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CARDIOVASCULAR DISEASE AND STROKE (P PERRONE-FILARDI AND S. AGEWALL, SECTION EDITORS)

Endothelial Dysfunction: Its Clinical Value and Methods of Assessment

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Abstract Endothelial dysfunction (ED) is a systemic disorder characterized by reduced production of nitric oxide. This pathologic condition, which impairs vascular homeostasis, leads to the loss of protective properties of endothelial cells and is related to the pathogenesis of cardiovascular diseases. ED may affect every vascular bed, accounting for several clinical implications, particularly when the coronary bed is affected. Although the reliability of ED as a cardiovascular disease surrogate is still debated, many methods for its assessment have been proposed. In this review, we underline the clinical value of ED in the cardiovascular field and summarize the principal methods currently available for its assessment.

Keywords Coronary artery disease · Endothelial dysfunction · Flow mediated dilation · Pulse amplitude tonometry

Introduction

In the past decades, frantic research activity focusing on the endothelium and its functions revealed that the endothelium is not an unresponsive layer of cells marking vessel boundaries but is a leading actor in vascular homeostasis, through its paracrine and autocrine properties. This concept arose from the studies of Ignarro et al. [1], who in the late 1980s first demonstrated that nitric oxide (NO) is produced by endothelial cells and that by activating the enzyme guanylyl cyclase, it is responsible for relaxation of the nearby smooth muscle cells. In particular, NO is released constitutively and in response to several stimuli, such as acetylcholine binding to muscarinic receptors, sympathetic stimulation, and a response to increased blood flow due to shear stress [2]. Taken together, these data demonstrate that NO plays a pivotal role in endothelium integrity and function, regulating its antiadhesive, anti-inflammatory, and vasodilator properties. This arginine-derived factor reduces the expression of adhesion molecules, such as CD11/CD18 [3] and P-selectin, in leukocytes and platelets, respectively; inhibits conformational changes in glycoprotein IIb/IIIa, which are essential for platelet binding to fibrinogen [4]; and, through nitrosylation of cysteine residues, inactivates nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB), one of the prominent mediators of inflammation [5]. Vascular tone may be defined as the tone of smooth muscle cells surrounding vessels, and it is the result of complex metabolic and autonomic control, the former being exerted by vasodilator and vasoconstrictor substances released locally and the latter by the parasympathetic and sympathetic systems in response to external stimuli. The endothelium produces and releases vasodilators, such as NO and prostacyclin, and vasoconstrictors, such as endothelin; therefore, it has a significant role in the control of vascular tone [6].

Endothelial dysfunction (ED) is a condition characterized by a decrease in NO levels due to both reduced synthesis and increased consumption, leading to endothelial function impairment. Because endothelium is a ubiquitous tissue, this pathologic condition is a systemic disorder, and is implicated in the pathogenesis of several diseases. In particular, impaired endothelial dilation has been ascribed as the ultimate cause of cardiovascular diseases (CVDs) such as coronary artery disease (CAD) and peripheral artery disease (PAD). The aim of this review is to underline the clinical value of ED

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and to evaluate the principal methods available for its assessment.

Endothelial Dysfunction and Cardiovascular Diseases

Endothelial Dysfunction and Atherosclerosis

The relationship between ED and atherosclerosis was demonstrated initially by the studies of Furchgott and Zawadzki [12••], who reported that acetylcholine, which is usually a potent vasodilator, causes vasoconstriction when endothelial cells are removed from vessels; in addition, a few years later, investigators found that arteries affected by atherosclerosis are more sensitive to vasoconstrictor stimuli [7]. Today, it is known that this opposite behavior depends on acetylcholine, which normally causes release of NO from endothelial cells; however, in atherosclerotic vessels, owing to ED, production and release of NO are decreased [8].

