

ANNA HULOK^{1, A, B, D–F}, KRZYSZTOF ŚCIBORSKI^{1, A, B, D–F}, JAKUB MARCZAK^{*, 2, 3, C–E},
TOMASZ BAŃKOWSKI^{1, A, C–F}, RAFAŁ PORĘBA^{4, C, E, F}, MARTA NEGRUSZ-KAWECKA^{1, A, B, D–F}

Soluble Cell Adhesion Molecules – Does Estimating sVCAM-1 and sICAM-1 Concentration Provide Additional Information About Cardiovascular Risk in Patients with Coronary Artery Disease?

¹ Department and Clinic of Cardiology, Wrocław Medical University, Poland

² Department and Clinic of Cardiac Surgery, Wrocław Medical University, Poland

³ Department of Cardiac Surgery, Medinet Heart Center Ltd., Nowa Sol, Poland

⁴ Department and Clinic of Internal and Occupational Diseases and Hypertension, Wrocław Medical University, Poland

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article; G – other

Abstract

Objectives. Cell adhesion molecules (CAM) are thought to have a great impact on endothelium functioning. Interaction between CAM and a receptor may lead to macrophage activation and the release of multiple enzymes such as elastases and collagenases. These enzymes can, in turn, play a role in atherosclerotic plaque destabilization and initiation of acute coronary syndrome (ACS). The main aim of this study was to assess the role of sVCAM-1 and sICAM-1 in the risk stratification of ACS.

Material and Methods. 63 patients – mean age 62.7 ± 9.5 years (26 women, 37 men) – were included in the study. Patients were divided into two groups: I – patients with acute coronary syndrome (ACS) diagnosed by coronary angiography (n = 45: 15 women; 30 men); and II – patients without apparent CAD in coronary angiography (n = 18: 11 women, 7 men). In both groups, samples required for sVCAM-1 and sICAM-1 level measurements were collected before the angiography.

Results. Mean age, prevalence of arterial hypertension, diabetes mellitus and chronic kidney disease did not differ between the groups. Levels of sVCAM-1 and sICAM-1 were significantly higher in group I (group I vs. group II: 850.3 ± 337.9 vs. 675.9 ± 178.8 ; $p = 0.02$ and 737.2 ± 353.5 vs. 428.5 ± 157.3 ; $p = 0.001$ respectively). ROC analysis revealed that there is significantly higher risk of ACS above the level of 700.15 ng/mL for sVCAM-1 and 407.8 ng/mL for sICAM-1. The level of sVCAM-1 was also found to be an independent risk factor of NSTEMI, OR 1.003 (95% CI: 1.0007–1.004); $p = 0.007$, but not of STEMI ($p > 0.05$).

Conclusions. Levels of sVCAM-1 and sICAM-1 were found to be negative predictors of acute coronary syndrome. Further studies should assess the relationship between sVCAM-1 and sICAM-1 levels and the survival of patients suffering from CAD (*Adv Clin Exp Med* 2014, 23, 5, 735–741).

Key words: sVCAM-1, sICAM-1, soluble cell adhesion molecules, coronary artery disease, STEMI, NSTEMI, acute coronary syndrome, risk factor.

Coronary artery disease (CAD) remains the major cause of death worldwide. According to WHO data, more than 50% of deaths are caused by CAD, globally [1]. It is therefore advocated that we pursue some new insights into the

pathomechanism of atherosclerosis, which is fundamental for the prophylaxis, diagnosis and treatment of related diseases. It is generally known that in the development of atherosclerosis, endothelium dysfunction plays a pivotal role. Not only do

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endothelial cells create a biological barrier on the inner side of the arterial wall but they are also an important factor in the regulation of homeostasis, structure, arterial wall tension and undisturbed blood flow [2]. Regional inflammation promotes migration and adhesion of leucocytes, especially monocytes, and its translocation into the subendothelial layer.

Cell adhesion molecules (CAM) are those responsible for the adhesion of different cells onto the endothelial surface. CAM are glycoproteins integral to the cell membrane and whose proteins meet the criteria of receptor proteins [3]. Their expression is observed on the surface of various cells as well as on the surface of atherosclerotic plaques [4]. The main role of cell adhesion molecules consists of the preservation of tissue continuity, mediation in cellular interactions and provision of extracellular matrix contact.

