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Endothelial function as a functional expression of cardiovascular risk factors

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Abstract

Traditional cardiovascular risk (CV) factors based on the Framingham study have been used to estimate the risk of CV events and determine target cholesterol levels for primary prevention. Recently published systematic reviews have, however, demonstrated that the Framingham risk score is limited in certain cohorts and requires adjustment. Indeed, traditional CV risk factors fail to predict the development of coronary heart disease in 25–50% of cases. This underscores the complex interplay between traditional CV risk factors, genetic predisposition and other atheroprotective factors present in individuals of different populations in predicting CV events. Endothelial dysfunction, a functional expression of the inherent atherosclerotic risk representing an integrated index of both the overall CV risk-factor burden and the sum of all vasculoprotective factors in an individual, may serve as the missing link between CV risk factors and atherosclerotic disease. Endothelial function measurements may aid in future prediction of CV events and help identify high-risk patients for targeted therapy as well as provide a primary therapeutic end point for clinical follow-up of these patients. Recently introduced reactive hyperemia peripheral arterial tonometry is emerging as a promising tool in endothelial function measurement and CV risk stratification.

Keywords

atherosclerosis; endothelial dysfunction; endothelium; surrogate marker; therapeutic end point; vascular health

This article focuses on endothelial function assessment as a universal tool for prediction of cardiovascular (CV) events in primary and secondary prevention. It also discusses endothelial function as a therapeutic end point to assess efficacy of treatment. Endothelial function is discussed in the context of traditional and nontraditional CV risk factors. Biomarkers from distinct biologic pathways used in CV risk assessment have previously been reviewed [1,2] and are not the subject of this article.

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Endothelium & endothelial function

The healthy endothelium is a monolayer of cells that is an active paracrine, endocrine and autocrine organ exerting many important homeostatic functions. It secretes molecules that have key functions in defense against atherosclerosis by regulating vascular tone, cellular adhesion, thromboresistance, smooth muscle cell proliferation and vessel wall inflammation [3]. Indeed, the importance of the endothelium was first recognized by its effect on vascular tone. In 1980, Furchgott and Zawadzski demonstrated an endothelium-derived relaxing factor that was subsequently demonstrated to be nitric oxide (NO) [4]. Other vasodilating and vaso-constricting factors have since been demonstrated to be produced by the endothelium [5]. A critical balance between endothelium-derived factors determines the regulation of vascular hemodynamic functions and cellular proliferation in health and in CV disease states, such as atherosclerosis.

Nitric oxide contributes to several other aspects of arterial health, including inhibition of platelet aggregation, monocyte adhesion to endothelial cells and abnormal smooth muscle cell proliferation, highlighting it as an important 'antiatherogenic' moiety [6]. NO is generated from L-arginine by the action of endothelial NO synthase in the presence of cofactors such as tetrahydrobiopterin [7]. Once NO is produced, it diffuses to the vascular smooth muscle cells and activates guanylate cyclase, which leads to cGMP-mediated vasodilatation.

There are several methods to assess the NO activity pathway and to gain information on the status of endothelium. Endothelial function, measured both in coronary vessels (using acetylcholine as the stimulus) and peripherally, (using shear stress as the stimulus) measures NO-induced vasodilation and, by implication, the ability of the endothelium to protect against the initiation and the progression of atherosclerosis. Thus, vascular tone is an important function of the endothelium and may serve as an index and a window to the underlying process of vascular injury and early atherosclerosis.

Endothelial dysfunction

Alteration of normal endothelial physiology constitutes endothelial dysfunction and is characterized by a reduction in the bioavailability of vasodilators, especially NO, leading to impaired endothelium-dependent vasodilation. NO mediates many of the protective functions of the endothelium by limiting vascular inflammation, vascular smooth-muscle proliferation, platelet aggregation and tissue-factor production [8,9]. These functions are compromised in endothelial dysfunction, a systemic disorder that is characterized by a proinflammatory, proliferative and procoagulatory milieu that favors all stages of atherogenesis [10]. Endothelial dysfunction is a marker of the inherent atherosclerotic risk, representing an integrated index of both the overall CV risk-factor burden and the sum of all vasculoprotective factors in an individual. Thus, it is regarded as the 'ultimate risk factor' indicating the existence of a specific atherogenic milieu and the lack of vascular repair [3].

