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Blann, Andrew

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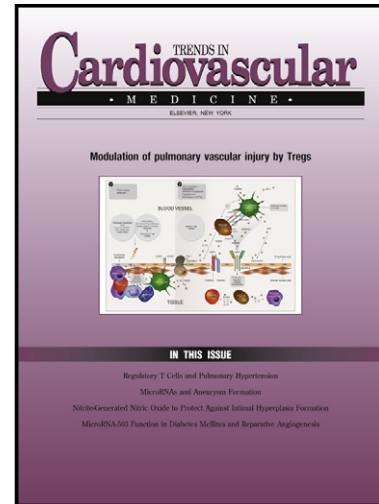
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A reliable marker of vascular function: does it exist?**Andrew Blann**

University of Birmingham Centre for Cardiovascular Sciences,
City Hospital, Birmingham, B18 7QH, UK.

Correspondence

Dr AD Blann PhD FRCPATH FRCPE

Tel/fax 0121 507 5076

Email a.blann@bham.ac.uk

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The major causes of mortality, and a great deal of morbidity, are cardiovascular disease and cancer. The endothelium, reputed to be the largest organ in the body (weighing about a kilogram and consisting of some $1-6 \times 10^{13}$ cells (1), is undoubtedly the primary target for the disease process of atherosclerosis (2). Epidemiological studies such as the Framingham Heart Study and others have unequivocally defined the importance of the four major risk factors for this disease. Their pathological link is that, either directly or indirectly, each of the risk factors independently cause damage to the endothelium, and of course in a clinical setting they overlap as, for example, many diabetics also have hypertension and dyslipidaemia (3). As regards cancer, the endothelium is important because of its role in angiogenesis (4). Furthermore, the endothelium is sensitive to cytotoxic chemotherapy, and this is perhaps why some forms of chemotherapy are successful in that they preferentially destroy those blood vessels feeding a tumour. In both disease groups a damaged endothelium loses its anticoagulant nature and becomes procoagulant, thereby providing a link with atherothrombosis in cardiovascular disease, and potentially with the increased risk of venous thromboembolism in cancer (especially during bolus chemotherapy)(5). A malfunctioning endothelium is unable to part-regulate blood pressure, leading to hypertension. Loss of the barrier function of the endothelium seem likely to be a contributor to oedema, whilst the increased expression of adhesion molecules (such as intercellular adhesion molecule [ICAM] and E selectin which recruit leukocytes) and release of cytokines such as IL-6 are likely contributors to inflammation (6,7). Consequently, the endothelium is of great interest to oncologists, cardiologists and hematologists, all of whom are keen to develop methods of assessing the integrity of this tissue. Candidate methods include those of plasma markers, techniques based on blood flow, and of cell biology.

The endothelium secretes and/or releases and/or expresses at its cell surface a variety of molecules (table 1). These molecules have a variety of functions, such as contributing to the

regulation of hemostasis (when released or expressed lumenally) and to vascular tone (when released into the vessel wall), some of which act as antagonistic pairs (7). Furthermore, several are easily measured in plasma by immunoassay, although not all are specific products of the endothelium. Endothelial integrity may also be assessed by changes in vascular tone, hypertension being a classic model, although endothelial-independent smooth muscle cell change may also be important in this disease. Nonetheless, endothelial function can be determined in a physiological setting by techniques such as flow mediated dilatation and arterial stiffness/pulse wave velocity, although these methods are slow and are strongly operator dependent (8-10).

The healthy endothelium adheres to the internal elastic lamina of the intima until it dies or is driven off by a disease process, at which time cells may be found in the plasma: hence circulating endothelial cells (CECs). Although described long ago (11,12), research on CECs took off once specific markers, such as CD146, were discovered (13). Thus armed, increased numbers of CECs were described in many cardiovascular, inflammatory and neoplastic diseases, the interpretation being that each disease process was (at least) partly responsible for this increase (14-16). However, others used alternative molecules to define CECs (17), and further confusion followed from the parallel discovery of bone marrow derived endothelial progenitor cells (EPCs), said to be a population that replaced the dead and dying CECs (18). Further confusion followed with the use of additional markers (many of which are expressed by non-endothelial cells (table 2)) such as CD34 and CD309 (19,20), and the use of intimately linked terms such as ‘circulating progenitor cell’ and ‘endothelial progenitor cells’, alone and in combination (21). The most recent development in this area is of endothelial microparticles (EMPs), exceptionally small particles of cytoplasm, increased numbers of which are, like CECs and plasma markers, increased in cardiovascular disease (22,23).

Schmidt et al have accurately summarised these issues in the present volume of the Journal (24).

Having agreed that the endothelium is an important organ/tissue whose status needs to be accessed, how should this be achieved? Clearly one of the more important arteries (if not the most important artery) is that of the epicardium, upon which the beating heart relies. Reminiscent of Koch's postulate for pathogenic organisms, Flammer et al (25), focussing on the heart, described nine criteria for an optimal endothelial function test, these being that it reflects the disease state, is reversible with interventions, mirrors coronary endothelial function, improves risk stratification, is reproducible, is operator independent, is non-invasive (with no or low risk for the patient), is easy to use and is inexpensive. Table 3 sets these nine criteria, and others, against a cross-section of methods (26). It is clear that none of the methods (as yet) comes anywhere near close to being a truly useful method, in the same way are the full blood count, urea and electrolytes and the electrocardiogram, for assessing coronary endothelial function. However, any of these methods may be useful in determining the state of other vascular systems, such as those of the brain. But in considering wider pathophysiological issues, an alternative use of these methods may be in determining global endothelial function and damage, and this may be important in other settings such as disseminated intravascular coagulation or in septicemia (27-30).

