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Reliable Endothelial Function Testing At Our Fingertips?

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Noninvasive testing for the purpose of cardiovascular risk stratification has been a “holy grail” of Cardiology for some time. As the endothelium plays such a key role in normal vascular health and endothelial dysfunction is such an early event in atherogenesis, the appeal of endothelial function testing for risk stratification is easy to understand. Nevertheless, the goal of developing a noninvasive, widely applicable, reproducible, and informative test for endothelial function has so far proven elusive.

Article p 2467

Why Endothelial Function Is Important

Although the healthy endothelium is only a monolayer of relatively simple cells that for many years was regarded as little more than a semipermeable barrier lining the vasculature, it is very well placed to exert many important homeostatic functions. The endothelium is exposed to a variety of blood-borne signals and intravascular stressors and has adapted to respond by secretion or modification of a large number of factors that regulate (inter alia) vascular tone, thromboresistance, and cellular adhesion. For example, the endothelium transduces the stimulus of increased shear stress into the response of vasorelaxation, facilitating one of the most basic cardiovascular homeostatic mechanisms of flow-mediated dilatation.

Furthermore, it transpires that some of the molecules secreted by a healthy endothelium have key functions in defense against atherosclerosis, including the important antiproliferative and antithrombotic molecules, nitric oxide (NO) and prostacyclin. In particular, the role of NO as an agent that not only vasodilates but inhibits platelet aggregation, monocyte adhesion to endothelial cells, and abnormal smooth muscle cell proliferation has highlighted the status of NO as an important “antiatherogenic” moiety.¹ By some serendipity, the first invasive tests (using acetylcholine as the stimulus)² and the first noninvasive tests for endothelial function assessment (using shear stress as the stimulus)³ both result predominantly in the release of NO from the large vessels. Assessing the degree of NO-induced dilatation is thus a “barometer” for several aspects of arterial health, beyond

the simple vasodilator response itself. “Endothelial dysfunction” thus defined has been used in clinical research to assess the ability of the endothelium to release NO (and by implication to protect against atherogenesis) in a variety of cross-sectional studies investigating the impact of risk factors on blood vessels⁴ and the ability of a number of potentially beneficial interventions to reverse early arterial damage.⁵

These studies on endothelium-dependent (and also smooth muscle-dependent) vasomotor properties of the vasculature in health and disease also serve to highlight another important concept in cardiovascular pathophysiology; that vessel function may play a very important role in determining cardiovascular risk, over and above the risk conveyed by a structural impediment to flow such as a large plaque. Indeed plaque size (and minimum lumen diameter) are relatively poor predictors of cardiovascular risk, whereas vessel function (particularly the propensity for vessels to constrict rather than dilate during conditions of physical and/or mental stress) appears to confer important risk in the pathophysiology of acute vascular events.⁶ Consistent with this suggested importance of vascular function in risk determination, a large number of studies have now been published (summarized in Deanfield et al⁷) correlating endothelial function measurements with prospectively determined risk of cardiovascular events during follow-up.

Problems With Endothelial Dysfunction: Semantic

The majority of studies examining “endothelial function” in vivo in the last decade have involved the measurement of endothelium-dependent dilatation, in large part because techniques have been developed to assess this particular aspect of endothelial physiology. The endothelium, however, has a multiplicity of functions beyond the regulation of vessel tone, as noted above. Thus, articles reporting on “endothelial function” and “dysfunction” on the basis of the measurement of endothelium-dependent dilatation give insights to only 1 aspect of endothelial physiology, albeit an important one. Little work has been done to date on how the various endothelial functions correlate with each other in disease states (for example, how impaired endothelium-dependent dilatation, regulation of endothelial cell adhesion molecule expression, and release of key hemostatic regulatory molecules relate to one another).