Endothelial dysfunction is also characterized by increased vasoconstrictors, especially endothelin (ET)-1. ET-1 is a 21-amino-acid peptide with both mitogenic and vasoconstricting properties [11]. A growing body of evidence suggests that ET-1 plays a significant role in the regulation of vascular tone not only under normal conditions, but also in pathophysiological states such as atherosclerosis. We demonstrated that circulating ET-1 is increased in patients with atherosclerosis [12], and in the coronary circulation of patients with early atherosclerosis and coronary endothelial dysfunction [13]. We also recently demonstrated that long-term administration of ET-1 receptor antagonist in humans improves coronary microvascular endothelial function, thus, supporting the role of the endogenous ET system in the regulation of endothelial function in early atherosclerosis in humans [Reriani

M, Raichlin E, Prasad A *et al.*, Long-term administration of endothelin receptor antagonist improves coronary endothelial function in patients with early atherosclerosis. Submitted]. These findings are underscored by the observations that ET-1 levels may serve as a marker for increased mortality in humans following myocardial infarction, and ET-1 receptor antagonists decrease the mortality in experimental models of myocardial infarction [14].

Measure of endothelial function

The majority of studies used in measuring endothelial function assess the degree of NO-induced vasodilation, which serves as an index for several aspects of arterial health beyond the simple vasodilator response itself [15]. Regulation of vessel tone varies importantly by vessel size, the site in the body and in response to different chemical agonists, such as acetylcholine, serotonin and bradykinin, which are widely used in endothelial function testing. Responses to these substances are themselves different from the response of the vascular endothelium to the more physiologic stimulus of shear stress [15].

Coronary endothelial function

Invasive coronary endothelial function

Invasive measurement of the change in coronary artery diameter, coronary blood flow and coronary vascular resistance to an intracoronary infusion of acetylcholine (ACh) is considered the gold standard in endothelial function measurements [16]. In coronary arteries with normal endothelium, the response to intracoronary ACh is epicardial and microvascular dilatation results in an increase in coronary blood flow [3]. When the endothelium is disrupted or dysfunctional, intracoronary ACh induces paradoxical vasoconstriction at the sites of vascular injury and a decrease in coronary blood flow as a result of a lack of NO-dependent vasodilation at the level of the coronary microcirculation. Thus, vasodilator response to ACh serves as a marker for the bioavailability of NO, indicating normal endothelial function.

Noninvasive myocardial endothelial function

Noninvasive imaging techniques, such as PET, can be used to assess myocardial blood flow to pharmacological and/or physiological stress. Coronary flow reserve may be assessed by the response of myocardial blood flow to pharmacological stress in the presence of various vasodilator agents, such as adenosine, adenosine triphosphate and dipyridamole. This technique permits the assessment of microcirculatory vascular function, including both vascular smooth muscle and endothelial function [17]. Coronary flow reserve is a ratio of near maximal myocardial blood flow during pharmacologically induced hyperemia to myocardial blood flow at rest. The coronary flow reserve, measured by PET, accurately reflects regional myocardial blood flow as measured by an intracoronary Doppler flow guide wire. Coronary endothelial function can be estimated by the response of myocardial blood flow to cold-pressor testing. Cold-pressor testing is useful for noninvasive evaluation of endothelial-dependent vasodilator function based on the activated sympathetic nervous system with increased catecholamine and an increment in shear stress on endothelial cells with the release of NO. The increase in global myocardial blood flow detected by this technology represents the endothelium-dependent and -independent increase in coronary blood flow and, thus, may be less specific for the evaluation of endothelial function.

Noninvasive peripheral endothelial function

Although quantitative angiography is considered the gold standard for the evaluation of endothelial function, it is an invasive and time-consuming procedure, thus, limiting its use [18]. There is a growing need for the development of a noninvasive method for the

assessment of vascular health and, specifically, endothelial function. In order for a test to be accepted as a clinical test it has to fulfill several criteria: it needs to be simple, noninvasive, reproducible, not operator-dependent, cheap, widely available and able to accurately predict endothelial function. There are currently several methods for assessing endothelial responses in the peripheral vessels. A summary of these is provided in Table 1, including their advantages and disadvantages relative to coronary endothelial function testing.

Venous occlusion plethysmography

The basic methodology of venous occlusion plethysmography was introduced by Hewlett and Van Zwaluwenburg approximately a century ago [19] and has remained the same since. The underlying principle involves the arrest of venous outflow from the forearm with uninterrupted arterial blood flow. This results in a linear increase in forearm volume over time, which is proportional to arterial blood flow and reflects forearm vascular resistance, which is a function of normal vascular endothelial function. Changes in the forearm volume are measured by a plethysmograph after occlusion of blood circulation to the hand. Vascular resistance changes after intravascular administration of vasoactive drugs, such as ACh, are measured and reflect endothelial function.

