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Analysis of risk-factors among patients with Crimean-Congo haemorrhagic fever virus infection: severity criteria revisited

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ABSTRACT

The aim of this study was to determine the predictors of mortality among patients infected with Crimean-Congo haemorrhagic fever (CCHF) virus. Among patients with acute febrile syndrome, characterised by malaise, bleeding, leukopenia and thrombocytopenia, who were admitted to hospital during the spring and summer of 2002–2004, 54 had positive IgM and/or PCR results for CCHF virus in blood or tissue. The overall case fatality rate was 7.4%. Among the fatalities, haematemesis (p 0.009), melaena (p 0.001) and somnolence (p 0.022) were more common, the median platelet count was significantly lower (10 600/mL vs. 20 000/mL; p 0.038), the mean prothrombin time (27 s vs. 16 s; p 0.002) and mean activated partial thromboplastin time (73 s vs. 44 s; $p < 0.001$) were longer, and the mean alanine transferase (ALT) level (1125 vs. 331; $p < 0.001$), the mean aspartate transferase (AST) level (3118 vs. 913; p 0.004) and the mean fibrinogen level (119 vs. 340; p 0.012) were higher. Serum IgM and IgG against CCHF virus was detected in 25% and 0%, respectively, of fatal cases, compared with 94% and 62%, respectively, of cases with favourable outcomes. Oral ribavirin was prescribed to 22 (41%) patients. Of the four fatal cases, it was the intention to prescribe ribavirin to three patients, but this was not possible because of haematemesis and melaena. Higher levels of AST (≥ 700 U/L) and ALT (≥ 900 U/L) are suggested for use as severity criteria. Oral ribavirin was not effective for patients with haematemesis, and intravenous ribavirin is necessary for treatment of CCHF.

Keywords Crimean-Congo haemorrhagic fever virus, diagnosis, ribavarin, risk-factors, severity criteria

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INTRODUCTION

Crimean-Congo haemorrhagic fever (CCHF) is a fatal viral infection described in parts of Africa, Asia, Eastern Europe and the Middle East [1]. The virus belongs to the genus *Nairovirus* in the Bunyaviridae family, and causes severe disease in humans, with a reported mortality rate of 15–30% [2]. Humans become infected through the bites of ticks, by contact with a patient with CCHF during the acute phase of infection, or by contact with blood or tissues from viraemic livestock [3].

Cases infected with CCHF virus (CCHFV) were first reported in Turkey in 2002 [4–6], although epidemics were reported before 2003 in neighbouring countries. CCHFV infection is an important public health issue in Turkey because of its high case fatality rate. Since 2002, 399 cases of CCHF have been reported to the Ministry of Health of Turkey, and 19 (4.7%) of these cases died. The present study analysed the risk-factors of 54 CCHF patients, based on epidemiological, clinical and laboratory findings. Based on this analysis, the predictors of fatality criteria, defined previously by Swanepoel *et al.* [7] in 1989, were reassessed.

MATERIALS AND METHODS

Patients with acute febrile syndrome, characterised by malaise, bleeding, leukopenia and thrombocytopenia, during the spring and summer of 2002, 2003 and 2004 were admitted to the Infectious Diseases and Clinical Microbiology Clinic of Ankara Numune Education and Research Hospital. Patients with positive IgM and/or PCR results for CCHFV in blood or

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tissue were included in the study. Recent or current CCHFV infection was confirmed by demonstrating seroconversion, or at least a four-fold increase in antibody titre in paired serum samples, or IgM antibodies with ELISA in a single sample [8]. ELISA methods are quite specific and much more sensitive than immunofluorescence and neutralisation tests [9]. RT-PCR is the method of choice for rapid laboratory diagnosis of CCHFV infection [10].

The presence of antibodies to *Leptospira*, Lyme disease, *Salmonella*, rickettsiae, *Brucella*, *Coxiella burnetii*, *Toxoplasma*, rubella virus, cytomegalovirus, herpes virus and hepatitis A, B and C viruses was investigated by serology. Acute and convalescent sera from all the acute cases were sent to the Refik Saydam Hygiene Center of Ankara, Turkey for ELISA and PCR tests [8,10]. Laboratory parameters were measured on a daily basis following hospital admission.

The patients were given erythrocytes, fresh frozen plasma and total blood preparations, depending on the state of their haemostasis. The patient characteristics were assessed according to the 90% fatality outcome criteria described by Swanepoel *et al.* [7]. According to these criteria, patients were defined as 'severe' when they had white blood cell (WBC) counts $\geq 10\ 000/mm^3$, platelet counts (PLT) $\leq 20\ 000/mm^3$, aspartate transferase (AST) levels ≥ 200 U/L, alanine transferase (ALT) levels ≥ 150 U/L, activated partial thromboplastin times (aPTT) ≥ 60 s, or fibrinogen levels ≤ 110 mg/dL during the first 5 days of illness. Various threshold levels were investigated with the aim of achieving higher sensitivity, specificity, positive and negative predictive values.

