sPECAM-1 and sVCAM-1: role in pathogenesis and diagnosis of chronic hepatitis C and association with response to antiviral therapy

Michal Kukla, **Krystyna Zwirska-Korczala**, **Andrzej Gabriel**, **Ewa Janczewska-Kazek**, **Agnieszka Berdowska**, **Andrzej Wiczkowski**, **Barbara Rybus-Kalinowska**, **Mariusz Kalinowski**, **Adam Ziolkowski**, **Elzbieta Wozniak-Grygiel**, **Marek Waluga and Blazej Nowak**

Abstract:

Aim: To analyze the relationship between pretreatment clinical or histological features and the levels of soluble platelet-endothelial cell adhesion molecule-1 (sPECAM-1) and soluble vascular cell adhesion molecule-1 (sVCAM-1), to determine their serum concentration in responders and nonresponders, to evaluate the behavior under antiviral therapy, to explain their relationship in response to therapy and to assess the association between these two molecules in chronic hepatitis C (CHC).

Methods: The study analyzed 65 CHC patients, including 50 patients (Group 1) with marked fibrosis treated with peginterferon plus ribavirin, 15 patients without fibrosis (Group 2) and 13 healthy volunteers (the control group, Group 3). sPECAM-1 and sVCAM-1 levels were assessed by an immunoenzymatic method (ELISA) before and after therapy.

Results: sVCAM-1 and sPECAM-1 serum concentrations increased significantly in CHC patients ($p < 0.001$). sPECAM-1 levels corresponded to inflammatory grade ($p = 0.03$) and fibrosis stage ($p = 0.01$). sVCAM-1 increased only in advanced fibrosis. After therapy, sPECAM-1 levels decreased significantly ($p < 0.001$) with no difference between responders and nonresponders. sPECAM-1 correlated positively with inflammatory activity ($p = 0.02$), fibrosis stage ($p < 0.001$), sVCAM-1 ($r = 0.56$, $p < 0.001$) and alanine aminotransferase activity ($r = 0.30$, $p = 0.05$). Receiver operating characteristic curve analysis showed a good discriminant power of serum sPECAM-1 concentrations for detection of liver fibrosis – stage 0 *versus* stage 1–3, AUC 0.81; cut-off 221.0 ng/ml and a fair discriminant power for distinguishing bridging fibrosis, AUC 0.78; cut-off 237.1 ng/ml.

Conclusions: Hepatitis C virus (HCV) infection results in upregulation of sPECAM-1 and sVCAM-1. sPECAM-1 levels are related to necroinflammatory activity and may also identify patients with advanced fibrosis. The sPECAM-1 value was decreased by therapy but its measurement cannot predict therapy outcome and confirm HCV persistence. sPECAM-1 may influence VCAM-1 expression.

Keywords: soluble platelet-endothelial cell adhesion molecule-1, soluble vascular cell adhesion molecule-1, chronic hepatitis C, fibrosis, inflammatory activity, antiviral therapy

Correspondence to:
Michal Kukla MD, PhD
Department of Physiology, Medical University of Silesia, Zabrze, Poland.
kuklamich@ poczta.onet.pl

Krystyna Zwirska-Korczala
Elzbieta Wozniak-Grygiel
Department of Physiology in Zabrze, Medical University of Silesia Katowice, Poland

Andrzej Gabriel
Adam Ziolkowski
Department of Pathomorphology in Zabrze, Medical University of Silesia, Katowice Poland

Ewa Janczewska-Kazek
Outpatients Clinic for Infectious Diseases Chorzów, Poland

Agnieszka Berdowska
Department of Microbiology and Biotechnology, Jan Długosz University Częstochowa, Poland

Andrzej Wiczkowski
Department of Biology in Zabrze, Medical University of Silesia, Katowice Poland

Barbara Rybus-Kalinowska
Department of Basic Sciences in Bytom Medical University of Silesia, Katowice, Poland

Mariusz Kalinowski
Department of Cardiology Silesian Center for Heart Diseases in Zabrze Medical University of Silesia, Katowice, Poland

Marek Waluga
Department of Gastroenterology and Hepatology, Medical University of Silesia Katowice, Poland

Blazej Nowak
Department of Internal Medicine, Regional Hospital in Nowa Sol Poland

Introduction

Direct virus influence on hepatocytes and host immune response play an essential role in the pathogenesis of liver injury [Ballardini *et al.* 1995; Cerny *et al.* 1995; Brillanti *et al.* 1993]. The normal liver contains an enormous number of lymphocytes such as specialized NK, NKT, CD4 and CD8 cells [Lalor *et al.* 2002]. During infectious liver disease [Lalor *et al.* 2002] and nonalcoholic steatohepatitis (NASH) [Ziółkowski *et al.* 2005] the number of lymphocytes in the liver increases. The extravasation of

leukocytes to the inflammatory tissue is the phenomenon that involves several sequential steps and requires a wide spectrum of adhesion molecules [Petri and Bixel, 2006; Engelhardt and Wolburg, 2004; Ley, 1996; Gonzalez-Amaro *et al.* 1998]. The migration requires not only molecular interaction involved in firm adhesion of leukocytes interfered by function antigen-1/intercellular adhesion molecule-1 (LFA-1/ICAM-1) and very late antigen-4/vascular cell adhesion molecule-1 (VLA-4/VCAM-1) complexes, but also receptors located at the endothelial cells (EC) junctions, such as platelet endothelial cell adhesion molecule-1 (PECAM-1) [Petri and Bixel, 2006; Hordjik, 2006].

