Endothelial Function in the Stress Echocardiography Laboratory

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26.1 Introduction

Endothelial dysfunction is an early stage of atherosclerotic disease [1], which may progress to impairment in coronary flow reserve in the intermediate stage and then to stress-induced dysfunction in the advanced stages (Fig. 26.1). The challenge of endothelial function can be obtained through a physical (postischemic dilation) and a pharmacological (nitrate-induced vasodilation) challenge. Technologically, the assessment of endothelial function employs the same basic echocardiography hardware of stress echocardiography testing, with a higher frequency transducer [1]. Similar know-how and training are also required for accurate measurements of two-dimensional (2D) echocardiography images and Doppler signals from the brachial artery. The additional technological and cultural burden required to implement the technique is high for a hypertension specialist or a cardiologist without echocardiography training and only modest for a cardiologist already skilled in echocardiography. The endothelial function is attractive for a cardiologist because of the potential it has to supply important pathophysiological, diagnostic, and prognostic information currently missed by our noninvasive testing modalities. Endothelial dysfunction is a key factor in the onset and development of atherosclerosis, hypertension, and heart failure, as it is also a serious candidate to bridge the gap between hemodynamic atherosclerotic burden and occurrence of clinical events [2]. It is placed exactly in the physiological scotoma of stress echocardiography, which somewhat measures the functional or hemodynamic impact of a coronary stenosis [3] but is unable to assess the status of endothelial function, allegedly responsible for many catastrophic cardiovascular events.

Endothelial dysfunction is also and mainly a biomarker of atherosclerosis. In 2001, a working group of the National Institutes of Health standardized the definition of a biomarker as a "characteristic that is objectively measured and established as an indicator of normal biological pathologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" [4]. A biomarker may be measured on a biosample (such as

E. Picano, *Stress Echocardiography*,

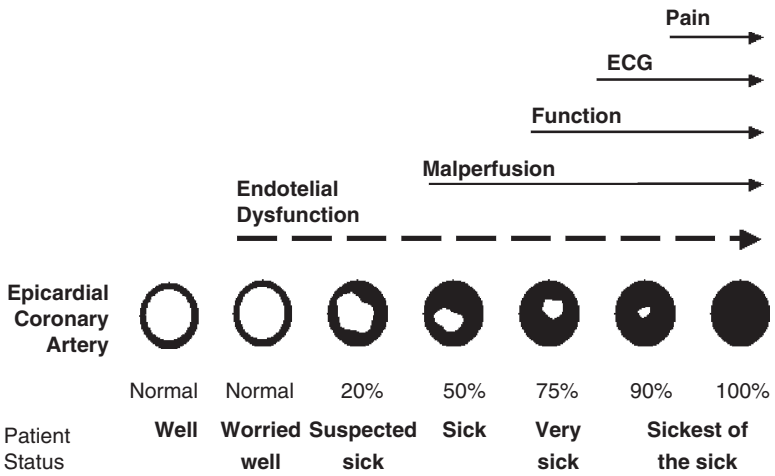

Fig. 26.1 The timeline of atherosclerosis. Endothelial dysfunction occurs early in the natural history of atherosclerosis

a blood test, for instance, the D-dimer as a biomarker of vulnerable blood) or it may be an imaging test (for instance, echocardiogram for vulnerable myocardium). A simplistic way to think of biomarkers (including endothelial dysfunction) is as indicators of a disease trait (risk factor or risk marker), a disease state (preclinical or clinical), or a disease rate (progression). Biomarkers may also serve as surrogate end points. Although there is limited consensus on this issue, a surrogate end point is one that can be used as an outcome in clinical trials to evaluate the safety and effectiveness of therapies in lieu of measurements of true outcome of interest. Surrogate end points (for instance, endothelial dysfunction in hypertensives in lieu of major cardiovascular events) have the advantage that they may be gathered in a shorter time frame and with less expense than end points such as morbidity and mortality, which require large clinical trials for evaluation. A biomarker will be of clinical value only if it is accurate, it is reproducibly obtained in a standardized fashion, it is acceptable to the patient, it is easy to interpret by the clinician, it has high sensitivity and specificity for the outcome it is expected to identify, and it explains a reasonable proportion of the outcome independent of established predictors (in case of atherosclerosis, Framingham Heart Study risk score) [4]. As a biomarker of atherosclerosis, endothelial dysfunction assessed by brachial ultrasound meets only some of these criteria (Table 26.1), and the deceptively simple methodology and pathophysiologically sweet appearance of the technique may harbor, at the present stage of technology and knowledge, substantial inaccuracies.

26.2 Historical Background

Endothelial surface totals about 27,000 m^2, an extension similar to a football field, and represents the largest epithelial surface of the body. It was long considered "little more than a sheet of nucleated cellophane," according to the definition of Florey, the Nobel

Table 26.1 Ultrasound biomarkers for identifying the vulnerable patient (adapted and modified from [4])

	Methodology standardized	Methodology available/ convenient	Linked to disease progression	Addictive to FHS risk score	Tracks with disease treatments
Arterial vulnerability					
• Structural markers (carotid IMT)	++	+	++	+	+
• Functional markers (endothelial dysfunction)	+	+	?	?	+
Myocardial vulnerability					
• Structural markers (LVH, LV dysfunction)	++	++	++	?	++
• Functional markers (stress echo)	++	++	++	++	++

++ Good evidence, + some evidence, *?* unknown or ambiguous data
LVH left ventricular hypertrophy, *LV* left ventricle, *FHS* Framingham heart study