To date, several cardiovascular risk factors have been shown to impair endothelial cell integrity and function, leading to ED and development of overt atherosclerotic plaques [9]. In particular, hypercholesterolemia, namely, the ratio between the levels of low-density lipoproteins (LDLs) and high-density lipoproteins, correlates strongly with ED severity [2]. Importantly, it is now clear that there is a hierarchic impairment of endothelial responses to three different vasodilator stimuli: acetylcholine, sympathetic activity, and augmented blood flow [10]. The first is impaired even if only hypercholesterolemia is present, without atherosclerotic plaques, whereas the other mechanisms of endothelial-mediated dilation are impaired with increasing atherosclerotic burden. Oxidized LDLs are a potent oxidative stimulus that enhances production of free oxygen radicals, especially superoxide anion, which reduces NO availability by reacting with it; conversely, administration of L-arginine may restore endothelial function [2]. Moreover, ED is also correlated with other cardiovascular risk factors, such as male sex, age, smoking, hypertension, diabetes, and a family history of CAD [11] (Fig. 1).

Reduced NO production, resulting in the loss of vascular bed integrity, determines a proinflammatory and proadhesive phenotype of endothelial cells, with higher activation of NF-κB and increased expression of adhesion molecules, such as intercellular and vascular cell adhesion molecules, thus promoting intima infiltration and plaque formation, which is the pathogenesis of atherosclerosis [3].

Coronary Artery Disease

The coronary artery bed is organized in vessels of three different sizes: epicardial arteries, which are conduit vessels;

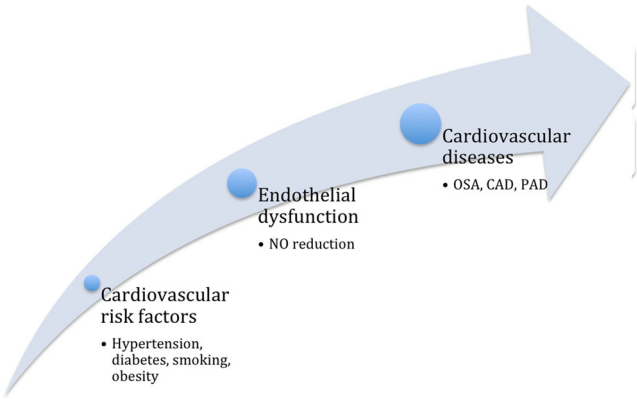

Fig. 1 From the causes to the consequences of endothelial dysfunction. *CAD* coronary artery disease, *OSA* obstructive sleep apnea, *PAD* peripheral artery disease

prearterioles, which regulate arteriole pressure, playing a major role in coronary resistance; and arterioles, very small vessels whose blood flow is regulated metabolically by oxygen demand [12••]. Myocardial ischemia is the result of an imbalance between oxygen demand and supply, so it can result from many disorders affecting both processes. The most frequent cause of impaired oxygen supply is coronary atherosclerosis, which primarily affects the large epicardial vessels; atherosclerotic plaques, which narrow the lumen of these vessels, reduce blood flow to downstream tissue. The clinical presentation of CAD includes stable angina, which occurs during physical exertion as the result of chronic lumen narrowing, and acute coronary syndrome (ACS), which occurs acutely when an occlusive thrombus forms as a consequence of plaque rupture or ulceration. It is noteworthy that the first step of CAD development is represented by ED; indeed, it has been reported that approximately 40 % of ACSs occur in people without angiographically demonstrated obstructive CAD, especially women [13]. These syndromes include Takotsubo cardiomyopathy and cardiac X syndrome, which mimics ACS, presenting with chest pain, electrocardiographic changes, and increased troponin levels but with no significant coronary narrowing on angiography. Nevertheless, studies on endothelial function have demonstrated its impairment in both conditions, resulting in myocardial perfusion defects; therefore, these conditions have been named microvascular angina (Fig. 2). Today, the prognosis for patients in this ACS subgroup remains controversial; although some investigators reported good outcomes for these patients [14], others demonstrated a higher incidence of cardiac events and a poorer prognosis compared with the control population [15]. In patients with obstructive coronary disease, ED has a key role in determining the severity of clinical consequences. Nevertheless, in stable CAD, the ischemic threshold is influenced by endothelial cell dysfunction. There is a constriction of prearterioles and arterioles, which leads to capillary derecruitment and maintains enough pressure to permit nutrient