Although the method has good reproducibility [20,21], it is relatively invasive and cumbersome, limiting its widespread use for the clinical assessment of vascular function.

Brachial artery reactivity test

Brachial artery imaging coupled with reactive hyperemia (RH; flow-mediated dilatation [FMD]) is one of the most popular techniques for the measurement of vascular function [22]. It is noninvasive, relatively repeatable, reproducible and correlates with findings in the coronary circulation of the same patients [23]. FMD involves measurement of the change in diameter of a conduit artery (the brachial or radial artery) in response to increased flow, typically induced by a period of ischemia in the distal circulatory bed [24]. FMD is predominantly NO-mediated and is depressed in endothelial dysfunction found in patients with CV risk factors [25,26]. Significant intra- and interobserver variation, however, remains in the measure of FMD, which limits its widespread use in risk stratification [15].

RH-peripheral arterial tonometry

Reactive hyperemia-peripheral arterial tonometry (PAT) is a noninvasive technique to assess peripheral microvascular endothelial function by measuring changes in digital pulse volume during RH [27]. The system (Itamar Medical Ltd; Caesarea, Israel) is a US FDA-approved method to measure vascular health and comprises a finger probe to assess digital volume changes accompanying pulse waves. It has been validated and used to assess peripheral arterial tone in different populations [28–29]. The RH-PAT experimental setup has been previously described [27,30]. The calculated ratio reflects the RH index (RHI).

The Framingham RHI (F-RHI) is the natural logarithm of the RHI, and they both represent the postdeflation:baseline pulse amplitude ratio in the hyperemic finger divided by the same ratio in the contralateral finger that served as control (Figure 1). Using the F-RHI, researchers from the Framingham Heart Study demonstrated that RH response (with PAT), as detected by the RHI, is related to multiple traditional and metabolic risk factors [31]. We have also previously reported the correlation between abnormal PAT results and coronary endothelial dysfunction as detected by invasive evaluation of coronary endothelial function (Figure 2) [32]. Assessment of pulse-wave amplitude response during RH, as assessed by PAT, has also been shown to demonstrate a profile similar to that of FMD [33]. Moreover,

recent data suggested that the F-RHI can predict CV events in a population with intermediate risk [34].

The benefits, limitation and utility of PAT testing in the clinical arena has recently been reviewed [35]. Assessment of vascular health using PAT has been demonstrated to be feasible in an outpatient setting [36] and its use is emerging as a clinical tool to identify patients at high risk of CV events.

Endothelial dysfunction & cardiac risk factors

Functional vascular abnormalities, such as endothelial dysfunction, are associated with most of the traditional and nontraditional CV risk factors, and are considered a key event in the initiation, progression and the complications of atherosclerosis [3]. Endothelial dysfunction has also been demonstrated in most disease conditions that predict high risk of CV events [37]. The association between endothelial dysfunction, different CV risk factors and disease conditions is complex and involves an interplay with individual genetic predisposition, environmental conditions, local factors and currently unknown factors. Indeed, many individuals do not develop atherosclerotic manifestations despite the presence of several risk factors and, conversely, many patients with only a few CV risk factors develop endothelial dysfunction [3]. Over half of the patients presenting with acute coronary syndrome (ACS), for example, have a normal lipid profile and blood pressure [38]. Patients with similar CV risk-factor profiles have also been demonstrated to have varying degrees of endothelial dysfunction, with some patients having a normal endothelial function (Figure 3).

Endothelial dysfunction as a functional expression of risk factors

Traditional risk factors established in the Framingham study are used in clinical guidelines for the primary prevention of coronary heart disease and to recommend a risk management based on the Framingham score. However, the performance of the Framingham score when applied in different populations has shown inconsistent results [39,40]. A systematic study addressing the relationship between predicted and observed event rates of the Framingham risk score in US and non-US populations found that in certain cohorts it needed adjustment. Indeed, results from the Seven Countries Study consisting of the first 20-year mortality data in 12 cohorts of six countries demonstrated that traditional risk factors, such as age, blood pressure, cholesterol, smoking habits, BMI and physical activity as risk factors only within certain cohorts [41]. Only age and mean blood pressure were universal predictors of CHD, suggesting a lack of homogeneity among the different cohorts in terms of predictive value of risk factors [41]. These studies underscore the complex interplay between CV risk factors, genetic predisposition and other atheroprotective factors present in an individual in predicting endothelial dysfunction and CV events. Thus, endothelial dysfunction represents the integrated functional expression of the CV risk-factor burden in an individual, indicating the existence of a specific atherogenic vascular milieu [42].