Prize winner for medicine for his work on penicillin. Actually, the endothelium not only serves as a nonthrombogenic diffusion barrier to the migration of substances in and out of the bloodstream, but also as the largest and most active paracrine organ of the body, producing potent vasoactive, anticoagulant, procoagulant, and fibrinolytic substances [4]. In 1992, the journal *Science* dedicated the cover page to nitric oxide (NO), referring to it as the molecule of the year. In that very same year, Celermejer proposed a novel method to assess endothelial function in a totally noninvasive way through ultrasound assessment of postischemic hyperemia in the forearm [5]. This postischemic flow-mediated vasodilation is largely mediated by NO. Clinical assessment of endothelial function shifted from the venous occlusion plethysmographic method, exclusively used by a few research-oriented centers mostly interested in hypertension and clinical pharmacology, to the widespread availability of the echocardiography laboratory, crowded by cardiologists, who expect clinically relevant information from the technique [6]. The plethysmographic technique is complex, time-consuming, technically demanding, and invasive, requiring highly skilled expertise and intra-arterial scalar administration of acetylcholine (to assess endothelial function) and nitroprusside (to assess endothelium-independent vasodilation) [6]. The ultrasonic technique immediately showed potential for much broader applications, repeated assessment and large-scale diagnostic and prognostic validations. Both plethysmographic and ultrasonic techniques assess endothelial function in the brachial artery. With invasive cardiac catheterization, endothelial function can be assessed in the coronary artery segments by measuring the vasoconstrictor response to intracoronary acetylcholine administration [4] (Table 26.2).

Table 26.2 Methods to assess endothelial function in humans

	Intracoronary angiography	Brachial artery ultrasound	Venous occlusion plethysmography
Target endothelium	Coronary	Systemic	Systemic
Arterial catheterization	Yes (coronary)	No	Yes (brachial)
Radiation exposure	Yes	No	No
Intra-arterial acetylcholine	Yes (intracoronary)	No	Yes (intrabrachial)
Endothelium-dependent stimulus	Pharmacological (acetylcholine)	Physical (postischemic hyperemia)	Pharmacological (acetylcholine)
Intra-arterial nitrates	Yes (intracoronary)	No	Yes (intrabrachial)
Endothelium-independent stimulus	Coronary nitrates	Sublingual nitrates	Intra-arterial nitroprusside
Risk	Yes	No	Yes
Dedicated hardware	No	No	Yes
Cost	Very high	Low	High
Time required	Hours	Minutes	Hours
Key parameter	Coronary diameter	Brachial diameter	Brachial resistance
Setting	Catheterization lab	Echocardiography lab	Clinical pharmacology
Interest	Pathophysiology	Clinical and Pathophysiology	Pathophysiology

26.3 Physiology of Normal Endothelium

The endothelium lies between the lumen and the vascular smooth muscle (Fig. 26.2). Although it is only one cell layer thick, it senses changes in hemodynamic forces, or blood-borne signals by membrane receptor mechanisms, and is able to respond to physical and chemical stimuli by synthesis or release of a variety of vasoactive and thromboregulatory molecules or growth factors [7]. These are secreted into the lumen or abluminally toward the smooth muscle, affecting vessel tone and growth (Fig. 26.2). In addition to its universal functions, the endothelium may have organ-specific roles (such as control of myocardial contractility by coronary artery and endocardial endothelium) that are differentiated for various parts of the body [7]. As a result of their unique location, endothelial cells experience three primary mechanical forces: pressure, created by the hydrostatic forces of blood within the blood vessel; circumferential stretch or tension, created as a result of defined intercellular connections between the endothelial cells that exert longitudinal forces on the cell during vasomotion; and shear stress, the dragging friction force created by blood flow [8]. Of these forces, shear stress appears to be a particularly important hemodynamic force because it stimulates the release of vasoactive substances (including NO) and changes gene expression, cell metabolism, and cell morphology (Fig. 26.3). Many blood

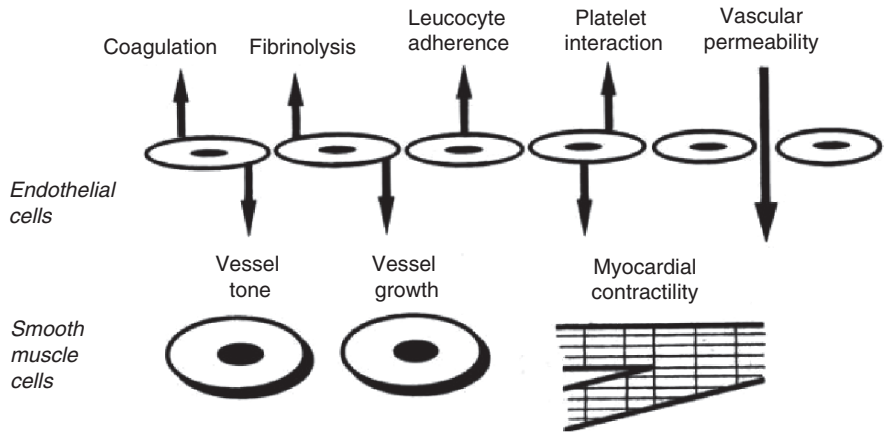

Fig. 26.2 The functional versatility of the endothelial cell. Factors secreted into the lumen (*upward arrows*) include prostacyclin and t-PA, which influence coagulation. Cell surface adhesion molecules (such as intercellular adhesion molecules-1, ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) regulate leukocyte adhesion. Factors secreted abluminally (toward the smooth muscle, *downward arrows*) may influence vessel tone and growth. Coronary artery and endocardial endothelium may also influence myocardial contractility. (From [7], with permission)