PECAM-1 (CD 31) is constitutively expressed on platelets, monocytes, neutrophils, NK and CD8 T cells. High expression exists on continuous endothelial cells at cell–cell borders [Prager and Stockinger, 2000; Newman, 1997] and weak expression on sinusoidal endothelial cells (SEC) [Katz *et al.* 2004; Xu *et al.* 2003; Neubauer *et al.* 2000].

PECAM-1 plays a putative role in the inflammatory process and leukocyte–endothelial interaction, especially in transmigration of leukocytes through intercellular junctions. PECAM-1 is capable of mediating both homophilic adhesion and heterophilic binding to other molecules [Prager and Stockinger, 2000; DeLisser *et al.* 1993]. PECAM-1 has been implicated in cell survival, angiogenesis [Newman and Newman, 2003; Graesser, 2002] and in NASH progression [Goel *et al.* 2007]. In addition PECAM-1 affects activation and regulates trafficking of integrins [Newman and Newman, 2003]. PECAM-1 enhances T lymphocyte ability to bind to $\beta 1$ integrin substrates, such as VCAM-1 [Newman and Newman, 2003].

VCAM-1 (CD 106) expression on EC is not constitutive, but can be caused by tumor necrosis factor alpha, interleukin-1 (IL-1), IL-4 and interferon gamma [Pober and Cotran, 1990]. There is low basal expression of VCAM-1 in the normal liver, both on sinusoidal and Kupfer cells [Lang *et al.* 1995]. As well as the membrane-bound forms, soluble forms – sVCAM-1 and sPECAM-1 exist in human plasma [Prager and Stockinger, 2000; Gearing and Newman, 1993].

Nowadays, liver biopsy seems to be the most suitable method to assess CHC progression.

As an invasive procedure, it is associated with high costs and clinical complications. An accurate prediction of the response to antiviral therapy is difficult. Therefore, additional criteria for precise, noninvasive diagnosis should be investigated, including adhesion molecules involved in pathogenesis of viral hepatitis. The exact role of adhesion molecule in viral hepatitis immunopathogenesis is still uncertain. To our knowledge, there is very little data regarding sPECAM-1 in CHC available and there is no data with respect to its relationship with other adhesion molecules and antiviral therapy.

The purposes of the study were to analyze the relationship between pretreatment clinical or histological features and the levels of sPECAM-1 and sVCAM-1, to determine their serum concentration in responders and nonresponders, to evaluate the behavior of these adhesins under antiviral therapy, to explain their relationship in response to combined antiviral therapy and to assess the association between these two molecules in CHC.

Methods

Patients selection and serological assays
The study was performed on 65 patients with chronic hepatitis C (CHC), infected with HCV genotype 1b. This included 50 patients (25F/25M) with marked fibrosis and persistently elevated alanine aminotransferase (ALT) activity for at least 6 months, aged between 32 and 65 years [average 52.0 (11.2) years, BMI 24.6 (3.2) kg/m^2] – Group 1, and 15 patients (8F/7M) with minimal inflammatory activity and without fibrosis, with normal ALT activity [average 48.9 (9.9) years, BMI 24.9 (2.8) kg/m^2] – Group 2. The duration (i.e. time since first diagnosis of HCV infection) ranged from 6 to 15 years, an average of 9.3 (4.3) years. Exclusion criteria included other virus genotypes, drug or alcohol abuse and autoimmune, neoplastic, thyroid or psychiatric diseases, hepatitis B or HIV coinfection, diabetes, renal and heart failure.

The diagnosis of CHC was confirmed by presence of serum HCV RNA assayed with reverse transcription polymerase chain reaction (RT-PCR) method (Amplicor Roche/Promega v.2 Diagnostic Test, New Jersey, USA). Virus genotype was assessed by a reverse-hybrydization line probe assay (LiPA Versant

Test Milwauke, USA). The control group (Group 3) comprised 13 healthy volunteers (7F/6M) without anti-HCV antibodies and with normal ALT.

sVCAM-1 and sPECAM-1 serum concentrations were assessed in duplicate by immuno-enzymatic method with commercially available ELISA kits (Quantikine Immunoassay, Bender MedSystem, Vienna, Austria, human sVCAM-1 ELISA, catalog number BMS232, sensitivity 0.9 ng/ml and human sPECAM-1 ELISA, catalog number BMS229, sensitivity 0.1 ng/ml).

A blood sample was withdrawn (fasting in the morning) from all subjects at the beginning of the study. The second blood collection was carried out in CHC patients upon completion of antiviral therapy. The samples were centrifuged and serum was frozen at -70°C until assay. The remaining biochemical parameters were measured with routine methods. The study was approved by the Ethical Committee of the Medical University of Silesia in Katowice and conformed to the ethical guidelines of the Declaration of Helsinki. Informed consent was obtained for the whole study series.

Treatment protocol
All patients from Group 1 were administered pegylated interferon α2b subcutaneously (Pegasys, Roche, New Jersey, USA) at a dose of 180 μg/week, together with ribavirin (Copegus, the same producer) orally at a dose dependent on body weight (1000–1200 mg/day) for 48 weeks. Patients were considered to be responders if serum ALT activity had reached normal values and HCVRNA was not detectable at the end of treatment – end of treatment response (ETR).

Liver histology
All studied patients had liver biopsy performed with Hepafix kit (B.Braun, Melsungen AG, Germany) before antiviral therapy, as part of the diagnostic routine. Biopsy samples included at least six portal tracts and were examined by two pathologists. Histopathological features were assessed according to modified Scheuer's scale [Gabriel and Ziółkowski, 1997; Scheuer, 1995].

