Testing Endothelial Vasomotor Function

Nitric Oxide, a Multipotent Molecule

Peter Ganz, MD; Joseph A. Vita, MD

The initial description in 1980 by Furchgott and Zawadzki of endothelium-derived vasodilator factor has stimulated more than 2 decades of intense research to delineate the basic biology of the endothelium and its importance in the clinical setting. Endothelium-derived vasodilator factor has been identified as nitric oxide (NO). It is formed in endothelial cells from the amino acid l-arginine by endothelial isoform of NO synthase (eNOS), which is the product of the NOS3 gene. In addition to producing NO constitutively, the enzyme may be stimulated to increase NO synthesis by a variety of physiological agonists, shear stress, and pharmacological agents. Although discovered as a vasodilator, NO mediates many of the protective functions of the endothelium. It limits vascular recruitment of leukocytes by inhibiting the expression of proinflammatory cytokines, chemokines, and leukocyte adhesion molecules. In addition to producing NO constitutively, the enzyme may be stimulated to increase NO synthesis by a variety of physiological agonists, shear stress, and pharmacological agents. Although discovered as a vasodilator, NO mediates many of the protective functions of the endothelium. It limits vascular recruitment of leukocytes by inhibiting the expression of proinflammatory cytokines, chemokines, and leukocyte adhesion molecules. It inhibits vascular smooth muscle proliferation and platelet adhesion and aggregation. NO also inhibits the production of tissue factor, a molecule that plays a critical role in the propensity of disrupted atherosclerotic plaques to cause intravascular thrombosis. In the setting of risk factors and experimental atherosclerosis, loss of the biological activity of endothelium-derived NO is accompanied by other alterations in endothelial phenotype that further increase the propensity for vasoconstriction, thrombosis, inflammation, and cellular proliferation in the vascular wall. Thus, endothelial dysfunction has the potential to contribute to key events in the course of human atherosclerosis.

NO in Health and Atherosclerosis: Relationship With Risk Factors and Hemodynamic Stress

The experimental observations of Furchgott and others have stimulated translational research to elucidate the importance of endothelium-dependent vasodilation in human coronary atherosclerosis. Accordingly, Ludmer and colleagues administered the endothelium-dependent dilator acetylcholine into the coronary arteries of subjects undergoing cardiac catheterization. Acetylcholine induced dilation of normal epicardial coronary arteries but induced abnormal vasoconstriction, indicative of endothelial dysfunction, in patients with angiographic evidence of atherosclerosis. The concept of endothelial vasodilator dysfunction in atherosclerotic human coronary arteries was reinforced by similar findings when other stimuli to NO release were tested, including flow-mediated dilation, sympathetic activation, serotonin, and adenosine diphosphate.

The loss of endothelium-dependent dilation occurs in the earliest stages of atherosclerosis. In fact, it has been linked to each of the known atherogenic risk factors, including several forms of dyslipidemia, hypertension, diabetes mellitus, cigarette smoking, aging, menopause, family history of premature atherosclerosis, and hyperhomocysteinemia. The endothelium is a direct, sensitive target for the damaging effects of atherogenic risk factors, as evidenced from the experimental introduction of risk factors into healthy subjects. For example, elevation of blood homocysteine by administration of its precursor methionine, generation of lipoprotein remnant...
particles by feeding a high-fat meal, or infusion of glucose to raise its plasma level to mimic hyperglycemia of diabetes mellitus leads to endothelial vasodilator dysfunction in a span of just a few hours. Endothelial dysfunction is also perturbed by abnormal hemodynamic stresses. Bifurcations in human coronary arteries, sites of predilection toward atherosclerosis, show impaired endothelium-dependent vasodilation before atherosclerosis is detected.

