Mean Platelet Volume and Neutrophil-to-Lymphocyte Ratio in Patients with Crimean–Congo Hemorrhagic Fever

Derya Kocer¹, Fatma M Sariguze², Funda Gözütk³, Dilek Yagci², Cigdem Karakucu², Ahmet Godekmerdan²

¹Department of Biochemistry, Training and Research Hospital, Kayseri, Turkey
²Department of Microbiology, Training and Research Hospital, Kayseri, Turkey
³Department of Infectious Diseases and Clinical Microbiology, Training and Research Hospital, Kayseri, Turkey

Abstract

Objectives: Crimean–Congo hemorrhagic fever (CCHF) is a tick-borne zoonotic infection caused by Crimean Congo hemorrhagic fever virus (CCHFV). The aim of the present study was to investigate the association between blood neutrophil-to-lymphocyte ratio (NLR) and mean platelet volume (MPV) which are simple markers of subclinical inflammation and CCHF. We also investigated the relationship of these markers with coagulation parameters.

Methods: Thirty-one suspected CCHF patients, who submitted to Training and Research Hospital, Kayseri, Turkey between 2009 and 2013, were evaluated retrospectively. Among thirty-one patients, nineteen were laboratory confirmed CCHF patients diagnosed by RT-PCR or CCHFV-specific IgM positivity. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatinine phosphokinase (CK), coagulation parameters, white blood cell counts (WBCs), and platelet counts of patient group were compared with twenty-five healthy individuals.

Results: MPV, AST, ALT, LDH, CK and coagulation parameters were significantly higher in patients with CCHF than the controls, whereas WBCs, neutrophil, lymphocyte, hemoglobin, platelet counts and NLR were significantly lower (p<0.05). We found no significant correlation between MPV, NLR and coagulation parameters.

Conclusions: Our study demonstrates that MPV and NLR may be beneficial markers in the diagnosis of CCHF. But these parameters should not be considered stand-alone tests for this use owing to nonspecificity with other diseases.

Keywords: Crimean–Congo hemorrhagic fever, mean platelet volume, neutrophil-to-lymphocyte ratio

Received: 16.12.2014  Accepted: 19.02.2015 doi: 10.15824/actamedica.50168

Introduction

Crimean–Congo hemorrhagic fever (CCHF) is a tick-borne zoonotic infection caused by Crimean Congo hemorrhagic fever virus (CCHFV), which is a member of the Nairovirus genus of the Bunyaviridae family (1). People living in endemic areas and working in farming, and the health employees working in institutes have the highest risk for the disease (2,3). The most commonly observed symptoms are fatigue, high fever, headache, myalgia, nausea, and vomiting (4). CCHF has been reported in more than 30 countries and the fatality rate varies between 3 and 30% (1). CCHFV infection is one of the important public health issues in Turkey, because of its high case fatality rate.

Despite increasing knowledge about hemorrhagic fever viruses, little is known about the pathogenesis of Crimean–Congo hemorrhagic fever. In viral hemorrhagic fevers, inflammatory processes are main elements of the immune response and it has been suggested that release of proinflammatory cytokines is related to the disease course (5,6).

Platelet volume is an indicator of platelet function and activation. Platelet activity and aggregation capacity can be easily determined by measuring mean platelet volume (MPV). The intensity of systemic inflammation can be seen as a distinctive factor for classifying conditions associated with large and small-sized circulating platelets. Large platelets have more granules, aggregate more rapidly with collagen, produce higher levels of thromboxane A2 and express more glycoprotein Ib and IIb/IIIa receptors than smaller ones. Platelet activation is a link in the pathophysiology of diseases prone to inflammation (7-9).