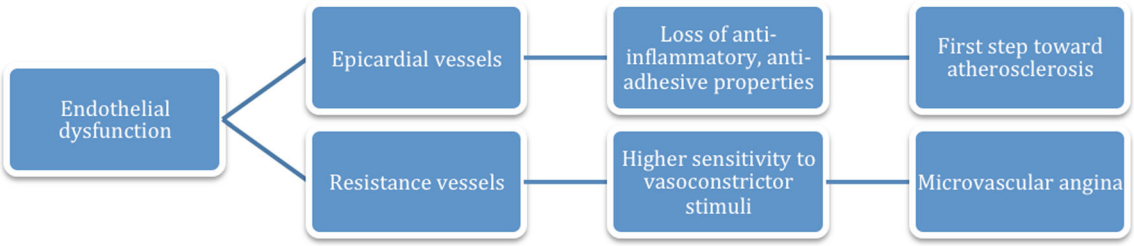

Fig. 2 Effects of endothelial dysfunction on the coronary vascular bed

exchange in the rest of the underperfused myocardium, decreasing the oxygen supply to the myocardium [16•]. Interestingly, ED is also involved in the harmful plaque destabilization that culminates in ACS. Because of the lack of NO, there is an inflammatory milieu associated with the plaque that is related to greater NF-κB activation and decreased production of plasminogen activator inhibitor. Moreover, ED of the vasa vasorum, which usually provides nutrients to the vascular wall, may lead to its ischemia and activation of neovascularization, which may result in intraplaque hemorrhage [16•]. In addition, in atherosclerotic vessels, ED leads to higher sensitivity to vasoconstrictor stimuli, such as exposure to cold or catecholamines, which may be explained by the reduced levels of NO and by increased production of endothelin 1, which favors vasoconstriction [12••].

Coronary revascularization is the gold standard in ACS treatment; however, vessel reopening is not without cost, because injury to arterial access may cause peripheral ED, and distal microembolization after percutaneous coronary intervention (PCI) may result in the no-reflow phenomenon [17]. The no-reflow phenomenon is the condition of inadequate myocardial reperfusion of a given coronary segment, without angiographic evidence of epicardial vessel obstruction, flow-limiting dissection, conduit vessel spasm, or in situ thrombosis [18]. It is well known that no reflow worsens the prognosis after PCI; however, recent findings demonstrate how coronary microvascular dysfunction affects prognosis in different ways. For example, patients with enhanced or severe ED undergoing elective PCI for stable CAD show greater platelet activity after receiving clopidogrel, a potent inhibitor of $P2Y_{12}$ platelet receptor, leading to increased platelet activation, which explains the unfavorable PCI outcomes in these patients [19]. In this regard, to underline the current clinical relevance of the topic, it has been shown that patients undergoing PCI and having impaired flow-mediated dilation (FMD)—defined as the ability of vessels to dilate in response to augmented shear stress—at 30 days after stent deployment had a higher risk of developing in-stent restenosis [20]. Furthermore, many studies have focused on the effects of coronary stent implantation on the coronary vascular bed, and overall they have found worse coronary endothelium impairment with sirolimus- and everolimus-eluting stents than with bare metal or zotarolimus-eluting or umirolimus-eluting stents, although it is unknown whether these findings reflect a poorer prognosis [21]. Taken together, these data suggest that ED may be considered a useful surrogate to guide the physician in the clinical management of patients with CVD.

Finally, besides the decrease in NO production that is the hallmark of ED, we must also consider the pivotal role of endothelial progenitor cells (EPCs) in vascular homeostasis. The endothelium continuously undergoes processes of injury and repair, in which EPCs are the key regulator. Previous studies showed a link between ED and EPCs. Decreased NO production leads to impairment in the ability of EPCs to repair vascular damage; however, a reduced number of EPCs homing to sites of vascular injury has been reported in cases of ED [16•, 22•].

Assessment of Endothelial Dysfunction

Evidence that coronary ED is the first step toward atherosclerosis has led to the development of newer strategies to assess it. Available methods include those assessing not only vasodilator properties but also vascular integrity and endothelial regenerative capacity.

