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Cellular adhesion molecules and peripheral arterial disease

Gregorio Brevetti, Vittorio Schiano and Massimo Chiariello

Abstract: Cellular adhesion molecules (CAMs), by mediating the recruitment of circulating leukocytes to the blood vessel wall and their subsequent migration into the subendothelial spaces, play a crucial role in all stages of atherosclerosis. Soluble forms of CAMs, probably derived from proteolytic shedding, are present in the circulation and their blood levels parallel the amount expressed on the cell surface. In patients with peripheral arterial disease (PAD), increased levels of soluble CAMs have been found during exercise-induced claudication, are associated with the presence, the severity and the extent of atherosclerosis in the arteries of the lower limbs, and portend a worse outcome. These findings have provided new insights into the pathophysiology of PAD and its consequences. However, further large population studies are needed to firmly establish whether increased levels of circulating CAMs give additive information to current risk assessment approaches, and to verify whether PAD patients with elevated levels of circulating CAMs would benefit from any specific therapy.

Key words: atherosclerosis; cellular adhesion molecules; intermittent claudication; peripheral arterial disease

Introduction

Over the past decade, enormous advances have occurred in the understanding of the cellular and molecular mechanisms involved in atherogenesis. In particular, it is now clear that one of the major pathogenic mechanisms of atherosclerosis is the recruitment of circulating leukocytes to the blood vessel wall, and their subsequent migration into the subendothelial spaces.¹ This process is predominantly mediated by cellular adhesion molecules (CAMs), which are expressed on both the vascular endothelium and the leukocytes in response to several atherogenic stimuli.^{1–4}

The contention that CAMs are causally related to the development of atherosclerosis is supported by the finding that knock-out mice deficient in E-selectin, P-selectin or intracellular adhesion molecule-1 (ICAM-1) have a reduced response to atherosclerotic stimuli.^{5,6} Furthermore, increased expression of adhesion molecules have been found in human atherosclerotic plaques.^{7,8} Thus, evaluation of CAMs' expression

may importantly contribute to improve our knowledge about the mechanisms responsible for the initiation, progression and clinical manifestations of atherosclerosis. Unfortunately, adhesion molecules are difficult to measure *in vivo*, but their soluble forms, probably resulting from proteolytic shedding,^{9,10} may be detected in serum or plasma, and are increased in a variety of conditions in which their blood levels parallel the amount expressed on the cell surface.^{11,12} On the basis of this information, numerous studies have investigated the relationships between the soluble forms of the adhesion molecules and the various aspects of the atherosclerotic disease. The scope of this article is to summarize the findings of studies which investigated the role of soluble CAMs in peripheral arterial disease (PAD), which represents a major manifestation of atherosclerosis.

Biology of CAMs

CAMs are transmembrane glycoproteins expressed on the surface of many cell types, which mediate cell–cell and cell–extracellular matrix interactions.¹ Cell adhesion occurs when plasma membrane adhesion receptors bind to their counter-receptors (ligands) placed on the neighbouring cell or in the extracellular matrix. Therefore, the process is controlled by the modulation of the number and type of the adhesion receptors, and by encounter with the corresponding

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Table 1 Adhesion molecules involved in atherosclerosis, their cellular function and their biological and pathological implications.

Adhesion molecules	Cellular function	Biological and pathological implications
Selectins	Leukocyte–endothelium interaction Leukocyte–platelet interaction	Leukocyte rolling and tethering
Integrins	Leukocyte–endothelium interaction Platelet–endothelium interaction	Firm adhesion, spreading, homing to inflamed tissue
Immunoglobulins	Leukocyte–leukocyte interaction Leukocyte–endothelium interaction	Firm adhesion, leukocyte extravasation, inflammatory and immune responses

ligands. Three families of CAMs play a crucial role in the pathogenesis of atherosclerosis: the selectins, the integrins and the immunoglobulin superfamily (Table 1). These different types of CAMs interact with CAM chemoattractant/activator molecules in a programmed, sequential manner (Figure 1) to form the so-called leukocyte–endothelial cell adhesion cascade.¹³

Selectins

The initial phase of the adhesion cascade (ie leukocyte tethering and rolling along the endothelium surface) is mediated by members of the selectin family. The three members of this family are named according to the cell type on which each of them was originally identified: E-selectin (endothelium), P-selectin (platelets) and L-selectin (lymphocytes), but they are not exclusively located on these cells.