Also, blood neutrophil-to-lymphocyte (NLR) ratio is a simple marker of subclinical inflammation that can be easily obtained from the differential WBC count. The NLR has been used to predict outcomes in patients with cancer and coronary artery disease (10,11). We can obtain information about two different immune pathways from the NLR. First of all about the neutrophils those are responsible for lasting inflammation and the second about the lymphocytes that demonstrate the regulatory pathway (12,13). To...
our knowledge, there is no report in the literature about the usefulness of NLR in patients with CCHF.

Thus, MPV and NLR are indicators of the inflammatory status of the body, and an alteration in MPV and NLR may be found in patients with CCHF. The aim of the present study is to investigate the relationship of NLR and MPV with CCHF and their relation with coagulopathy parameters (activated partial thromboplastin time [aPTT], prothrombin time [PT], international normalized ratio [INR]).

Materials and Methods

Thirty-one suspected CCHF patients, who submitted to Training and Research Hospital, Kayseri, Turkey between 2009 and 2013, were evaluated retrospectively. Among thirty-one patients, nineteen were laboratory confirmed CCHF patients. The definitive diagnosis of CCHF infection was done by the detection of CCHFV-specific IgM by enzyme-linked immunosorbent assay (ELISA) or of genomic segments of the CCHFV by reverse-transcription polymerase chain reaction (RT-PCR) in Department of Microbiology Reference Laboratories, Public Health Institute of Turkey. None of patients had any systemic disease, such as chronic obstructive pulmonary disease, peripheral and cerebral vascular disease, diabetes mellitus, other hematological disorders, cirrhosis, portal hypertension, malignancies, hypertension, hypercholesterolemia, obesity, coronary heart disease, metabolic syndrome, statin and antihypertensive use and atrial fibrillation. Age and gender matched 25 healthy individuals were included as control group.

The blood samples of patients were taken at admission of the hospital. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatinine phosphokinase (CK), INR, PT, aPTT, white blood cell counts (WBCs), and platelet counts of patient group were determined by examining the records of Kayseri Training and Research Hospital Clinical Biochemistry Laboratory. In our study period neutrophil, lymphocyte, platelet count and MPV determinations were done by Mindray BC-6800 (Shenzhen Mindray Biomedical Electronics, Nanshan, P.R.China) a multi-parameter automated hematology analyzer. This is a retrospective study in which the data were obtained from a computerized patient registry database. As routine complete blood counts are analyzed in EDTA containing tubes, MPVs were also calculated in those tubes as a part of routine blood counting. However, our laboratory makes the measurements within maximum two hours; even samples which reach laboratory after that time are refused. So we think that the changes in MPV of patients and controls were minimum and similar. Reference value of MPV in our laboratory is 7.2 to 11.1 fL. NLR was calculated as the ratio of the total count of neutrophils divided by the total count of lymphocytes.

The Institution Review Board of Kayseri Training and Research Hospital approved the study protocol (No: 2014/28). The study was performed in accordance with the Declaration of Helsinki.

Statistical Analysis

Statistical evaluation was carried out with the SPSS® 20.0 (Statistical Packages for Social Sciences; SPSS Inc, Chicago, Illinois, USA). For comparison of all the variables between patient and control groups, independent t test was used. Pearson correlation analysis was used to explore correlations between the variables. Data were presented as “mean with their standard deviation” (mean ± SD). Non-normally distributed data were presented as “median” (min-max). P value less than 0.05 was considered as statistically significant.

Results

In 19 of 31 prediagnosed as CCHF patients, RT-PCR and/or Ig M antibody were positive. Laboratory results of these 19 patients (6 females and 13 males) were compared with control group. There was no statistically significant difference between the genders and ages of the study participants (p>0.05).

As shown in Table 1, WBCs, neutrophil, lymphocyte, hemoglobin and platelet counts were significantly lower (p<0.001), although levels of INR, PT, aPTT, AST, ALT, LDH and CK were significantly higher in CCHF patients.

Although MPV levels are within the normal range in both patient and control group, a statistically significant increase in MPV was observed in patients with CCHF compared with healthy controls (9.9±1.4 fL vs 8.8±0.7 fL, p=0.003). NLR were significantly lower in patients with CCHF than the controls (1.4±1.1 vs 2.2±0.9, p=0.011).