Problems With Endothelial Dysfunction: Biological

The above comments notwithstanding, the measurement of endothelium-dependent dilatation has given important insights into the temporal relationship between major cardiovascular risk factors and early arterial abnormalities, while providing important information in studies of reversibility.^{3,4} Nevertheless, the

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process of endothelium-dependent dilatation is highly complex, with several putative mediators including NO, vasodilator and vasoconstrictor prostanoids (such as the potent dilator prostacyclin), and the elusive endothelium-derived hyperpolarizing factor or factors.⁸ It is almost certain that a different balance of these dilator and/or constrictor substances are of varying importance in different situations in health and disease. Regulation of vessel tone varies importantly by vessel size (for example, between epicardial arteries and myocardial resistance vessels); by site in the body (for example, between coronary and pulmonary circulation), and importantly in response to different chemical agonists. Thus, different pharmacological substances such as acetylcholine, serotonin, and bradykinin, which are widely used in endothelial function research, stimulate different amounts of vasodilator substances. Responses to these substances are themselves different from the response of vascular endothelium to the more physiological stimulus of shear stress.

Flow-Mediated Dilatation: An Appealing Test

Although the first descriptions of endothelial function measurements in humans² involved measuring the response of the epicardial arteries to infused acetylcholine, such testing is clearly not applicable for either large-scale studies of endothelial function nor for use in asymptomatic subjects, particularly children and young adults at risk of atherosclerosis. Such investigations awaited the development of noninvasive endothelial function testing, which was first described in 1992.³ This testing involved measuring the diameter of an artery by noninvasive ultrasound before and after increasing shear stress (provided by reactive hyperemia), with the degree of dilatation reflecting (in large part) arterial endothelial NO release.⁹

Measurement of ultrasound-based flow-mediated dilatation (FMD) in the brachial artery has intrinsic appeal, as it is noninvasive, relatively repeatable and reproducible, reflects important biology, has some data to support its predictability, and is useful in serial studies of disease reversibility (such as Woo et al⁵). Brachial artery endothelium-dependent dilatation is also significantly correlated with findings in the coronary circulation in the same patients.¹⁰

Nevertheless, the measurement of FMD by ultrasound has also proven problematic for risk stratification in individuals. The method is technically demanding, requiring specific training. Furthermore, FMD is very sensitive to a number of intercurrent factors that may influence vascular function transiently but may not have great importance for long-term atherosclerosis risk. For example, FMD can be acutely lowered by an intercurrent viral illness, can be transiently impaired after a meal, and varies in a circadian pattern.

In part reflecting these difficulties, different laboratories have used different variations in technique for the measurement of FMD (for example, placing the occluding pneumatic cuff around the upper arm or lower arm) and consequently have reported different ranges of normal values of FMD. Efforts to standardize FMD technique and results have recently been published in an attempt to redress this situation.¹¹ Nevertheless, the measurement of FMD by arterial ultrasound is not yet ready for “prime time” for widespread risk stratification.

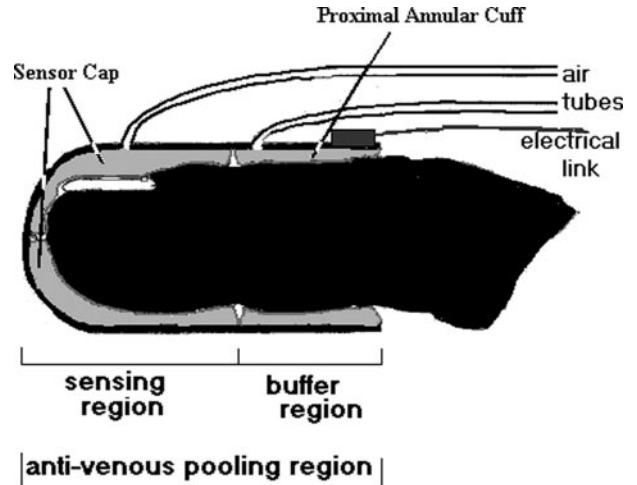


Figure. Cross-sectional view through the PAT finger probe; the amplitude of the finger pulse is sensed distally (image courtesy of Itamar Medical Ltd).

Digital Vascular Function: A New Option?