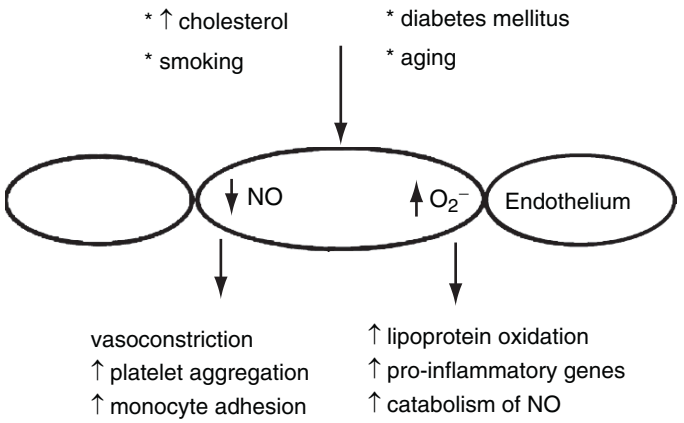

Fig. 26.3 A radical view of endothelial dysfunction. In the presence of certain risk factors, endothelial cells may produce less nitric oxide (NO) or more oxygen-derived free radicals (such as O_2^-) or both. This may lead to a variety of proischemic or proatherogenic effects. (From [7], with permission)

vessels respond to an increase in flow, or more precisely shear stress, by dilating (Fig. 26.3). This phenomenon is designated flow-mediated dilation. The principal mediator of flow-mediated vasodilation is endothelium-derived NO produced by endothelial nitric oxide synthase (eNOS), although other mediators such as endothelium-derived prostanoids or the putative endothelium-derived hyperpolarizing factor can cause vasodilation if NO

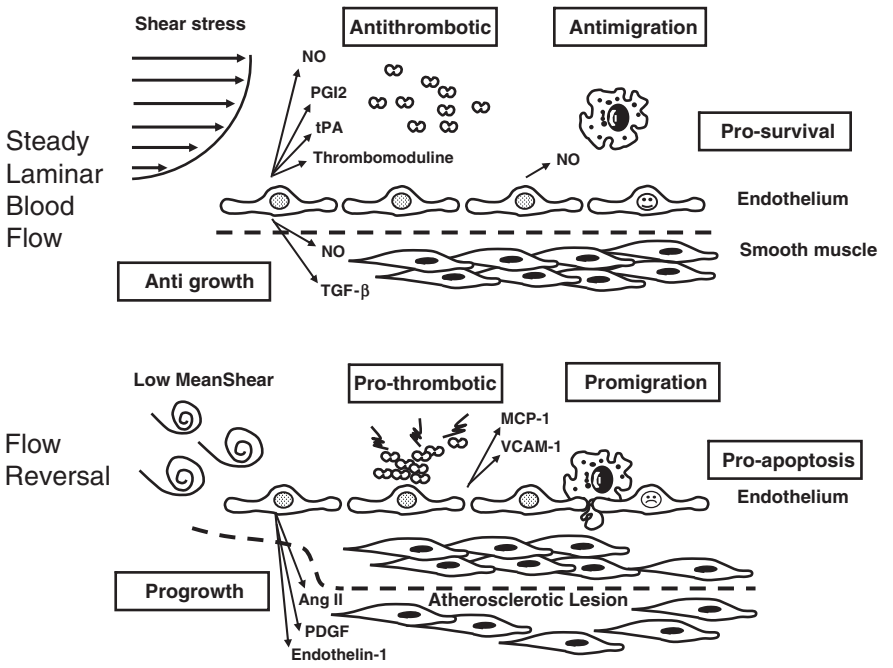

Fig. 26.4 Endothelial cell biology and shear stress. Steady laminar shear stress promotes release of factors from endothelial cells that inhibit coagulation, migration of leukocytes, and smooth muscle proliferation, while simultaneously promoting endothelial cell survival. Conversely, low shear stress and flow reversal favor the opposite effects, thereby contributing to the development of atherosclerosis. (From [8], with permission)

is deficient. The biological link between endothelial damage and atherosclerosis may be related to the decreased arterial bioavailability of NO. In the presence of certain risk factors, endothelial cells may produce less NO or more oxygen-derived free radicals (such as O_2), or both. These changes may in turn result in certain proischemic or proatherogenic effects (Fig. 26.4). The reduced bioavailability of NO translates into impaired flow-mediated vasodilation, which becomes a biomarker of depressed endothelial function.

26.4 Methodology of Endothelium-Dependent Flow-Mediated Vasodilation

The ultrasound technique for assessing endothelial function is attractive because it is non-invasive and allows repeated measurements. However, it also has technical and interpretative limitations [9, 10]. Until recently, the clinical instability of the technique had been magnified by absolute methodological deregulation on how to collect and interpret data. When evaluating endothelial function, these important factors should be taken into consideration:

1. Location of the occlusion device (upper vs. lower arm)
2. Duration of the brachial artery occlusion (5 min vs. 10 min)
3. Timing for detection of peak hyperemia
4. Portion of cardiac cycle during which brachial diameter should be measured
5. Time of day
6. Dominant or nondominant arm testing
7. Ongoing vasoactive medications
8. How best to evaluate vessel diameter
9. What form of nitrates should be used
10. Which are the normal reference values

In 2002, this methodological tower of Babel was replaced by the guidelines issued by the International Brachial Artery Reactivity Task Force [9], which aimed to minimize the sources of variability associated with patient, acquisition, analysis, and interpretation (Fig. 26.5). Because the magnitude of brachial artery diameter change is a fraction of a millimeter, the technique requires extreme accuracy in the methodology. According to these guidelines,

