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PRESEPSIN LEVELS OF PATIENTS WITH CRIMEAN-CONGO HEMORRHAGIC FEVER

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Summary

The presepsin level (a soluble CD14 subtype) is thought to increase in cases of bacterial infection. It has been stated that CD 14 also plays a role in the pathogenesis of various viral diseases. Crimean-Congo Hemorrhagic Fever (CCHF) is an arboviral zoonotic infection. Our study focuses on the presepsin level as a biomarker for CCHF. Serum presepsin levels in CCHF group (n=59) and control (n=28) have been compared. Patients with CCHF have been classified as mild, moderate and severe groups (group 1, 2 and 3 respectively) in terms of the Severity Grading Score (SGS). Presepsin levels were measured in serum samples using a commercial ELISA. The mean presepsin levels were found to be significantly different in the CCHF and healthy group (1499.46±411.96 pg/ml and 430.68±61.21 pg/ml, respectively). The mean presepsin levels were found to be significantly different in the CCHF groups (1, 2, 3) and healthy group (1204.53±371.18, 1464.21±338.37, 2007.36±82.18 and 430.68±61.21 pg/ml, respectively) (p<0,05). We have also found out that as the severity of the disease
increases, the presepsin value increases. We are of the opinion that presepsin values could be used as a supportive biomarker for diagnosis and follow-up of the disease.

**Introduction**

CCHF is a tick-borne human disease caused by the negative-strand RNA virus of the Bunyaviridae family, Nairovirus genus (1). Crimean-Congo Hemorrhagic Fever (CCHF) is a zoonotic acute viral disease causing high mortality and seen in many countries in Africa, Asia, Europe and the Middle East (2). The mortality rate among adults is between 3-30% in countries where the disease has been observed (3, 4).

The limited information available about the pathogenesis of the disease has been obtained by analyzing the changes in the liver biopsies and blood tests (5). The most common pathological findings are increase in capillary fragility, endothelial damage, platelet aggregation and degranulation and similar haemostatic disorders.

The development of bleeding, which is an important predictive clinical symptom of disease progression, is not necessarily a result of the interaction between virus and cells, but can also occur as a result of the effect of proinflammatory cytokines released in response to infection (2, 6). It is stated that, natural immunity, TNF alpha, type I interferon, IL 1,6,10 and similar cytokines and Toll-like receptors (TLRs) play an important role in disease pathogenesis (7-9).

Cluster of differentiation 14 (CD14) is a receptor with glycoprotein structure that is expressed on the surface of monocyte and macrophages. CD14 activates TLR4 causing release of some proinflammatory cytokines and the initiation of the antiimmune response to microorganisms. In the course of inflammation, the soluble form of CD14 (sCD14) is degraded by plasma proteases and fragments called presepsin are formed (10). An increase in the levels of presepsin have been observed due to gram negative microorganisms, particularly in septic patients (11). In recent studies, it has been indicated that CD14 plays a role in the pathogenesis of various viral diseases. However, no study has been found on the presepsin levels in CCHF patients.

**Material and methods**
Study population

This prospective study was carried out in Cumhuriyet University Hospital. The protocol was approved by the Cumhuriyet University Ethical Committee. The study included 59 (64.41% male) CCHF patients and 28 (60.71% male) healthy controls. Diagnosis was made using clinic and laboratory findings. Only those patients whose CCHF diagnosis were confirmed at the national reference Virology laboratory of Refik Saydam Hygiene Center in Ankara, Turkey, were enrolled in the study. The demographic and clinical data of patients, such as age, sex, occupation, city of residence, history of tick bite or of tick removal, smoking, and their outcome were obtained from the hospital information system. The most frequently observed clinical symptoms were fever, which was found in 42 (71.19%) of patients. Other clinical findings were fatigue, myalgia, headache, tonsillopharyngitis, nausea, vomiting, somnolence and agitation. 35 (59.3%) patients were found to have hemorrhage had petechial-purpura and ecchymosis, 11 patients (18.6%) had mucosal hemorrhage and 2 (3.4%) patients had bleeding in their body cavity. 1 (1.7%) patient had hepatomegaly and 1 (1.7%) had splenomegaly. No patients showed organ failure and mortality.

Patients with autoinflammatory disease, chronic diseases such as chronic renal failure and liver disease, malignancy and pregnant patients were excluded. Controls and patients were similar in terms of age and gender (p>0.05). Control subjects were randomly recruited from a group of healthy volunteers who had been admitted to the hospital for routine checks. Basic Laboratory tests (routine biochemistry analyses, complete blood count and coagulation tests) and physical examination findings of controls showed no pathological characteristics. The exclusion criteria in the study included clinical suspicion of infections (body temperature outside the range of 36-38 °C, heart rate > 90 rate/minute, respiratory rate > 20/minute, white blood count > 12.000/mm³ or < 4000 mm³), presence of liver disease, kidney disease, malignancy, pregnancy and smoking for healthy controls.