Mean comparisons for continuous variables were performed using independent group *t*-tests. Proportion comparisons for categorical variables were performed using chi-square tests, with Fisher's exact test being used when data were sparse. A multivariate analysis was performed for the prediction of fatality. Physical findings, haematemesis, melaena, somnolence, and the laboratory tests (AST, ALT, PLT, WBC count, lactate dehydrogenase (LDH), creatine phosphokinase (CPK), prothrombin (PT), aPTT and fibrinogen) were included in the model as independent variables. Significance was set at $p < 0.05$ using two-sided comparisons. The STATA v.8.0 software package (Stata Corp., College Station, TX, USA) was used for the analyses.

Results of diagnostic tests were not available for up to 4 weeks; therefore, all ribavirin therapy was initiated before laboratory evidence of CCHF infection. Oral ribavirin was administered, within a mean 5.5 days following the onset of symptoms, at the dosage recommended by the WHO (4 g four times daily for 4 days; 2.4 g four times daily for 6 days) [11]. The intravenous form of ribavirin was not available in Turkey at this time. Written consent was obtained from the patients or their family members.

RESULTS

Fifty-four patients were admitted from various regions of north-eastern Anatolia and the southern parts of the Black Sea region; all of these patients were involved in animal husbandry. Four (7.4%) cases were fatal with massive haemorrhage. The age, gender and number of days from the appearance of symptoms to admission were similar between favourable and fatal cases ($p > 0.05$). Fever lasted for an average 4.3 (± 2.5) days. Among the clinical findings, haematemesis (p 0.009), melaena (p 0.001) and somnolence (p 0.022) were more common among fatal cases (Table 1).

Forty-eight (89%) of the 54 patients were IgM-positive, 31 (57%) were IgG-positive during the acute or convalescent phase, and 22 (41%) patients were PCR-positive (Table 2).

All the patients had leukopenia, thrombocytopenia, and elevated AST, ALT, LDH and CPK levels (Table 3). On admission, 31 patients had thrombocytopenia of $\leq 20\ 000/mm^3$, 45 patients had AST levels ≥ 200 U/L, 40 patients had ALT levels ≥ 150 U/L, 16 patients had aPTT of > 60 s. The fibrinogen level of one patient was ≤ 110 mg/dL. None of the patients had leukocytosis, with the exception of one fatal case whose leukocyte count increased to $12\ 000/mm^3$ on the final day (day 10 since the onset of disease). Among the fatal cases, the median PLT was significantly lower (10 600/mL vs. 20 000/mL; p 0.038), the mean PT (27 s vs. 16 s; p 0.002) and

Table 1. Demographic characteristics of 54 patients with Crimean-Congo haemorrhagic fever

	Favourable cases $n = 50$ (%)	Fatal cases $n = 4$ (%)	p
Number of females	26 (52)	2 (50)	0.939
Mean age (SD), years	43 (18)	54 (11)	0.280
Days from symptoms to admission	6	4	0.074
Symptoms			
Nausea-vomiting	41 (82)	4 (100)	0.471
Myalgia	31 (62)	4 (100)	0.285
Fever	38 (76)	4 (100)	0.576
Headache	35 (70)	3 (75)	0.684
Physical findings			
Fever (Temperature > 38 °C)	21 (42)	2 (50)	0.755
Bleeding			
Haematemesis	13 (26)	4 (100)	0.009
Melaena	7 (14)	4 (100)	0.001
Epistaxis	20 (40)	3 (75)	0.301
Haemoptysis	4 (8)	1 (25)	0.341
Haematuria	8 (16)	0 (0)	0.509
Maculopapular rash	15 (30)	1 (25)	0.640
Conjunctival injection	21 (42)	1 (25)	0.638
Somnolence	17 (34)	4 (100)	0.022
Jaundice	5 (10)	1 (33)	0.330
Diarrhoea	17 (34)	2 (50)	0.607
Hepatomegaly	17 (35)	0 (0)	0.293

SD, standard deviation.

Table 2. Serology and PCR results for 54 patients with Crimean-Congo haemorrhagic fever

	Favourable cases $n = 50$ (%)	Fatal cases $n = 4$
IgM-positive	47 (94)	1 (25)
IgG-positive	31 (62)	0 (0)
PCR-positive	19 (48)	3 (75)

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Table 3. Laboratory findings for 54 patients with Crimean-Congo haemorrhagic fever