Cell adhesion proteins are thought to be specialized structures capable of information reception, its transformation and transfer as a specific signal to several cellular structures such as macrophages. As a consequence of macrophage activation mediated by CAM, macrophageal degranulation is observed with the release of elastase and collagenase. These two enzymes are responsible for the degradation of the collagen cups of atherosclerotic plaques, which may result in their rupture.

The basic structure of a cellular adhesion molecule is universal – an amino acid chain with short intracellular domain, an intramembranous fragment and a complex extracellular domain. CAMs exist in several different forms such as membrane CAM (mCAM) and soluble forms not attached to cellular membrane (sCAM).

There are several groups among CAMs such as: cadherins, selectins, CD44 particles and immunoglobulin superfamily CAMs. The latter consist of proteins related to antibodies. This group further divides into intracellular cell adhesion molecules (ICAM), CD54, vascular cell adhesion molecules (VCAM), and platelet-endothelial cell adhesion molecules (PECAM).

ICAM are one of the most extensively studied proteins. They contain 5 immunoglobulin-like extracellular domains attached to integrin ligands. These are usually found on the surface of lymphocytes B and T, monocytes and endothelium [5, 6]. VCAM consist of a single amino acid chain whose extracellular part is divided into 7 immunoglobulin-like domains. Its expression is found in both macrophages and the endothelium [6]. Therefore, our hypothesis was that the increased concentration of CAM may have a significant relationship to coronary artery disease, including acute coronary

syndrome (ACS). The main aim of this study was to assess the role of sVCAM-1 and sICAM-1 in the risk stratification of ACS.

Material and Methods

The study involved 63 patients hospitalized in the Department and Clinic of Cardiology, Wrocław Medical University, Poland, between 2009 and 2010. The mean age of the population studied was 62.7 ± 9.5 years (26 women and 37 men). The patients were divided into 2 groups: I – patients with coronary artery disease diagnosed by coronary angiography ($n = 45$: 15 women, 30 men), and II – patients who had undergone coronary angiography due to coronary artery disease ($n = 18$; 11 women, 7 men) with a negative result. In both groups, the coronary angiography was performed from the femoral access. During angiography, no less than 2 catheters were used for the examination (usually left and right coronary catheters). The coronary angiography was done with the use of Iomeron or Optiray contrast solutions. The examination used several projections such as right and left oblique, and caudal or cranial projections where necessary. Initial presentation with acute coronary syndrome was an indication for coronary angiography in group I. Group II was based on patients who did not have acute coronary syndrome but had had a clinical diagnosis of coronary artery disease based on a high probability of CAD or a positive or non-diagnostic ECG cardiac stress test. Patients with ACS were treated according to the guidelines for ACS of the European Society of Cardiology, whereas patients without ACS were treated in accordance with their co-morbidity treatment standards. Stent type was dependent on the morphology of the lesion within the coronary artery.

In patients from both groups, blood samples necessary for the assessment of sICAM-1 and sVCAM-1 concentration were collected before the coronary angiography and in group I also 6 months after discharge.

sICAM-1 and sVCAM-1 levels were measured with the use of commercially available Biovendo Kits. Transthoracic echocardiogram (TTE) was done with the use of Vivid 4 ultrasound. The left ventricular ejection fraction was measured using the Simpson's rule in 2D projections.

The collected data was analyzed with the use of Statistica 9 software. The character of distribution was assessed by Shapiro-Wilk test. When normally distributed, data was compared by means of a Student *t*-test, ANOVA *F*-test for independent variables and Pearson's coefficient of correlation.