Although the selectins are closely related in structure and function, their patterns of expression are quite different. The expression of E-selectin appears to be largely restricted to activated endothelial cells, as this molecule is almost absent from resting endothelium.¹⁴ Once transcriptionally induced following cytokine stimulation, E-selectin, by interacting with their counter-receptors, participates in the adhesion of neutrophils, monocytes, and of a subpopulation of memory T lymphocytes to the endothelium.^{14,15} Eosinophils and basophils may also bind to endothelium through E-selectin.^{16,17} P-selectin is stored in the α -granules of platelets and in Weibel–Palade bodies of the endothelium. From these intracellular pools, after activation by thrombin and other mediators, P-selectin can be rapidly mobilized to the cell surface, where it

binds neutrophils and monocytes,¹⁸ interacting with the P-selectin glycoprotein ligand (PSGL-1). Thus, P-selectin mediates platelet–leukocyte and endothelial cell–leukocyte adhesion. Of the three selectins, only L-selectin is constitutively expressed at the cell surface.¹⁴ It is present in almost all leukocytes, and is shed from the cell surface within minutes after leukocyte activation. Soluble forms of E-selectin, P-selectin and L-selectin may be detected in serum and plasma.

Integrins

Although rolling is a prerequisite for the eventual firm adherence to blood vessels, the selectin-dependent adhesion of leukocytes does not lead to firm adhesion and transmigration unless another set of adhesion molecules is engaged. For neutrophils, firm adhesion requires activation of the integrin family, resulting in binding to one of the adhesion molecules on the surface of the endothelial cells.

The integrins comprise a superfamily of heterodimer transmembrane proteins composed of non-covalently associated α - and β -subunits.¹⁹ Different cell types assemble and express different $\alpha\beta$ complexes. Beta-integrins are distributed in various kinds of cells, and mediate cell adhesion to proteins found in extracellular matrix. Among the $\beta 1$ subunits, the $\alpha_4\beta_1$ integrin,²⁰ expressed on resting lymphocytes, may be the most relevant to atherosclerosis, as it has been shown to modulate the evolution of ischemic stroke.²¹ Beta-2 integrins are strictly expressed in leukocytes and interact with immunoglobulin superfamily members (ICAM-1, ICAM-2, and ICAM-3) to induce a firm arrest of the white cells at the surface of the activated endothelium. The $\beta 3$ subfamily of integrins are expressed in platelets. The $\alpha_2\beta/\beta_3$ integrin, also known as glycoprotein (GP) IIB/IIIa, is specific for this cell type and its activation by thrombin and other agonists represents the final common pathway for platelet aggregation. Furthermore, GP IIB/IIIa also binds fibronectin and von Willebrand factor, thus mediating platelet adhesion to the endothelium and subendothelial matrix, and enabling the formation of a platelet thrombus with attachment to the vessel wall.¹ Different from GP IIB/IIIa, $\alpha V\beta_3$ integrin is more widely expressed and is involved in cell migration and proliferation.²²

Immunoglobulin superfamily

Transmigration of leukocytes, ie the final step of the leukocyte–endothelial cell cascade (Figure 1), is mediated by certain members of the immunoglobulin superfamily.^{23,24} These molecules are membrane glycoprotein receptors containing a variable number of extracellular immunoglobulin-like domains.¹

The ICAM family comprises five members, but ICAM-4 and ICAM-5, which are expressed in erythroid cells and in the brain, respectively, are out of the scope of this review. ICAM-1 and ICAM-2 are present