Statistical analysis
The values were expressed as the mean and standard deviation ($\pm$SD). The Shapiro–Wilk test was used to evaluate the distribution. Because of non-Gaussian distribution, nonparametric methods were used. Differences in studied variables between groups were tested using U Mann–Whitney and ANOVA rang Kruskal–Wallis tests for independent groups and by means of Wilcoxon's matched-pairs signed-rank test for the dependent groups. Correlations were analyzed with the Spearman rank correlation coefficient. A p value of less than 0.05 was considered to be statistically significant. Analysis of receiver operating characteristic curves (ROC) was performed for assessing the discriminant power of sVCAM-1 and sPECAM-1 for fibrosis stage and inflammatory activity.

Results

Comparison of clinical and biochemical parameters among analyzed groups
The comparison of analyzed clinical and biochemical parameters between investigated groups is shown in Table 1.

Histopathologic examination of liver tissue samples
Results revealed by histopathologic examination of biopsy specimens of CHC patients belonging to Group 1 and Group 2 are shown in Table 2.

Assessment of sVCAM-1 and sPECAM-1 serum concentrations before antiviral therapy and the association with liver histology
sVCAM-1 serum concentration in Group 1 was significantly higher only in comparison with Group 3 (2197.2 *versus*, 1144.9 ng/ml, $p < 0.001$) (Table 3; Figure 1). sPECAM-1 serum concentration in Group 1 was significantly increased when compared with Group 2 (252.7 *versus* 166.1 ng/ml, $p = 0.009$) and Group 3 (252.7 *versus* 86.4 ng/ml, $p < 0.001$) (Table 3, Figure 2). Both parameters were significantly elevated in Group 2 when compared with Group 3 (Table 3).

Patients from Group 1 and Group 2 were connected in one group and subsequently divided into subgroups according to grade of inflammatory activity. Minimal activity was found in 15 patients, mild in 29 and moderate in 21. A significant difference was found with respect to sPECAM-1 values (166.1 *versus* 253.9 *versus* 252.1 ng/ml, $p = 0.03$), AST and ALT ($p < 0.001$) activity, but not sVCAM-1 ($p = 0.9$) (Table 4).

Table 1. The comparison of clinical and biochemical parameters among analyzed groups before therapy.

Parameter	Group 1 (n=50)	Group 2 (n=15)	p*	Group 3 (n=13)	p*	p**
	Mean (SD)	Mean (SD)		Mean (SD)		
Age (years)	52.0 (11.2)	48.9 (9.9)	NS	47.9 (11.2)	NS	NS
Weight (kg)	70.6 (9.5)	71.1 (10.3)	NS	69.4 (10.1)	NS	NS
Height (cm)	174 (6.5)	171.8 (9.9)	NS	173.6 (8.4)	NS	NS
BMI (kg/m^2)	24.6 (3.2)	24.9 (2.8)	NS	23.0 (3.3)	NS	NS
AST (IU/l)	122.6 (68.2)	26.7 (6.1)	<0.001	24.9 (6.0)	<0.001	NS
ALT (IU/l)	136.6 (58.2)	24.7 (7.3)	<0.001	22.8 (8.0)	<0.001	NS
BIL (μmol/l)	21.1 (4.9)	15.8 (4.2)	NS	10.1 (4.8)	<0.001	NS
PLT (G/l)	218.9 (79.9)	257 (69.5)	0.02	278.1 (71.2)	0.025	NS
WBC (G/l)	6.7 (1.8)	7.1 (1.3)	NS	7.0 (1.6)	NS	NS
RBC (T/l)	4.5(0.3)	4.3 (0.4)	NS	4.4 (0.5)	NS	NS
FA (IU/l)	91.4 (24.1)	82.1 (20.8)	NS	78.1 (19.8)	0.057	NS
TP (g/l)	76.0 (4.2)	75 (4.5)	NS	74 (5.1)	NS	NS
ESR (mm/h)	11.6 (8.7)	6.7 (4.3)	0.02	5.9 (3.8)	<0.001	NS
HGB (mmol/l)	8.1 (0.5)	8.6 (0.5)	NS	8.7 (0.5)	NS	NS

p, according to Mann Whitney U-Test, *p**, values *versus* Group 1; *p***, Group 2 *versus* Group 3; BMI, Body Mass Index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; BIL, total serum bilirubin; FA, alkaline phosphatase; TP, total protein; HGB, hemoglobin, RBC, red blood cells; WBC, white blood cells; PLT, platelets; ESR, erythrocyte sedimentation rate; NS, not significant.

Table 2. The results of histopathologic examinations (according to Scheuer's scale) of liver biopsy specimens of CHC patients (Group 1 and Group 2).

Inflammatory activity	Group 1	Group 2
	Number of patients (%)	Number of patients (%)
Grade 1 (minimal)	0	15 (100)
Grade 2 (mild)	29 (58)	0
Grade 3 (moderate)	21 (42)	0
Fibrosis		
Stage 1 (portal)	6 (12)	NF
Stage 2 (periportal)	20 (40)	NF
Stage 3 (septal/bridging)	24 (48)	NF

NF: no fibrosis was found in histopathologic examination.

According to the fibrosis stage, Group 1 was divided into two subgroups: subgroup 1 included stage 1 and 2 (26 patients) while subgroup 2 included stage 3 (24 patients). The concentrations of sVCAM-1 and sPECAM-1 were noticeably higher in patients with more advanced fibrosis (2070.7 *versus,* 2308.0 ng/ml, *p* = 0.47; 224.1 *versus* 280.0 ng/ml, *p* = 0.01, respectively) but the difference was significant only for sPECAM-1.