Laboratory analysis

All measurements were performed in venos blood specimens collected from patients and controls. The blood samples of the patients were taken on the first morning of admission. An empty
A tube with gel was used for presepsin measurement and routine biochemistry analysis, a citrated tube was used for coagulation test analysis, and a tube containing K$_2$EDTA was used for complete blood count (all tubes are product of Becton Dickinson, Oxon UK). Plasma and serum specimens were obtained after centrifugation of the blood samples. Routine biochemistry analyses, complete blood counts and coagulation tests were immediately carried out in specimens. Serum samples for presepsin analysis were aliquoted, frozen and kept at −20 °C until they were tested.

The Presepsin levels were determined in serum samples by enzyme-linked immunosorbent assay (Abbexa Ltd, Cambridge Science Park, Cambridge, UK) on a Triturus Analyser (Diagnostics Grifols, Spain). The measurement range of the assay was 65 -3000 pg/ml.

The Aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), blood urea nitrogen (BUN) and creatinine levels were determined in serum samples by spectrophotometricaly on an AU5800 auto analyzer (Beckman Coulter, USA).

The prothrombin time (PT), activated partial thromboplastin time (aPTT), international normalized ratio (INR) values were determined in plasma samples by means of clotting time assay on a ACL TOP 300 CTS analyzer (Instrumentation Laboratory, USA). The D-dimer values were determined in plasma samples by turbidimetric assay on a ACL TOP 300 CTS analyzer (Instrumentation Laboratory, USA).

The leukocyte (WBC), thrombocyte (PLT), hemoglobin (Hb) and monocyte counts were determined in whole blood samples on a hematology auto analyzer (Mindray BC 6800, China).

**Statistical analysis**

All analyses were conducted using SPSS for Windows 22.00. Continuous variables were expressed as mean ± standard deviation (S.D). The Kolmogorov–Smirnov test was used to check the normality of distribution. In intergroup comparisons, the Student’s t, One-Way Anova and Tukey test, which are parametric tests, and the Man-Whitney-U and chi-square test, which are non-parametric tests, were used. The Pearson correlation analysis was used to determine correlation. A P-value < 0.05 was considered as significant.
Results

Basic characteristics of the study population and laboratory results are given in Table 1. The mean presepsin level was determined to be 1499.46 ± 411.96 (between 621 and 2109) pg/ml and 430.68 ± 61.21 (between 345 and 559) pg/ml in the patient and control groups, respectively. The presepsin level was found to be significantly different between the patients and control subjects (p< 0.05) (Table 1). Patients with CCHF were classified as mild (group 1), moderate (group 2) and severe group (group 3) in terms of the SGS (12); the serum presepsin levels were compared across these three groups and controls. A significant difference across the three patient groups and control subjects was found in terms of presepsin (p<0.001) (Table 2).

It was also found that there was a statistically significant positive correlation between the presepsin level and the AST, LDH, INR, PT, aPTT, D-dimer values (p = 0.003, r:0.38; p = 0.001, r:0.523; p = 0.01,r:0.317; p = 0.02, r: 0.3; p = 0.002, r:0.4; p = 0.001, r:0.5; respectively). In addition, a negative correlation was observed between the presepsin level and PLT values (p = 0.001, r: -0.571) in the acute phase (Figure 1). No correlation was observed between presepsin levels and the creatine and BUN value.

Discussion

In this study, we have found that the presepsin levels in CCHF patients were higher compared to the healthy control group. In addition, when CCHF patients were grouped in terms of disease severity, a significant inter-group difference was found with respect to presepsin levels (12). These results suggest that the evaluation of presepsin levels in patients with CCHF should be taken into consideration.

CD14 is a myeloid cell receptor that binds to bacterial lipopolysaccharide, ensures intracellular transfer of endotoxin and thus stimulates the inflammatory response (13). The soluble form of CD 14 is contained in the blood and its production is thought to increase in case of infection. In a healthy population, the serum concentration of sCD14 is at microgram level (10). sCD14 is pointed out as an indicator of macrophage and monocyte activation. Presepsin is an indirect sepsis marker that forms
with degradation of sCD14 (11,14). Elevated levels of presepsin were determined in various infectious diseases such as AIDS (15), meningitis (16), hepatitis (17), sepsis (11), periodontitis (18) and malaria (19).

In viral diseases with hemorrhagic fever, including CCHF, the virus proliferates in the regional lymphatic glands and local tissues after entering the body and spreads to other organs, in particular the spleen and liver, by means of monocytes (20). The main inflammatory cells, which participate in these diseases, are the monocytes and neutrophils. A systematic inflammatory response occurs by the interaction of macrophages and endothelial cells in particular (8, 21, 22). It was stated that, cytokines such as TNF-alpha and interferon gamma, which are released from activated lymphocytes could lead to co-activation in the macrophages in the course of the disease (1, 5, 23-25). With respect to the current study, higher levels of presepsin observed in CCHF patients compared to healthy controls is likely to be associated with macrophage and monocyte hyper activation which develop in disease pathogenesis. According to the results of some studies on viral meningitis and hepatitis, elevated levels of sCD14 were stated to be likely associated with macrophage activation (16, 26).