Although atherosclerotic stenoses in large arteries can restrict blood flow, second-to-second regulation of flow in response to metabolic and other stimuli is generally accomplished in resistance arterioles. Although atherosclerosis is typically absent from these small vessels, atherogenic risk factors also impair endothelium-dependent vasodilation at these sites and thereby contribute to myocardial ischemia. In addition, endothelial dysfunction has been detected in several peripheral vascular beds. This has led to the concept of generalized, "systemic" nature of endothelial dysfunction and has facilitated endothelial function testing in readily accessible vascular beds.

Assessment of Endothelium-Dependent Vasodilator Function in Humans
As originally described by Ludmer and colleagues, endothelial function in human coronary arteries can be assessed by measuring the vasomotor responses of epicardial arteries by quantitative coronary angiography in response to graded concentrations of acetylcholine or other agonists. Endothelial function in coronary resistance vessel responses can be assessed at the same time by Doppler flow measurements. This method has been considered the "gold standard" against which other tests of endothelial function have been compared. It has been particularly useful for developing a framework that relates disturbed coronary pathophysiology to myocardial ischemia in patients with coronary artery disease. This invasive method is of necessity restricted to patients undergoing clinically indicated cardiac catheterization.

Endothelial function of forearm resistance vessels can be assessed by measurement of forearm blood flow using strain-gauge plethysmography in conjunction with intra-arterial infusion of endothelium-dependent agonists and selective pharmacological probes. This approach has been used primarily in studies intended to elucidate the basic mechanisms that underlie endothelial dysfunction in humans. The general applicability of this technique to broader populations is limited by the requirement for an intra-arterial catheter.

Assessment of endothelium-dependent, flow-mediated dilation of the brachial artery using high-resolution ultrasound has provided an entirely noninvasive approach to evaluating endothelial function. This technique uses increased hemodynamic shear stress during reactive hyperemia as a stimulus for the release of NO. The ultrasound approach has permitted studies of endothelial function in populations of asymptomatic subjects in whom cardiac catheterization is not indicated. The same atherogenic risk factors that impair coronary endothelial function similarly affect endothelial function in brachial arteries. However, the concordance between coronary and brachial endothelial responses when both tests are performed in the same patients is only modest. Moreover, the magnitude of the flow-mediated dilation depends on the specific protocol used to elicit reactive hyperemia, which differs from center to center at the present time. Finally, the methodology is associated with a relatively poor signal-to-noise ratio, which reflects variability in brachial artery size and the current resolution of vascular ultrasound. Despite these limitations, the methodology has proven to be particularly valuable for comparing different populations of patients from single centers and for assessing responses to therapeutic interventions over time.

Given the limitations of ultrasound-determined flow-mediated dilation, there is continuing interest in the development of better noninvasive approaches to test endothelial function. For example, emerging studies suggest that other noninvasive methods provide information about endothelium-dependent vasodilation, including fingertip pulse arterial tonometry and measures of arterial stiffness.

Endothelial Function and Clinical Outcomes
The postulated antiatherogenic role of NO has been supported by clinical studies. Higher rates of myocardial ischemia or infarction have been reported in humans with polymorphisms of eNOS that reduce the activity of the enzyme. Among human cardiac transplant recipients, coronary endothelial dysfunction after transplantation has been associated with accelerated coronary arteriosclerosis.

Several studies have investigated whether endothelial function testing predicts clinical complications associated with atherosclerosis (Table 1). Using several different methods to test endothelial function in coronary and peripheral arteries, patients with endothelial dysfunction had a far greater incidence of adverse cardiovascular events in follow-up compared with patients with preserved endothelial function. This ability of endothelial function testing to predict events was independent of other known risk factors. Although intriguing, these observations have definite limitations. They are largely a retrospective examination of clinical outcomes in patients enrolled in various research protocols in a few laboratories and hence may not be applicable to the population at large. Moreover, as the number of events in each study is small, composite end points consisting of a hard and a soft end point typically have been used. Nonetheless, the totality and consistency of these studies suggest that assessment of endothelial function has the capacity to provide useful prognostic information about future cardiovascular events.