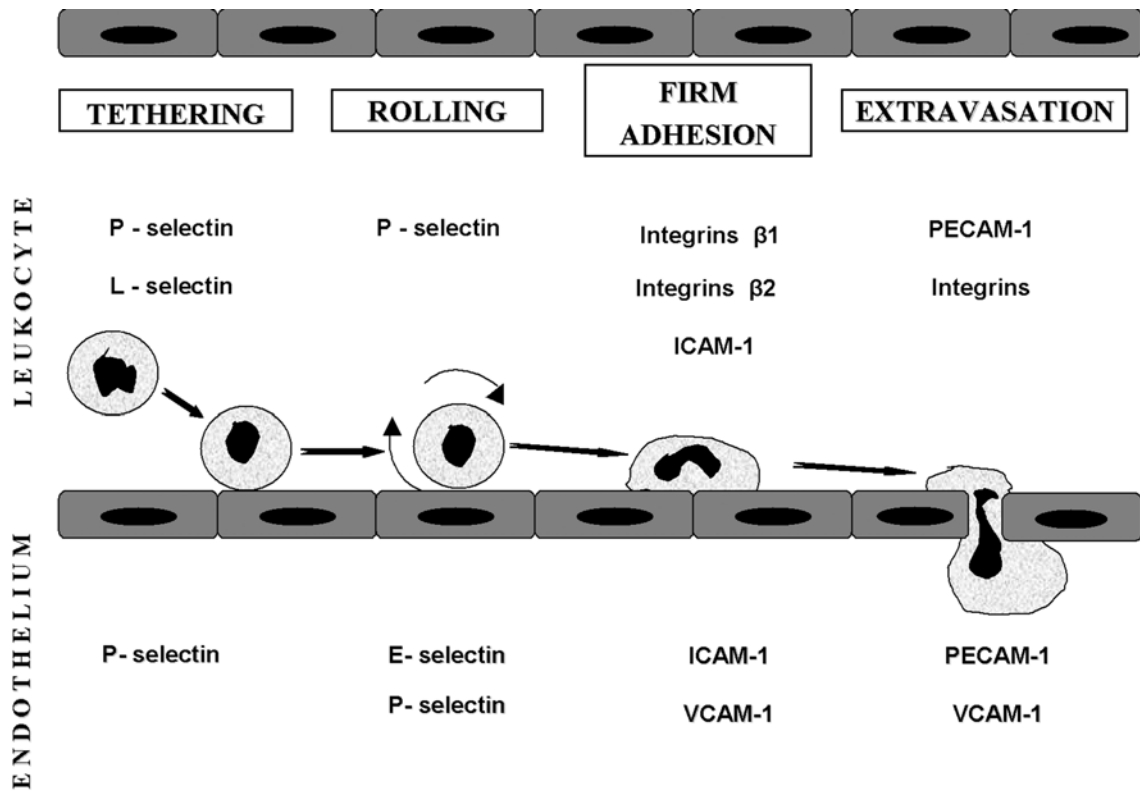


Figure 1 Schematic representation of the leukocyte tethering/rolling, adhesion and transmigration across the endothelium, and the cellular adhesion molecules involved in these processes.

constitutively on endothelial cells and leukocytes; however, the first one is upregulated by proinflammatory cytokines, whereas constitutive expression of ICAM-2 is relatively stable.¹³ ICAM-3 is found in endothelial cells and leukocytes, and it is the only ICAM present on neutrophils. Each ICAM may bind more than one ligand. Unlike ICAMs, vascular cell adhesion molecule-1 (VCAM-1) is mainly restricted to the endothelial cells, although it may be present in several non-vascular cell types.²⁵ Through an interaction with the integrin $\alpha_4\beta_1$, endothelial VCAM-1 mediates the adhesion of lymphocytes and monocytes. Adhesion of eosinophils and basophils to activated endothelium also appears to involve VCAM-1, which, in addition, participates in the process of leukocyte migration.²⁶ Another important member of the immunoglobulin superfamily is the platelet–endothelial cell adhesion molecule-1 (PECAM-1), which is expressed at relatively low levels on the surface of leukocytes and platelets, but at higher levels on the endothelium.¹³ Its location at the junctions between endothelial cells has suggested a role in transendothelial migration of leukocytes.²⁴ In addition, PECAM-1 participates in homophilic binding between adjacent cells.²⁷ Soluble forms of ICAM-1, ICAM-3, VCAM-1 and PECAM-1 may be measured in serum and plasma.