Comparison of sVCAM-1 and sPECAM-1 serum concentrations before and after antiviral therapy and the association with therapy effectiveness

In Group 1 sVCAM-1 serum concentrations decreased insignificantly after therapy (2197.2 *versus,* 1803.4 ng/ml, *p* = 0.17), while sPECAM-1 decreased significantly (252.7 *versus* 175.9 ng/ml, *p* < 0.001) (Table 5). ETR was achieved in 27 patients. Thus, according to therapy outcome Group 1 was divided into two subgroups: responders (27 patients) and nonresponders (23 patients). sVCAM-1 and sPECAM-1 serum levels between responders and nonresponders both before (1967.9 *versus,* 2397.9 ng/ml, *p* = 0.15 and 243.1 *versus* 262.2 ng/ml, *p* = 0.24, respectively) (Table 6, Figures 1 and 2) and after therapy (1747.1 *versus,* 1869.2 ng/ml, *p* = 0.70 and 164.1 *versus* 190.1 ng/ml, *p* = 0.46, respectively) differed insignificantly (Table 7, Figures 3 and 4). Nevertheless, mean values appeared to be higher in nonresponders. The comparison of soluble adhesion molecule levels in Group 1, responders and nonresponders to untreated patients with minimal inflammatory activity and healthy controls is presented in Figures 3 and 4. The significant difference both in responders (243.1 *versus* 164.1 ng/ml, *p* = 0.004) and nonresponders (262.2 *versus* 190.1 ng/ml, *p* < 0.001) for values before and after therapy was observed only for sPECAM-1 (Table 8).

Analysis of ROC curve of sVCAM-1 and sPECAM-1 for inflammatory activity and fibrosis

sPECAM-1 specificity and sensitivity for the detection of liver fibrosis stage – stage 0 *versus* stage 1–3 is shown in Figure 5. ROC curve analysis showed a good discriminant power of serum

Table 3. The comparison of sVCAM-1 and sPECAM-1 between analyzed groups before antiviral therapy.

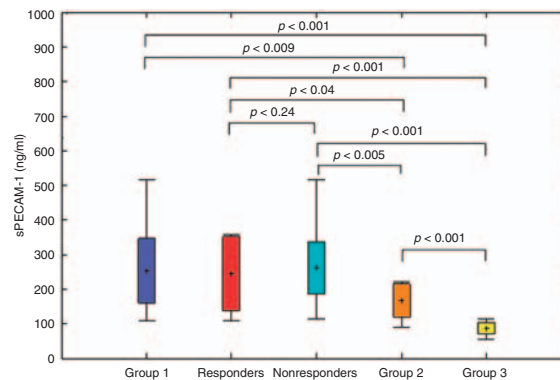| Parameter	Group 1 (n = 50)	Group 2 (n = 15)	p*	Group 3 (n = 13)	p*	p**
	Mean (SD)	Mean (SD)		Mean (SD)		
sVCAM-1 (ng/ml)	2197.2 (793.1)	2358.8 (958.2)	0.27	1144,9 (253.2)	<0.001	0.004
sPECAM-1 (ng/ml)	252.7 (92.)	166.1 (48.0)	0.009	86.4 (17.3)	<0.001	<0.001

p, according to Mann–Whitney U test, *p**, values *versus* Group 1; *p***, Group 2 *versus* Group 3.

Figure 1. The comparison of sVCAM-1 serum levels before therapy in all analyzed groups.
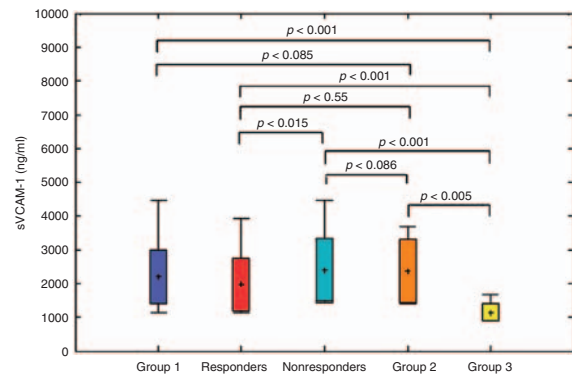
Figure 2. The comparison of sPECAM-1 serum levels before therapy in all analyzed groups.

sPECAM-1 concentrations for liver fibrosis detection with an area under curve (AUC) 0.81. The best cut-off was identified as 221.0 ng/ml (sensitivity 62.8% and specificity 100%). Figure 6 shows sPECAM-1 ROC curve for groups with fibrosis stages 0–2 *versus* stage 3. AUC 0.78 indicated a fair discriminant power for liver fibrosis differentiation, with cut-off set for 237.1 ng/ml (sensitivity 77.7% and specificity 87.5%). Unfortunately, ROC curves analysis showed no discriminant power of sVCAM-1 concentrations for liver fibrosis (stage 1 *versus* stage 2-3, AUC 0.52, sensitivity 66% and specificity 50%) and both analyzed molecules for inflammation (data not shown).

Correlations among analyzed variables
Significant mutual correlation appeared between serum concentrations of sVCAM-1 and sPECAM-1 before therapy ($r = 0.56$, $p = 0.001$) (Figure 7). sPECAM-1 serum concentrations correlated positively with fibrosis stage ($r = 0.35$, $p = 0.02$), inflammatory grade ($r = 0.53$, $p < 0.001$), AST ($r = 0.31$, $p = 0.048$) and ALT activity ($r = 0.30$, $p = 0.049$), whereas sVCAM-1 correlated with the duration of CHC ($r = 0.34$; $p = 0.047$). AST and ALT serum activities correlated with fibrosis stage ($r = 0.42$, $p = 0.006$; $r = 0.36$, $p = 0.02$, respectively) and

inflammation grade ($r = 0.60$, $p < 0.001$; $r = 0.53$, $p < 0.001$, respectively).

