

Original Article

Crimean-Congo Hemorrhagic Fever: Prognostic Factors and the Association of Leukocyte Counts with Mortality

Aliye Bastug*, Bircan Kayaaslan, Sumeyye Kazancioglu, Halide Aslaner, Ayse But, Esragul Akinci, Meltem Arzu Yetkin, Selim Eren, and Hurrem Bodur

Ankara Numune Training and Research Hospital, Department of Infectious Diseases and Clinical Microbiology, Ankara, Turkey

SUMMARY: We aimed to determine the relationship between leukocyte counts and the survival of patients with Crimean-Congo hemorrhagic fever (CCHF), a life-threatening illness. This is the first study to do so. A total of 220 patients with CCHF were evaluated retrospectively. The mortality rate was 16.4%. Analysis of the relationship between leukocyte counts and mortality rates provided insight into the pathogenesis of CCHF. Receiving operating curve analysis revealed that leukocyte counts $\geq 2,950/\text{mm}^3$ on the day of admission predicted mortality rate with 62.1% sensitivity. The mean hospitalization stay in patients with fatal disease was 4.3 days; therefore, leukocyte counts were compared on the day of admission and day 3 of the hospital stay. Increases in neutrophil levels and decreases in lymphocyte and monocyte levels were identified as significant risk factors for mortality ($P = 0.01, 0.037$, and 0.001 , respectively). The mortality risk was 7–12 fold higher in patients whose levels of leukocytes ($2,950 \mu\text{L}$), lactate dehydrogenase (967.5 U/L), and alanine aminotransferase ($> 119.5 \text{ U/L}$) and activated partial thromboplastin time (42.4 s) exceeded the cut-off values; these were identified as independent predictors of mortality. Depletion of monocytes and lymphocytes and accumulation of neutrophils correlated with poor outcome. These results highlight the importance of the mononuclear immune response for the survival of patients with CCHF.

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 range from 5 to 30% 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 2 days later (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

Received December 10, 2014. Accepted April 6, 2015.
J-STAGE Advance Publication June 12, 2015.

DOI: 10.7883/yoken.JJID.2014.566

*Corresponding author: Mailing address: Ankara Numune Training and Research Hospital, Department of Infectious Diseases and Clinical Microbiology, Anafartalar Mh., Talatpaşa Bulvarı No:5, post code: 06030 Altındağ/Ankara, Turkey. Tel: +9 05056814223, Fax: +9 0312 5084838, E-mail: dr.aliye@yahoo.com

using SPSS 20.0 software for Windows. Descriptive statistics are presented as mean, standard deviation, median, minimum, and maximum for quantitative variables, and as number and percentage for categorical variables. For numerical comparisons of paired independent groups, t-test was used when distribution was normal, and Mann-Whitney U test was used when it was not. For numerical comparisons of paired dependent groups, the paired t-test was used when distribution was normal, and the Wilcoxon signed-rank test was used when it was not. The chi-square test and Fisher's exact test were used for categorical comparisons of paired groups. Receiver operating curve (ROC) analysis was performed to determine cut-off values. Logistic regression analysis was performed to identify the risk factors for categorical variables. The statistical level of significance was set at $P < 0.05$.

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-

Table 1. Demographical, epidemiological and clinical characteristics of patients with CCHF

Characteristic	Total case (%) <i>n</i> = 220	Non-fatal case (%) <i>n</i> = 184	Fatal case (%) <i>n</i> = 36	<i>P</i> -value
Age (yr, mean) \pm SD	50.2 \pm 17.0	49.4 \pm 17.2	54.0 \pm 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
Bite/contact history with tick	140 (63.6)	119 (64.7)	21 (58.3)	0.470
Time from tick bite to onset of symptoms (days)	3.8 \pm 3.0	3.8 \pm 2.9	4.0 \pm 3.9	0.765
Duration of symptoms before hospitalization (days)	5.0 \pm 3.3	5.0 \pm 3.4	4.8 \pm 2.4	0.949
Length of hospital stay (days)	6.8 \pm 3.5	7.3 \pm 3.3	4.3 \pm 3.0	<0.001
Symptoms				
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
Clinical findings				
Fever, temperature $> 38^{\circ}\text{C}$	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
Splenomegaly	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

toma, and infusions (fresh, previously frozen plasma; platelets; and erythrocytes) ($P < 0.001$ for all variables) (Tables 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 (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). Leukocyte 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 $\geq 2,950/\text{mm}^3$ 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 ($> 2,950/\mu\text{L}$), ALT ($> 119.5 \text{ U/L}$),