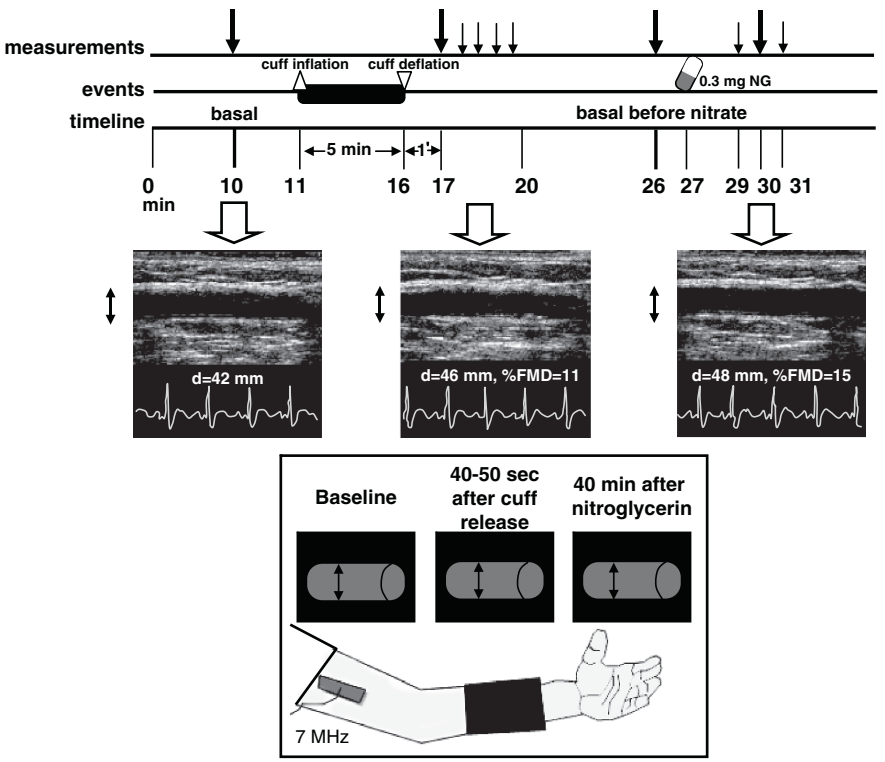

Fig. 26.5 Schematic drawing of the ultrasound imaging of the brachial artery. *Upper panel*: Timeline of events. *Middle panel*: Ultrasound imaging of the brachial artery. *Lower panel*: Cuff and transducer position. (Modified from [10], with permission)

the patient should fast for at least 8 h before the study. All vasoactive medications should be withheld for at least four half-lives, if possible. A linear array transducer with a minimum frequency of 7 MHz is used to acquire images with sufficient resolution for subsequent analysis. The brachial artery is imaged above the antecubital fossa in the longitudinal plane. A segment with clear anterior and posterior intimal interfaces between the lumen and vessel wall is selected for continuous 2D gray-scale imaging. After baseline rest image acquisition, arterial occlusion is created by cuff inflation to suprasystolic pressure, typically 50 mmHg above systolic pressure for 5 min. Lower-arm occlusion is preferred, since upper-arm occlusion is technically more challenging for accurate data acquisition, because the image is distorted by collapse of the brachial artery and shift in soft tissue. At least 10 min of rest is needed after reactive hyperemia before another image is acquired to reflect the reestablished baseline conditions. An exogenous NO donor, such as a single high dose (0.4 mg) of nitroglycerin spray or sublingual tablet is administered. Peak vasodilation occurs 3–4 min after nitroglycerin administration. Nitroglycerin should not be given to individuals with clinically significant bradycardia or hypotension. Variability during analysis is lowest when there is an average of three diameter measurements along a segment of the vessel. Such measurements should be obtained at baseline, during hyperemia (at least at 60 s, better every 30 s from 30 to 120 s after release, to circumvent the problem of temporal variability of response), again at baseline and 4 min after exogenous nitrates. The available technology now also makes it possible to acquire multiple images of the brachial artery automatically, using the ECG signal as a trigger. Arterial diameter is measured automatically using computer edge-detection techniques, making it possible to examine the entire time course of brachial dilation in response to reactive hyperemia (Fig. 26.6). In addition to errors related to improper techniques, it is important to be aware of a host of factors that cause intrinsic

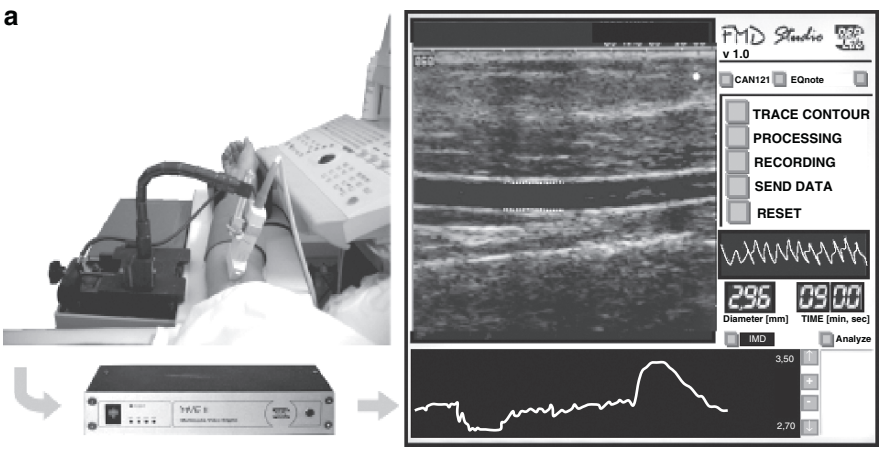

Fig. 26.6 Showing the automated edge-detection system (a), with on-line visual feedback on the quality of the detected signal. Brachial artery flow-mediated vasodilation is obtained with a brachial artery diameter measured using an operator-independent, automated software (Prototype by Marcello Demi, Institute of Clinical Physiology, Pisa, Italy). In panel b, two examples are shown, of a normal (*upper panel*) and an abnormal (*lower panel*) endothelial function

