Assessment of Endothelial Dysfunction: Focus on Atherothrombotic Disease

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Key Words

Endothelial cell · von Willebrand factor · Flow mediated dilatation · Soluble E-selectin · Circulating endothelial cells

Abstract

As the endothelium is crucial to cardiovascular disease, the accurate assessment of this organ is a valuable tool, especially if such assessments are clinically relevant. As functions of the endothelium focus on haemostasis and the maintenance of correct vascular tone, and dysfunction results in changes that promote thrombosis and hypertension, thus assessment of endothelial function therefore follows these processes. Foremost in the plasma markers of vascular function is von Willebrand factor, a molecule that interacts with platelets. Lack of nitric oxide results in poor blood pressure control that can be quantified by impaired flow mediated dilatation. More recently, increased numbers of circulating endothelial cells have been described that indicate severe damage to the endothelium. Unsurprisingly, these three markers correlate with each other and former two predict adverse outcome in long-term follow up studies. The assessment of vascular damage is becoming recognised as having an increasingly prominent part in the pathophysiology of cardiovascular disease.

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Introduction

If collected together, the endothelium is an organ weighing perhaps 1 kilogram, consisting of 1 to 6 x 10¹³ cells and covering an area of 1 to 7 m² [1]. Elsewhere, this has been equated to a mass of five normal hearts and in area to half a dozen tennis courts per standard 70 kg man [2]. Good performance of this organ is essential if the development and progression of atherothrombotic disease is to be resisted. Indeed, (pathological) damage to the endothelial cell (EC) and the resulting (physiological) dysfunction links health, the major risk factors such as diabetes, and the presence of clear disease as suggested by stroke and myocardial infarction (Figure 1).

Our approach to endothelial dysfunction considers firstly normal EC functioning such as in providing an anti-coagulant and anti-platelet surface, regulating fluid movement from the blood to the tissues, and correctly regulating blood vessel tone. The former is mediated by the release of molecules such as nitric oxide and prostacyclin and the presence of anti-coagulant molecules such as ectonucleotidase (CD39) and thrombomodulin (CD141) at the cell surface. However, ECs also produces pro-coagulants such as von Willebrand factor (vWf), coagulation factor V, and tissue factor. Correct vascular tone is regulated by a balance of vasodilators (such as nitric oxide and prostacyclin), and vasoconstrictors (such as endothelin). A healthy response to

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1424-8832/04/0336-0256\$21.0/0

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Fig. 1. Position of the endothelium in the pathogenesis of atherothrombotic disease.

a localised inflammation is the transient increase in the expression of cell adhesion molecules such as E-selectin (CD62E) that aid the process of trans-endothelial migration by leukocytes. Thus a dysfunctional endothelium that fails in these tasks instead (respectively) promotes thrombosis, oedema, hypertension and increased leukocyte adhesion.

Assessment of endothelial dysfunction therefore follows these leads and focus on three techniques: (i) the release of molecules into the plasma, (ii) the impaired vasodilator responses to an appropriate stimulus, and (iii) increased numbers of ECs in the plasma (i.e. circulating endothelial cells, CECs). Loss of endothelial functioning in regulating fluid movement (resulting in oedema) is difficult to quantify.

Plasma Markers of Endothelial Function

The endothelium produces/releases a large number of molecules, although few are both specific for this organ and easy to measure in the clinic or the laboratory (Table 1). Of those that remain, vWf, soluble thrombomodulin (sTM) and

soluble E selectin have been extensively studied by ELISA. The latter two molecules are normally cell surface constituents that can be cleaved or digested away to yield forms measurable in the plasma. Although tissue plasminogen activator is EC-specific, a variable proportion circulates bound to plasminogen activator inhibitor, leading to difficulties in interpretation if defined simply by structural ELISA [3]. Numerous groups have reported that raised vWf is a risk factor for coronary artery disease (CAD) and stroke on a variety of backgrounds although this relationship may not be causal [4-7]. Early work suggested that raised sTM may be useful in predicting atherothrombotic events [3], and although this has been questioned most recent data suggests it predicts stroke, not myocardial infarction [8, 9]. The Atherosclerosis Risk in Communities Study (ARIC), recruiting 792 subjects, demonstrated a significant association between levels of soluble E-selectin and occurrence of carotid artery atherosclerosis [10]. Curiously, in a study of hyperlipidemic patients who went on to experience a cardiovascular event, high levels of vWf, but low levels of soluble E-selectin, predicted a poor outcome [11]. In a differ-