Soluble adhesion molecules in PAD

Although the origin and function of the soluble forms of CAMs remain to be clarified, elevated serum and plasma (circulating) levels of these molecules have been found to be associated with the atherosclerotic burden, severity of PAD, exercise-induced claudication, restenosis after percutaneous transluminal angioplasty and increased cardiovascular risk.

Circulating levels of soluble CAMs in PAD patients vs controls

Circulating levels of sE-selectin, sP-selectin, sL-selectin, sICAM-1 and sVCAM-1 have all been shown to be increased in PAD, although the findings of different studies are not consistent (Table 2). Elevated levels of sE-selectin have been detected in PAD patients with diabetes mellitus,^{28,29} but conflicting results were reported in non-diabetics.^{30–32} Similarly, although P-selectin has been consistently found to be overexpressed in platelets of PAD patients,^{33–35} increased levels of the soluble form of this molecule were observed in some studies^{31,36} but not in others.^{30,37} With respect to the members of the immunoglobulin superfamily, sICAM-1 has been reported to be increased in PAD^{29–32,38,39} with one exception.²⁸ For sVCAM-1, the findings are less consistent.^{28,30–32,38–40}

The explanation for these heterogeneous results remains obscure. Increased circulating levels of the adhesion molecules are due to a variety of molecular and cellular factors, including the increased transcription of genes, alteration of mRNA stability, changes in translation, production of alternatively spliced forms and enhanced proteolytic cleavage from the cell surface.⁴¹ None of these factors was investigated in the studies reported in Table 2. In addition, little is known about the clearance and catabolism of soluble CAMs. This lack of knowledge of the mechanisms determining the blood levels of these molecules is obviously a barrier in understanding between study discrepancies, which may be due also to differences in study populations and diagnostic criteria.^{28,30,32,40}

Soluble CAMs and atherosclerotic burden in PAD

Given the systemic nature of atherosclerosis, individuals with PAD are often affected by concomitant coronary and/or carotid artery disease.⁴² A recent study⁴³ has shown that patients with both peripheral and coronary artery disease have plasma levels of sICAM-1 and sVCAM-1 similar to those affected only by coronary artery disease. Similarly, in two separate studies, Blann et al found that, compared with patients with arterial disease at one site, those with disease at two or more sites had similar circulating levels of sP-selectin⁴⁴ and sE-selectin.⁴⁵ These findings indicate that soluble forms of the adhesion molecules do not differentiate the extent of atherosclerosis. However, in 52 PAD patients, Peter et al⁴⁰ observed that serum levels of sVCAM-1 significantly correlated with the extent of atherosclerosis angiographically detected in infrarenal aorta and in the arteries of the lower limbs. Therefore, although the association of the adhesion molecules with the atherogenic process is well established, the associations between the circulating levels of the soluble forms and the atherosclerotic burden are less robust. This may in part be explained by the complex interaction of effects on protein synthesis and rate of shedding, which could be responsible for

differences in associations between the plasma levels of different adhesion molecules and the extent of atherosclerosis.

Soluble CAMs and PAD severity

From a clinical point of view, PAD patients are classically divided into four stages according to the Fontaine's classification: stage I, asymptomatic PAD; stage II, intermittent claudication; stage III, pain at rest; stage IV, trophic lesions. Interestingly, Peter et al⁴⁰ found that serum levels of sVCAM-1, although showing a high correlation with leg atherosclerosis, did not reflect clinical stages of PAD. Similarly, a more recent study reported that plasma levels of both sVCAM-1 and sICAM-1 did not differentiate stage II from stage I patients.⁴⁶ However, when stage I and stage II patients were considered collectively, plasma levels of these two adhesion molecules showed a significant, albeit weak, negative relationship with the ankle/brachial index (ABI),⁴⁶ which is a marker of PAD severity.⁴⁷ With respect to the relationship between selectins and PAD severity, patients with ischemic rest pain (stage III) have been found to have similar levels of sP-selectin than those less severely affected.⁴⁸ Taken together, these findings suggest that plasma levels of sCAMs, while not reflecting clinical stages, correlate with more objective measures of PAD severity, such as the ABI and the extent of atherosclerosis in the arteries of the lower limbs.