Table 1. Population characteristics

Data	Group I (n = 45)	Group II (n = 18)	p
demographic data			
Gender	w: 15 (33.3%) m: 30 (66.7%)	w: 11 (61.1%) m: 7 (38.9%)	0.043
Age	64.1 ± 9.3	60.0 ± 9.7	0.14
admission			
STEMI	22 (48.9%)	0 (0%)	0.001
NSTEMI	23 (51.1%)	0 (0%)	0.001
Obesity	3 (6.7%)	3 (16.7%)	0.46
Nicotine abuse	4 (8.9%)	6 (33.3%)	0.04
Arterial hypertension	30 (66.7%)	12 (66.7%)	0.77
Arterial hypertension I stage	8 (17.8%)	6 (33.3%)	0.31
Arterial hypertension II stage	17 (37.8%)	6 (33.3%)	0.97
Arterial hypertension III stage	5 (11.1%)	0 (0%)	0.34
Atrial fibrillation	7 (15.6%)	2 (11.1%)	0.95
Heart failure	2 (4.4%)	0 (0%)	0.91
Diabetes mellitus type 2	8 (17.8%)	2 (11.1%)	0.79
biochemical markers			
LDL [mg/dL]	142.4 ± 48.2	139.2 ± 39.1	0.82
HDL [mg/dL]	42.4 ± 11.3	53.1 ± 12.3	0.08
Total cholesterol [mg/dL]	215.4 ± 51.6	223.6 ± 43.8	0.57
Triglycerides [mg/dL]	135.4 ± 82.3	146.2 ± 50.2	0.31
Creatinine clearance [mg/dL]	1.4 ± 0.91	1.35 ± 0.77	0.27
eGFR [mL/min/1.73m ²]	74.0 ± 21.9	73.7 ± 19.3	0.95
sVCAM-1 [ng/mL]	850.3 ± 337.9	675.9 ± 178.8	0.02
sICAM-1 [ng/mL]	737.2 ± 353.5	428.5 ± 157.3	0.001
WBC [10 ³ /uL]	10.6 ± 3.6	7.4 ± 1.9	0.001
miscellaneous data			
LVEF [%]	54.9 ± 1.2	66.8 ± 8.1	0.001

When not normally distributed, the data was analyzed using the Mann-Whitney test, nonparametric ANOVA F-test and calculation of the Spearman correlation coefficient. Additional analysis of the receiver-operator curves (ROC) was conducted where appropriate. For the qualitative variables, a chi-square test was used. Statistical significance was assumed at $p < 0.05$.

Results

The groups did not differ as far as age and gender are concerned. The prevalence of arterial hypertension, diabetes, heart failure and atrial fibrillation was equal among the groups. Patients in group I (ACS) showed a lower prevalence of nicotine abuse ($p = 0.04$), higher white blood count ($p = 0.001$) and lower left ventricular ejection fraction ($p = 0.001$) in comparison to patients in group II (non-ACS). In group I, a higher concentration of sVCAM-1 ($p = 0.02$) and sICAM-1

Table 2. Relationship between concentration of cytokines in the acute and the chronic phase of ACS

Parameter	Acute phase (n = 45)			Chronic phase (n = 45)			Percentage v < V	Z	p
	mean	median	SD	mean	median	SD			
sVCAM-1	850.3	794.4	337.9	1086.0	1040.9	394.0	80	2.8	0.01
sICAM-1	737.2	642.5	353.5	1098.9	865.8	608.7	56	0.4	0.69

Table 3. The Spearman correlation matrix – the concentration of sVCAM-1 and sICAM-1 and several biochemical markers

Relationship	Patients – group I (n = 45)		Arterial hypertension subgroup (n = 30)		Diabetes mellitus subgroup (n = 8)	
	R	p	R	p	R	p
sVCAM-1 & WBC	-0.48	0.001	-0.62	0.001	-0.90	0.001
sVCAM-1 & PLT	-0.28	0.09	-0.23	0.22	-0.56	0.15
sVCAM-1 & LDL	0.14	0.40	0.002	0.98	-0.04	0.93
sVCAM-1 & HDL	0.14	0.41	0.18	0.34	-0.18	0.67
sVCAM-1 Tot. Chol.	0.08	0.65	-0.06	0.76	0.04	0.93
sVCAM-1 & TG	-0.18	0.27	-0.20	0.30	-0.30	0.47
sVCAM-1 & LVEF	-0.03	0.84	-0.20	0.29	-0.08	0.84
sICAM-1 & WBC	-0.11	0.51	-0.22	0.25	-0.21	0.61
sICAM-1 & PLT	-0.02	0.88	0.08	0.68	-0.62	0.10
sICAM-1 & LDL	0.26	0.12	0.11	0.56	-0.10	0.82
sICAM-1 & HDL	0.02	0.93	0.07	0.72	-0.12	0.78
sICAM-1 Tot. Chol.	0.15	0.39	0.00	0.99	-0.26	0.53
sICAM-1 & TG	-0.32	0.05	-0.41	0.03	-0.79	0.02
sICAM-1 & LVEF	-0.21	0.21	-0.31	0.10	-0.57	0.14

($p = 0.001$) were observed. In patients suffering from ACS, sVCAM-1 increased during the transition from the acute to the chronic phase of acute coronary syndrome ($p = 0.01$), whereas the concentration of sICAM-1 remained at the same level ($p = 0.69$).