ent follow-up of hypertensive patients, both vWf and soluble E-selectin levels decreased with the control of blood pressure, but only vWf was a predictor of the appearance or progression of atherosclerosis [12]. These two studies (and others) demonstrate a difference in vWf and soluble E-selectin responses, suggesting the two molecules reflect different aspects of vascular physiology. For example, perhaps raised soluble E-selectin is related more to increased endothelial cell regeneration after cell damage caused by the progression of the disease. Galen has recently concluded that although soluble E-selectin may reflect vascular dysfunction during hypertension, it is not a factor of prognostic value concerning the development of target organ damage [13]. Notwithstanding high baseline levels of soluble E-selectin found in type 2 diabetic patients, this molecule failed to predict death after a mean of 9 years [14], although several other molecules were predictors. Despite high levels of both soluble E-selectin and sTM in myocardial infarction patients, only the latter (said to reflect endothelial injury) was able to predict a poor outcome after 49 months of follow-up [15].

Table 1. Products of the endothelium

Anticoagulant/antithrombotic	thrombomodulin
	heparin
	protein C
	protein S
	tissue plasminogen activator
	prostacyclin
Procoagulant/prothrombotic	nitric oxide
	von Willebrand factor (vWf)
	factor V
	plasminogen activator inhibitor
	thromboxane
	tissue factor
Immunological	E selectin
	Inter-cellular adhesion molecule
	Vascular cell adhesion molecule
	Interleukins 1, 6 and 18
	Tumour necrosis factor
	Monocyte chemotactic factor-1

In patients admitted acutely with suspicion of CAD, soluble E-selectin was not a significant predictor of early outcome at three months [16,17]. Similar results were obtained after 6 months of follow-up, from patients suffering from stable [18] and unstable angina (and non-Q wave myocardial infarction) [19]. In a small cross-sectional study, soluble E selectin failed to discriminate between 60 patients with angiographically proven coronary artery disease and 60 individuals without evidence of stenosis [20]. In a large cohort of 1246 patients with angiographically-proven CAD, soluble E-selectin levels (and also C-reactive protein and fibrinogen) were significantly related to future cardiovascular death, although this disappeared on adjustment for clas-

sical risk factors, age, sex and other markers [22]. In the smaller British Regional Heart Study of 643 men, Malik et al, reported an odds ratio of 1.13 (95% CI 0.78-1.62) for adjusted soluble E-selectin, concluding that measurement of this molecule is unlikely to add much predictive information to that provided by more established risk factors [22]. A definitive, large and therefore well powered, head-to-head study of these three molecules will define which has most to offer.

Flow Mediated Dilatation

Evidence of the importance of the endothelium in regulating blood pressure was stimulated in 1980 by Furchgott and Zadowski with their discovery of endothelium-derived relaxing factor in an animal in-vitro model, a property subsequently shown to be due to nitric oxide [23]. These observations took on more importance when altered coronary artery vasoreactivity to infused acetylcholine was demonstrated by quantitative angiography in patients with atherosclerosis [24]. However, this dangerously invasive process remained of limited research value until the development by Celermajer and colleagues of the ultrasonic and non-invasive assessment of EC dysfunction, currently evolved into impaired blood flow after a temporary arterial occlusion [25]. In this technique, the response of downstream (i.e. forearm) arteries to the increase in blood flow following the release of a tourniquet over the upper arm is to dilate to facilitate this increased flow of blood, hence flow mediated dilatation (FMD).

Impaired brachial artery FMD is common in atherosclerosis and all its major risk factors, correlates with dysfunction of coronary arteries, and is reversible upon successful treatment of the particular risk factors (e.g. statins for hypercholesterolaemia) for atherosclerosis [26-28]. Notable, this pattern of findings is identical to that of vWf [3-7]. The similarity with this molecule also extends to the ability of impaired FMD to predict the development of major adverse cardiovascular events [28]. Thus, given the close association, albeit in completely different groups of patients and studies, between raised vWf and impaired FMD, it is no surprise that the two have been inversely correlated, i.e. those with the highest vWf have the most impaired FMD, whereas those with low vWf have preserved FMD [29] (Figure 2). Thus a dysfunctional or damaged endothelium not only fails to adequately regulate blood pressure but also releases increased amounts of pro-thrombotic vWf.