Adhesion molecules and exercise-induced claudication

Although in PAD the primary determinant of inadequate blood supply to the affected limb during exercise is a flow-limiting lesion of a conduit artery, increased leukocyte adhesivity and platelet aggregation, by altering blood rheology, may contribute to the pathophysiology of intermittent claudication.⁴⁹ Indeed, acute exercise affects various components of the immune system, including the function and number of leukocytes,⁵⁰ and the surface expression of

Table 2 Studies showing an increase (YES) or not (NO) in plasma levels of adhesion molecules in PAD patients versus controls.

	sE-selectin	sP-selectin	sL-selectin	sICAM-1	sVCAM-1
Boulbou et al ²⁸	YES (d)	–	–	NO (d)	NO (d)
Elhadd et al ²⁹	YES (d)	–	–	YES (d)	–
Brevetti et al ³⁰	NO	NO	–	YES	NO
Signorelli et al ³¹	YES	YES	YES	YES	YES
Blann and McCollum et al ³²	NO	–	–	YES	NO
Blann et al ³⁶	NO	YES	NO	–	–
Kirkpatrick et al ³⁷	–	YES	–	–	–
Blann et al ³⁸	–	–	–	YES	YES
Brevetti et al ³⁹	–	–	–	YES	NO
Peter et al ⁴⁰	–	–	–	–	YES

(d), patients with diabetes mellitus.

adhesion molecules may importantly contribute to such responses by changing patterns of cell trafficking.⁵¹ In PAD patients, in whom intermittent claudication is associated with neutrophil activation⁵² and systemic endothelial dysfunction,⁵³ exercise has been shown to induce reduction in the number of leukocytes expressing the $\alpha M/\beta_2$ and $\alpha X/\beta_2$ integrins.⁵⁴ This indicates that leukocyte–endothelial interactions occur during intermittent claudication. In addition, plasma levels of both sICAM-1 (the endothelial counter-receptor of $\alpha M/\beta_2$ integrin) and sVCAM-1 have been shown to increase after maximal exercise in claudicants, but not in control subjects.^{30,31} A more recent study⁵³ reported that the increase in sICAM-1 levels paralleled an increase in thiobarbituric acid-reactive substances (TBARS), and was prevented by intravenous administration of antioxidant vitamin C. Furthermore, such an increase was not observed after a submaximal exercise not heavy enough to induce TBARS elevation. Collectively, these findings indicate that changes in sICAM-1 observed in claudicants performing a maximal exercise are presumably due to increased oxidative stress. Also P-selectin levels have been found to increase with exercise,^{31,37} thus indicating that platelet activation occurs during claudication.

Adhesion molecules, percutaneous transluminal intervention and restenosis

Because percutaneous transluminal angioplasty (PTA) has low procedural risk, PAD patients with lifestyle-limiting claudication are often considered for this therapeutic approach. However, the long-term benefit of PTA continues to be compromised by the recurrence of stenotic lesion.

A number of animal studies suggest that adhesion molecules may play a role in the pathophysiology of peripheral vessel restenosis. In experimental models, the absence of $\alpha M/\beta_2$ integrin and blockade of both VCAM-1 and its ligand $\alpha_4\beta_1$ reduced intimal hyperplasia after balloon-induced carotid artery injury.^{55–57} Similarly, P-selectin-deficient mice were protected from neointimal hyperplasia after transluminal endothelial injury of the femoral artery.⁵⁸ Furthermore, in rabbit models of balloon-induced injury of the iliac artery, antibody blockade of the $\alpha M/\beta_2$ integrin, and treatment with the oligosaccharide SLe^x, which blocks the selectin effects, resulted in a lower intimal/media ratio and a smaller area of stenosis.^{59,60} In humans, only two studies reported an association between adhesion molecules and restenosis in PAD.^{61,62} Tsakiris et al found that those patients who developed restenosis 6 months after PTA had higher circulating levels of sP-selectin and sVCAM-1.⁶¹ Similarly, in 42 patients with intermittent claudication, increased plasma levels of sE-selectin were found to be predictive of restenosis at 1 year.⁶² Taken together, animal and human studies indicate that all three families of CAMs may be involved in the process of restenosis