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) (median, min-max)	Fatal case (n) (median, min-max)	P-value
WBC ($/\mu\text{L}$)	2,300 (200–16,500)	3,500 (700–23,900)	0.006
Leukocytosis ($> 10,800/\mu\text{L}$) (n, %)	1 (0.5)	4 (11.1)	< 0.001
Leukopenia ($< 4,800/\mu\text{L}$) (n, %)	161 (88.0)	23 (63.9)	< 0.001
Neutrophils ($/\mu\text{L}$)	1,400 (130–9,000)	2,550 (400–21,200)	0.001
Lymphocytes ($/\mu\text{L}$)	600 (0–8,000)	700 (100–6,000)	0.499
Monocytes ($/\mu\text{L}$)	200 (0–1,600)	100 (0–1,000)	0.142
Platelets ($/\mu\text{L}$)	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 (8–1,278)	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		
	Non-fatal case (median, range)	Fatal case (median, range)	P-value	Non-fatal case (median, range)	Fatal case (median, range)	P-value
WBC ($/\mu\text{L}$)	2,300 (200–16,500)	3,500 (700–23,900)	0.006	2,900 (500–24,900)	3,400 (1,100–11,600)	0.495
Neutrophil ($/\mu\text{L}$)	1,400 (130–9,000)	2,550 (400–21,200)	0.001	1,200 (110–14,300)	1,950 (400–10,100)	0.01
Lymphocyte ($/\mu\text{L}$)	600 (0–8,000)	700 (100–6,000)	0.499	1,100 (200–16,000)	720 (100–3,140)	0.037
Monocyte ($/\mu\text{L}$)	200 (0–1,600)	100 (0–1,000)	0.142	500 (0–2,300)	200 (0–1,300)	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		
	First day (median, range)	Third day (median, range)	P-value	First day (median, range)	Third day (median, range)	P-value
WBC ($/\mu\text{L}$)	2,300 (200–6,500)	2,900 (500–24,900)	< 0.001	3,500 (700–23,900)	3,400 (1,100–11,600)	0.431
Neutrophil ($/\mu\text{L}$)	1,400 (130–9,000)	1,200 (110–14,300)	0.043	2,550 (400–21,200)	1,950 (400–10,100)	0.141
Lymphocyte ($/\mu\text{L}$)	600 (0–8,000)	1,100 (200–16,000)	< 0.001	700 (100–6,000)	720 (100–3,140)	0.083
Monocyte ($/\mu\text{L}$)	200 (0–1,600)	500 (0–2,300)	< 0.001	100 (0–1,000)	200 (0–1,300)	0.022

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

	Level ¹⁾	Sensitivity	Specificity	PPV	NPV	AUROC	P-value
WBC (/μL)	≥2,950	62.1	33.1	26.9	90.0	0.662	0.006
Neutrophils (/μL)	≥1,760	62.1	36.5	25.0	89.5	0.695	0.001
ALT (U/L)	>119.5	75.0	34.5	30.7	92.8	0.751	<0.001
AST (U/L)	>304.5	77.1	27.7	35.5	94.1	0.821	<0.001
LDH (U/L)	>967.5	75.0	16.7	46.2	94.6	0.828	<0.001
CPK (U/L)	>443.5	71.0	44.3	23.9	90.7	0.709	<0.001
aPTT (s)	>42.4	88.2	17.9	50.8	97.1	0.914	<0.001
PT (s)	>14.0	67.6	20.4	41.8	91.9	0.786	<0.001
INR	>1.12	64.7	22.7	37.3	91.3	0.714	<0.001

¹⁾ Normal range of laboratory parameters; WBC (4,800–10,800), 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

	Odds ratio	95% CI	P-value
In admission:			
WBC > 2,950/μL	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 than 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

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 significantly increased in the nonfatal group, but not in the fatal group. This finding suggests that leukocyte, lymphocyte, and monocyte levels return to normal levels in patients with nonfatal disease, but do not improve in patients with fatal disease. The cut-off levels of 4 laboratory parameters (leukocytes >2,950 μL, ALT >119.5 U/L, LDH >967.5 U/L, and aPTT >42.4 s) may be useful for predicting the prognosis of CCHF. The risk of mortality may be 7–12 fold higher in patients whose values for these parameters exceed the cut-off levels, which were identified as independent predictors of mortality in the present study.

