INTRODUCTION

Crimean-Congo hemorrhagic fever (CCHF) is a zoonotic viral disease caused by the CCHF virus, a member of the Nairovirus genus in the Bunyaviridae family (1). Hyalomma ticks are the main vectors of this disease; blood and body secretions of viremic patients and livestock are also transmission vehicles. CCHF is widespread throughout Africa, Central Asia, Southeast Europe, and the Middle East (2–5). It was first observed in Turkey in 2002 and has since become a major public health threat in this country owing to its high mortality rate, especially in rural areas (3,6,7). Mortality rates depending on the geographic region and transmission route (8,9). The incubation period of the CCHF virus is 3 to 7 days depending on its titer and transmission route (8,10). Fever, chills, myalgia, severe headache, dizziness, nausea, vomiting, diarrhea, and abdominal pain are the nonspecific symptoms of CCHF. Hemorrhagic manifestations range from petechia to ecchymosis, epistaxis, and melena, which are detected in severe cases 3–6 days after the onset of the disease (10). There is no effective antiviral treatment for CCHF, although supportive treatment has been suggested (11,12). The epidemiologic features, pathogenesis, and clinical characteristics of CCHF and its severity criteria have been described (2,8,13,14). In this study, we aimed to determine the association between leukocyte, neutrophil, lymphocyte, and monocyte levels and the survival of patients with CCHF. To our knowledge, this is the first study to do so.

MATERIALS AND METHODS

Study design, setting, and patients: This retrospective case control study was carried out at the Ankara Numune Education and Research Tertiary Care Hospital in Turkey. The medical records of patients diagnosed with CCHF and followed-up at this hospital between 2002 and 2013 were examined. Patients with a decisive diagnosis of CCHF based on clinical manifestations and the presence of viral RNA (determined via reverse transcription-polymerase chain reaction and/or by using an anti-IgM antibody) were enrolled in the study. Data regarding the demographical, clinical, and laboratory characteristics and outcomes of the patients were extracted from the medical records. Leukocyte, lymphocyte, neutrophil, and monocyte levels on the first day of admission and day 3 of the hospital stay were compared between patients with fatal and nonfatal disease to determine their relationship to mortality. Levels were measured by using a Beckman Coulter LH 750 Hematology Analyzer (Fullerton, CA, USA). Our study did not require informed consent from patients or approval from the ethics committee because it was retrospective.

Statistical analysis: Statistical analysis was performed
Table 1. Demographical, epidemiological and clinical characteristics of patients with CCHF