Therapeutic Interventions and Endothelial Function
Because of the pivotal role that endothelial dysfunction plays in atherosclerosis and its complications, numerous strategies of reversing endothelial dysfunction have been investigated. Short-term studies have shown that mechanistically diverse interventions improve endothelial function, including
TABLE 1. Clinical Studies Supporting the Prognostic Value of Endothelial Vasomotor Function Testing

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Vascular Bed</th>
<th>Test of Endothelial Function</th>
<th>No. of Patients</th>
<th>Follow-Up, mo</th>
<th>Clinical Events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al Suwaidi et al</td>
<td>CAD</td>
<td>Coronary resistance vessels</td>
<td>Acetylcholine</td>
<td>157</td>
<td>28</td>
<td>MI, cardiovascular death, revascularization, CHF</td>
</tr>
<tr>
<td>Schächinger et al</td>
<td>CAD</td>
<td>Epicardial coronary arteries</td>
<td>Acetylcholine</td>
<td>147</td>
<td>92</td>
<td>MI, cardiovascular death, revascularization, unstable angina, ischemic stroke</td>
</tr>
<tr>
<td>Halcox et al</td>
<td>CAD</td>
<td>Epicardial coronary arteries</td>
<td>Acetylcholine</td>
<td>308</td>
<td>46</td>
<td>MI, cardiovascular death, unstable angina, stroke</td>
</tr>
<tr>
<td>Perticone et al</td>
<td>Hypertension</td>
<td>Forearm resistance vessels</td>
<td>Acetylcholine</td>
<td>225</td>
<td>32</td>
<td>Cardiac, cerebrovascular, peripheral vascular</td>
</tr>
<tr>
<td>Heitzer et al</td>
<td>CAD</td>
<td>Forearm resistance vessels</td>
<td>Acetylcholine</td>
<td>281</td>
<td>54</td>
<td>MI, cardiovascular deaths, ischemic stroke,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>revascularization</td>
</tr>
<tr>
<td>Neunteuffi et al</td>
<td>CAD</td>
<td>Brachial arteries</td>
<td>Flow-mediated dilation</td>
<td>73</td>
<td>60</td>
<td>MI, revascularization</td>
</tr>
<tr>
<td>Gokce et al</td>
<td>PAD</td>
<td>Brachial arteries</td>
<td>Flow-mediated dilation</td>
<td>187</td>
<td>1</td>
<td>Cardiovascular death, MI, unstable angina, stroke</td>
</tr>
<tr>
<td>Modena et al</td>
<td>Hypertension, postmenopausal</td>
<td>Brachial arteries</td>
<td>Flow-mediated dilation</td>
<td>400</td>
<td>67</td>
<td>Cardiovascular events</td>
</tr>
<tr>
<td>Gokce et al</td>
<td>PAD</td>
<td>Brachial arteries</td>
<td>Flow-mediated dilation</td>
<td>199</td>
<td>14</td>
<td>Cardiovascular death, MI, unstable angina, stroke</td>
</tr>
<tr>
<td>Targonski et al</td>
<td>Risk factors, but normal coronary arteries</td>
<td>Coronary resistance vessels</td>
<td>Acetylcholine</td>
<td>503</td>
<td>16</td>
<td>Cerebrovascular events</td>
</tr>
</tbody>
</table>

CAD indicates coronary artery disease; PAD, peripheral arterial disease; and MI, myocardial infarction.

Correction of lipid abnormalities, inhibition of angiotensin-converting enzyme or angiotensin II receptor, smoking cessation, exercise, and various dietary interventions. Interestingly, all of these interventions have also been shown to reduce cardiovascular events in clinical outcome studies.