Although the mechanism of presepsin release is not fully understood, one stimulus that increases its level is the process of phagocytosis (1). In a study on human cell culture, phagocytosis is stated to stimulate presepsin release from monocytes (27). According to a study by Karti et. al., reactive hemophagocytosis can develop in the course of CCHF (28).

The clinical severity of CCHF can change from mild to the manifestation of disseminated intravascular coagulation (DIC). It is stated that, in order to be able to make a judgement about the severity of the disease, the factors such as old age, elevated AST, ALT and LDH levels, increased WBC count, and the presence of bleeding and organ failure should be evaluated altogether (12). In our study, AST, ALT and LDH, WBC levels were found higher compared to the control group. Moreover, a positive correlation was found between presepsin levels and AST, LDH, INR, PT, aPTT, D-dimer values whereas a negative correlation was found between presepsin levels and platelet values. We found that if the cases in the acute period of the disease are grouped based on the severity index, presepsin level is observed to increase significantly as the severity score increases. When evaluating
the patient in the acute period, evaluating presepsin levels prior to obtaining all other laboratory results can help save time in the clinical approach to disease.

There is no available study investigating the usefulness of presepsin level in assessing the patient with CCHF. When the laboratory findings of CCHF patients were analyzed, it was determined that there were no observed change in the levels of trombosit, WBC, ALT, AST in some cases (29). Thus, in addition to routine laboratory tests used for the diagnosis and follow-up of CCHF patients, we are of the thought that using presepsin as a biomarker for diagnosis and follow-up of the disease could be beneficial. On the other hand, in order to obtain certain information and to reveal the association between presepsin levels in CCHF patients, studies with a higher number of patients should be conducted.

Acknowledgements

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

References


**Figure 1 legend:** Scatter plot matrix with pearson correlation between presepsin and AST, LDH, PT, aPTT, INR, DDIMER and platelet values.
### Table 1. Baseline characteristics of study groups

<table>
<thead>
<tr>
<th>Study marker</th>
<th>CCHF (n=59)</th>
<th>Control (n=28)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presepsin (pg/ml)</td>
<td>1499.46±411.96</td>
<td>430.68±61.21</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

**Baseline characteristics**

- **Age (years)**: 49.3±13.52 (between 22 and 71) vs 49.5±11.94 (between 27 and 71), >0.05
- Male/Female: 38/21 vs 17/11, >0.05
- Additional disease (hypertension): 7(%11.86) vs 17/11, >0.05
- The presence of tick exposure: 41(%69.5) vs 17/11, >0.05
- The presence of livestock exposure: 59(%100) vs 17/11, >0.05
- Duration of symptoms (days): 4.44±1.94 vs 4.44±1.94, >0.05

**Laboratory Analysis**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CCHF (IU/l)</th>
<th>Control (IU/l)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td>235.6±210.28</td>
<td>23.57±8.37</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>ALT</td>
<td>122.98±103.16</td>
<td>19.21±9.06</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>LDH</td>
<td>692.54±541.39</td>
<td>195.46±31.86</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>17.27±12.72</td>
<td>14.34±3.2</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>CREATININE (mg/dl)</td>
<td>0.89±0.41</td>
<td>0.82±0.15</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>14.13±1.62</td>
<td>14.05±1.06</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>PLT (×10^3 cells/ul)</td>
<td>766±7.97±45214.22</td>
<td>250500±43780.17</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>WBC (×10^3 cells/ul)</td>
<td>3890±51±2375.73</td>
<td>4354±64±676.59</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>MON (×10^3 cells/ul)</td>
<td>0.18±0.14</td>
<td>0.16±0.13</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>aPTT (s)</td>
<td>40.51±12.37</td>
<td>31.04±4.63</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>PT(s)</td>
<td>14.78±2.89</td>
<td>11.68±0.6</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>INR</td>
<td>1.15±0.30</td>
<td>1.03±0.08</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Results are n (%) and mean ± Standard deviation.

CCHF: Crimean-Congo hemorrhagic fever; ALT: alanine amino transferase; AST: aspartate aminotransferase; LDH: lactate dehydrogenase; BUN: Blood urea nitrogen; WBC: white blood cell; Hb: hemoglobin; PLT: platelet; MON: Monocyte Count; aPTT: activated partial thromboplastin time; PT: prothrombin time; INR: international normalized ratio.
Table 2. The presepsin level of CCHF patients and control group.

<table>
<thead>
<tr>
<th></th>
<th>GRUP 1 CCHF* (n=15)</th>
<th>GRUP 2 CCHF* (n=33)</th>
<th>GRUP 3 CCHF* (n=11)</th>
<th>Control (n=28)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presepsin level</td>
<td>1204.53±371.18</td>
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<td>&lt;0.001</td>
</tr>
<tr>
<td>(pg/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* According to SGS system developed by Bakır et.al., patients with CCHF are classified in mild, moderate and severe groups (group 1, 2 and 3 respectively)
Figure 1. Scatter plot matrix with pearson correlation between presepsin and AST, LDH, PT, aPTT, INR, DDIMER and platelet values.