Discussion
HCV infection leads to inflammatory processes of different grades that involve activation of adhesion molecules and cytokines which facilitate recruitment of leukocytes to inflammatory areas. The evidence supporting this suggestion is the increase of sVCAM-1 and sPECAM-1 serum concentrations, as observed from the performed study. Consistent with other authors [Dejica *et al.* 2002; Fukuda *et al.* 1998; Lo Iacono *et al.* 1998; Kapllanski *et al.* 1997; Marui *et al.* 1996], we observed significantly higher serum sVCAM-1 levels in CHC patients. Dejica *et al.* showed a weak expression of VCAM-1 on cell borders of SEC in normal livers [Dejica *et al.* 2002]. Volpes *et al.* [1992], using immunohistochemical methods, demonstrated the expression of VCAM-1 during hepatitis on endothelial cells of portal tracts, part of SEC and some Kupffer cells. sVCAM-1 may be released from cytokine-activated endothelial cells and the serum levels may reflect activity of these cells [Bruno *et al.* 2005; Dejica *et al.* 2002].

Table 4. The comparison of sPECAM-1, sVCAM-1 and aminotransferase activity in patients with chronic hepatitis C (Group 1 + Group 2) before therapy with respect to the inflammatory activity grade.

Parameter	Inflammatory activity (number of patients)			p
	Grade 1 (n = 15)	Grade 2 (n = 29)	Grade 3 (n = 21)	
sPECAM-1 (ng/ml)	166.1 (48.0)	253.1 (92.3)	252.1 (95,5)	0.03
sVCAM-1(ng/ml)	2358.8 (958.2)	2182 (821.2)	2217.1 (999.1)	0.9
AST (IU/l)	26.7 (6.1)	102.2 (59.9)	146.8 (72.4)	<0.001
ALT (IU/l)	24.7 (7.3)	119.5 (57.3)	157.0 (76.7)	<0.001

p, according to ANOVA Kruskal–Wallis test.

Table 5. The comparison of serum concentrations of sPECAM-1, sVCAM-1 and other analyzed parameters in Group 1 before and after therapy.

Parameter	Before therapy	After therapy	p
	Mean (SD)	Mean (SD)	
sVCAM-1 (ng/ml)	2197.2 (793.1)	1803.4 (648,3)	0.17
sPECAM-1 (ng/ml)	252.7 (92.6)	175.9 (83.7)	<0.001
AST (IU/l)	122.6 (68.2)	49.0 (25.1)	<0.001
ALT (IU/l)	136.6 (58.2)	53.0 (32.4)	<0.001
Bilirubin (μmol/l)	21.1 (4.9)	19.8 (5.8)	0.6
HGB (mmol/l)	8.1 (0.5)	7.1 (0.4)	<0.001
RBC (T/l)	4.5 (0.3)	3.7 (0.3)	0.02
WBC (G/l)	6.7 (1.8)	3.9 (0.9)	0.003
PLT (G/l)	218.9 (79.9)	133.1 (46.5)	0.001

p, according to Wilcoxon's matched-pairs signed-rank test.

Table 6. The comparison of sVCAM-1 and sPECAM-1 serum concentrations as well as some analyzed biochemical parameters in responders and non-responders before therapy.

Parameter	Responders (n = 27)	Nonresponders (n = 23)	p
	Mean (SD)	Mean (SD)	
sVCAM-1(ng/ml)	1967.9 (787.8)	2397.9 (955.0)	0.15
sPECAM-1(ng/ml)	243.1 (107.6)	262.2 (75.3)	0.24
AST (IU/l)	95.9 (52.1)	147.8 (74.2)	0.13
ALT (IU/l)	114.6 (55.2)	157.4 (74.7)	0.28
FA (IU/l)	84.7 (20.1)	98.0 (27.4)	0.30

p, according to Mann–Whitney U test.

Table 7. The comparison of sVCAM-1 and sPECAM-1 serum concentrations in responders and nonresponders after therapy.

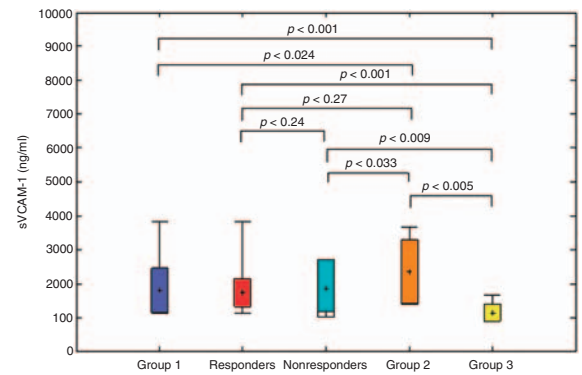| Parameter	Responders	Nonresponders	p
	Mean (SD)	Mean (SD)	
sPECAM-1 (ng/ml)	164.1 (86.6)	190.1 (80.8)	0.46
sVCAM-1 (ng/ml)	1747.1 (422.3)	1869.2 (857.8)	0.70

p, according to Mann–Whitney U test.

