Ratio of neutrophil to lymphocyte counts in Crimean Congo Hemorrhagic Fever

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Özet
Bakteriyel ve viral enfeksiyonlarda yeni belirteçlerin klinik uygulamada kullanımları onların maliyetleri sınırlandırılmaktadır. Bu çalışmada kırım kongo kanamalı ateş (KKKH) hastalığında nötrofil lenfosit oranının (NLO) değerlendirilmesi amaçlandı. NLO kanama gözlenmeyen 77 hasta ve kanama gözlenen 27 hasta olmak üzere toplam 104 KKKA hastasında ve 61 sağlıklı yetişkinlerde ölçüldü. Hastaların NLO değerleri, hastaneye başvurunun birinci ve üçüncü gününde ölçüldü. Medyan NLO değeri kontrol grupunda 4.10 iken hasta grubunun 1. gününde 4.02 ve 3. gününde 2.02 olarak tespit edildi. 3. gündeki NLO değerleri (p <0.01) kanama gözlenen grupta 1.16 (0.05-3.40) ve kanama gözlenmeyen grupta 2.32 (0.35-19.45) olarak bulundu. NLO değeri kene ile temasın 3. gününden itibaren, hastalığın klinik seyri ve tedavinin belirlenmesinde yol gösterici bir işaret gibi görülmektedir.

Anahtar Kelimeler
KKKA; Nötrofil Lenfosit Oranı; Acil Servis

Abstract
The implementation of new markers of bacterial and viral infection into clinical practice is hindered by their costs. In this study we assessed the potential use of the neutrophil to lymphocyte count ratio (NLCR) in crimean congo hemorrhagic fever (CCHF). NLCR values were measured in total 104 patients with CCHF with 77 patients in whom bleeding was observed and 27 patients without bleeding observed and in 61 healthy adults. NLCR values of patients were measured in the first and 3rd days in which the patients admitted to the hospital. Median NLCR value was found as 4.02 in the patient group on the 1st day and 2.02 on the 3rd day, while this value was 4.10 in the healthy adults. NLCR values of the 3rd day were found as 1.16 (0.05-3.40) in the patients with bleeding and 2.32 (0.35-19.45) in the patients without bleeding (p<0.01). NLCR value seems to be a marker guiding in determination of the clinical course and treatment from the 3rd day of the contact with ticks.

Keywords
CCHF; Ratio of Neutrophil to Lymphocyte Counts; Emergency Service

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Introduction
Crimean-congo hemorrhagic fever (CCHF) is a zoonotic viral disease caused by a tick-borne virus of the Nairovirus genus in the Bunyaviridae family [1,2]. CCHF has a fatality rate of 3-30% of cases and the pathogenesis of the disease is not well understood [3]. CCHF characteristically occurs in humans via a tick bite or from contact with the blood or tissues of infected livestock [4]. The incubation period for CCHF ranges from 2 to 12 days after the tick bite. This period ranges from 3 to 10 days in nosocomial cases [5,6]. The hemorrhagic period develops rapidly and usually begins between the fifth and seventh days of disease [7]. Patients may show signs of progressive hemorrhagic diathesis, such as petechiae, mucous membranes hemorrhage, conjunctival hemorrhage, nosebleed, hemoptysis, hematuria, hematemesis, and melena [7-9]. It has been reported that mononuclear phagocytes, hepatocytes, and endothelial cells are major targets of CCHF virus infection [10]. Despite increasing knowledge about hemorrhagic fever viruses, little is known about the pathogenesis of CCHF [2].

Absolute lymphocytopenia (lymphocyte count < 1.0 × 10⁹/L) in the course of the immune response to systemic infection is a relatively unknown phenomenon to physicians [11-13]. Initially, lymphocytopenia has been described in case reports concerning infectious emergencies such as toxic shock syndrome [14]. Later, Zahorec demonstrated in a prospective longitudinal observational study the correlation between the severity of the clinical course and lymphocytopenia in patients treated for severe sepsis and septic shock in an oncologic intensive care unit (ICU) [15]. Crimean-Congo hemorrhagic fever disease is a viral infection. However, it shows a course similar to sepsis with development of DIC manifestation, especially in severe cases. Therefore, in this study we aimed to measure the prognostic value of NLCR in the diagnosis and treatment of Crimean Congo hemorrhagic fever disease which is a viral systemic disease and manifests with bleeding.

Material and Method
One hundred four consecutive patients (40 male and 40 female, mean age 45±19 years) with a laboratory confirmed diagnosis of CCHF were prospectively enrolled in the study over a period of two years (April 2011 and October 2013). Sixty one age and sex matched healthy volunteers were included in the study as controls. Patients using anticoagulants and platelet aggregators (35 male and 26 female, mean age 43±15) in the control group. Gender and age were similar in the CCHF groups and healthy volunteers groups (Table 1).

Results
The study group consisted of one hundred four (64 male and 40 female, mean age 45±19 years). There were 61 healthy volunteers (35 male and 26 female, mean age 43±15) in the control group. Gender and age were similar in the CCHF groups and healthy volunteers groups (Table 1).