Fig. 2. Correlation between von Willebrand factor and FMD.

Circulating Endothelial Cells (CECs)

In 1991 George and her colleagues reported a monoclonal antibody to cell surface antigen CD146 on ECs and used it to quantify blood-borne ECs in the blood (i.e. circulating ECs, CECs) in a variety of conditions including rickettsial infection, sickle cell disease, thrombotic thrombocytopenia and acute coronary syndromes (reviewed in [30]).

Table 2. Reports of increased number of CECs

Condition	Reference
Sickle cell anaemia	[31]
Acute coronary syndromes	[32]
Chronic venous insufficiency	[33]
Aortoarteritis	[34]
Cytomegalovirus infection	[35, 36]
Septic shock	[37]
Breast cancer and lymphoma	[38]
Systemic lupus erythematosus	[39]
Renal transplant recipients	[40, 41]
Inflammatory vasculitis	[42]
Pulmonary hypertension	[43]
Peripheral vascular disease	[44]
Allogeneic bone marrow transplantation	[45]

Solovey et al also used a monoclonal antibody against CD146 to detect CECs in sickle cell anaemia [31]. More recently, various groups have reported increased numbers of CECs in many diverse disease and conditions including cardiovascular disease, cancer, and inflammatory connective tissues disease [32-45] (Table 2). However, these reports are diverse not only in disease studies but also in precise methodology, so that caution in interpretation is necessary.

Perusal of the list of diseases and conditions in table 2 will draw the conclusion that the majority are associated with a severe and injurious pathology. It seems reasonable, therefore, that increased numbers of CECs in the blood are the product of a disease process that irreversibly damages the endothelium. This theory is intrinsically attractive, given

the knowledge of the pathophysiology of conditions in which CECs were found, such as established vascular damage in cardiovascular disease and the 'response to injury' theory of atherosclerosis [46].

As previously discussed, numerous epidemiological studies have shown that increased levels of plasma markers such as vWf, tissue plasminogen activator, and (to a lesser extent) soluble E selectin are taken to imply some degree of disturbance of the endothelium, be it damage, dysfunction or activation. The inverse relationship between vWf and FMD has been mentioned [29]. Therefore, if increased numbers of CECs do indeed reflect a damaged endothelium, such levels should correlate with plasma markers and with FMD. Therefore, perhaps unsurprisingly, Kas-Deelen et al [35] found that CECs strongly ($p<0.001$) correlated with vWf but not soluble E selectin, whilst Makin et al [44] also correlated CECs with vWf ($p=0.002$). Since vWf is essentially the gold standard plasma marker for endothelial damage [3], this is strong evidence that CECs also reflect severe vascular disturbance. As yet no complete studies of CECs and FMD have been reported but doubtless this data is currently being generated.

Conclusions

As good endothelial function is desirable and poor endothelial function (i.e. dysfunction) undesirable, therefore assessment of this cell is valuable. Of the three methods, vWf is certainly easiest (ELISA) but the link with pathophysiology is indirect. FMD is only assessable with complex investigations but provides more direct information on the regulation of vascular tone. The presence of increased numbers of CECs is unequivocal demonstration of severe endothelial damage. Thus although all offer different perspectives on vascular physiology, it is unclear which will prove the most useful in helping to unravel the disease process and so provide better patient care.

Note Added In Proof

Qulici et al [47] have shown that CEC count can be used as an early, specific, independent marker of non-ST-elevation acute coronary syndrome (ACS). Similarly Lee et al [48] found that raised CECs predicted poor cardiovascular outcome at day 30 and 1 years post admission. vWf was not as accurate a predictor as CECs, and these cells largely stained positive for nitric oxide synthase. These two papers clearly demonstrate the potential of CECs as markers of future adverse events. Chong et al [49] reported raised CECs in congestive heart failure that correlated inversely with flow mediated dilatation, positively with vWf, but not at all with another putative EC marker, soluble thrombomodulin.

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