Figure 3. The comparison of sVCAM-1 serum levels after therapy in Group 1, responders and non-responders with Group 2 and the control group.
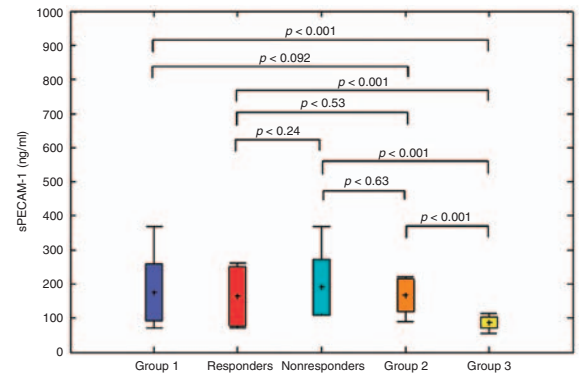

Figure 4. The comparison of sPECAM-1 serum levels after therapy in Group 1, responders and nonresponders with Group 2 and the control group.

Another possible source of sVCAM-1 may be activated dendritic cells localized in inflamed portal tracts [Garcia-Monzon *et al.* 1996]. Moreover, CD8 lymphocytes infiltrating the liver during CHC, express VLA-4 on its cell border, confirming participation of this molecule in cellular adhesion [Garcia-Monzon *et al.* 1996]. Binding T cells by VLA-4/VCAM-1 complex may cause T-cell activation and proliferation leading in consequence to damage of the surrounding hepatocytes [Dejica *et al.* 2002].

Unfortunately, our study showed the similar concentrations of sVCAM-1 in CHC patients with more advanced and minimal inflammatory activity. Bruno *et al.* [2005] affirmed that sVCAM-1 levels were significantly higher in a case of severe inflammatory activity and liver cirrhosis when compared to mild inflammatory activity and portal fibrosis. Moreover, sVCAM-1

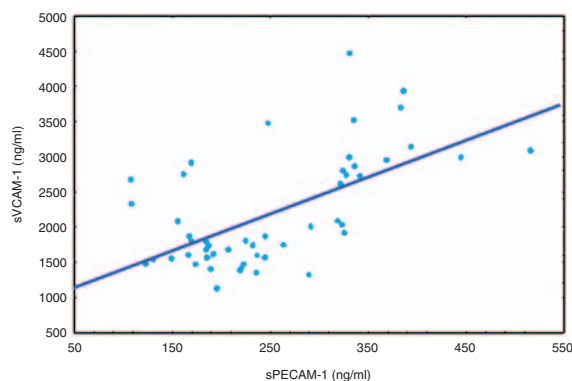**Table 8.** sVCAM-1 and sPECAM-1 serum concentrations in responders and nonresponders before and after therapy.

Parameter	Before therapy Mean (SD)	After therapy Mean (SD)	p
Responders			
sVCAM-1 (ng/ml)	1967.9 (787.8)	1747.1 (422.3)	0.34
sPECAM-1 (ng/ml)	243.1 (107.6)	164.1 (86.6)	0.004
Nonresponders			
sVCAM-1 (ng/ml)	2397.9 (955.0)	1869.2 (857.8)	0.3
sPECAM-1 (ng/ml)	262.2 (75.3)	190.1 (80.8)	<0.001

p, according to Wilcoxon's matched-pairs signed-rank test.
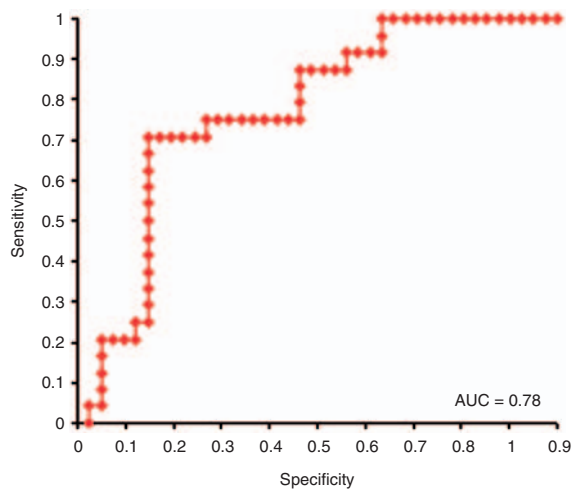
Figure 5. Receiver operating characteristic curve analysis for sPECAM-1. Sensitivity and specificity for the detection of liver fibrosis – stage 0 *versus* stage 1–3. Area under the curve = 0.81. The best cut-off was set as 221.0 ng/ml with sensitivity 62.8% and specificity 100%.
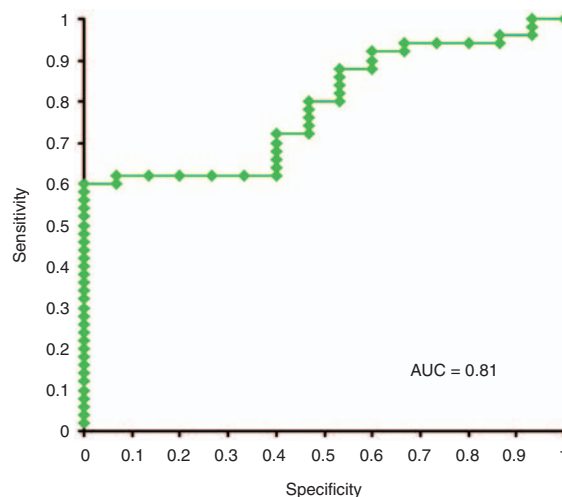
Figure 6. Receiver operating characteristic curve analysis for sPECAM-1. Sensitivity and specificity for the detection of liver fibrosis stage – stage 0–2 *versus* stage 3. Area under the curve = 0.78. The best cut-off was set as 237.1 ng/ml with sensitivity 77.7% and specificity 87.5%.

concentration was marginally higher in patients with mild CHC activity when compared with healthy subjects. Fukuda *et al.* [1998] did not observe significant sVCAM-1 difference between healthy volunteers and asymptomatic CHC carriers with minimal inflammatory grade. Nevertheless, differences between patients with minimal inflammatory activity but elevated aminotransferases and those with normal aminotransferase activity were showed. Kapllanski *et al.* [1997] did not observe association between inflammatory activity and sVCAM-1 concentration.