When the patient and control groups were compared on the first day; a significant difference was observed between the groups in terms of ALT, AST, LDH, leukocytes, neutrophils, platelets, INR, lymphocytes, white blood cells and platelet counts (p<0.01). Of these values, ALT, AST, LDH and INR increased, while a decrease was found in terms of leukocytes, neutrophils, platelets, lymphocytes, and platelet counts. While NLCR was insignificant in the patient group compared to the controls on the first day [4.02 (0.07-27.6) vs 4.10 (2.17-8.11) respectively; P>0.05], this value was the only significant biomarker on the 3rd day [4.02 (0.07-27.6) vs 2.02 (0.05-19.05) respectively; P<0.01] (Table 2).

Statistical analysis
Statistical analysis was performed using SPSS software, version 15 (SPSS, Chicago, IL). Distribution of continuous variables was tested by the Kolmogorov–Smirnov test. Continuous variables were expressed as means±standard deviation or medians and 25th-75th percentile values (Interquartile range: IQR) (normally and not normally distributed, respectively). Statistical differences among groups were tested by independent –samples Kruskal-Wallis for nonparametric variables, respectively. Univariate linear regression model was used to adjust differences in mortality for age and sex in CCHF patients and controls.
Table 3. Characteristics laboratory values of the patients classified according to the severity of disease.

<table>
<thead>
<tr>
<th></th>
<th>Non-severe (n:58)</th>
<th>Severe (n:46)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT, U/L</td>
<td>56.62±40.89</td>
<td>72.71±47.61</td>
<td>0.067</td>
</tr>
<tr>
<td>AST, U/L</td>
<td>101.31±82.26</td>
<td>140.58±96.47</td>
<td>0.027</td>
</tr>
<tr>
<td>LDH, U/L</td>
<td>360 (112-921)</td>
<td>531 (151-933)</td>
<td>0.001</td>
</tr>
<tr>
<td>Platelets, x103/mm3</td>
<td>93.43±39.31</td>
<td>73.76±50.86</td>
<td>0.028</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>267.46±36.30</td>
<td>242.78±45.96</td>
<td>0.003</td>
</tr>
<tr>
<td>NLCR</td>
<td>3.36 (0.42-14.85)</td>
<td>4.85 (0.07-27.63)</td>
<td>0.084</td>
</tr>
<tr>
<td>Neutrophils, x103/mL</td>
<td>1.95 (0.44-5.85)</td>
<td>2.58 (0.40-5.85)</td>
<td>0.040</td>
</tr>
<tr>
<td>Lymphocytes, x103/mL</td>
<td>0.78±0.40</td>
<td>0.74±0.46</td>
<td>0.627</td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>11.86±1.21</td>
<td>11.96±1.15</td>
<td>0.654</td>
</tr>
<tr>
<td>Leukocytes, x103/mL</td>
<td>3310.15 (1500-7640)</td>
<td>3378.26 (600-6541)</td>
<td>0.858</td>
</tr>
<tr>
<td>INR</td>
<td>1.01±0.08</td>
<td>1.05±0.07</td>
<td>0.518</td>
</tr>
<tr>
<td>D-dimer</td>
<td>1114.05 (88-5289)</td>
<td>2821.86 (116-5247)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

AST: Aspartate aminotransferase, ALT: alanine aminotransferase, LDH; lactate dehydrogenase, PT; prothrombin time, INR; international normalized ratio, Plt; platelet count, wbc; white blood cell, NLCR; neutrophil to lymphocyte count ratio.

Table 4. Characteristics laboratory values of the patients classified according to the mortality of disease.

<table>
<thead>
<tr>
<th></th>
<th>Non fatal (N:93)</th>
<th>Fetal (N:11)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT, U/L</td>
<td>60.82±42.85</td>
<td>88.36±52.52</td>
<td>0.052</td>
</tr>
<tr>
<td>AST, U/L</td>
<td>110.47±85.64</td>
<td>188.09±104.94</td>
<td>0.007</td>
</tr>
<tr>
<td>LDH, U/L</td>
<td>403.04 (112-933)</td>
<td>710.81 (156-929)</td>
<td>0.001</td>
</tr>
<tr>
<td>Platelets, x103/mm3</td>
<td>90.53±43.09</td>
<td>35.65±37.35</td>
<td>0.001</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>262.41±37.77</td>
<td>206.90±49.24</td>
<td>0.001</td>
</tr>
<tr>
<td>NLCR</td>
<td>3.85 (0.14-25.21)</td>
<td>5.47 (0.07-27.63)</td>
<td>0.243</td>
</tr>
<tr>
<td>Neutrophils, x103/mL</td>
<td>2.01 (0.14-5.87)</td>
<td>4.12 (0.04-5.85)</td>
<td>0.008</td>
</tr>
<tr>
<td>Lymphocytes, x103/mL</td>
<td>0.72±0.39</td>
<td>1.11±0.57</td>
<td>0.050</td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>11.89±1.18</td>
<td>12.04±1.27</td>
<td>0.692</td>
</tr>
<tr>
<td>Leukocyte, x103/mL</td>
<td>3179.66 (610-7640)</td>
<td>4698.18 (600-7535)</td>
<td>0.012</td>
</tr>
<tr>
<td>INR</td>
<td>1.04±0.08</td>
<td>1.07±0.06</td>
<td>0.291</td>
</tr>
<tr>
<td>D-dimer</td>
<td>1630.24 (88-5289)</td>
<td>3891.63 (219-5247)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