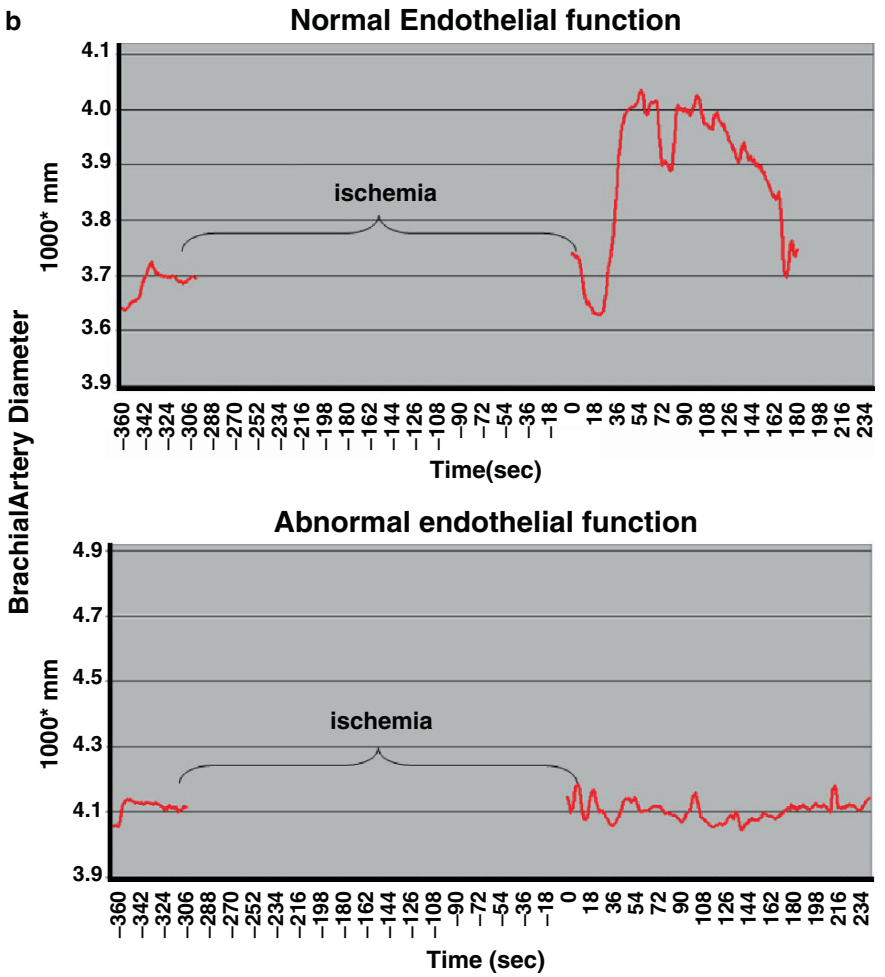

Fig. 26.6 (continued)

variability in flow-mediated vasodilation, including mental or physical stress, recent intake of a meal, medications including vitamins, exogenous hormones, cyclic changes related to the menstrual cycle in females, age, and body weight [10].

26.5 Diagnostic Value of Endothelial Dysfunction for Detection of Coronary Artery Disease

The integration of endothelial function in the stress testing laboratory has already provided some clinically relevant information. The electrocardiographic ischemic response during stress testing is in fact highly predictive of an altered systemic endothelial

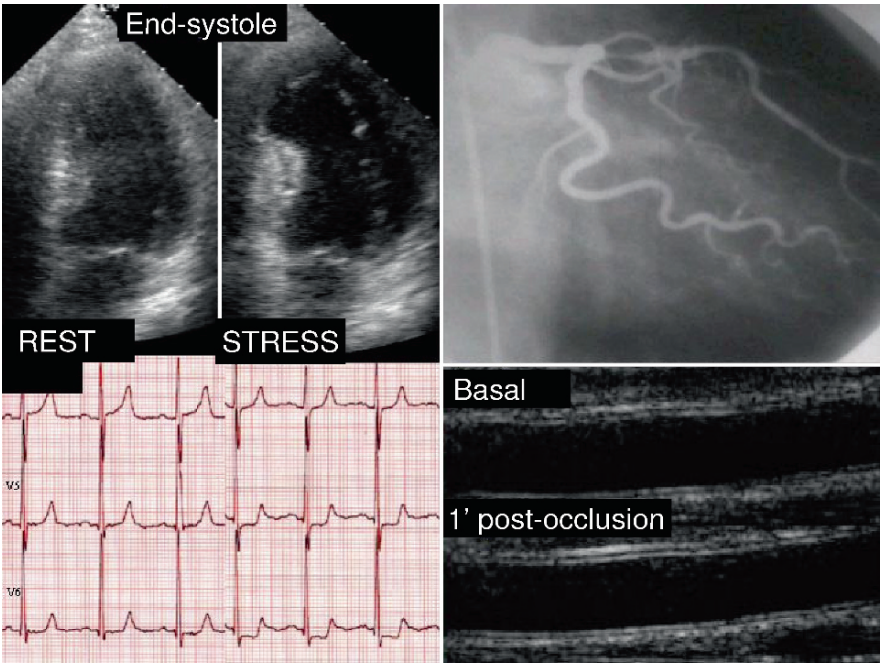

Fig. 26.7 Illustrative example of a typical pattern of test results in a patient with significant proximal stenosis of the left anterior descending artery (*right upper panel*). Exercise stress echocardiography testing (with representative end-systolic frames) reveals a dyskinetic septoapical segment (*left upper panel*) and significant ST-segment depression at peak stress (*lower left panel*); depressed brachial artery flow-mediated vasodilation (FMD) is also displayed on the *lower right panel*. (From [13], with permission)

dysfunction. This endothelial dysfunction can occur with normal (Fig. 26.7) or stenotic (Fig. 26.8) coronary arteries. The electrocardiographic information is therefore considered a misleading false-positive response compared to an angiographic standard, but a true-positive result when a physiologically relevant gold standard such as endothelial dysfunction is considered [11–16]. However, the diagnostic accuracy of endothelial dysfunction for noninvasively predicting coronary artery disease is poor, and there is no correlation between presence and extent of angiographically assessed coronary artery disease and percent flow-mediated vasodilation (Fig. 26.9). This cannot be surprising since flow-mediated vasodilation is impaired, independently of underlying coronary artery disease, in patients with coronary risk factors such as hypercholesterolemia [17], hypertension [18], smoking [19], diabetes mellitus [20], hyperhomocysteinemia [21], and aging [22]. In addition, lipid-lowering therapy [23], antioxidants [24], estrogen replacement [25], and treatment with angiotensin-enzyme inhibitors or receptor blockers [26] have each been shown to improve the flow-mediated vasodilation response, but cannot affect anatomically significant coronary artery disease.