A number of new techniques have recently been proposed as potentially applicable screening tools for endothelial testing in humans. Each novel test proposed should meet several criteria, if it is to be clinically useful for assessing cardiovascular risk in individual subjects. The test should:

- Be simple, noninvasive, and widely applicable.
- Be reproducible, with low interobserver error.
- Be standardizable between laboratories and have population normal data to inform interpretation.
- Be able to predict risk.
- Add to the predictive value of established risk factor measurement, particularly in intermediate-risk subjects.
- Be able to demonstrate that improvement with the new test predicts a reduction in subsequent cardiovascular risk.

Candidates for noninvasive vascular testing proposed in recent years include pulse wave analysis, pulse wave velocity measurement, and pulse amplitude tonometry (PAT). Interest has recently grown particularly in testing endothelial vasomotor function after reactive hyperemia by PAT (RH-PAT), as measured in the fingertips, a test that is examined by Hamburg et al in the current issue of *Circulation*.¹²

Measuring digital RH-PAT involves quantifying arterial pulsatile volume at rest and during a condition of increased shear stress that results in the release of NO (and other mediators). It is performed with the use of a novel finger plethysmograph, essentially a longitudinal socket in the form of a split thimble, closed off at the proximal end (the Figure). The probe has an internal membrane surrounded by an outer rigid wall and is pressurized to provide a uniform field over the distal index finger by applying near-diastolic external pressure; thus, the RH-PAT probe is able to unload arterial wall tension, which increases the signal-to-noise ratio. Systemic influences are controlled by simultaneous measurement of PAT without RH in the contralateral index fingertip.

Although the distal fingertip is not an intuitively obvious place to look for endothelial dysfunction as a marker of atherosclerosis risk, the peripheral vascular beds located at

the distal part of the limbs are major sites of sympathetic α -adrenergic vasoconstrictor activity and hence play an important role in circulatory regulation.¹³ It appears that endogenous NO-mediated vasoregulation is particularly prominent in the AV anastomoses in the human fingertips,¹⁴ and indeed Gerhard et al have shown that approximately 60% of the RH-PAT response is mediated by NO release.¹⁵

Studies using the PAT probe have demonstrated altered endothelial function in children at cardiovascular risk because of type 1 diabetes mellitus,¹⁶ have shown that impaired RH-PAT responses are present in patients with coronary microvascular endothelial dysfunction measured in vivo,¹⁷ and, as in the case of Fisher et al,¹⁸ have investigated reversibility of peripheral endothelial dysfunction with interventions such as flavanol-rich cocoa.

The current study reported by Hamburg et al gives further insights into the potential role of RH-PAT for the noninvasive assessment of endothelial function in asymptomatic subjects. Digital pulse amplitude was assessed in a group of nearly 2000 subjects in the Framingham Third Generation Cohort study. The authors provide important physiological information about the time dependency of the increase in fingertip pulse amplitude after reactive hyperemia and carefully document the relationship between this novel vascular measure and multiple traditional and metabolic cardiovascular risk factors, revealing a significant although relatively weak relationship between RH-PAT and most cardiovascular risk factors (r values ≈ 0.4). Some findings were counterintuitive, however, such as the lack of a significant relationship between hypertension and digital vasomotor responses and a small but paradoxically positive relation between older age and the RH-PAT. Furthermore, much of the variability in the digital pulse amplitude remained unexplained by measured clinical factors. In this cross-sectional study, Hamburg et al were also unable to investigate whether RH-PAT measurement gave any independent prognostic information, although this will be a fruitful area for further study as the subjects in this cohort are followed up.

Thus, much work remains to be done in evaluating the potential role of RH-PAT in risk stratification for individuals. Data on circadian variation, changes after meals and during intercurrent illnesses, relationship to other tests of vasomotor function known to have importance, and information about the prognostic value of this novel index above and beyond traditional risk factors require further investigation.

Future Directions

The noninvasive identification of subjects at higher risk of cardiovascular events than might be predicted from traditional risk factors alone is still vitally needed to facilitate effective targeted prevention. Even though none of the currently available methods for measuring endothelium-dependent dilatation fulfill all the desiderata for clinical assessment of individual risk, several promising novel technologies, including RH-PAT, are being actively investigated. The measurement of endothelial function, as an integrative marker of arterial health in response to the effects of risk factors on the vessel wall, shows great promise. Nevertheless, much work remains to be done to incorporate functional assessments of nonvasomotor aspects of

endothelial physiology, as well to define which testing modalities give the best readily obtainable prognostic information.