So using one or more of these tests, suppose we have identified a patient at high risk of an adverse cardiovascular event by virtue of poor endothelial function – how should we proceed? Inasmuch as the four major risk factors are cytotoxic to the endothelium, and that reversal of the factors restores endothelial integrity, then the strategy is clear. However, the process of treating the risk factors for atherosclerosis, whether by formal pharmaceutical intervention (statins, ACE inhibitors, hypoglycemics) or by simply adopting a healthy lifestyle (no smoking, a diet rich in fresh fruit and vegetables, regular exercise, avoidance of

overweight and obesity) has been known for decades as effective in reducing major cardiovascular events (31,32). Furthermore, vascular dysfunction is not the only pathophysiology that contributes to atherosclerosis. Suppression of platelet function by aspirin is probably the most successful single cardiovascular intervention of the 20th century, and in addition reversal of the risk factors is also likely to reduce inappropriate platelet and coagulation activation independently of any effect on the endothelium (33,34).

Clinical research is as sensitive to Darwinian mechanisms as any other field: our area of study is littered with disappointments, an excellent example being the hope of using viral plasmid as therapeutics (35). Similarly, endothelial progenitor cells have (as yet) not translated from the laboratory to the bedside (36), although more time may be needed. Two decades ago, I speculated that plasma markers may be useful in some settings, whilst a decade ago, Hwa et al drew attention to a bench-to-bedside gap that has still to be closed (37,38). Although plasma von Willebrand factor adds to risk-factor scores for predicting outcome in atrial fibrillation (39), and despite its ease of measurement, much more work is required before even this one molecule is adopted as a routine laboratory marker. Although a daunting task, the introduction into routine pathology of brain natriuretic peptide as marker of heart failure provides a model (40). However, perhaps our focus on one single marker is short-sighted. With an organ as complex and widespread as the endothelium perhaps a panel of markers representing different aspects of vascular physiology and pathology may be fruitful (7,41). Such a panel may well include CECs and/or endothelial microparticles as Schmidt and colleagues promote (24), but much work needs to be done, especially in the adoption of an international consensus on methodology.

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Table 1: Products of the endothelium

Anti-coagulant/vasorelaxive Anti-inflammatory	Pro-coagulant/vasoconstrictive Pro-inflammatory
Nitric oxide	Endothelin
Prostacyclin	Thromboxane
Tissue plasminogen activator	Plasminogen activator inhibitor
Protein C	Tissue factor
Heparin	Von Willebrand factor
Thrombomodulin	Factor V
	Interleukins/cytokines
	Adhesion molecules

Table 2: Endothelial markers and their expression on non-endothelial cells

Marker	Antigen name	Expression on non-endothelial cells
PECAM-1	CD31	Platelets, leucocytes
ICAM-1	CD54	Leucocytes
Endoglin	CD105	Macrophages, activated monocytes, erythroid progenitors, pre-B lymphocytes
VCAM-1	CD106	Stromal cells, smooth muscle cells, fibroblasts
Thrombomodulin	CD141	Platelets, monocytes, neutrophils, keratinocytes
E-cadherin	CD144	Fetal liver cells
PIH12, S-endo-1	CD146	pericytes, bone marrow fibroblasts, nerve fibres, activated T-lymphocyte, malignant cells
VEGF receptor 1, KDR	CD309	Hematopoietic cells, progenitor cells
von Willebrand factor		Platelets

Table 3: Criteria for an Optimal Endothelial Function Test

Criterion	Plasma markers *	CECs	EMPs	FMD	PWV/AS	Coronary epicardial vasoreactivity
Reflects disease state	Probably	Possibly	Possibly	Probably	Probably	Yes
Reversible with interventions	Yes	Unclear, probably	Possibly	Yes	Probably	Yes
Reflects coronary endothelial function	No	No	No	Indirectly	Possibly	Yes (Gold Standard)
Improves risk stratification	Possibly	Unclear	Possibly	Possibly	Possibly	Probably
Reproducibility	Good	Poor	Moderate /poor	Moderate	Moderate	Moderate
Operator independent	Yes	Yes	Yes	No	No	No
Invasive	No	No	No	No (but inconvenient)	No	Very
Easy to use	Yes	No	No	No	No	No
Inexpensive	Yes	No	No	No	No	No
Consensus on definition	Yes	Weak	Weak	Yes	Yes	Yes
Consensus on method	Yes	No	Weak	Modest	Modest	Yes
Potential as a Global marker	Yes	Yes	Yes	Possibly	Possibly	No

*for example, von Willebrand factor, soluble thrombomodulin. CECs = circulating endothelial cells, EMPS = endothelial microparticles, FMD = flow mediated dilatation, PWV/AS = pulse wave velocity/ arterial stiffness.