Endothelial dysfunction & atherosclerosis

Endothelial dysfunction is considered an early marker for atherosclerosis, preceding angiographic or ultrasonic evidence of atherosclerotic plaque. In established coronary artery disease (CAD), endothelial dysfunction is a better predictor of acute vascular events compared with the risk conveyed by the structural barrier to blood flow by atherosclerotic plaques [15]. Indeed, plaque size and minimum lumen diameter are a relatively poor predictor of CV risk [43,44]. This was corroborated by a recently published study on the utility of peripheral endothelial function measured by RH-PAT in predicting obstructive and nonobstructive CAD in women. It demonstrated that RH-PAT accurately predicted CAD and had the potential to identify high-risk patients for ischemic heart disease [45]. When

compared with the Reynolds Risk Score, RH-PAT was a superior predictor of nonobstructive CAD in women with angina-like symptoms lacking angiographic CAD [46].

Acute coronary syndrome

Endothelial dysfunction plays a fundamental role in the pathogenesis of ACS, such as unstable angina and acute myocardial infarction [47]. Plaque destabilization, the detrimental process that predisposes the plaque to rupture, results from a complex interplay of inflammatory effects that involve cellular plaque components and various proinflammatory mediators [48]. A dysfunctional endothelium may contribute to plaque destabilization owing to its reduced anti-inflammatory potential. We have recently demonstrated that segments with coronary endothelial dysfunction are associated with tissue cauterization of venerable plaques, such as necrotic cores, as detected by intravascular ultrasound [49].

Endothelial dysfunction is also characterized by a reduction in the anticoagulatory potential of the endothelium and an increase in endothelial production of procoagulatory mediators (e.g., tissue factor and plasminogen activator inhibitor) [50], resulting in a thrombogenic vascular environment. In addition, platelet-derived mediators, such as serotonin, induce vasoconstriction in the presence of a dysfunctional endothelium [51]. In summary, endothelial dysfunction contributes to enhanced plaque vulnerability, may participate in the process of plaque rupture, favors thrombus formation and, thus, may be viewed as an important contributory factor for several aspects of ACS and sudden cardiac death.

Endothelial dysfunction & cardiac events

The clinical implication of endothelial dysfunction and the association between endothelial cell dysfunction and cardiac events are well established and reviewed [9,52]. A meta-analysis of the studies reporting an association between coronary or peripheral endothelial function and CV events demonstrated that endothelial dysfunction is strongly and independently associated with CV events [53]. The analysis further underscored the systemic nature of endothelial dysfunction as endothelial function, measured remotely from the coronary site, was able to accurately predict CV events.

The prognostic impact of endothelial dysfunction in peripheral, easily accessible arteries suggests that assessment of peripheral endothelial function may represent an additional means for risk stratification in patients at possible risk for CV events.

Endothelial dysfunction & biomarkers in the prediction of CV events

Several studies evaluating conventional and novel biomarkers for the prediction of CV events have demonstrated that several of these biomarkers may be useful in CV risk stratification. However, large epidemiological studies have shown that the gains over conventional risk factors are minimal [1,2]. Recent evaluation by the Framingham Heart Study demonstrated that the use of ten contemporary biomarkers added only small increases in the ability to risk-stratify individual patients [1]. A cohort study of over 5000 participants in Sweden, followed for a median duration of 12 years, demonstrated that the utility of contemporary biomarkers, when added to conventional risk factors, for predicting future CV risk to be minimal [2].

Kita *et al.* demonstrated that improvements in endothelial function in response to risk-factor reduction had a positive prognostic value. In a multivariate analysis of the study, only diabetes among the conventional risk factors, in addition to persistent impairment of endothelial vasomotor function, were retained in the model predicting adverse CV events [54]. To date, no study has evaluated the prognostic value of therapeutic improvements in endothelial function and biomarkers in predicting CV events. Studies are needed to assess

the utility of putative biomarkers in assessing CV risk when compared with endothelial function. Given the relatively small incremental value of current biomarkers over conventional risk factors, one would speculate that biomarkers in such studies would still play a minimal role in CV risk stratification.