The Spearman correlation coefficient was also calculated for the concentration of sICAM, sVCAM and several biochemical markers (Table 3).

There was an inverse proportion found between sVCAM-1 concentration and WBC count in patients from group I (ACS). This was also seen in patients with hypertension and diabetes mellitus. A similar trend was observed between PLT count and sVCAM-1 concentration in patients of group I and those with hypertension and diabetes. An inversely proportional trend was observed in left ventricular ejection fraction and sICAM-1 concentration. This last observation was valid in all studied patients with ACS.

A receiver-operator curves analysis was performed in order to provide a specific value above which there is a significant increase in the risk for ACS. It was shown that above the cut-point of 700.15 ng/mL sVCAM-1, a statistically significant increase in the risk of ACS is observed (sensitivity 71.1%; specificity 77.8%) (Fig. 1); the same observation could be drawn for sICAM-1 with the cut-point above 407.8 ng/mL (sensitivity: 86.7%; specificity: 66.7%) (Fig. 2).

In ACS patients, 71.1% had sVCAM concentrations above the cut-point (22.2% in controls reached the cut-point concentration, $p < 0.001$). Similarly, 86.7% of patients suffering from ACS reached the sICAM cut-point level (only 33.3% of controls had a concentration higher or equal to the cut-point, $p < 0.001$).

Using ROC analysis, we found a sVCAM-1 concentration above which there is a significantly higher risk of ACS: NSTEMI and STEMI

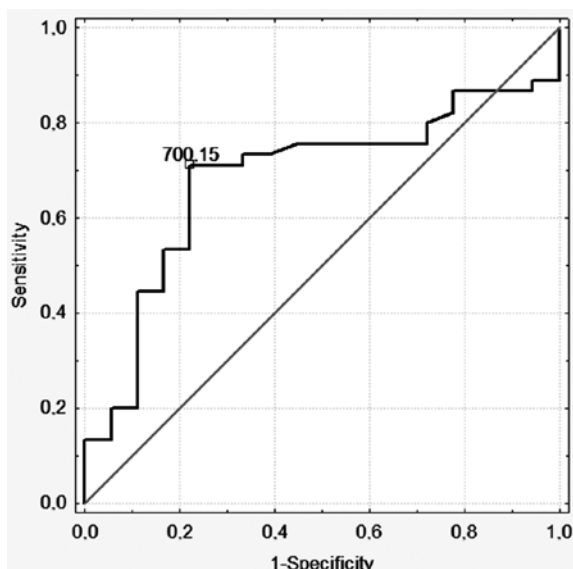


Fig. 1. ROC analysis of sVCAM-1 and the risk of ACS

(1478.4 ng/mL and 1781.2 ng/mL, respectively) – this was burdened with very low sensitivity (25% and 0.2%, respectively). Therefore, these cannot be used in the ACS NSTEMI and STEMI risk stratification.

Univariate analysis of logistic regression did not find sICAM-1 concentration as a risk factor for ACS: STEMI or NSTEMI ($p > 0.05$). ROC analysis found that above 1210.5 ng/mL and 837.1 ng/mL of sICAM-1 there is a higher risk for NSTEMI and STEMI respectively, yet with very low sensitivity, 18.8% and 27.1%.

In the univariate model of logistic regression, a concentration of sVCAM-1 higher than or equal to 700.15 ng/mL was associated with a significant increase in the risk of ACS: (OR 8.61 (95% CI 2.3–31.9); $p = 0.001$). The effect was even larger for sICAM-1 at or above the limit of 407.8 ng/mL – (OR 13 (95% CI 3.4–49.1); $p = 0.0001$).

