Original Article
Association of susceptibility to septic shock with platelet endothelial cell adhesion molecule-1 gene Leu125Val polymorphism and serum sPECAM-1 levels in sepsis patients

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Abstract: Sepsis is a systemic inflammatory response to infection and includes severe sepsis, septic shock and death. Platelet endothelial cell adhesion molecule-1 (PECAM-1) is one cell adhesion molecule expressed on platelets and leukocytes. It regulates platelet activation and mediates transendothelial migration of leukocytes, thus maintaining the integrity of the vasculature. There are some animal experiments associated with the protective role of PECAM-1 against septic shock. However few host genetic risk factors have been identified for sepsis severity and susceptibility to septic shock. A case-control study was conducted, which included 217 patients with sepsis and 90 control subjects recruited from our hospital. One single nucleotide polymorphisms (SNP) of PECAM-1 gene Leu125Val (C373G) was analyzed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. Serum soluble PECAM-1 (sPECAM-1) levels were determined by enzyme-linked immunosorbent assay (ELISA). Our results showed that the CG and GG genotypes of SNP in Leu125Val of PECAM-1 (rs668: C>G) was significantly associated with increased susceptibility to septic shock compared with CC genotype in sepsis patients (CG genotype, OR: 2.493, 95% CI: 1.175~5.287, P = 0.016; GG genotype: OR: 3.328, 95% CI: 1.445~7.666, P = 0.004). The serum levels of sPECAM-1 in the sepsis patients (47.1 ± 20.9 ng/ml) were significantly higher than those in the healthy controls (61.3 ± 20.9 ng/ml, P<0.01). Among sepsis patients, the serum levels of sPECAM-1 were significantly higher in CG and GG genotype than in CC genotype. In septic shock patients, nonsurvivors (83.7 ± 12.6 ng/ml, n = 69) had a significantly higher serum sPECAM-1 level than the survivors (76.9 ± 12.7 ng/ml, n = 53) (P<0.01). In conclusion, PECAM-1 Leu125Val polymorphism and its sPECAM-1 levels are associated with sepsis severity and susceptibility to septic shock.

Keywords: Sepsis, septic shock, platelet endothelial cell adhesion molecule-1 (PECAM-1), single nucleotide polymorphisms (SNPs)

Introduction
Sepsis is a systemic inflammatory response to infection and constitutes the leading cause of death in intensive care units (ICUs). Septic shock is severe form of sepsis with refractory hypotension and demonstrates high mortality rate caused by multiple organ dysfunction syndrome (MODS) [1]. A variety of studies have confirmed that individuals demonstrate different susceptibility to sepsis or septic shock. Genetic polymorphisms, such as those in E-selectin, apolipoprotein E, C-reactive protein, interleukin-10 and CD14 alleles, have been reported to determine the risk and outcome of sepsis [2-4]. In sepsis endothelium is activated and subsequent endothelial dysfunction lead to inflammation response, impaired microvascular barrier integrity, tissue edema, shock and MODS, thereby contributing to the morbidity and mortality of sepsis [5]. In septic shock the endothelial barrier function is mostly impaired, which might contribute to its adverse outcomes [6]. Therefore, endothelial barrier repair is a promising treatment strategy for sepsis. Furthermore, molecules that could regulate endothelial integrity may participate in the pathophysiology of sepsis and their genetic polymorphisms may influence the risk and outcome of sepsis.
Platelet endothelial cell adhesion molecule-1 (PECAM-1, CD31) is a 130-kDa cell adhesion molecule that is expressed on platelets and leukocytes and is highly enriched in endothelial cell intercellular junctions [7]. As a signaling adhesion molecule, PECAM-1 shows diverse roles in vascular biology, including platelet activation, thrombosis, angiogenesis, endothelial cell response to shear stress, and transendothelial migration of leukocytes [8]. Recently, there is growing evidence that PECAM-1 functions as one regulator of endothelial junctional integrity [9]. PECAM-1-overexpressing endothelial cells exhibit increased steady-state barrier function and more rapid restoration of barrier integrity induced by thrombin perturbation [10]. Furthermore, PECAM-1 deficient mice showed reduced survival in endotoxic shock induced by LPS, which was associated with enhanced vascular permeability [11]. The increased LPS-induced mortality in PECAM-1 deficient mice was caused by reduced expression of PECAM-1 at endothelial cell-cell junctions [12]. The SNP of PECAM-1 gene have been reported to be correlated with atherosclerosis and myocardial infarction [13, 14]. However, currently it remains unclear about the association between PECAM-1 gene SNP and sepsis.