Pearson correlation analysis showed no significant correlation between MPV, NLR and coagulation parameters.
### Table 1. Clinical and laboratory characteristics of CCHF patients and controls

<table>
<thead>
<tr>
<th>Variables</th>
<th>CCHF (n:19)</th>
<th>Controls (n:25)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>45.8±10.6</td>
<td>41.7±13.6</td>
<td>NS</td>
</tr>
<tr>
<td>Gender (F/M)</td>
<td>6/13</td>
<td>13/12</td>
<td>NS</td>
</tr>
<tr>
<td>White blood cell, x10³/µL</td>
<td>2.5 (1.0-13.8)</td>
<td>7.38 (3.64-10.19)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Neutrophil, x10³/µL</td>
<td>0.9 (0.4-6.9)</td>
<td>4.17 (2.36-8.22)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Lymphocyte, x10³/µL</td>
<td>1.2±0.8</td>
<td>2.2±0.6</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>8.7±1.5</td>
<td>12.4±0.8</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Platelet count, x10³/µL</td>
<td>58.5±28.1</td>
<td>246.6±57.8</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>MPV, fl</td>
<td>9.9±1.4</td>
<td>8.8±0.7</td>
<td>0.003</td>
</tr>
<tr>
<td>NLR</td>
<td>1.4±1.1</td>
<td>2.2±0.9</td>
<td>0.011</td>
</tr>
<tr>
<td>PT (sn)</td>
<td>15.1±4.3</td>
<td>11.4±0.8</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>INR</td>
<td>1.2±0.3</td>
<td>0.9±0.1</td>
<td>0.002</td>
</tr>
<tr>
<td>aPTT (sn)</td>
<td>48.1±28.0</td>
<td>24.0±1.6</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>AST (IU/mL)</td>
<td>202.4±144.2</td>
<td>21.8±6.1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>ALT (IU/mL)</td>
<td>102.8±58.5</td>
<td>24.6±13.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>LDH (IU/mL)</td>
<td>462.7±229.2</td>
<td>175.4±32.9</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CK (IU/mL)</td>
<td>562.1±359.6</td>
<td>80.2±46.9</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

NS: Nonsignificant

### Discussion

The CCHF is a viral tick-borne zoonosis that causes severe illness and hemorrhages in humans. The disease generally appears in Asia, the Middle East, Africa, and southeastern Europe (14). At present, Turkey is experiencing an increase in outbreaks of the disease, especially in Central Anatolia. According to Turkish Ministry of Health records, the fatality rate is reported to be around 5% in Turkey (15).

For preventing the outbreaks and decreasing mortality rates, early diagnosis of CCHF is essential. The certain diagnosis is made initially by an in-house RT-PCR method and those which were found negative with RT-PCR, were then studied by in-house ELISA method in terms of CCHFV-IgM antibodies in Department of Microbiology Reference Laboratories, Public Health Institute of Turkey (16). As seen, these laboratory tests can only be done in reference laboratories which are not routinely available in most areas. Furthermore, these tests are expensive and determination of tests is quite hard. For this reason, in this study we aimed to investigate the utility of MPV and NLR, as simple and readily available predictors in CCHF.

The MPV and NLR are simple, noninvasive, inexpensive measures and available in a majority of the healthcare centers. NLR could be an important measure of subclinical inflammation (10,11). To our knowledge, prior studies have never analyzed the association between NLR and CCHF as an inflammation indicator. In this study we demonstrated statistically significant lower NLR in patients with CCHF when compared healthy controls. As shown in Table 1, lymphocyte counts were observed to be relatively higher in patient group. Since lymphocyte counts increase in viral infections, we thought this may be the cause of decreased NLR values in CCHF patients. For this reason, even though NLR is a marker of subclinical inflammation, it has no clinical significance in CCHF.