Disclosures

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References

- Cooke JP, Tsao PS. Is NO an endogenous anti-atherogenic molecule? *Arterioscler Thromb*. 1994;653–655.
- Ludmer PL, Selwyn AP, Shook TL, Wayne RR, Mudge GH, Alexander RW, Ganz P. Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. *N Engl J Med*. 1986;315:1046–1051.
- Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, Lloyd JK, Deanfield JE. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet*. 1992;340:1111–1115.
- Celermajer DS, Sorensen KE, Bull C, Robinson J, Deanfield JE. Endothelium-dependent dilation in the systemic arteries of asymptomatic subjects relates to coronary risk factors and their interaction. *J Am Coll Cardiol*. 1994;24:1468–1474.
- Woo KS, Chook P, Yu CW, Sung RY, Qiao M, Leung SS, Lam CW, Metreweli C, Celermajer DS. Effects of diet and exercise on obesity-related vascular dysfunction in children. *Circulation*. 2004;109:1981–1986.
- Lerman A, Zeiher AM. Endothelial function: cardiac events. *Circulation*. 2005;111:363–368.
- Deanfield JE, Halcox JP, Rabelink TJ. Endothelial function and dysfunction: testing and clinical relevance. *Circulation*. 2007;115:1285–1295.
- Cohen RA. The endothelium-derived hyperpolarizing factor puzzle: a mechanism without a mediator? *Circulation*. 2005;111:724–727.
- Joannides R, Haefeli WE, Linder L, Richard V, Bakali EH, Thuillez C, Lüscher TF. Nitric oxide is responsible for flow-dependent dilatation of human peripheral conduit arteries in vivo. *Circulation*. 1995;91:1314–1319.
- Anderson TJ, Uehata A, Gerhard MD, Meredith IT, Knab S, Delagrange D, Lieberman EH, Ganz P, Creager MA, Yeung AC. *J Am Coll Cardiol*. 1995;26:1235–1241.
- Corretti MC, Anderson TJ, Benjamin EJ, Celermajer DS, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D, Vallance P, Vita J, Vogel R; International Brachial Artery Reactivity Task Force. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol*. 2002;39:257–265.
- Hamburg NM, Keyes MJ, Larson MG, Vasan RS, Schnabel R, Pryde MM, Mitchell GF, Sheffy J, Vita JA, Benjamin EJ. Cross-sectional relations of digital vascular function to cardiovascular risk factors in the Framingham Heart Study. *Circulation*. 2008;117:2467–2474.
- Burton AC. The range and variability of the blood flow in the human fingers and the vasomotor regulation of body temperature. *Am J Physiol*. 1939;127:437–453.
- Noon JP, Haynes WG, Webb DJ, Shore AC. Local inhibition of nitric oxide generation in man reduces blood flow in finger pulp but not in hand dorsum skin. *J Physiol*. 1996;490:501–508.
- Nohria A, Gerhard-Herman M, Creager MA, Hurley S, Mitra D, Ganz P. Role of nitric oxide in the regulation of digital pulse volume amplitude in humans. *J Appl Physiol*. 2006;101:545–548.
- Haller MJ, Stein J, Shuster J, Theriaque D, Silverstein J, Schatz DA, Earing MG, Lerman A, Mahmud FH. Peripheral artery tonometry demonstrates altered endothelial function in children with type 1 diabetes. *Pediatr Diabetes*. 2007;8:193–198.
- Bonetti PO, Pumper GM, Higano ST, Holmes DR, Kuvin JT, Lerman A. Noninvasive identification of patients with early coronary atherosclerosis by assessment of digital reactive hyperemia. *J Am Coll Cardiol*. 2004;44:2137–2141.
- Fisher ND, Hughes M, Gerhard-Herman M, Hollenberg NK. Flavanol-rich cocoa induces nitric oxide-dependent vasodilation in healthy humans. *Hypertension*. 2003;21:2281–2286.

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