In this study, we investigated the relationship between SNP in PECAM-1 gene and the serum concentration of sPECAM-1 in sepsis patients. We evaluated whether the SNP and serum sPECAM-1 influence the severity and outcome of sepsis.

Materials and methods

Subjects

A total of 217 patients with sepsis were included in this study between January 2013 and December 2014 in our hospital. All patients were admitted to intensive care unit (ICU) and treated following sepsis management protocol. The average age of all patients were 58.2 ± 14.5 (male: female (M: F) = 131:86). All 217 patients were divided into two groups: the severe sepsis group (mean age 59.6 ± 14.5 years; M: F = 56:39) and the septic shock group (mean age 57.1 ± 14.5 years; M: F = 75:47). The severe sepsis or septic shock were diagnosed based on the criteria proposed at the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference in 1992 [15]. Patients were followed until death or hospital discharge. A total of 90 healthy blood donors were recruited as control subjects with matched age and sex ratios. Informed written consent was obtained from all subjects or patients’ surrogates. This study was approved by the Research Ethics Committee of our hospital.

SIRS (systemic inflammatory response syndrome) was defined as the presence of at least two symptoms in the following: (1) fever or hypothermia (core temperature >38°C or <36°C); (2) tachycardia (>90 beats/min); (3) tachypnea or hyperventilation (breaths/min >20 or PaCO₂ <32 mmHg); (4) leukocytosis (WBC >12,000 mm³) or leucopenia (WBC <4,000 mm³). Severe sepsis was defined as SIRS and organ dysfunction secondary to infection, and septic shock was defined as severe sepsis complicated with refractory arterial hypotension which need fluid replacement and vasopressors.

APACHE II (Acute Physiology and Chronic Health Evaluation II) score was used to evaluation illness severity [16]. SOFA (Sequential Organ Failure Assessment) score was used to evaluate organ dysfunction [17]. The clinical data, including demographic details and age were collected from each subject. The APACHE II score and SOFA score were obtained on ICU admission day of severe sepsis or septic shock patients. Length of ICU stay was recorded for severe sepsis or septic shock patients. Mortality was defined as death of patients during total hospital stay, and correspondingly divided patients into survivors and nonsurvivors. Within 24 hours of the onset of severe sepsis or septic shock, blood samples were collected for further evaluations on PECAM-1 polymorphism and serum sPECAM-1 levels.

Determination of PECAM-1 genotype

Peripheral venous blood was obtained from severe sepsis patients, septic shock patients and healthy controls. Genomic DNA was extracted from leucocytes by a DNA extraction kit (Qiagen, Crawley, UK), according to the manufacturer’s protocols, and was stored at -70°C until use. The genotypes of Leu125Val were determined by polymerase chain reaction-restriction fragment length polymorphism method (PCR-RFLP). The PCR primers were designed based on the GenBank reference
Increased susceptibility to septic shock with SNP of PECAM-1

Table 1. Baseline characteristics of the patients at day one of severe sepsis or septic shock