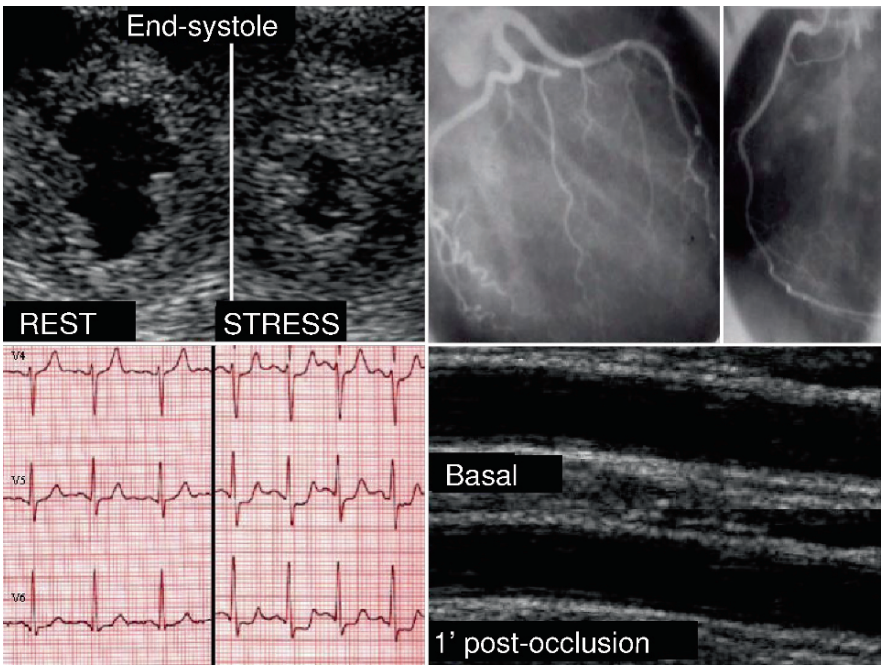

Fig. 26.8 Illustrative example of a typical pattern of test results in a patient with an anginal syndrome and normal coronary angiogram (*right upper panel*). Dipyridamole stress echocardiography testing (with representative end-systolic frames) reveals hyperkinetic wall motion response at peak stress (*left upper panel*), but significant ST-segment depression at peak stress (*left lower panel*); brachial artery FMD confirmed systemic endothelial dysfunction (*right lower panel*). (From [13], with permission)

26.6 Prognostic Value of Endothelial Dysfunction

The prognostic value of endothelial dysfunction is founded on a strong pathophysiological basis but supported, at present, by only weak clinical evidence, at least in patients with known or suspected coronary artery disease. From the pathophysiological viewpoint, the mechanism by which endothelial dysfunction may lead to cardiac events is multifactorial. One possible mechanism is myocardial ischemia secondary to endothelial dysfunction, even in the absence of obstructive coronary artery disease. Patients with abnormal coronary endothelial function often show a positive stress perfusion scintigraphy [14, 27, 28]. Another possible mechanism by which coronary endothelial dysfunction may contribute to cardiac events is through acceleration of coronary atherosclerosis, as evidenced by the development of obstructive coronary artery disease. This is also supported by the observation that in cardiac transplant patients, coronary endothelial dysfunction precedes the development of coronary atherosclerosis [29]. A number of studies have examined the prognostic value of endothelial assessment in predicting subsequent cardiovascular event risks, and ten of them were pooled in a 2005 meta-analysis [30] (Fig. 26.10). Studies have

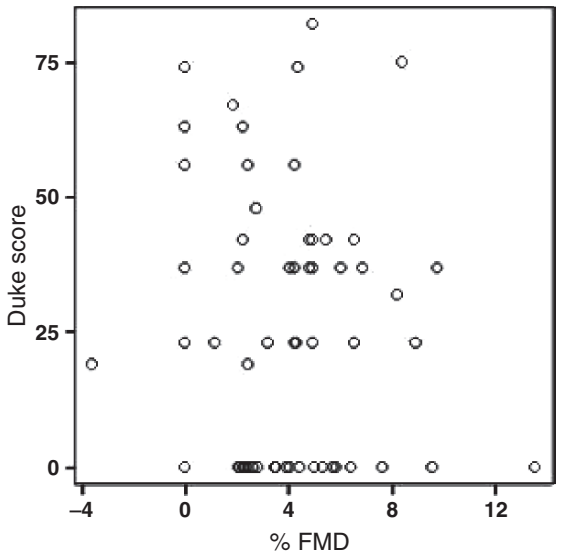

Fig. 26.9 Scatter plot diagram for angiographically assessed Duke score (*y-axis*) and percent flow mediated vasodilation (% FMD, *x-axis*) fails to show any significant relationship. (From [13], with permission)

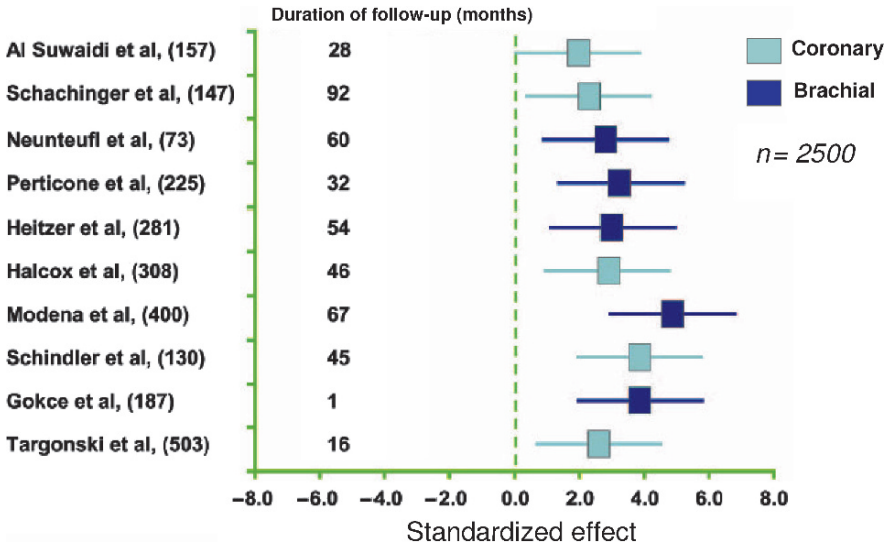

Fig. 26.10 A 2005 meta-analysis showing the capability of endothelial dysfunction to predict future cardiovascular events. In parenthesis, number of patients in each study (From [30])