after PTA, and suggest that the blockade of some of them may be a potential therapeutic strategy to decrease restenosis. In this regard it is noteworthy that cilostazol, by inhibiting leukocyte $\alpha M/\beta_2$ integrin, the transcardiac expression of which is increased during percutaneous coronary intervention,⁶³ leads to a potential reduction in restenosis after coronary stent implantation.⁶⁴ Unfortunately, although cilostazol is effectively used in PAD to improve claudication,⁶⁵ no study has investigated its ability to prevent post-PTA restenosis in these patients.

sCAMs and risk prediction

Although the mechanisms that modulate the circulating levels of sCAMs remain unclear, the value of these molecules for the prediction of vascular risk has been assessed in apparently healthy subjects^{66–69} and in patients with CAD.^{70,71} With respect to PAD, the Physician's Health Study, conducted among middle-aged healthy men, showed that increased plasma levels of sICAM-1 but not sVCAM-1 were associated with a threefold higher risk of developing symptomatic atherosclerosis in the lower limbs.⁷² Noteworthy, this predictive power was maintained after adjustment for classic risk factors and C-reactive protein. The finding of the Physician's Health Study seems to be consistent with the observation that polymorphism of the EE genotype of the ICAM-1 gene significantly increases the risk of PAD.⁷³

Furthermore, a recent study⁷⁴ has shown that sICAM-1 is a significant predictor of lower-extremity atherosclerotic progression measured by ABI over 12 years of follow-up independently of cardiovascular risk factors. In the setting of secondary prevention, Blann et al found that increased circulating levels of sICAM-1 were a significant predictor of adverse cardiovascular events in a group of patients with ultrasound-proven atherosclerosis (stenosis > 70%) of carotid, iliac or femoral arteries.³² Different results were observed in another study.⁷⁵ In a well-defined population of patients with obliterative atherosclerosis of the lower limbs, plasma levels of sVCAM-1 were 618 ± 258 ng/ml in those with and 496 ± 164 ng/ml in those without an event ($p = 0.016$). The corresponding sICAM-1 values were 344 ± 239 ng/ml and 275 ± 99 ng/ml ($p = 0.079$). Thus, an increased cardiovascular risk was associated with increased plasma levels of sVCAM-1 (relative risk = 4.06; 95% confidence intervals = 1.53–10.74), but not of sICAM-1.⁷⁵ The discrepancy between the two studies is difficult to explain. The expression of ICAM-1 is strongly up-regulated by inflammatory cytokines in endothelial cells, fibroblasts, epithelial cells and multiple cells of hematopoietic lineage.⁴¹ By contrast, VCAM-1 is more anatomically restricted to activated endothelial cells in lesion-prone areas,⁷⁶ and is more prominent on intimal neovasculature than on the arterial luminal surface of complex lesions.⁷⁷

Thus, although ICAM-1 and VCAM-1 are structurally and functionally similar, plasma levels of sICAM-1 may be considered a marker of a more generalized inflammatory process, whereas VCAM-1 seems to be an indicator of plaque burden or activity. Probably as a consequence of these differences, sVCAM-1 emerges as a risk predictor in patients with advanced atherosclerosis,^{70,71,75} whereas sICAM-1 is a better prognostic indicator in healthy individuals.^{66,67} It is noteworthy that, in PAD, the predictive value of sVCAM-1 is independent of classic risk factors, previous myocardial infarction and stroke,⁷⁵ which are conditions associated with increased circulating levels of adhesion molecules.^{78,79} Even more important, sVCAM-1 measurement adds to the predictive value of ABI (Figure 2), which is to date the most powerful prognostic risk indicator in PAD.^{80,81} In particular, among patients with an ABI < median (ie patients at higher cardiovascular risk), those with sVCAM-1 > median had a 3.4-fold increased risk of feature events as compared to patients with sVCAM-1 < median. A similar additive prognostic value has been reported for reduced brachial artery flow-mediated dilation.⁸² Collectively, these findings indicate that markers of endothelium activation/dysfunction are strong predictors of cardiovascular risk in PAD.