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Severe sepsis (n = 95)</th>
<th>Septic shock (n = 122)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>59.6 ± 14.5</td>
<td>57.1 ± 14.5</td>
<td>0.218</td>
</tr>
<tr>
<td>Gender (Male %)</td>
<td>58.9</td>
<td>61.5</td>
<td>0.588</td>
</tr>
<tr>
<td>APACHE II score</td>
<td>19.3 ± 1.9</td>
<td>26.6 ± 2.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SOFA score</td>
<td>8.9 ± 1.1</td>
<td>12.5 ± 1.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Length of ICU stay</td>
<td>4.2 ± 1.2</td>
<td>9.9 ± 2.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mortality</td>
<td>32.6</td>
<td>56.6</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Note: Data are expressed as the mean ± standard deviation (SD). APACHE II = Acute Physiology, Age, and Chronic Health Evaluation II; NS = not significant; SOFA = Sequential Organ; ICU = intensive care unit.

sequence (accession no. NC-000017) and primer sequences were as follows. Leu125Val: sense: 5'-GCTCCATCTGCTTGCCTGT-3'; antisense: 5'-TGTCAGCACCACCTCTACG-3'. The cycling conditions for PCR were as follows: after initiation at 95°C for 5 min, 30 cycles were performed by denaturation at 95°C for 30 sec, annealing at 60°C for 5 sec and extension at 72°C for 10 sec, with a final extension step at 72°C for 7 min. After overnight incubation with restriction endonuclease PvuII, the amplified PCR products were separated on 8% polyacrylamide gel electrophoresis. PvuII digestion produced 245 bp fragment in Leu125Leu (C373C) homozygous genotype, 52+193+245 bp fragment in Leu125Val (C373G) heterozygous genotype, and 52+193 bp fragment in Val125Val (G373G) homozygous genotype.

Determination of sPECAM-1 level

Venous blood collected from severe sepsis and septic shock patients within 24 hours after disease onset, or from healthy controls. Serum was acquired by centrifugation was performed at 1000 g for 10 min, and was stored in aliquots at -70°C until use. The serum sPECAM-1 were measured by Human sPECAM-1 enzyme-linked immunosorbent assay kit (ELISA) (Bender MedSystems, Vienna, Austria), according to the manufacturer’s protocol. The developed color reaction was determined at OD450 wavelength by an ELISA plate reader (Ricso RK201, Shenzhen Ricso Technology Co., Ltd, Shenzhen, Guangdong, China). The serum concentrations of sPECAM-1 was determined by standard curve which was constructed with the kit’s standards (Range: 0~1000 ng/ml).

Statistical analysis

All quantitative data were expressed in mean ± standard deviation (SD). The statistical analysis was performed by the commercially available software SPSS 19.0 (SPSS Inc., Chicago, IL, USA). The comparisons between two groups were determined by an independent t-test. Chi-squared test or a Fisher’s exact test was applied to compare categorical data. The consistency of genotype frequencies among patients and controls was checked by Hardy-Weinberg equilibrium separately. The differences in the genotype frequencies were compared using Chi-squared test between controls and sepsis, and between severe sepsis and septic shock patients. The odds ratios (ORs) and 95% confidence intervals (95% CIs) were determined by unconditional logistic regression models. A probability value of P<0.05 was considered as statistically significant difference.

Results

Demographics of the subjects

The clinical characteristics of the patients at the time of ICU admission are shown in Table 1. There are no statistically significant differences in age and gender between severe sepsis group and septic shock group (P>0.05), which indicates the two groups were matched in age and gender. The septic shock group showed higher APACHE-II score, higher SOFA score and increased length of ICU stay compared with the severe sepsis group (P<0.001). The overall mortality rate at 28 days was 40.1% among all 217 sepsis patients. The septic shock group had higher mortality rate compared with the severe sepsis group (56.6% vs. 32.6%; P<0.01).