Higher sVCAM-1 concentration in patients with minimal inflammatory grade without fibrosis compared with healthy subjects observed in our study suggests that sVCAM-1 increases may

Figure 7. Correlation between sVCAM-1 and sPECAM-1 serum concentrations in all patients with chronic hepatitis C (Group 1 and Group 2) before antiviral therapy.

reflect an ongoing inflammatory process and persistence of viremia. Nevertheless, Lo Iacano *et al.* [1998] and Radu *et al.* [2003] did not establish a relationship between sVCAM-1 concentration and viral load. Our results correspond to other authors [Bruno *et al.* 2005; Dejica *et al.* 2002; Granot *et al.* 2001] and demonstrate the lack of association between sVCAM-1 concentration and aminotransferase activity, which may be an intermediate marker of inflammatory activity in the liver. Only Lo Iacono *et al.* [1998] demonstrated the significant correlations between ALT, gamma-glutamyltranspeptydase and sVCAM-1 concentrations.

Until now, unequivocal results that defined the role of PECAM-1 in pathogenesis of inflammatory process during CHC had not been obtained. Some authors [Katz *et al.* 2004; Xu *et al.* 2003; Neubauer *et al.* 2000; Garcia-Monzon *et al.* 1996] observed expression of PECAM-1 in mononuclears and SEC from healthy human or rat livers. The marked differences in cell adhesion molecules expression on EC in the liver appeared to be due to the differential structure of the liver vasculature [Volpes *et al.* 1992].

Significantly higher sPECAM-1 concentrations in patients with more advanced hepatitis alongside positive association between sPECAM-1 levels and grade of inflammatory activity suggests that PECAM-1 may reflect disease progression and its expression can be induced by an active inflammatory process. Correlation between sPECAM-1 and aminotransferase activity – a suitable marker of inflammatory process – also supports this suggestion. Moreover, the lowest concentrations in patients with minimal inflammatory activity were still significantly higher than in healthy subjects, which indicates that sPECAM-1 also points to the ongoing inflammatory process. Unfortunately, ROC curve analysis showed the uselessness of sPECAM-1 and distinguish various grades of inflammation (data not shown).

Liver fibrosis follows hepatic inflammation and results from hepatocyte injury [Gressner and Weiskirchen, 2006]. Interactions between hepatic stellate cell (HSC) membrane receptors and the extracellular matrix proteins is regulated by adhesion molecules [Friedmann, 2003; Rachwal and Brigstock, 2003]. These interactions may influence on many signal pathways in cells important for collagen synthesis and metalloproteinases activation, leading to the increase of synthesis

and/or the decrease of degradation of liver connective tissue. It is thought that VCAM-1 can play a certain role in these processes [Friedmann, 2003]. Consistent with Granot *et al.* [2001], in our investigation no association between fibrosis stage and sVCAM-1 concentration was observed, although slightly higher levels were found in patients with bridging fibrosis. In the above-mentioned study, the highest sVCAM-1 levels occurred in patients with liver cirrhosis. On the other hand, a positive relationship between sVCAM-1 concentration and fibrosis stage was found by Kapllanski *et al.* [1997] and Lo Iacono *et al.* [1998] with significantly higher sVCAM-1 values in case of bridging fibrosis/cirrhosis compared with portal/periportal fibrosis. This association may result from VCAM-1 synthesis by activated endothelial [Steinhoff *et al.* 1993] and dendritic cells [Garcia-Monzon *et al.* 1996]. Additionally, Lo Iacono showed correlation between sVCAM-1 and procollagen type-III amino-terminal propeptide, the biochemical marker of fibrosis progression [Lo Iacono *et al.* 1998]. Bruno *et al.* [2005] found elevated sVCAM-1 concentration only in cirrhotic patients. Moreover, sVCAM-1 increased significantly in patients with Child-Pugh C liver cirrhosis when compared with class B. A notable fact also was that the lowest sVCAM-1 concentration in patients with liver cirrhosis was above the highest one for those with low fibrosis progression. It can be assumed that serum sVCAM-1 levels increase is rather associated with stage of liver fibrosis than liver inflammation. The above observations may also suggest that sVCAM-1 levels markedly increase when fibrosis has already become irreversible. Unfortunately, the study by Bruno *et al.* [2005] did not include patients with periportal/bridging fibrosis, whereas all studies except for ours included cirrhotic patients. Thus, the complete interpretation and comparison with our results is difficult and the diversity in results may be due to selection of patients.

The evaluation of usefulness of sVCAM-1 determination, with respect to differentiation of fibrosis stage by the assessment of area under ROC curve for sVCAM-1 concentrations in our study, gave negative results. Contrary to our findings analysis of area under ROC curve by Lo Iacono *et al.* [1998] demonstrated usefulness of sVCAM-1 determinations to differentiate septal fibrosis/cirrhosis from lower fibrosis stages, with the cut-off point set at, 1280 ng/ml.