AST: Aspartate aminotransferase, ALT: alanine aminotransferase, LDH; lactate dehydrogenase, PT; prothrombin time, INR; international normalized ratio, Plt; platelet count, wbc; white blood cell, NLCR; neutrophil to lymphocyte count ratio.

A significant difference was found in neutrophil count, lymphocyte count and neutrophil-lymphocyte ratio (P < 0.01). ALT, AST, LDH and lymphocyte count increased, while a decrease was observed in the number of neutrophils, platelets, white blood cell and neutrophil lymphocyte ratio.

When the 3rd day of the patient and control group was compared, ALT, AST, LDH, leukocytes, neutrophils, platelets, INR, the number of lymphocytes, neutrophil to lymphocyte count ratio and platelet white blood cell count showed a significant difference. Of these values, ALT, AST, LDH and INR increased, while kocytes, neutrophils, platelets, the number of lymphocytes, neutrophil to lymphocyte count ratio and platelet white blood cell count decreased.

When the patients having bleeding at anywhere were compared with the patients having not complaints of bleeding; there was a significant difference between both the groups in terms of neutrophil to lymphocyte count ratio. Mean NLCR value was found as 2.51 (0.07-6.26) in the bleeding patients and 4.55 (0.42-27.63) in the non-bleeding patients on the 1st day (p: 0.04). Mean NLCR value was found as 1.16 (0.05-3.40) in the bleeding patients and 2.32 (0.35-19.45) in the non-bleeding patients on the 3rd day (p<0.01).

Patients were classified according to the severity of the disease. Significant differences were observed between severe and non-severe patients with respect to AST, LDH, fibrinogen, neutrophil and d-dimer values. LDH, neutrophil and d-dimer were correlated with the severity of disease in the regression analysis of these significant values.(table 3)

Patients were classified according to the severity of disease. Significant differences were observed between severe and non-severe patients with respect to AST, LDH, platelets, fibrinogen, neutrophil leukocytes and d-dimer values. Platelets, fibrinogen, neutrophil leukocytes and d- dimer were correlated with the mortality of disease in the regression analysis of these significant values.(table 4)

Discussion

To the best of our knowledge, this study is the first in the published literature to investigate the predictive value of admission NLRc on the severity of CCHF. We found that NLRc was significantly decreased in CCHF patients. Furthermore even after controlling for confounding parameters we found that less NLRc was strongly associated with disease severity.

Previous studies have reported that decreased levels of thrombocytes and increased levels of white blood cells, AST,ALT, LDH, as well as a prolonged APTT were associated with a poor outcome in CCHF patients [16,17].

Lymphocytopenia has been described in case reports concerning infectious emergencies such as toxic shock syndrome [14]. Later, Zañorec demonstrated in a prospective longitudinal observational study the correlation between the severity of the clinical course and lymphocytopenia in patients treated for severe sepsis and septic shock in an oncologic intensive care unit (ICU) [15].

In study of Incl’s leukocytes and platelets values were found in CCHF patients to be significantly lower than the control group [18]. In our study, while AST, LDH neutrophil leukocytes and d-dimer levels were significantly higher in fetal group, platelets and fibrinogen levels were significantly lower.

Any of the following clinical pathologic values during the first 5 days of illness were found to be >90% predictive of fatal outcome in a series of South African CCHF patients: platelet counts <20 × 109/L, ALT >150 U/L, AST >200 U/L, leukocyte counts < 10 × 109/L, fibrinogen <110 mg/dl and aPTT >60 s [17]. In a study from Turkey, higher ALT and AST levels (>700 and >900 IU/l, respectively) were found to have higher sensitivity for severe cases [19]. AST, LDH, platelets, fibrinogen, neutrophil leukocytes and d-dimer were significant in our study. NLCR might be a finding which could be expected to decrease in CCHF that is a viral disease, but decreases observed in the follow-up suggest that the bleeding may occur. In our study, NLCR began to be significant for CCHF disease compared to the control group on the 3rd day of the onset of clinical complaints. Viral replication is likely to be more common in these patients because of the acute period. Both neutrophils and lymphocytes number and variation of the ratio of these values can give us a suggestion about the clinical course.
Acknowledgements

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Conflict of interest

No conflict of interest and no funding source to declare.

Ethical approval

The study was performed in accordance with the Declaration of Helsinki for Human Research, and was approved by the institutional ethics committee.

References