Discussion

Elevated plasma concentration of soluble forms of cellular adhesion molecules have already been seen in patients suffering from advanced cerebrovascular and coronary atherosclerosis, as well as in patients suffering from diabetes mellitus. The Physicians Health Study (PHS) and Atherosclerosis Risk in Communities Study (ARIC) found that elevated plasma concentration of sICAM-1 precedes actual complications associated with atherosclerosis. A 9 year follow-up of patients qualified to the PH Study showed that ICAM-1 concentration above 260 ng/mL was associated with 80% higher risk of ACS, and that the risk was independent from other factors [7, 8]. In our study, we reported that

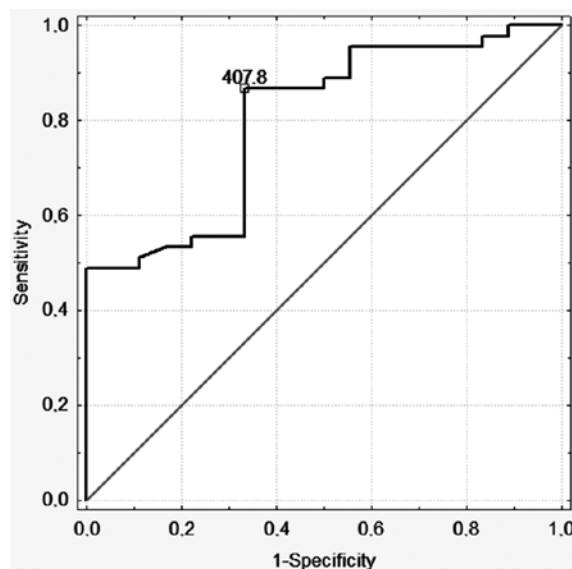


Fig. 2. ROC analysis of sICAM-1 and the risk of ACS

sICAM-1 concentration higher than 700.15 ng/mL translates into an 8-times higher risk of ACS. Another important observation, in our opinion, was the negative correlation between the left ventricular ejection fraction and the concentration of the above-mentioned adhesins that, in turn, can provide a context for the higher risk of ACS.

To date, no clear-cut association between the concentration of sVCAM-1 and the risk of MACCE has been drawn. Nevertheless, clinical data exists that elevated serum VCAM-1 can be observed in patients with arterial hypertension and the relation can be drawn between the serum concentration of VCAM-1 and the advancement of atherosclerosis [9]. The studies of Semaan and Mulvihill documented coronary artery disease coupled with elevated concentration of VCAM-1 [10, 11]. Our analysis confirmed that VCAM-1 concentration is elevated in patients suffering from coronary artery disease. We have also found that the concentration of VCAM-1 above the specific cut-point increases the risk of ACS thirteenfold. The VCAM-1 molecule therefore may play a significant role in the risk assessment of cardiovascular adverse events.

Our study confirmed a negative correlation between the white blood cell count and the VCAM-1 concentration [12]. A similar trend was observed as far as platelet count was concerned. A coronary artery disease pathomechanism may explain such observations. The elevated expression of cellular adhesion molecules on the atheromatous plaques correlates positively with their soluble forms and can be linked with elevated chemotaxis of macrophages and platelets to the arterial wall, which in turn can be associated with their decrease in the complete blood count. This observation may serve as an explanation to the association between

the higher risk of acute coronary syndrome that was seen for VCAM-1, OR = 13, but not seen for sICAM, OR = 8.4. It is believed that sVCAM-1 level is prognostic for the development of coronary artery disease, though several authors documented its diagnostic role only in the stable phase of CAD or as soon as after the 6th month following ACS [13]. In our analysis, after 6 months following ACS and optimal medical therapy, sVCAM-1 concentration increased ($p = 0.01$). There are studies heralding the role of VCAM-1 in the optimization of CAD therapy, as it was shown that a decrease in the expression of VCAM-1 can be observed along with the administration of antioxidants and polyunsaturated fatty acids as well as in patients chronically treated with ACE inhibitors, statins and fibrates [13–16]. The elevation of sVCAM-1 seen in our study following the six-month-long optimal medical therapy might require further explanation. The reason for this elevation may be patient

non-compliance, but also such phenomenon as resistance to antiplatelet treatment should be taken into account. This, in turn, may enable early isolation of high risk ACS patients.

The authors concluded that levels of sVCAM-1 and sICAM-1 were found to be negative predictors of acute coronary syndrome. Moreover, sVCAM-1 significantly correlated with the level of post-infarction left ventricular injury. The assessment of cell adhesion molecules in patients with coronary artery disease may bring additional information as far as the risk of ACS is concerned. sVCAM-1 level assessment could also tell us which patients may be unresponsive to standard optimal medical therapy.

Limitations

The main limitation was the small number of the study group.

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Address for correspondence:

Tomasz Bańkowski
Department and Clinic of Cardiology
Wroclaw Medical University
Borowska 213
50-556 Wroclaw
Poland
E-mail: tba0@poczta.onet.pl

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