Prediction of cardiovascular risk using soluble cell adhesion molecules

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Introduction

Inflammation is now considered a critical pathogenic component of the atherosclerotic process[1,2]. This is characterized histopathologically by leukocyte infiltration of the vascular endothelial wall. The vascular endothelium is a dynamic organ system, which has natural antiadhesive and anticoagulant properties. However, in response to injury the endothelium promotes coagulation and mediates the binding and transendothelial migration of activated leukocytes[3–5]. These pathological processes are believed to be critical to the initiation and progression of atherosclerosis as well as the pathogenesis of acute coronary syndromes and are mediated by a group of molecules referred to collectively as cell adhesion molecules.

Cell adhesion molecules are a diverse system of proteins and adhesive receptors, which orchestrate vital biological phenomena such as embryogenesis, cell growth and differentiation as well as inflammatory processes. Over the past decade there has been considerable progress in our understanding of adhesion molecules and their complex interactions[6]. Among the many types of adhesion receptors on the cell surface, four main families are described (Fig.1).

(1) Immunoglobulin superfamily

This is the most abundant family of cell surface adhesion molecules, accounting for 50% of leukocyte membrane glycoproteins which have evolved to serve many different functions including receptors for growth factors, receptors for the Fc region of immunoglobulins and they are the cellular counter receptors for integrins. Examples include intercellular adhesion molecule-1 (ICAM-1) (CD54) and vascular cell adhesion molecule-1 (VCAM-1).

(2) Integrins

These are membrane glycoproteins with two principle subunits designated α and β. The β3 subfamily are expressed on endothelial cells and platelets and include the platelet glycoprotein IIb/IIIa receptor which is expressed by platelets and undergoes conformational change when platelets are activated promoting platelet aggregation by cross-linking fibrinogen via the activated IIb/IIIa receptor.

(3) Selectins

The selectin family of cell adhesion molecules interact with carbohydrate ligands on leukocytes and endothelial cells. The selectins are named according to the cell type on which they were originally identified: endothelial (E-selectin), platelet (P-selectin) and leukocyte (L-selectin).

(4) Cadherins

These molecules establish links between adjacent cells. They form zipper-like structures at adherens junctions, membrane regions where a cell makes contact with another cell.

The evidence that cell adhesion molecules have a key role in atherosclerosis comes from a variety of sources. The process of leukocyte extravasation across the vascular endothelium is critically dependent on both selectin and immunoglobulin adhesion molecules on endothelial cells interacting with integrin receptors on leukocytes[3]. Immunohistochemical analysis of
human atherosclerotic coronary arteries has demonstrated expression of ICAM-1 and VCAM-1 on endothelial cell macrophages and smooth muscle cells within the plaque\cite{7,8}. Interestingly in genetically engineered mice with significantly reduced expression of VCAM-1 there is less development of early atherosclerotic lesions, suggesting that VCAM-1 is important in the initiation of atherosclerotic lesions\cite{9}. The critical role of cell adhesion molecules in atherosclerosis is evidenced by the fact that mice deficient in cell adhesion molecules develop significantly less atherosclerotic plaques than wild-type mice\cite{10}, and administration of monoclonal antibodies to cell adhesion molecules decreases intimal hyperplasia and the vascular inflammatory response following balloon induced arterial injury\cite{11}.

The investigation of the genetics of atherosclerosis has confirmed the importance of cell adhesion molecules to this disease process\cite{12}. An E-selectin polymorphism mediating increased cellular adhesion has been found to be associated with premature coronary artery disease\cite{13}. A polymorphism at position 715 of the P-selectin gene is associated with a reduced risk of myocardial infarction for carriers\cite{14,15}. Furthermore, Barbaux et al. have reported that a number of P-selectin gene polymorphisms (including the polymorphism at position 715) significantly affects soluble P-selectin levels in the carriers\cite{16}. These data provide a broad evidence base implicating the importance of cell adhesion molecules in the initiation and progression of atherosclerotic disease.

Although increased cell surface expression of these molecules is difficult to quantify in vivo, soluble forms have been identified and can be measured in serum. The exact biological role of these soluble cell adhesion molecules (sCAM), if any, is unclear. They are released into the circulation from the cell surface by proteolytic cleavage of the extracellular portion\cite{17}. This shedding of the extracellular portion of cell adhesion molecules may be a means of controlling cell adhesion. Intense interest has been generated by the discovery of strong associations between levels of soluble cell adhesion molecules and coronary heart disease events. Investigators have examined the relationship between coronary heart disease event rates and levels of these soluble inflammatory markers in three principal groups: (1) asymptomatic, apparently healthy individuals, (2) patients with clinically stable disease and (3) those with acute coronary syndromes.