Association of the PECAM-1 gene polymorphisms with susceptibility to septic shock

We performed PCR-RFLP assay to determine the genotype at Leu125Val of PECAM-1 gene. The restriction endonuclease PvuII cleaved sequence in 373G allele but not in 373C allele, therefore produced one DNA fragment (245 bp) in 373CC homozygous subjects, three DNA fragments (52, 193 and 245 bp) in 373CG heterozygous genotype, and two DNA fragments (52 and 193 bp) in 373GG homozygous subjects (Figure 1). The 52 bp fragment was too small to
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be visible in the electrophoresis gel. In Leu125Val of PECAM-1, the frequencies of the 373CC, 373CG and 373GG genotypes were 23.3%, 55.6%, and 21.1% in healthy controls, and were 18.0%, 53.0%, and 29.0% in all sepsis patients, respectively. There was no significant difference in the genotype frequencies between controls and sepsis patients (Table 2). The frequencies of the 373CC, 373CG and 373GG genotypes were 26.3%, 50.5%, 23.2% in severe sepsis group, and were 11.5%, 54.9%, 33.6% in septic shock group, respectively (Table 3). Compared with the 373CC genotype, patients who were heterozygous (373CG) or homozygous (373GG) for the Leu125Val polymorphism were more likely to have septic shock in all sepsis patients (373CG, OR: 2.493, 95% CI: 1.175–5.287, P = 0.016; 373GG, OR: 3.328, 95% CI: 1.445–7.666, P = 0.004). The general genotype and allele frequencies did not differ from the values expected by the Hardy-Weinberg model among the controls, severe sepsis and septic shock groups (Table 4).

Association of serum sPECAM-1 levels with PECAM-1 gene Leu125Val genotypes

To investigate the association between serum sPECAM-1 levels and Leu125Val genotype, we performed ELISA assay to measure serum sPECAM-1 levels in three genotypes of controls, severe sepsis group and septic shock group. The serum sPECAM-1 levels were closely associated with various PECAM-1 genotypes. In controls, severe sepsis group and septic shock group, the serum sPECAM-1 levels was significantly higher in subjects with heterozygous 373CG genotype (Controls: 69.5 ± 12.0 ng/ml; severe sepsis: 70.2 ± 11.0 ng/ml, 81.1 ± 12.2 ng/ml) or homozygous 373GG genotype (Controls: 72.3 ± 11.3 ng/ml; severe sepsis: 74.5 ± 11.2 ng/ml, 85.4 ± 12.1 ng/ml) than subjects with homozygous 373CC genotype (Controls: 59.1 ± 8.6 ng/ml; severe sepsis: 63.3 ± 9.4 ng/ml, 65.4 ± 6.9 ng/ml. P< 0.01, respectively). However, there were no significant differences in serum sPECAM-1 levels between 373CG subjects and 373GG subjects in controls, severe sepsis group and septic shock group, respectively (Figure 2).

Associations of serum sPECAM-1 levels with outcome

The serum sPECAM-1 level was significantly higher in the septic shock group (80.8 ± 13.0 ng/ml, n = 122) than the severe sepsis (69.4 ± 11.3 ng/ml, n = 95) or control groups (67.3 ± 12.1 ng/ml, n = 90) (P<0.001) (Figure 3). However, there was no significant difference in the serum sPECAM-1 level between the severe sepsis and control groups. We further divided severe sepsis and septic shock patients into survivors and nonsurvivors respectively. Our results showed that nonsurvivors in septic shock patients (87.4 ± 10.4 ng/ml, n = 69) had a significantly higher serum sPECAM-1 level than the survivors (72.1 ± 10.9 ng/ml, n = 53) (P<0.01) (Figure 4). There was no significant difference in serum sPECAM-1 level between survivors and nonsurvivors in severe sepsis patients (P>0.05).
In this study, we show that the SNP in Leu125Val of PECAM-1 may be associated with increased susceptibility to septic shock in sepsis patients. The serum levels of sPECAM-1 in sepsis patients were significantly higher in CG and GG genotype than in CC genotype. In septic shock patients, nonsurvivors had a significantly higher serum sPECAM-1 level than the survivors. However, we did not observe associations of SNP in Leu125Val of PECAM-1 with the risk of sepsis. Our data suggest that PECAM-1 may play protective roles in the development of septic shock, and Leu125Val polymorphism of PECAM-1 gene may serve as a novel genetic marker for susceptibility to septic shock in sepsis patients.