Potential significance of adhesion molecules in clinical practice

As our knowledge of the atherosclerotic process improves, so will our working concepts of the strategies for defining and reducing the cardiovascular risk. Therefore, studies of the adhesion molecules, by providing new insights into the pathophysiology of PAD and its consequences, have potential clinical implications.

First, circulating levels of sCAMs could be used to gauge the cardiovascular risk of PAD patients. However, at the present, sCAMs' assay does not fulfill all the conditions required because a diagnostic test enters into clinical practice.⁸³ Nevertheless, the finding that in PAD, sVCAM-1 measurement improves the stratification of prognosis provided by a well-established risk factor such as the ABI,⁷⁵ encourages further efforts in this field.

Second, the observation that increased preprocedural levels of sVCAM-1, sP-selectin and sE-selectin are associated with restenosis after PTA^{61,62} suggests that these markers may be of some value in the selection of patients for PTA and could constitute a new therapeutic target. Indeed, the phosphodiesterase III inhibitor cilostazol prevents restenosis after percutaneous coronary angioplasty,^{64,84} probably by inhibiting leukocyte $\alpha M/\beta_2$ integrin.⁶⁴ Future studies will clarify whether cilostazol is effective also in preventing peripheral vessel restenosis after PTA.

Finally, for sICAM-1 and sVCAM-1, which are considered important markers of inflammation and endothelial dysfunction, another potential use that might be considered is that of monitoring therapies aimed at reducing the inflammatory status and improving endothelial function. If plasma levels of these molecules do not fall, treatment might be intensified. Large clinical trials are needed to verify whether PAD patients with elevated levels of circulating CAMs would benefit from any specific therapy.

Conclusions

Studies of adhesion molecules provide a substantial insight into the pathophysiology of PAD. However, the clinical utility of measuring the circulating forms of these markers remains uncertain. First, to have a clinical role, the assay of these molecules should be less expensive. Second, there must be a greater number of prospective studies demonstrating consistently that adhesion molecules, in PAD, add substantially to our ability to predict risk beyond that achievable by use of the ABI and traditional risk factors. Finally, of critical need are a large number of studies showing that a reduction in the plasma levels of adhesion molecules, obtained through specific interventions, results in a reduction of cardiovascular risk. Therefore, although extraordinarily valuable as research tools, we must await the completion of prospective evaluations of various panels of these molecules before introducing their use into daily clinical practice.

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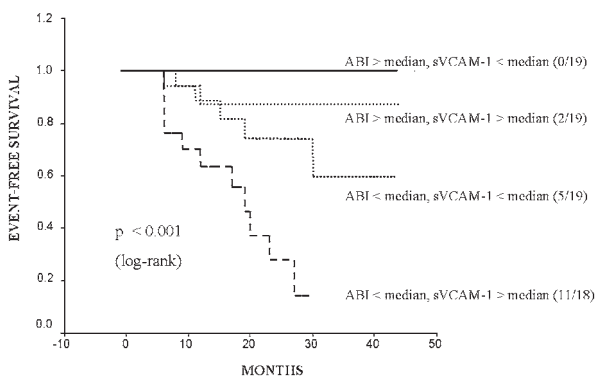


Figure 2 Kaplan–Meier analysis of event (cardiac + cerebrovascular)-free survival curves refers to a subgroup of patients categorized as being above or below the median values for sVCAM-1 and ABI. The combination of sVCAM-1 and ABI below the median was associated with the highest cardiovascular event incidence. (Adapted from ref.⁷⁵).

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