In conclusion, our study showed that, in addition to

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.

REFERENCES

1. Bishop DH. Biology and molecular biology of bunyaviruses. In: Elliot RM. editor. *The Bunyaviridae*. New York: Plenum Press; 1996. p. 19-61.
2. Sisman A. Epidemiologic features and risk factors of Crimean-Congo hemorrhagic fever in Samsun province, Turkey. *J Epidemiol* 2013;23:95-102.
3. Bodur H, Akinci E, Ongürü P, et al. Detection of Crimean-Congo hemorrhagic fever virus genome in saliva and urine. *Int J Infect Dis*. 2010;14:e247-9.
4. Adam IA, Mahmoud MAM, Aradaib IE. A seroepidemiological survey of Crimean Congo hemorrhagic fever among cattle in North Kordufan State, Sudan. *Virology J*. 2013;10:178.
5. Leblebicioglu H. Crimean-Congo haemorrhagic fever in Eurasia. *Int J Antimicrob Agents*. 2010;36:S43-6.
6. Bodur H, Akinci E, Ascioglu S, et al. Subclinical infections with Crimean-Congo hemorrhagic fever virus, Turkey. *Emerg Infect Dis*. 2012;18:640-2.
7. Fisgin NT, Fisgin T, Tanyel E, et al. Crimean-Congo hemorrhagic fever: five patients with hemophagocytic syndrome. *Am J Hematol*. 2008;83:73-6.
8. Akinci E, Bodur H, Leblebicioglu H. Pathogenesis of Crimean-Congo hemorrhagic fever. *Vector Borne Zoonotic Dis*. 2013;13:429-37.
9. Yilmaz GR, Buzgan T, Irmak H, et al. The epidemiology of Crimean-Congo hemorrhagic fever in Turkey, 2002–2007. *Int J Infect Dis*. 2009;13:380-6.
10. Whitehouse CA. Crimean-Congo hemorrhagic fever. *Antiviral Res*. 2004;64:145-60.
11. Leblebicioglu H, Bodur H, Dokuzoguz B, et al. Case management and supportive treatment for patients with Crimean-Congo hemorrhagic fever. *Vector Borne Zoonotic Dis*. 2012;12:805-11.
12. Elaldi N, Bodur H, Ascioglu S, et al. Efficacy of oral ribavirin treatment in Crimean-Congo haemorrhagic fever: a quasi-experimental study from Turkey. *J Infect*. 2009;58:238-44.
13. Cevik MA, Erbay A, Bodur H, et al. Clinical and laboratory features of Crimean-Congo hemorrhagic fever: predictors of fatality. *Int J Infect Dis*. 2008;12:374-9.
14. Swanepoel R, Gill DE, Shepherd AJ, et al. The clinical pathology of Crimean-Congo hemorrhagic fever. *Rev Infect Dis*. 1989;11: S794-800.
15. Iowa State University: The Center for Food Security and Public Health. Crimean-Congo hemorrhagic fever. Available at <http://www.cfsph.iastate.edu/Factsheets/pdfs/crimean_congo_hemorrhagic_fever.pdf> Accessed August 29, 2009.
16. Duran A, Küçükbaşrak A, Ocak T, et al. Evaluation of patients with Crimean-Congo hemorrhagic fever in Bolu, Turkey. *Afr Health Sci*. 2013;13:233-42.
17. Yilmaz GR, Buzgan T, Torunoglu MA, et al. A preliminary report on Crimean-Congo haemorrhagic fever in Turkey, March–June 2008. *Euro Surveill*. 2008;13:pii:18953.