Therapeutic strategies to improve endothelial function

Although several strategies aimed at reducing CV risk factors are associated with an improvement in endothelial health, to date, no single agent has been approved for the treatment of endothelial dysfunction *per se* [24]. This has, in part, been owing to the fact that a standardized and reproducible technique for the measurement of peripheral, easily accessible, endothelial function in order to monitor the effects of therapy, has been difficult to establish [22]. Endothelial dysfunction is a reversible disorder that follows the nature of the CV disease, and strategies aimed at reducing CV risk factors also translate into an improvement in endothelial health [3]. Several studies reported the beneficial effect of statins on peripheral and coronary endothelial function [55–59]. Statin therapy is associated with a significant improvement in both peripheral and coronary endothelial function. Statin therapy has also been demonstrated to decrease CV morbidity and mortality in both hypercholesterolemic patients and those with a normal cholesterol profile.

The striking reduction in mortality in patients with a wide range of cholesterol levels cannot be attributed to their cholesterol-lowering effect alone. Indeed, Rosuvastatin was recently shown to significantly reduce the incidence of major CV events in patients with lipid levels at baseline, well below the threshold for treatment according to current prevention guidelines [60]. Some of the potential benefits of statins may be mediated through an improvement of endothelial function, which is independent of the improvement in cholesterol level. Thus, it may be speculated that the assessment of endothelial function may provide a therapeutic end point in individualized CV risk assessment and provide a basis for more aggressive medical therapy.

Similar meta-analysis on the effect of folic acid [61] and L-arginine [62] have also demonstrated an improvement in endothelial function as measured by FMD. Multiple studies on the effect of antihypertensive therapies using β -blockers, angiotensin-converting enzyme inhibitors, calcium channel blockers, smoking cessation and estrogen replacement therapy in postmenopausal women have also demonstrated an improvement in endothelial function.

Nonpharmacological approaches may also promote the preservation of a healthy endothelium. Exercise training improves coronary endothelial function [63]. Several studies on mind–body therapy demonstrated a trend towards improving FMD [64–66]. We have previously shown that enhanced external counter-pulsation improves peripheral endothelial function with beneficial clinical effects persisting in patients with symptomatic coronary disease at 1-month follow-up [27]. The observation that several therapeutic approaches may improve endothelial function may support the hypothesis that these therapies operate on a common mechanism, such as the reduction of oxidative stress and improvement of NO bioavailability.

Clinical relevance of therapeutic improvement of endothelial function

Until recently, there has also been a lack of direct evidence that therapeutic improvement in endothelial function translates into lower CV morbidity and mortality [54,67]. Thus, to date, endothelial function has not been considered a primary therapeutic target in the prevention of atherosclerotic disease. Modena *et al.* demonstrated that improvement in endothelial function in hypertensive postmenopausal women was associated with an improvement in

CV prognosis [67]. Recently, Kitta *et al.* also demonstrated that persistent impairment of endothelial function in patients with CAD and endothelial dysfunction, despite optimized medical therapy, was a strong independent predictor of adverse CV events [54]. The study suggested that measurement of endothelial vasomotor function, a comprehensive analysis of atherosclerotic burden, provided a better predictive value of future CV events than the analysis of each of the traditional risk factors alone. Thus, the assessment of endothelial function may not only serve as a tool for the diagnosis of vascular injury and early atherosclerosis but also as a therapeutic target and a method to follow the success of the intervention.

Conclusion

Traditional risk factors, as identified in the Framingham study, have shown inconsistent results in predicting CV when applied to different populations and predicate the development of coronary heart disease in only half of cases. Endothelial dysfunction is an independent predictor of adverse CV outcome and may provide a better predictive value of future CV events than each of the traditional risk factors alone. Endothelial dysfunction is a reversible process and several therapeutic strategies have been shown to improve it. Improvement in endothelial function has recently been linked to improved CV morbidity and mortality. Endothelial function may thus be regarded as the integrated vascular expression of the overall CV risk-factor burden in a given individual and its measurement is emerging as a promising clinical tool for individualized CV risk assessment and may provide a basis for more aggressive medical therapy.