Venous Occlusion Plethysmography

Venous occlusion plethysmography was the first method used to assess peripheral ED, and for a long time it was considered the gold standard. This technique uses two cuffs inflated at the upper arm and wrist to block venous drainage, and combined with cannulation of the brachial artery for vasodilator infusion, it allows a plethysmograph to measure forearm volume changes, which depend on arterial blood flow. This method has been abandoned not only because of its invasiveness, but also because of its poor reproducibility and because it measures the endothelial function of conduit vessels, which does not reflect that of coronary resistance vessels [23].

Quantitative Coronary Angiography and Doppler Flow Wire

Quantitative coronary angiography and the Doppler flow wire measure coronary diameter changes during angiographic procedures. Intracoronary infusion of a vasodilator, such as acetylcholine or salbutamol, is used to assess endothelial-

dependent vasodilation, whereas nitroglycerin is used to assess the endothelial-independent component. Several studies have shown that in the presence of atherosclerosis, vasodilation is blunted in response to these stimuli, leading to altered vasomotor balance [8].

Coronary Flow Reserve

Coronary flow reserve (CFR) is the ratio between the maximal increase of coronary blood flow and its value at rest, and it depends on the function of small resistance vessels. A CFR less than 2 suggests microvascular dysfunction. This parameter may be evaluated noninvasively with various methods, such as positron emission tomography, MRI, and echocardiography, and it represents an indirect measure of coronary microcirculation vasodilator properties [24].

Thrombolysis in Myocardial Infarction Frame Count and Myocardial Blush Grade

The thrombolysis in myocardial infarction (TIMI) frame count (TFC) and myocardial blush grade (MBG) are grading systems used during PCI to assess the efficiency of the procedure as well as one of its most harmful consequences: the no-reflow phenomenon. TFC is a measure of the number of cineangiographic frames needed by the dye to opacify the vessel from a proximal segment to a distal landmark: the lower the TFC, the better the clinical outcome [25]. MBG is a measure of myocardial perfusion after PCI; even when complete vessel recanalization has been obtained, distal embolization and endothelial edema may impair myocardial reperfusion. For PCI to be considered successful, a TFC of 3 and an MBG of 2 or 3 should be reached; MBG also correlates with ejection fraction and infarct size, which are among the most powerful predictors of prognosis [26].

Flow-Mediated Dilation

Since it was demonstrated that peripheral circulation may be used as a surrogate for the coronary vascular bed, several methods have emerged to assess ED noninvasively. FMD is the most widely used technique, thanks to its ease of use and reproducibility, although it requires some expertise. Measuring brachial artery diameter after stimulation by a vasodilator , such as reactive hyperemia due to the cold pressor test or 5-min sphygmomanometer insufflation at the forearm, FMD investigates the endothelial vasodilator properties at a peripheral site, which should reflect coronary endothelial function [27]. It was reported that in PAD patients with no history of CAD or chest pain, poorer FMD is also related to impairment of coronary endothelial function, as detected by a decreased CFR [28]. More importantly, other studies reported that FMD in PAD patients is an independent predictor of cardiovascular events, adding value to the ankle–brachial prognostic index [29], and is related to CAD severity [30]. Nevertheless, several undeniable differences exist between the coronary and peripheral vascular beds that increase the variability of this method and raise doubts about its validity. First, FMD is influenced by hormones, especially in women, in whom values differ in the same subject depending on the menstrual cycle phase [31]. Second, technical aspects of the measurement may explain the wide variety in absolute values across studies, especially the location and duration of the occlusion [32]. Third, increased sympathetic tone may falsify FMD values, so this interaction should be taken into account when considering patients with heart failure, renal failure, or hypertension [33].