Pancytopenia is one of the most important findings of CCHF. In the pathogenesis of pancytopenia observed in CCHF, hemophagocytosis is thought to be responsible. A study conducted in Turkey reported reactive hemophagocytosis in 50% of patients infected with CCHFV, suggesting that it can also play a role in the pathogenesis of CCHF (17). The possible
mechanism of hemophagocytosis is increased activation of monocytes which is stimulated by high levels of proinflammatory cytokines (1,18). As seen in Table 1, we observed significantly decreased neutrophil, lymphocyte, hemoglobin and platelet counts which were lower than the reference values in patient group.

Mean platelet volume is the geometric mean of the transformed lognormal platelet volume data in impedance technology systems. In some optical systems MPV is the mode of the measured platelet volume (19). In the literature, there are numerous studies suggesting the important role of MPV as a marker of inflammation, and disease activity in inflammatory disorders (20-22). The results for infectious diseases seem to be conflicting. While some studies demonstrated a negative correlation between MPV and inflammatory activity, other investigators have reported an association between increased MPV and disease severity. Beyazıt et al. demonstrated that MPV is decreased in acute pancreatitis (23). Zubcevic et al. reported that one of the most reliable indicators of Crohn’s disease activity was MPV; however, it was not sensitive enough to distinguish the relationship between moderate and severe disease (19).

Thrombocytopenia was classified as hypoproductive and hyperdestructive. Hypoproductive thrombocytopenia is commonly secondary to the decreased production of the platelets in bone marrow and platelets with a lower MPV are expected in hypoproductive thrombocytopenia. Generally, hyperdestructive thrombocytopenia is a result of extramedullary destruction of the platelets; this is seen in patients with condition such as immune thrombocytopenic purpura (ITP), secondary ITP, and disseminated intravascular coagulopathy (DIC) (24-26). Conversely, recent studies have demonstrated that patients with hyperdestructive thrombocytopenia are likely to have higher values of MPV when compared with normal reference values (24).

In this study we aimed to search if MPV could be a biomarker for the assessment of disease course in CCHF. To our knowledge, an association between CCHF and MPV levels has been reported only by Ekiz et al’s (27). In accordance with Ekiz et al’s study, our study showed that patients with CCHF have significantly higher MPV when compared to control subjects. But we found no significant correlation between MPV, NLR and coagulation parameters in contrast to Ekiz et al’s study (27). In the pathogenesis of CCHF, endothelial damage contributes to hemostatic failure by stimulating platelet aggregation and degranulation, with consequent activation of the intrinsic coagulation cascade which leads to DIC (1). During CCHF infection, inflammatory processes are main elements of the immune response. In the literature it has been suggested that levels of proinflammatory cytokines increase in CCHF and they are responsible for the maturation of thrombopoietic cells and induction of platelet production (28,29). For that reason increased MPV can be the result of an increased proportion of young platelets in the circulation in CCHF.

**Conclusion**

CCHF is an important infectious disease that may cause loss of labor force and death, and is still an epidemic disease in our country. Although molecular and serological evaluation is essential on certain diagnosis of CCHF infection, MPV value may give an opinion and may be a beneficial biomarker in the diagnosis of CCHF. But, since MPV does not have specificity among the other diseases, this test alone should not be considered as a determining factor. For using it as biomarker further studies with consideration of mentioned limitations, larger sample size and determination of its sensitivity, specificity and cut-off point is necessary. According to our findings, in contrast to MPV, NLR is not a clinically useful marker for assessment of disease progression. Further studies are needed to explain the mechanism of increased MPV levels in CCHF.

**References**

5. Villinger F, Rollin PE, Brar SS, Chikkala NF, Winter J, Sundstrom JB, et al. Markedly elevated levels of interferon (IFN)-gamma, IFN-alpha, interleukin (IL)-2, IL-10, and tumor...


