariate analysis.31

Perticone and colleagues32 studied 225 hypertensive subjects who underwent Ach testing in the forearm with plethysmography. After correcting for blood pressure, subjects with the lowest tertile of endothelial function had an increase in cardiovascular events over a 3-year follow-up. The most robust of the prognostic studies was reported by Heitzer et al.,33 who studied 281 subjects with coronary disease undergoing forearm Ach studies. Two key observations were made. First, subjects with attenuated responses had more events. Second, subjects with a greater acute improvement of endothelial function with vitamin C (suggesting more oxidative stress) had a worse outcome.

Small studies have also suggested a prognostic role for the brachial ultrasound assessment of endothelial function.34 A recently reported prospective study demonstrated that abnormalities of flow-mediated dilation were predictive of postoperative complications in patients undergoing noncardiac vascular surgery.35

All the studies done to date are too small to be definitive. Studies with thousands of subjects are underway36 with brachial ultrasound to determine if a single measure of vasoreactivity in an individual patient predicts the development of atherosclerosis or its complications (J. Vita, MD, personal communication, 2002).

Prognostic Implications of Endothelium-Dependent Vasomotion

<table>
<thead>
<tr>
<th>Author and Reference No.</th>
<th>No. Patients Studied</th>
<th>No. of End Points (Events)</th>
<th>Parameter Associated With Event</th>
<th>Study Design</th>
<th>Population Studied</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schachinger et al 39</td>
<td>147</td>
<td>16</td>
<td>Epicardial dilator response to Ach, CPT, FMD, NTG</td>
<td>Retrospective</td>
<td>Minimal CAD</td>
</tr>
<tr>
<td>Suwaidi et al 30</td>
<td>157</td>
<td>10 (in 6 patients)</td>
<td>Epicardial and resistance vessel function to Ach</td>
<td>Retrospective</td>
<td>Minimal CAD</td>
</tr>
<tr>
<td>Halcox et al 31</td>
<td>308</td>
<td>35</td>
<td>Epicardial/resistance vessel function to Ach</td>
<td>Retrospective</td>
<td>With and without CAD</td>
</tr>
<tr>
<td>Perlicone et al 32</td>
<td>225</td>
<td>29</td>
<td>Forearm blood flow response to Ach</td>
<td>Retrospective</td>
<td>Never-treated hypertension</td>
</tr>
<tr>
<td>Heitzer et al 33</td>
<td>281</td>
<td>91</td>
<td>Forearm blood flow response to Ach (Ach + vitamin C)</td>
<td>Retrospective</td>
<td>Coronary disease</td>
</tr>
<tr>
<td>Neunteuff et al 34</td>
<td>73</td>
<td>6</td>
<td>Brachial FMD</td>
<td>Retrospective</td>
<td>Chest pain</td>
</tr>
<tr>
<td>Gokce et al 35</td>
<td>187</td>
<td>45 (after vascular surgery)</td>
<td>Brachial FMD</td>
<td>Prospective</td>
<td>Elective vascular surgery</td>
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CPT indicates cold pressor testing; FMD, flow-mediated vasodilation; NTG, nitroglycerin; and CAD, coronary artery disease.
factor-α, soluble P selectin, and soluble intercellular adhesion molecule-1. However, high-sensitivity CRP has emerged as the most powerful predictor of future cardiovascular events. In both healthy men and women, CRP levels in the upper quartile increase risk of adverse clinical events by 2- to 4-fold and are at least as prognostic as lipid parameters. Of great interest is the fact that subjects with elevated levels of CRP seem to gain more benefit from pharmacological therapies such as aspirin or statin therapy. Given the direct effects of CRP to destabilize eNOS mRNA in endothelial cells, it is logical to propose that CRP is a sensitive marker of endothelial dysfunction; it remains to be determined whether the predictive value of endothelial vasomotion assessments will be independent of CRP levels.

An alternative approach to measuring endothelial cell function in response to assorted agonists is to measure changes in endothelial cell biocompatibility. In support of this possibility, Brister et al. found that the lipoxygenase pathway–derived monohydroxide, 13-hydroxyoctadecadienoic acid (13-HODE) decreases with age in patients with coronary artery disease undergoing coronary artery bypass grafting and that this decrease is associated with increased endothelial cell thrombogenicity. More recently, 13-HODE plasma levels have been shown to dramatically increase in patients with confirmed atherosclerosis and were higher in coronary artery bypass grafting patients who suffered a cardiovascular event during the 2-year follow-up.

Several other surrogate markers of endothelial cell activation and inflammation are being investigated, including lipoprotein-associated phospholipase A₂ (PLA₂), CD40 receptor/ligand interaction, LOX-1, and measurement of circulating endothelial progenitor cells. Implications for Practice

Atherosclerotic vascular disease remains the leading cause of morbidity and mortality among adults in developed countries and is increasing at an alarming rate in developing nations. The concept of risk introduced by the Framingham Heart Study more than 50 years ago serves as the “gold standard” in risk assessment. Enthusiasm for risk assessment and prevention are based on the demonstration that aggressive medical therapy reduces the likelihood of recurrent coronary events in patients with established coronary heart disease. A similar potential exists for primary prevention.

Patients at high risk (those with diabetes, other vascular disease, or multiple risk factors) clearly benefit from pharmacological therapy. In addition, patients without traditional risk factors are at low risk of events over both the short and long term. It is estimated however, that 40% to 50% of adults fall into the intermediate risk group according to National Health and Examination Survey (NHANES) III data. Patients in this intermediate risk group do not currently qualify for the most intensive risk factor interventions. The problem with this approach is that the treatment algorithms take into account short-term (10 years) rather than long-term risk (30 years), and there is a wide range of risk within this large group. It is this group that could potentially benefit from further risk stratification with endothelial function testing. If prospective studies confirm the predictive nature of endothelial markers for cardiovascular outcomes, then incorporation of these measures into risk factor models would lead to more effective prevention. A positive test in a subject at low to moderate risk would identify an individual whose risk would warrant pharmacological treatment. Other proposed markers of risk, including coronary calcium score (electron beam computed tomography), carotid intimal-medial thickness, ankle-brachial index, and stress testing, are also being evaluated carefully. The optimal diagnostic/therapeutic approach is not known at this time but will be better defined in the next decade. These data will come from ongoing prospective studies of endothelial function and the National Institutes of Health–sponsored MESA study (Multi-Ethnic Study of Atherosclerosis). Given the increasing atherosclerosis pandemic, advances in diagnostic modalities, and health economics issues, the time is right to define the most effective prevention strategy for subjects at risk of atherosclerotic events.

Summary

At the present time, measures of endothelial cell function and biocompatibility have advanced our understanding of the pathophysiology of atherosclerosis and its treatment. They are quickly becoming well-established surrogates of disease activity; however, the ideal test(s) of endothelial function have yet to be established. As we incorporate new biomarkers into global risk assessment, the endothelium is the logical target of study, given its unique position as both a sensor and participant in the atherosclerosis process. Recent evidence suggests that the mechanistic basis for the powerful predictive value of inflammatory biomarkers such as CRP may also reside at the level of the endothelium. Although endothelial function testing remains a research tool at the present time, it is our contention that this technology will figure prominently in risk assessment strategies in the future.

References


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