Pulse Amplitude Tonometry

Quantitative analysis of the arterial pressure pulse waveform, recorded noninvasively and reproducibly by applanation tonometry, allows measurement of pulsatile arterial function at rest and after stimulation by a vasodilator [34]. There is much evidence that the arterial pulse contour changes with cardiovascular risk factors, such as diabetes [35], hypertension, and cigarette smoking. Nevertheless, this parameter is subject to many fluctuations; therefore, the use of a novel assessment method called pulse amplitude tonometry (PAT), which is based on a similar principle, is increasing. This technique uses a fingertip plethysmograph (finger probe) to quantify the arterial pulse volume at rest and during conditions of hyperemia due to increased shear stress [36]. The finger probe is pressurized, and with uniform application of near-diastolic external pressure reduces arterial wall tension. To counterbalance systemic influences, the pulse amplitude is also measured at rest at the contralateral fingertip [37]. The reactive hyperemia index (RHI) is a comparable parameter used in many studies assessing ED with PAT, and it results from the ratio of the PAT value at rest and the value after reactive hyperemia. As with FMD, the most frequently used stimulus to provoke augmented shear stress and reactive hyperemia is 5-min insufflation of a sphygmomanometer cuff at the forearm level. A strong inverse relation has been demonstrated between the RH-index and male sex, body mass index, total/high-density lipoprotein cholesterol, diabetes, smoking, and lipid-lowering treatment [38]. The RH-index in patients without obstructive CAD but with a reduced CFR is lower than that in patients with a normal CFR, suggesting that this noninvasive method might be used to assess the early stages of coronary atherosclerosis [39]. Recently, the prognostic role of the RHI was also evaluated; a lower value was associated with a greater number of adverse cardiac events during a 7-year follow-up in a cohort of asymptomatic patients, highlighting the role of the RHI as an independent predictor [40]. Despite this convincing evidence, the reliability and reproducibility

of this technique remains a source of debate, as great intraindividual variability and poor ability in detecting short-term changes in endothelial function have been proven [41].

Arterial Glycocalyx

Glycocalyx is a complex of glycosaminoglycans and proteoglycans covering the surface of endothelial cells. It functions as a transducer of mechanical stimuli, such as shear stress, and at sites of augmented shear stress, it causes NO release [42], exerting protective properties and influencing interactions between leukocytes and the endothelium [43]. In recent years, it has been widely demonstrated that oxidized LDLs, cigarette smoking, and diabetes cause glycocalyx thinning [44]. Consequently, sites of low shear stress, where NO availability is reduced, are more exposed to leukocyte interaction and immobilization, leading to inflammation and vascular injury, and are prone to atherosclerotic plaque formation [45, 46]. Lately, glycocalyx has been measured in vivo by comparing the distribution volume of a glycocalyx-permeable tracer, dextran 40, with that of a glycocalyx-impermeable tracer in erythrocyte blood samples. Studies demonstrate a reduction in glycocalyx volume due to hyperglycemia, reflecting ED assessed by FMD and determining a greater coagulation activation [47]. These findings suggest that a thinner layer of proteoglycans might represent a marker of ED.

Endothelial Microparticles

As mentioned earlier, endothelial integrity plays a pivotal role in vascular homeostasis. In atherosclerotic vessels, ED leads to a decreased regenerative ability of EPCs, with an increased number of endothelial apoptotic cells releasing bloodstream endothelial microparticles (EMPs), detected by flow cytometry [48]. In particular, increased numbers of EMPs have been observed in patients with CAD, and among these patients, greater amounts have been seen in those with ACS than in those with stable CAD [49]. Moreover, in patients with CAD, the number of EMPs correlates inversely with endothelial function assessed by FMD [48]. This correlation was also confirmed in diabetic patients [50]. In the aforementioned studies, EMP release seems to be a consequence of ED; however, recently it was shown that EMP release precedes the onset of ED in healthy subjects with a family history of premature CAD, and it remains unclear which event occurs first [51].

Conclusions

ED, a systemic disorder affecting cardiovascular homeostasis, plays a pivotal role in the development of atherosclerosis and other CVDs. To date, several investigations have shown that ED might represent the pathophysiologic basis of microvascular angina and may affect the severity and prognosis of CAD, implicating ED as a surrogate for CVD. Thus, the assessment of ED, although not currently suggested by guidelines, may be a useful tool for physicians in the clinical decision-making process for patients with CVD.

Compliance with Ethics Guidelines

Conflict of Interest Teresa Strisciuglio, Stefania De Luca, Ernesto Capuano, Rossella Luciano, Tullio Niglio, Bruno Trimarco, and Gennaro Galasso declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- •• Of major importance

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