| Characteristic                      | Total case (%) | Non-fatal case (%) | Fatal case (%) | P-value |
|-------------------------------------|----------------|--------------------|----------------|---------|
|                                     | n = 220        | n = 184            | n = 36         |         |
| Age (yr, mean) ± SD                 | 50.2 ± 17.0    | 49.4 ± 17.2        | 54.0 ± 16.0    | 0.172   |
| Male                                | 123 (55.9)     | 104 (56.5)         | 19 (52.8)      | 0.679   |
| Comorbidities                       | 32 (14.5)      | 24 (13.0)          | 8 (22.2)       | 0.174   |
| Living in rural area                | 171 (77.7)     | 144 (78.3)         | 27 (75.0)      | 0.667   |
| Handling livestock/farming          | 165 (75.0)     | 138 (75.0)         | 27 (75.0)      | 1.000   |
| Time from tick bite to onset of symptoms (days) | 3.8 ± 3.0 | 3.8 ± 2.9 | 4.0 ± 3.9 | 0.765 |
| Duration of symptoms before hospitalization (days) | 5.0 ± 3.3 | 5.0 ± 3.4 | 4.8 ± 2.4 | 0.949 |
| Length of hospital stay (days)      | 6.8 ± 3.5      | 7.3 ± 3.3          | 4.3 ± 3.0      | <0.001 |
| Fever                               | 194 (88.2)     | 161 (87.5)         | 33 (91.7)      | 0.585   |
| Lack of appetite                    | 174 (79.1)     | 144 (78.3)         | 30 (83.3)      | 0.494   |
| Headache                            | 133 (60.5)     | 110 (59.8)         | 23 (63.9)      | 0.645   |
| Nausea                              | 129 (58.6)     | 103 (56.0)         | 26 (72.2)      | 0.070   |
| Vomiting                            | 79 (35.9)      | 64 (34.8)          | 15 (41.7)      | 0.431   |
| Hemorrhage                          | 65 (29.5)      | 41 (22.3)          | 24 (66.7)      | <0.001 |
| Diarrhea                            | 64 (29.1)      | 48 (26.1)          | 16 (44.4)      | 0.027   |
| Cough                               | 37 (16.8)      | 34 (18.5)          | 3 (8.3)        | 0.137   |
| Fever, temperature > 38°C           | 97 (44.1)      | 81 (44.0)          | 16 (44.4)      | 0.963   |
| Somnolence                          | 25 (11.4)      | 8 (4.3)            | 17 (47.2)      | <0.001 |
| Hepatomegaly                        | 19 (8.6)       | 16 (8.7)           | 3 (8.3)        | 1.000   |
| Spleenomegaly                       | 11 (5.0)       | 10 (5.4)           | 1 (2.8)        | 0.697   |
| Skin lesions                        |                |                    |                |         |
| Petechia                            | 47 (21.4)      | 34 (18.5)          | 13 (36.1)      | 0.018   |
| Ecchymosis                          | 35 (15.9)      | 21 (11.4)          | 14 (38.9)      | <0.001 |
| Maculopapular rash                  | 25 (11.4)      | 22 (12.0)          | 3 (8.3)        | 0.774   |
| Type of hemorrhage                  |                |                    |                |         |
| Epistaxis                           | 35 (15.9)      | 25 (13.6)          | 10 (27.8)      | 0.033   |
| Melena                              | 26 (11.8)      | 11 (6.0)           | 15 (41.7)      | <0.001 |
| Gum bleeding                        | 23 (10.5)      | 12 (6.5)           | 11 (30.6)      | <0.001 |
| Hematuria                           | 14 (6.4)       | 6 (3.3)            | 8 (22.2)       | <0.001 |
| Hematemesis                         | 11 (5.0)       | 4 (2.2)            | 7 (19.4)       | <0.001 |
| Hemoptysis                          | 6 (2.7)        | 2 (1.1)            | 4 (11.1)       | 0.007   |
| Hematoma                            | 3 (1.4)        | 0 (0.0)            | 3 (8.3)        | 0.004   |

RESULTS

Our study consisted of 220 patients with CCHF; the mean age was 50.21 ± 17.07 years (range, 15–85 years), and 123 patients (55.9%) were men. The mortality rate was 16.4% (n = 36). At the time of disease, 171 patients (77.7%) lived in a rural area, and 165 patients (75%) dealt with livestock. Most patients (n = 189, 85.9%) were admitted to the hospital in May, June, or July, and 140 (63.6%) patients had a history of tick bites. The mean incubation period after a tick bite was 3.8 ± 3.0 days. The most frequent symptoms were fever (88.2%), lack of appetite (79.1%), and myalgia (75%); other symptoms included hemorrhage (n = 65, 29.5%) and somnolence (n = 25, 11.4%). Age, sex, comorbidities (diabetes mellitus, hypertension, and chronic obstructive pulmonary disease), length of the incubation period, and length of the symptomatic period before admission did not differ significantly between patients with fatal and nonfatal disease (Table 1). No patients received ribavirin treatment; all patients received only supportive care. The mean length of hospitalization was 6.42 ± 3.06 days; it was significantly shorter in the fatal group (P < 0.001). In a univariate analysis, the following parameters were significantly higher in the fatal group than in the nonfatal group: leukocytosis, hemorrhage, somnolence, melena, ecchymosis, petechia, gum bleeding, hematuria, hematemesis, hemoptysis, hema-
levels were measured 2 days after admission (day 3): analysis (nonfatal group on day 1, as determined via univariate analysis (Table 1 and 2). Patients with fatal disease had significantly higher rates of elevated alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and creatine phosphokinase (CPK) levels and thrombocytopenia on the day of admission (day 1) (P<0.001 for all variables). The median values of the laboratory parameters are provided in Table 2.