During drug development, clinical outcome trials typically require thousands of patients and many years to complete and are associated with prohibitive costs. Hence, surrogate end points such as endothelial function testing have had much appeal in helping to decide which drugs to include in large clinical trials. The observation that an intervention improves endothelial function in a group of patients suggests that the intervention will also reduce cardiovascular risk and holds the promise that endothelial function testing may differentiate responders to treatment from nonresponders. In support of such a role for endothelial function testing, Modena and colleagues observed that improvement in brachial artery flow-mediated dilation after initiation of antihypertensive therapy coincided with a reduction in cardiovascular risk compared with patients with persistent endothelial dysfunction. However, that study lacked a standardized intervention, and further studies will be required to confirm the utility of endothelial function testing for this purpose.

Finally, endothelial function does not always correctly predict long-term outcome. For example, hormonal replacement therapy in postmenopausal women is consistently associated with improved endothelial function in peripheral and coronary arteries, but primary and secondary prevention clinical trials have proven negative. Given the complex causal mechanisms of atherosclerosis and the diverse effects of potential interventions, it is likely that no single surrogate end point will be completely predictive of clinical outcome. Accordingly, drug development in the field of atherosclerosis will have to rely on a broad panel of surrogate end points that test the impact of therapy on each key aspect of this disease, including endothelial function, inflammation, thrombosis, and plaque regression.

Endothelial Function Testing in the Coronary Risk Assessment of Generally Healthy Subjects

Another area of great interest is the potential use of endothelial function to stratify risk in individual subjects. Traditional and newly recognized risk factors account for only a portion of estimated risk for cardiovascular events such as myocardial infarction or coronary heart disease death. It is likely that genetic factors and other unrecognized environmental factors also play a role. Because the endothelium may be a target that integrates the damaging effects of the traditional and unknown risk factors, it has been proposed as a potential “barometer” of atherosclerosis risk, and as such, studying endothelial function may guide risk assessment and therapy for individuals. C-reactive protein, a biomarker of inflammation, recently received a limited endorsement to serve in that capacity.

Routine use of a biomarker for screening has profound implications for healthcare benefits and costs. Accordingly, each potential biomarker must pass rigorous tests, such as those we propose in Table 2. Currently, invasive and noninvasive endothelial function testing may reasonably used to investigate mechanisms of vascular disease and gain insight into the potential utility of new therapies in studies involving groups of patients. However, endothelial function testing does not meet most of the criteria in Table 2 as a biomarker for use in individual patients, so much work remains. Fortunately, simpler methods for endothelial function testing are currently in development and may prove helpful in that regard.

<table>
<thead>
<tr>
<th>Nature of the test</th>
</tr>
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<tbody>
<tr>
<td>● Test should be simple, noninvasive, commercially available, and cost-effective.</td>
</tr>
<tr>
<td>● Test should be reproducible and standardized internationally.</td>
</tr>
</tbody>
</table>

Ability to predict risk

| ● Ability to predict risk should be established in prospective studies. |
| ● Test should have population norms to guide results. |
| ● Test results should be generalized to various population groups. |

Relationship to established tests of risk

| ● New test should add to the predictive value of established tests. |
| ● New test should be particularly useful in refining the risk of medium- or low-risk subjects. |

Function as a surrogate end point

| ● New test should be useful as a screen for drug development. |
| ● New test should identify individual patients who will benefit from therapy. |
| ● An improvement in the new test should predict a reduction in the risk of clinical events. |

Summary

Basic observations by Furchgott and others have stimulated widespread interest in investigations of endothelial function in humans. This “translational research” has come to fruition. Much progress has been made in elucidating the biological mechanisms of human endothelial dysfunction, its relationship to atherosclerosis, and its clinical manifestations. Evidence for the importance of endothelial function has been strengthened by studies that relate endothelial dysfunction to future clinical events. Endothelial function testing may provide a useful target in the discovery and development of new therapies for the pharmaceutical industry. Further research is needed to establish endothelial function testing in the risk stratification and decisions regarding treatment of generally healthy subjects.

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