differences in the method (brachial artery ultrasound, venous plethysmography with intrabrachial injection, or intracoronary Doppler flow wire), cohort of patients studied (those with established atherosclerosis vs. those with risk factors for cardiovascular disease), and design (with or without comparison with established clinical or echographic risk predictors, such as stress-induced wall motion abnormalities or carotid intima-media thickness). Taken together, these studies suggest the presence of a pathogenetic and prognostic link between (coronary or systemic) endothelial dysfunction and cardiovascular disease. In particular, the patients with relatively preserved endothelial function have a very low risk, a finding consistent with the growing evidence that endothelial dysfunction contributes to the pathogenesis of cardiovascular disease. However, these studies have also revealed that, in general, measures of endothelial function do not have additional prognostic yield in patients at high risk [31–45] (Fig. 26.11).

The ability of flow-mediated vasodilation to provide prognostic information in individuals of intermediate to low risk, independent of more standard risk-specific approaches, remains to be established. As a matter of fact, there are conceptual and pragmatic limitations in the use of endothelial dysfunction as a marker of risk. First, there is only a weak (r = 0.36), albeit significant, relationship between endothelial function (assessed by ultrasound in the brachial artery) and coronary endothelial function (assessed invasively by intracoronary acetylcholine and quantitative coronary angiography) [46]. Second, endothelial responses are heterogeneous within the same coronary artery or within the

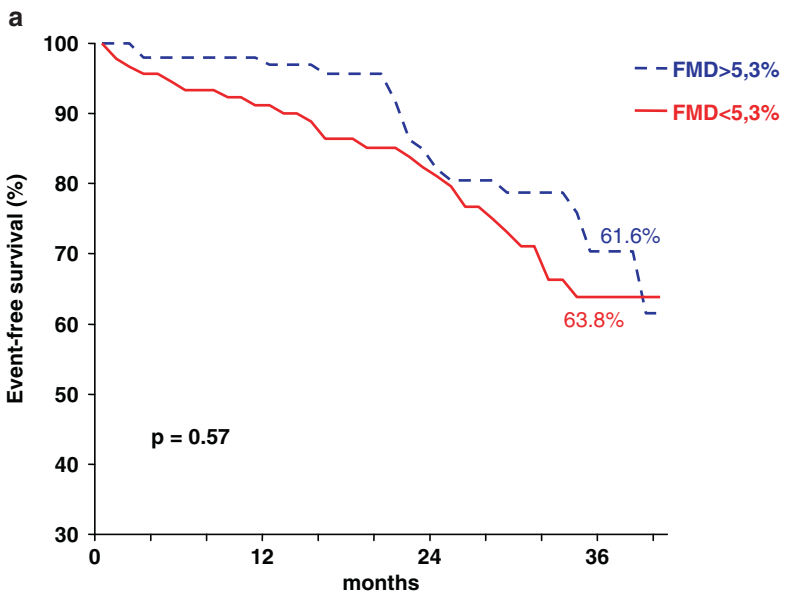

Fig. 26.11 Kaplan–Meier survival curves in patients with known or suspected coronary artery disease, whose prognosis cannot be separated on the basis of FMD values (**a**), but is clearly distinguished on the basis of echocardiographically assessed left ventricular hypertrophy (LVH, **b**) and ejection fraction (FE, **c**). (Adapted from [45])