White blood cell (WBC) counts and neutrophil levels were significantly higher in the fatal group than in the nonfatal group on day 1, as determined via univariate analysis (P = 0.006 and 0.001, respectively). Neutrophil levels were measured 2 days after admission (day 3): neutrophil levels were significantly higher (P = 0.01) and lymphocyte and monocyte levels were significantly lower (P = 0.037 and 0.001 respectively) in fatal group (univariate analysis, Table 3). Leukocyte, lymphocyte, and monocyte levels significantly increased between days 1 and 3 in the nonfatal group (P<0.001 for all variables) but not in the fatal group (univariate analysis, Table 4). The cut-off levels of the laboratory parameters on day 1 for predicting mortality were determined and are summarized in Table 5.

An ROC analysis revealed that a WBC count ≥2,950/mm³ on day 1 predicted mortality with 62.1% sensitivity and 33.1% specificity (Table 5). In a multivariate analysis of laboratory parameters on day 1, levels of leukocytes (≥2,950 μL), ALT (>119.5 U/L), and hemoglobin (≤13.6 g/dL) were significantly associated with mortality. The Effect of Leukocyte Counts on Mortality in CCHF

### Table 2. Univariate analysis of the first admission-day laboratory findings for fatal and non-fatal cases with Crimean Congo Hemorrhagic fever

|                      | Non-fatal case (n) | Fatal case (n) | P-value |
|----------------------|-------------------|----------------|---------|
| WBC (/μL)            | 2,300 (200–16,500)| 3,500 (700–23,900)| 0.006   |
| Leukocytosis (>10,800/μL) (n, %) | 1 (0.5) | 4 (11.1) | <0.001 |
| Leukopenia (<4,800/μL) (n, %) | 161 (88.0) | 23 (63.9) | <0.001 |
| Neutrophils (/μL)    | 1,400 (130–9,000) | 2,550 (400–21,200) | 0.001   |
| Lymphocytes (/μL)    | 600 (6–8,000)     | 700 (100–6,000) | 0.499   |
| Monocytes (/μL)      | 200 (0–1,600)     | 100 (0–1,000) | 0.142   |
| Platelets (/μL)      | 61,000 (4,000–189,000) | 20,000 (4,000–242,000) | <0.001 |
| Hemoglobin (g/dL)    | 13.9 (8.4–18.4)   | 13.6 (6.3–18.9) | 0.871   |
| ALT (U/L)            | 82 (8–1,270)      | 221 (24–3,080) | <0.001 |
| AST (U/L)            | 158 (15–4,202)    | 673 (30–11,870) | <0.001 |
| LDH (U/L)            | 526 (109–9,237)   | 1,405 (145–8,624) | <0.001 |
| CPK (U/L)            | 354 (41–7,150)    | 825 (56–3,904) | <0.001 |
| aPTT (s)             | 33.8 (20.4–154)   | 60.2 (31.6–113) | <0.001 |
| PT (s)               | 12.3 (10.0–27.0)  | 14.8 (10.2–39.8) | <0.001 |
| INR                  | 1.0 (0.75–3.9)    | 1.16 (0.25–3.0) | <0.001 |
| Fibrinogen (mg/dL)   | 261 (115–599)     | 198 (104–539) | 0.018   |

WBC, white blood cell; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CPK, creatine phosphokinase; LDH, lactate dehydrogenase; aPTT, activated partial thromboplastin time; PT, prothrombin time; INR, international normalized ratio.