The PECAM-1 gene is located on human chromosome 17q23, including 16 exons. Currently, a number of SNP have been identified in PECAM-1 gene [18], among which 3 SNP, including Leu125Val (C373G), Asn563Ser (T1688C) and Gly670Arg (C2008T), have been reported to be associated with some diseases [19]. Leu125Val polymorphism is located in the coding region (exon 3), causing a mutation of leucine to valine. The Leu125Val polymorphism in PECAM-1 gene has been reported to be associated with coronary artery disease [20, 21], ischemic stroke [22], atherosclerotic cerebral infarction [23], bronchial asthma [24] and Deep vein thrombosis [25]. However, though PECAM-1 deficient mice showed higher susceptibility to septic shock and higher mortality [11, 12], no studies has investigated the association between PECAM-1 gene polymorphisms and septic shock in sepsis patients.

In the present study, the genotypes of Leu125Val were not associated with the risk of sepsis. However, compared with 373GG (125 Leu/Leu) homozygous genotype, 373GC (125 Leu/Val) heterozygous genotype and 373GG (125 Val/Val) homozygous genotype for the PECAM-1 were significantly associated with the progression from

| Table 2. The genotype frequencies for Leu125Val in PECAM-1 between controls and sepsis patients |
| Genotype | Controls (n = 90) | Sepsis (n = 217) | OR (95% CI) | P value |
| Leu/Leu | 21 (23.3) | 39 (18.0) | 1 |
| Leu/Val | 50 (55.6) | 115 (53.0) | 1.238 (0.662, 2.316) | 0.503 |
| Val/Val | 19 (21.1) | 63 (29.0) | 1.785 (0.854, 3.735) | 0.122 |

Note: OR = odds ratio; CI = confidence interval.

| Table 3. The genotype frequencies for Leu125Val in PECAM-1 between severe sepsis and septic shock patients |
| Genotype | Severe sepsis (n = 95) | Septic shock (n = 122) | OR (95% CI) | P value |
| Leu/Leu | 25 (26.3) | 14 (11.5) | 1 |
| Leu/Val | 48 (50.5) | 67 (54.9) | 2.493 (1.175, 5.287) | 0.016 |
| Val/Val | 22 (23.2) | 41 (33.6) | 3.328 (1.445, 7.666) | 0.004 |

Note: Compared with the Leu/Leu genotype, the Leu/Val and Val/Val genotypes were significantly correlated with an increased risk of septic shock. OR = odds ratio; CI = confidence interval.

| Table 4. Hardy-Weinberg equilibrium of PECAM-1 genotype for the study population (healthy controls and septic patients) |
| Groups | Genotype | Observed value | Predicted value | χ² | P value |
| Controls | Leu/Leu | 21 (23.3) | 23.5 (26.1) | 1.12 | 0.289 |
| | Leu/Val | 50 (55.6) | 45.0 (50.0) | | |
| | Val/Val | 19 (21.1) | 21.5 (23.9) | | |
| Sepsis patients | Leu/Leu | 39 (18.0) | 42.9 (19.8) | 1.16 | 0.282 |
| | Leu/Val | 115 (53.0) | 107.2 (49.0) | | |
| | Val/Val | 63 (29.0) | 66.9 (30.8) | | |
| Severe sepsis | Leu/Leu | 25 (26.3) | 25.3 (26.6) | 0.01 | 0.910 |
| | Leu/Val | 48 (50.5) | 47.4 (49.9) | | |
| | Val/Val | 22 (23.2) | 22.3 (23.5) | | |
| Septic shock | Leu/Leu | 14 (11.5) | 18.5 (15.2) | 2.93 | 0.087 |
| | Leu/Val | 67 (54.9) | 58.0 (47.5) | | |
| | Val/Val | 41 (33.6) | 45.5 (37.3) | | |