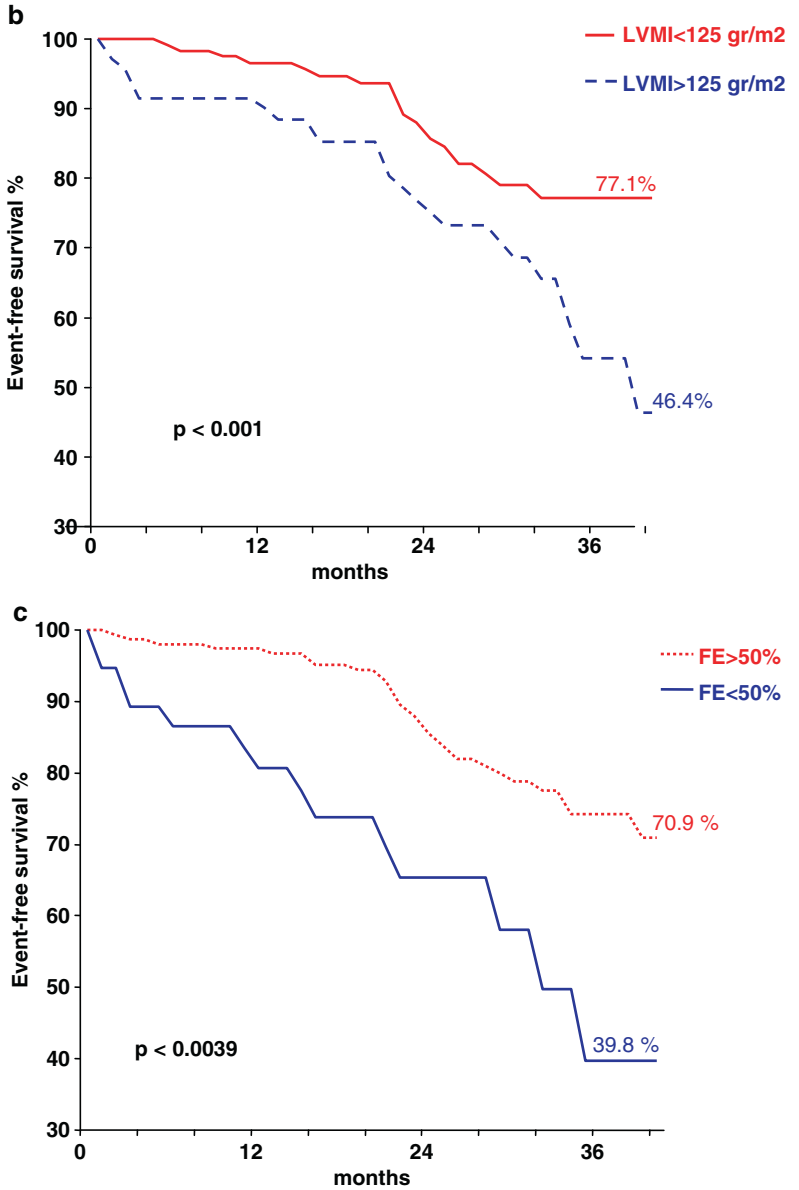

Fig. 26.11 (continued)

same patient [47], and a brachial artery endothelial function cannot be realistically considered a good predictor of endothelial function of the entire coronary tree, and much less of the endothelial function in the vulnerable coronary plaque. In the football field of endothelial layer, we are not measuring the endothelial function in the box, which is the critical region for events, but rather near the sidelines, far away from the core of the

clinical action. Third, some of these studies were retrospective in nature [33]; others included highly selected patient populations with a high number of adverse events when compared to the population usually enrolled in trials [34]; in still others the same prognostic value of coronary endothelial dysfunction was shown by much simpler – and theoretically much less robust – assessment of endothelium-independent vasodilation by nitrates [31]. In one study, the prognostic value of systemic endothelial dysfunction was lost after adjusting for presence and extent of angiographically assessed coronary artery disease [35]. In another study [39], there was no prognostic difference between patients with severely (<4%) to only mildly (4–8%) depressed flow-mediated vasodilation, although both these groups had worse prognosis than patients with preserved (>8%) endothelium-dependent vasodilation. Fourth, at present, there is no clear prospective evidence for prognostic benefit after improving endothelial function, although a recent study in postmenopausal hypertensive women shows that a significant improvement in endothelial function may be obtained after 6 months of antihypertensive therapy and clearly identifies patients who possibly have a more favorable prognosis [37].

26.7 Clinical Implications and Future Perspectives

Much more data are needed at this point to establish the clinical place, if any, of endothelial function in our diagnostic and prognostic flow charts. Despite its simple appearance, ultrasound assessment of brachial artery reactivity is technically challenging and has a significant learning curve [10]. The technique has the potential to offer an individual biological dosimeter of risk exposure through endothelial function [48], to identify early stages of atherosclerotic process [49], and to monitor interventions or therapy-induced changes in endothelial function in patients with heart disease [50], but it also skill- and labor-intensive and not easily used in routine clinical practice. Furthermore, interreader variability has led to difficulties replicating data and quantifying the real magnitude of the response [10]. For the clinical purpose of identifying asymptomatic patients at high risk who might be candidates for more intensive, evidence-based medical interventions that reduce cardiovascular disease risk, the evaluation of carotid intima-media thickness [51] might be a more robust option in the setting of carotid ultrasonography, which is already established in the cardiovascular ultrasound laboratory, traditionally used to evaluate the presence of obstructive atherosclerosis in the setting of symptomatic cerebrovascular disease or asymptomatic carotid bruit [10]. The carotid scan is presently recommended for risk assessment on patients at intermediate cardiovascular risk, i.e., patients with a 6–20% 10-year risk of myocardial infarction or coronary heart disease who do not have established coronary heart disease. In the near future, an effort should be made in order to study endothelial function in clinically critical districts, such as coronary, cerebral, and pulmonary circulation. This will make the base of the current diagnostic pyramid of atherosclerosis even more solid and attractive (Fig. 26.12), which makes it possible to track the natural history of atherosclerosis at an early stage [52, 53], certainly more susceptible to a reversal than a flow-limiting, ischemia-producing plaque determining stress echocardiographic positivity.

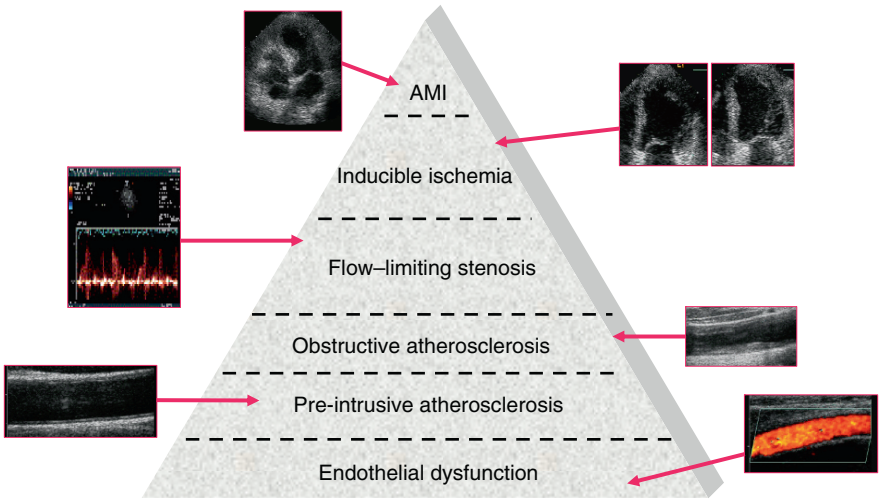

Fig. 26.12 The pyramid of atherosclerosis and the ultrasound imaging tools devoted to each of the segments of the disease: from the asymptomatic, clinically silent large base of the pyramid (endothelial dysfunction by brachial artery ultrasound) to the clinically obvious tip of the pyramid, represented by the baseline regional left ventricular dysfunction. *AMI* acute myocardial infarction

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