### Table 3. Univariate analysis of the first and third admission-days leucocytes counts for fatal and non-fatal cases

|                      | First admission-day | Third admission-day | P-value |
|----------------------|---------------------|---------------------|---------|
| Non-fatal case (median, range) | 2,300 (200–16,500) | 3,500 (700–23,900) | 0.006   |
| Fatal case (median, range)     | 2,900 (500–24,900) | 3,400 (1,100–11,600) | 0.495   |
| Neutrophils (/μL)            | 1,400 (130–9,000)  | 2,550 (400–21,200) | 0.001   |
| Lymphocytes (/μL)            | 600 (0–8,000)      | 700 (100–6,000)    | 0.499   |
| Monocytes (/μL)              | 200 (0–1,600)      | 100 (0–1,000)      | 0.142   |
| Neutrophils (/μL)            | 1,400 (130–9,000)  | 2,550 (400–21,200) | 0.001   |
| Lymphocytes (/μL)            | 600 (0–8,000)      | 700 (100–6,000)    | 0.001   |
| Monocytes (/μL)              | 200 (0–1,600)      | 100 (0–1,000)      | 0.001   |

### Table 4. Evaluation of the first and third admission-day laboratory values in non-fatal and fatal cases with Crimean Congo Hemorrhagic fever in univariate analysis

|                      | Non-fatal case | Fatal case | P-value |
|----------------------|---------------|------------|---------|
| WBC (/μL)            | 2,300 (200–6,500) | 3,500 (700–23,900) | <0.001 |
| Neutrophils (/μL)    | 1,400 (130–9,000) | 2,550 (400–21,200) | 0.043   |
| Lymphocytes (/μL)    | 600 (0–8,000)     | 700 (100–6,000) | <0.001   |
| Monocytes (/μL)      | 200 (0–1,600)     | 100 (0–1,000) | <0.001   |

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In the present study, the mortality rate was 16.4% of other transmission routes owing to higher viral loads. Transmission of CCHF has a higher mortality rate that availability of supportive care facilities (7). Nosocomial are thought to reflect the means of transmission and the important impact on mortality (2,8). Mortality rates are well as living in rural areas (77.7%). The male/female ratio in our study (1.3:1) was similar to the ratio reported in a previous study of patients with CCHF (16). The percentage of patients with a history of tick bites (63.6%) corresponded to the percentage reported by the Turkish Ministry of Health (60%) (17). The mean time for the occurrence of clinical manifestations was 3.8 ± 3.0 days after the tick bite. Similar to previous reports, the most frequent symptoms were fever (88.2%), lack of appetite (79.1%), and myalgia (75%). Additionally, all parameters associated with disease severity were significantly higher in the fatal group than in the nonfatal group; these parameters included thrombocytopenia, prolonged aPTT, prothrombin time, and international normalized ratio, decreased fibrinogen level, and elevated ALT, AST, LDH, and CPK levels. The incidence of somnolence, diarrhea, all types of hemorrhages, and skin lesions was also significantly higher in the fatal group (8,13). On average, hemorrhages have been reported in 25% of patients with CCHF in Turkey (9). In our study, 66.7% of the patients had hemorrhages, which may explain why our mortality rate was higher than the average mortality rate in Turkey.

Analysis of the relationship between leukocyte counts and mortality rates provided insight into the pathogenesis of CCHF. WBC and neutrophil levels on day 1 were significantly higher in the fatal group than the nonfatal group. Neutrophil levels were also significantly higher in the fatal group on day 3, whereas lymphocyte and monocyte levels were significantly lower. In patients with fatal disease, it is possible that neutrophil accumulation causes an excessive release of cytokines and that lymphocyte and monocyte depletion attenuates humoral immunity and antibody responses. As determined via comparison of day 1 and day 3 values, leukocyte, lymphocyte, and monocyte levels were significantly lower. In patients with fatal disease, which is higher than the average mortality rate in Turkey.