Note: To test the Hardy-Weinberg equilibrium for consistency, the genotype frequencies of Leu125Val were compared with those of expected values among healthy controls cases (n = 90), sepsis patients (n = 27), severe sepsis (n = 95) and septic shock (n = 122) separately.
Increased susceptibility to septic shock with SNP of PECAM-1

We then investigated the relationship between serum sPECAM-1 level and SNP in Leu125Val. Serum sPECAM-1 levels in 373CG subjects and 373GG subjects were significantly higher than those in 373CC subjects among healthy controls, severe sepsis and septic shock, respectively. This indicates that serum sPECAM-1 is influenced by genotypes in Leu125Val and G
Increased susceptibility to septic shock with SNP of PECAM-1

Figure 4. Comparison of serum sPECAM-1 levels between survivors and nonsurvivors in severe sepsis and septic shock groups. The nonsurvivors had a higher serum sPECAM-1 level than the survivors in the septic shock group. Data are expressed as the mean and standard deviation (SD). Significant difference from the survivors group is denoted by “*” (P<0.001).

allele can increase the serum sPECAM-1 levels universally in healthy and sepsis subjects. The association between higher circulating sPECAM-1 levels and G allele in Leu125Val has been reported in coronary artery disease [20], ischemic stroke [22], atherosclerotic cerebral infarction [23] and Deep vein thrombosis [25]. sPECAM-1 can be cleaved at the cell surface of PECAM-1 protein [31] and show competitive inhibition on the function of membrane-bound PECAM-1 [32]. Leu125Val is located in the first loop of the extracellular domain of PECAM-1 protein, and leucine to valine mutation may facilitate the cleavage of sPECAM-1 from cell surface, thereby increasing the serum sPECAM-1 level and decreasing the endothelium barrier function of PECAM-1. It deserves further study whether increased susceptibility to septic shock in subjects with G allele is caused by competitive inhibition of the membrane-bound PECAM-1 by serum sPECAM-1, or caused by reduced function of PECAM-1 protein with mutated extracellular domain. Our results also showed that there were no significant differences in serum sPECAM-1 levels between 373CG subjects and 373GG subjects. This means one mutated Val is enough to decrease the function of PECAM-1. Indeed, homophilic interactions of PECAM-1 proteins are essential for maintaining integrity of endothelial cell junctions [10]. Whether dominant negative inhibi-

tion effect is involved in 373CG heterozygous subjects in septic shock remains further investigation.

Our results showed a higher serum sPECAM-1 level in the septic shock group than in the severe sepsis group and control group, with no significant difference between severe sepsis group and control group. This further confirmed sPECAM-1 is a serum marker for sepsis severity and susceptibility to septic shock, rather than sepsis risk. Furthermore, we also found that nonsurvivors had a significantly higher serum sPECAM-1 level than the survivors in septic shock patients, but not in severe sepsis patients. Therefore, serum sPECAM-1 may act as one prognostic factor on ICU admission day of sepsis patients that elevated serum sPECAM-1 can predict poor survival for septic shock patients.

In conclusion, we found the subjects with 373G allele of PECAM-1 gene have an increased susceptibility to septic shock and increased serum sPECAM-1 levels. Septic shock patients show higher serum sPECAM-1 levels than severe sepsis patients and healthy controls. In septic shock patients, higher serum sPECAM-1 levels are associated with poor survival. Our study will provide Leu125Val SNP of PECAM-1 gene and serum sPECAM-1 as a prognostic marker to predict septic shock susceptibility and mortality.

Disclosure of conflict of interest

None.

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References


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