Future perspective

Endothelial function as a universal tool for the detection & prediction of future CV events

Given the central role that endothelium plays in vascular biology, it seems attractive to consider integrating endothelial function testing as a universal tool for CV risk stratification in primary and secondary prevention. Previous techniques for the assessment of endothelial function have, however, not been ideal for this due to either significant intra- and interobserver variation or their invasive nature limiting their widespread use in risk stratification. Recently introduced RH-PAT has generated a lot of interest and may emerge as a universal clinical tool for non-invasive assessment of endothelial function. The integration of these novel tools into our clinical practice requires a shift in our thinking and approach to patients from the traditional risk factors and biomarkers to functional risk factors.

Endothelial function assessment as a primary therapeutic end point

Traditional CV risk factors used in risk stratification for primary prevention are also used as therapeutic end points to assess the efficacy of treatment. However, many patients presenting with ACS only have a few traditional risk factors despite having impaired endothelial function. Indeed, many of the guidelines for secondary prevention following ACS recommend therapies aimed at particular risk modifications despite many of the patients having a normal risk-factor profile (e.g., statins in patients with normal cholesterol or angiotensin-converting enzyme inhibitors and β -blockers in normotensive patients). Over 20% of patients presenting with ACS have been shown to have adverse CV outcomes at 2 years despite being on an optimized medical therapy regimen. Integration of endothelial function, as a surrogate measure to assess the effectiveness of interventions that prevent CV events, may provide a better measure of therapeutic success of intervention and provide a basis for more intensive therapy to patients with an otherwise optimized traditional risk-factor profile.

Executive summary

- Traditional cardiovascular (CV) factors based on the Framingham study do not adequately predict future CV events.
- Endothelial dysfunction is an integrated functional expression of the CV risk-factor burden in an individual, indicating the existence of a specific atherogenic vascular milieu.
- Invasive measurement of coronary endothelial function is gold standard in endothelial function measurements.
- Reactive hyperemia peripheral arterial tonometry is a noninvasive measurement of endothelial function and is emerging as a promising tool in CV risk stratification.
- Several therapeutic strategies have been shown to improve endothelial function.
- Improvements in endothelial function has recently been linked to improved CV morbidity and mortality.

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Figure 1. Reactive hyperemia peripheral arterial tonometry recordings of subjects with normal and abnormal hyperemia response

Normal response is characterized by a distinct increase in the signal amplitude after cuff release compared with baseline.

Figure 2. Using reactive hyperemia peripheral arterial tonometry, digital pulse volume changes during reactive hyperemia were assessed in 94 patients without obstructive coronary artery disease and either normal ($n = 39$) or abnormal ($n = 55$) coronary microvascular endothelial function

The receiver operated characteristic curve analysis was carried out to identify the reactive hyperemia peripheral arterial tonometry index value for optimal discrimination between the presence/absence of coronary endothelial dysfunction, reactive hyperemia peripheral arterial tonometry index of 1.35 discriminates best between presence/absence of coronary endothelial dysfunction.

AUC: Area under the curve.

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Figure 3. Distribution of traditional cardiovascular risk factors in patients with normal endothelial function, mild or severe endothelial dysfunction of the coronary (A) or peripheral (B) vasculature

In these studies, prevalence of hypercholesterolemia, hypertension and smoking status were similar in all groups irrespective of the status of endothelial function, indicating that the presence of known risk factors is not the only determinant of endothelial function.

NS: Not significant.

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Table 1

Techniques to assess vascular endothelial function.

Name of the test	Vascular bed	Outcome measured	Advantages	Disadvantages
Coronary endothelial function testing	Coronary arteries	Change in diameter and blood flow of coronary artery in response to infusion of acetylcholine	Gold standard and reproducible	Invasive procedure, costly and procedural risk
PET	Coronary microcirculatory vasculature	Change in myocardial blood flow in response to adenosine/dipyridamole	Noninvasive	Measures both endothelium-dependent and -independent function and is, thus, less specific for the evaluation of endothelial function
Brachial artery reactivity test	Brachial artery	Change in diameter of brachial artery in response to shear stress	Noninvasive and easy to use	Intra- and interoperator variability, difficulty to standardize results
Venous occlusive plethysmography	Forearm resistance vessels	Increase in forearm blood flow as measured by change in forearm circumference	Accurate and reproducible	Invasive and procedural risks
Peripheral arterial tonometry	Peripheral arteries	Change in postocclusion pulse wave amplitude in relation to the baseline	Noninvasive, easy to use, not user dependent, automatic analysis, reliable and reproducible	Expense of disposable finger probes