There is very little data available with respect to the relationship between sPECAM-1 and liver fibrosis in CHC. Weak immunoreactivity for PECAM-1 observed on SEC and endothelial cells of vessels in portal tracts in normal liver increases significantly in the case of cirrhosis [Shah *et al.* 1999]. SEC isolated from fibrotic rat livers demonstrated increased expression of PECAM-1 on cell borders and in cytoplasm [DeLeve *et al.* 2004]. The expression was increased during neoangiogenesis in portal tracts [Garcia-Monzon *et al.* 1996] the phenomenon, which is markedly intensified in CHC [Kapllanski *et al.* 1997] and contributes to fibrosis progression [Garcia-Monzon *et al.* 1996; Bhunchet and Fujieda, 1995]. The pattern of PECAM-1 on endothelial cells was always similar in enlarged portal tracts acquiring a characteristic form of capillary tube formation [Garcia-Monzon *et al.* 1996]. PECAM-1 expression on endothelial cell–cell borders seems to play a fundamental role in leukocyte migration [Scalia and Lefer, 1998]. The above-mentioned changes suggest a difference in leukocyte migration between normal and fibrotic liver [DeLeve *et al.* 2004; Katz *et al.* 2004]. Our results showed the highest sPECAM-1 concentrations in patients with the most advanced fibrosis stage. Moreover, the lowest concentrations in patients with portal fibrosis were significantly increased in comparison with patients without fibrosis. These observations corresponded to data by DeLeve *et al.* [2004], who revealed significant PECAM-1 expression increase on cell–cell borders and in cytoplasm of SEC isolated from rat livers with advanced fibrosis. Unfortunately, on the basis of our study, it was difficult to unequivocally assess the association between fibrosis stage and sPECAM-1 concentration due to the lack of cirrhotic patients. Nevertheless, more distinct variation in sPECAM-1 levels compared with sVCAM-1 may result from changes in expression pattern of PECAM-1 not only in leukocytes but also in endothelial cells of constituent and new-formed vessels.

Our study showed sPECAM-1 concentration was positively associated with fibrosis stage. ROC curve analysis confirmed the feasibility of sPECAM-1 serum determinations in CHC to differentiate patients with respective, histopathologically confirmed fibrosis stages. The value 0.81 of area under the ROC curve for sPECAM-1 concentrations confirms the test's usefulness in differentiating patients with and without fibrosis. A sPECAM-1 concentration of 221.0 ng/ml should be taken as the cut-off point. Similarly, the value 0.78 of area under the ROC curve suggests feasibility of this parameter to differentiate patients with bridging fibrosis from lower stages, with the cut-off point set at 237.1 ng/ml. Antiviral therapy reduces extent of inflammatory process and therefore contributes to downregulation of PECAM-1 and VCAM-1 expression and subsequent significant decrease of sPECAM-1, and lesser degree of sVCAM-1, both in responders and nonresponders.

In our study sVCAM-1 concentration did not change significantly after therapy, which was in accordance with results of Lo Iacono *et al.* [1998] and Simpson *et al.* [1994]. Nevertheless, sVCAM-1 concentrations in both responders and nonresponders after therapy were slightly lower compared with initial values. Interestingly, Dejica *et al.* [2002] observed unchanged or even increased sVCAM-1 levels in some nonresponders after therapy. In contrast, Marui *et al.* [1996] showed that after antiviral monotherapy, serum sVCAM-1 concentrations in responders decreased significantly in comparison with nonresponders. However, the analyzed group was small and sVCAM-1 decreases were obvious only when initial levels were very high, which may suggest that results were ambiguous.

On admission, sVCAM-1 values did not differ between responders and nonresponders and these results correspond with data by Dejica *et al.* [2002] and Granot *et al.* [2001]. Similarly, sPECAM-1 concentrations did not differ between these groups. The results indicate that initial adhesion molecules measurement is useless in predicting therapy outcome. However, after antiviral therapy sVCAM-1 and sPECAM-1 levels, although insignificantly, seemed to be lower in responders. Nevertheless, the lack of significant difference between responders and nonresponders indicates that the persistence of viremia has no influence on PECAM-1 and VCAM-1 expression and therefore they are not a feasible indicator of virus persistence. With respect to sVCAM-1, the results are comparable to Dejica *et al.* [2002] and Granot *et al.* [2001]. Moreover, our study confirmed observations of Granot *et al.* [2001] which revealed the lack of association between sVCAM-1 concentrations and ALT activity during antiviral therapy.

It is thought that PECAM-1, as a signal transducer in cells, can be responsible for upregulation of integrins expression, their binding to other molecules and the formation of VLA-4/VCAM-1 complex [Engelhardt and Wolburg, 2004; Newman and Newman, 2003; RayChaudhury *et al.* 2001]. A positive correlation between both these molecules, observed in our research, additionally suggests that PECAM-1 may influence VCAM-1 expression.

Conclusion

Infection with HCV results in upregulation of sPECAM-1 and sVCAM-1. Our findings are consistent with the importance of PECAM-1 in CHC pathogenesis and suggest that its soluble form is related to necroinflammatory activity as assessed by biochemistry and histological score. Therefore, serum sPECAM-1 may be an additional feasible marker of cytonecrotic activity. Our analysis has shown that serum sPECAM-1 measurement was useful in distinguishing between patients with or without fibrosis, and enabled us to identify patients with advanced fibrosis as well. On the other hand, high sVCAM-1 levels may point to advanced and probably irreversible fibrosis. Antiviral therapy, by reducing the inflammatory activity, decreases markedly sPECAM-1 and to a lesser degree sVCAM-1 concentrations. However, measurement of these adhesion molecules does not allow us to predict therapy outcome and confirm HCV persistence. PECAM-1, as a signal transducer in cells, may influence VCAM-1 expression. Further studies on a greater number of patients are necessary to better determine the role of sPECAM-1 and sVCAM-1 in the pathogenesis, diagnosis and management of CHC.

Conflict of interest statement

None declared.

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