**Apparantly healthy individuals**

Ridker and colleagues examined levels of soluble ICAM-1 in patients enrolled into the Physicians Health study\cite{18}. They reported that baseline sICAM-1 levels are raised among apparently healthy men at increased risk of future myocardial infarction (Fig. 2). Controlling for lipid and non-lipid risk factors did not modify this increased risk. Similarly, levels of sICAM-1 were found to correlate with risk of future cardiovascular events in apparently healthy women in the Women’s Health study\cite{19}. These data supports the hypothesis that endothelial activation and inflammation occurs early in the atherosclerotic process and that the inflammatory component of the disease is important in influencing future cardiovascular events.

The same investigators also recently reported, in a similar prospective epidemiological evaluation of apparently healthy women, that elevation of baseline soluble P-selectin levels was associated with increasing risk of future myocardial infarction, stroke, coronary revascularization and cardiovascular death\cite{20}. This association was again independent of age and persisted after additional control for several lipid and non-lipid cardiovascular risk factors. Similarly, in the Atherosclerosis Risk in Communities (ARIC) study, levels of sICAM-1 were predictive of both carotid atherosclerosis and
future coronary heart disease events and there was also a significant association between levels of sE-selectin and carotid atherosclerosis\[21\]. In contrast to the strong association between serum levels of sICAM-1 and future cardiovascular events in apparently healthy men and women, the data concerning sVCAM-1 as a marker of future cardiac risk in asymptomatic individuals are less clear. There was no association between sVCAM-1 and subsequent cardiac risk in analysis from the Physicians Health study\[22\]. However, when assessed as a marker of future cardiovascular mortality in a cohort of type 2 diabetics, there was a strong independent association between the levels of sVCAM-1 and future cardiovascular mortality\[23\].

Recently data from Malik et al. confirmed the association of sICAM-1 with future coronary heart disease events in middle aged British men (OR=1.63); however, this link between levels of sICAM-1 and incident cardiac events was attenuated after adjustment for cardiovascular risk factors, socio-economic factors and other indices of inflammation and infection. The authors found weak associations between future cardiac events and levels of sVCAM-1, sE-selectin and sP-selectin, which were further attenuated after adjustment for the factors referred to above\[24\].

Established coronary heart disease

Recent data from a large cohort of patients with angiographically documented coronary artery disease revealed that baseline levels of sVCAM-1, sICAM-1 and sE-selectin are elevated among patients with future cardiovascular events\[25\]. Of all the inflammatory markers measured, including high sensitivity C-reactive protein and lipid values measured, sVCAM-1 levels were the strongest independent predictor of future death from cardiovascular disease. Again this association was independent of classical risk factors. Interestingly, the measurement of sVCAM-1 added to the predictive value of C-reactive protein in determining risk of future fatal cardiovascular events. Even patients with low levels of C-reactive protein were found to be at significantly higher risk with elevated sVCAM-1 levels, whereas by contrast, elevation of C-reactive protein alone without elevation of sVCAM-1 levels were not predictive of future fatal events (Fig. 3).

Acute coronary syndromes

There have been a number of reports of elevated levels of soluble cell adhesion molecules in acute coronary syndromes. Shyu et al. reported elevated levels of sICAM-1 in patients with acute coronary syndromes\[26\]. Ikeda et al. demonstrated elevated levels of sP-selectin after an episode of chest pain in patients with unstable angina, but not in those with stable angina or controls\[27\]. Recently, O’Malley et al. reported elevated
levels of sICAM-1 in the majority of patients within hours of the onset of cardiac chest pain\textsuperscript{[28]}. Our own group have reported that levels of sICAM-1, sVCAM-1 and sP-selectin remain elevated throughout the first 72 h after presentation in unstable angina and non-Q-wave myocardial infarction, whereas there is a fall in the levels of sE-selectin during this time\textsuperscript{[29]}. We have also reported that elevation of sVCAM-1 at the time of presentation in patients with unstable angina and non-Q-wave myocardial infarction is predictive of future cardiovascular events (Fig. 4)\textsuperscript{[30]}. Acute coronary syndromes are characterized by persistent instability for weeks to months after the resolution of the clinical symptoms resulting in either recurrent episodes of unstable angina, myocardial infarction or death\textsuperscript{[31]}. There is evidence of sustained vascular inflammation following an acute coronary syndrome by the finding of persistent elevation of sICAM-1, sVCAM-1, sE-selectin and sP-selectin for up to 6 months after unstable angina or non-Q-wave myocardial infarction before a return to levels seen in a stable angina population\textsuperscript{[32]}. Further recent data suggest that the inflammatory process persists after resolution of the clinical syndrome. C-reactive protein levels remain elevated at the time of discharge and at 3-month follow-up in up to 50\% of patients who presented with Braunwald class IIIIB unstable angina. This finding of persistent elevation of C-reactive protein after an episode of unstable angina was associated with frequent hospital readmission for recurrent instability\textsuperscript{[33]}. These data suggest an association between recurrent ischaemic episodes and persistent inflammatory stimuli after an acute coronary event.