In conclusion, our study showed that, in addition to

### Table 5. Cut off levels of first admission-day laboratory parameters as a prognostic factor for predicting mortality

| Parameter | Level\(^{(1)}\) | Sensitivity | Specificity | PPV | NPV | AUROC | \(P\)-value |
|-----------|----------------|-------------|------------|-----|-----|-------|-----------|
| WBC (\(\mu L\)) | \(\geq 2,950\) | 62.1 | 33.1 | 26.9 | 90.0 | 0.662 | 0.006 |
| Neutrophils (\(\mu L\)) | \(\geq 1,176\) | 62.1 | 36.5 | 25.0 | 89.5 | 0.695 | 0.001 |
| ALT (U/L) | \(\geq 119.5\) | 75.0 | 34.5 | 30.7 | 92.8 | 0.751 | <0.001 |
| AST (U/L) | \(\geq 304.5\) | 77.1 | 27.7 | 35.5 | 94.1 | 0.821 | <0.001 |
| LDH (U/L) | \(\geq 967.5\) | 75.0 | 16.7 | 46.2 | 94.6 | 0.828 | <0.001 |
| CPK (U/L) | \(\geq 443.5\) | 71.0 | 44.3 | 23.9 | 90.7 | 0.709 | <0.001 |
| aPTT (s) | \(\geq 42.4\) | 88.2 | 17.9 | 50.8 | 97.1 | 0.914 | <0.001 |
| PT (s) | \(\geq 14.0\) | 67.6 | 20.4 | 41.8 | 91.9 | 0.786 | <0.001 |
| INR | \(\geq 1.12\) | 64.7 | 22.7 | 37.3 | 91.3 | 0.714 | <0.001 |

\(1\): Normal range of laboratory parameters: WBC (4,000–10,800), Neutrophils (1,800–7,700), ALT (3–50), AST (4–50), LDH (25–248), CPK (10–171), aPTT (27.2–36.5), PT (10–12.7), INR (0.9–1.17).

### Table 6. The effect of possible risk factors on survival with multivariate logistic regression analysis

| Parameter | Odds ratio | 95% CI | \(P\)-value |
|-----------|------------|-------|------------|
| WBC > 2,950/\(\mu L\) | 8.86 | 1.55–50.62 | 0.014 |
| LDH > 967.5 U/L | 8.23 | 1.45–46.56 | 0.017 |
| aPTT > 42.4 s | 11.68 | 2.40–56.90 | 0.002 |
| ALT > 119.5 U/L | 7.26 | 1.12–47.27 | 0.038 |

LDH (\(> 967.5\) U/L), and activated partial thromboplastin time (aPTT, >42.4 s) that exceeded the cut-off values were identified as independent predictors of mortality (odds ratios: 8.86, 7.26, 8.23, and 11.68, respectively) (Table 6).

### DISCUSSION

CCHF has been a public health concern in Turkey since 2002 because of its severity and widespread distribution throughout this country. Reported mortality rates range from 5% to 30% (7,9,15). Geographic region and transmission route appear to have an important impact on mortality (2,8). Mortality rates are higher in China (80%) and the United Arab Emirates (73%) (15). Geographical differences in mortality rates are thought to reflect the means of transmission and the availability of supportive care facilities (7). Nosocomial transmission of CCHF has a higher mortality rate that of other transmission routes owing to higher viral loads. In the present study, the mortality rate was 16.4%. Because patients with severe CCHF are referred to our hospital for intensive supportive therapy, this rate is higher than the average rate in Turkey (5%) (9,13). The current study examined the association between leukocyte, neutrophil, lymphocyte, and monocyte levels and survival, as well as known predictors of mortality.

Living in a rural area is considered a risk factor for CCHF. Most of the patients in our study contracted the disease between March and July. This time period coincides with increased activity in agriculture and animal husbandry, which usually peaks in June and July (16). In our study population, 85.9% of the patients were admitted to the hospital in May or June. Our findings agree with previous findings (2) showing a strong correlation between CCHF and livestock/farming (75%), as well as living in rural areas (77.7%). The male/female

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previously reported severity risk factors, decreases in monocyte and lymphocyte counts and increases in neutrophil counts correlate with poor outcome in CCHF patients. Our results emphasize the importance of the mononuclear immune response for the survival of patients with CCHF.

Conflict of interest  None to declare.

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