**How should these data be interpreted?**

There is as yet no defined biological role for soluble cell adhesion molecules but there is widespread belief that the levels reflect the vascular inflammatory component of the atherosclerotic process in apparently healthy individuals as well as those with both stable and unstable coronary artery disease. There is evidence that levels of soluble cell adhesion molecules in the coronary circulation directly reflect those levels from peripheral veins, thus supporting the working hypothesis that levels of soluble cell adhesion molecules measured from a peripheral vein directly reflect levels of soluble cell adhesion molecules within the coronary circulation at the site of endothelial inflammation\textsuperscript{[34]}. However, it remains to be elucidated why sICAM-1 is the most powerful predictor of future events in the asymptomatic population whereas sVCAM-1 is most accurate as a prognostic marker in patients with established atherosclerotic disease.

ICAM-1 is constitutively expressed at low levels by a variety of cell types including endothelial cells and leukocytes. The higher levels of sICAM-1 in apparently healthy individuals who progress to have cardiovascular events may represent more aggressive low grade inflammation and the elevated levels may be analogous to elevated levels of C-reactive protein which provide similar prognostic data in the primary prevention setting\textsuperscript{[35]}. Thus sICAM-1 appears most useful in the setting of primary prevention, identifying those asymptomatic individuals at increased risk of future cardiovascular events. Expression of VCAM-1, however, occurs only on activated endothelial and vascular smooth muscle cells and thus sVCAM-1 may serve as a specific marker of plaque burden or activity, underlying its potential use as a prognostic marker in the secondary preventative setting. This hypothesis is supported by the finding of the strong associations between levels of sVCAM-1 and future cardiac events in patients with activated endothelium (diabetic patients)\textsuperscript{[23]} and those with already established atherosclerotic disease\textsuperscript{[25]}. Furthermore, levels of sVCAM-1 are prognostically important in acute coronary syndromes\textsuperscript{[30]}.

It remains to be seen if levels of inflammatory biomarkers, including soluble cell adhesion molecules, become used as part of routine biochemical risk factor assessment in both primary and secondary prevention of cardiovascular disease. It must be remembered that half of all myocardial infarctions occur in patients in whom plasma lipid levels are normal and thus current biochemical screening programmes fail to identify a significant at-risk population\textsuperscript{[36]}. The recently presented results of the Heart Protection Study and the previously reported AFCAPS/TexCAPS Study showed how treatment with HMG CoA reductase inhibitors significantly reduced the occurrence of future cardiovascular events even in those with LDL cholesterol levels considered ‘normal’. In the AFCAPS/TexCAPS study, the use of C-reactive protein identified a higher risk group among those with ‘normal’ LDL levels and also identified patients who benefited from treatment with lovastatin\textsuperscript{[37,38]}.

The studies referred to in this review all confirm an association between levels of soluble cell adhesion molecules and future cardiovascular events, but they do not
provide any mechanistic insight to explain the findings. Despite a lack of a clear cause and effect relationship between levels of soluble cell adhesion molecules and cardiovascular morbidity and mortality, these associations are significant and independent of conventional risk factors. The critical importance of these molecules in the atherosclerotic process is evident in the animal ‘knock-out’ models where a genetically engineered lack of cell adhesion molecules is associated with an inhibition of atherosclerosis[10].

**Limitations**

To date, most studies reporting associations between levels of soluble cell adhesion molecules and cardiovascular events have employed commercially available enzyme immunoassays. There is currently no standardized assay available to measure these markers and this significantly limits any widespread application of these markers into primary and secondary prevention strategies. Variations between available assays may account for some of the heterogeneity of results from different studies. Finally C-reactive protein has been widely studied and validated as an independent marker of cardiovascular risk in the primary preventative setting, and its application into widespread clinical use may only be a matter of time. Thus levels of soluble cell adhesion molecules may be most useful in a secondary preventative setting assisting in the assessment of asymptomatic disease in high-risk patients and established atherosclerotic disease in those who have had events.

**Future direction**

Whatever the fate of soluble cell adhesion molecules as markers of cardiovascular risk, the study of the molecules has significantly enhanced our understanding of the complex process of atherosclerosis. Perhaps the most important and exciting developments related to cell adhesion molecules and cardiovascular disease is the recognition of a novel target for therapeutics. Therapeutic trials are already underway using targeted antiinflammatory therapy in animal models with impressive results. Wang et al. recently reported that the use of recombinant soluble P-selectin glycoprotein ligand significantly reduced neointimal hyperplasia and the inflammatory and thrombotic responses following balloon induced endothelial injury[11]. Modulation of the inflammatory process at the level of the vascular endothelium by using targeted monoclonal antibody therapy to cell adhesion molecules is an exciting prospect which could revolutionize treatment of coronary artery disease.

**References**


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