	Favourable cases Mean (min–max) $n = 50$	Fatal cases Mean (min–max) $n = 4$	p
Longest prothrombin time, s	16 (10–42)	27 (18–33)	0.002
Longest activated partial thromboplastin time, s	44 (25–74)	73 (55–92)	< 0.001
Lowest platelet count, platelets/mm^3	27 000 (3500–108 000)	11 000 (6000–15 000)	0.038
Lowest WBC, WBCs/mm^3	1880 (150–13 000)	1800 (1200–3000)	0.595
Highest alanine transferase level, U/L	331 (38–1443)	1125 (219–1785)	< 0.001
Highest aspartate transferase level, U/L	913 (65–7150)	3200 (773–7700)	0.004
Highest lactic dehydrogenase level, U/L	2545 (283–26 000)	4019 (1980–9480)	0.478
Highest creatinine phosphokinase level, U/L	1277 (10–21 189)	1823 (1388–2164)	0.736
Lowest fibrinogen level	340 (165–780)	119 (86–140)	0.012

WBC, white blood cell count.

mean aPTT were longer (73 s vs. 44 s; p < 0.001), the mean ALT (1125 vs. 331; p < 0.001) and the mean AST (3118 vs. 913; p 0.004) were higher, and the mean fibrinogen level was lower (119 vs. 340; p 0.012) (Table 3).

The components of severity criteria defined by Swanepoel *et al*. [7] were reassessed (Table 4). Higher AST levels (≥ 700 U/L instead of ≥200 U/L) predicted fatality with greater sensitivity, specificity, positive and negative predictive values. Higher ALT levels (≥ 900 U/L instead of 150 U/L) increased the test performance, with the exception of positive predictive value (Table 5). The AST/ALT ratio was higher among severe cases than among mild-moderate cases for the first 3 days following admission (p 0.022, 0.003 and 0.047, respectively). In multivariate analysis, an increased ALT was associated with a fatal outcome (OR 1.003; 95% CI 1.001–1.005; p 0.007). LDH and CPK levels among severe cases were higher than among mild-moderate cases, although this was not statistically significant (p > 0.05). Laboratory tests, including complete blood count, and biochemical tests returned to normal levels within 5–9 days.

Oral ribavirin was prescribed for 22 (41%) of 45 severe cases. In three of the four fatal cases, it was the intention to prescribe oral ribavirin, but these patients had haematemesis and melaena, and their length of stay in the clinic was limited to 12, 36 and 48 h, respectively. Fatalities received significantly more fresh frozen plasma (p 0.001) and thrombocyte suspensions (p < 0.001) than patients with favourable outcomes. No adverse events related to ribavirin therapy were noted.

Table 4. Predictors of fatality among 54 patients with Crimean-Congo haemorrhagic fever

Criteria	Swanepoel *et al.* [7]	Present study	p
Increased WBC count ≥ 10 000/mm^3	Yes	No	0.595
Decreased platelet count	Yes	Yes	0.038
Elevated aspartate transferase level	Yes	Yes	0.004
Elevated alanine transferase level	Yes	Yes	< 0.001
Prolonged activated partial thromboplastin time	Yes	Yes	< 0.001
Prolonged thromboplastin time	No	Yes	0.002
Decreased fibrinogen ≤110 mg/dL	Yes	Yes	0.012
Melaena	No	Yes	0.001
Haematemesis	No	Yes	0.009
Somnolence	No	Yes	0.022

Table 5. Aspartate transferase (AST) and alanine transferase (ALT) levels used as severity criteria (95% CI)

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
AST				
≥ 200 U/L, Swanepoel *et al.* [7]	9	100	100	18
≥ 700 U/L, alternative	16	100	100	58
ALT				
≥ 150 U/L, Swanepoel *et al.* [7]	10	100	100	28
≥ 900 U/L, alternative	43	98	75	92

DISCUSSION

CCHFV infection causes a fatal haemorrhagic syndrome, which is a leading threat to public health in endemic countries. Ribavirin is the drug of choice as an antiviral agent for infected patients [7,12]. However, use of ribavirin remains uncommon for several reasons. First, ribavirin use has not been tested in randomised clinical trials, since ethical constraints do not allow such a study design, although its effectiveness has been described in observational studies [4,11,13]. Second, a decision concerning which CCHFV-infected patients should be given ribavirin may be difficult. Since severe cases need to be treated [4], categorisation of the patients according to the severity of the infection is an essential first step in deciding whether to initiate antiviral therapy [4].

The severity criteria defined by Swanepoel *et al*. [7] did not fit perfectly to the patients in the present study. In particular, AST and ALT levels were found to be higher among fatal cases in the present study, and higher threshold values for

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defining severity are suggested (Table 5). Leukocytosis was one of the criteria described by Swanepoel *et al*. [7], but was observed only in one patient, who died on day 8 following admission. Further observations with larger numbers of patients are necessary to clarify this discrepancy. In contrast, an aPTT of >60 s was found to be compatible with the observations in the present study. Since the mean time for hospital admission was 5 days among patients in the present study, measurement 'on admission', instead of for the first 5 days of illness, was practical and appropriate.

In a separate study, a high AST/ALT ratio was suggested as a prognostic factor for severity among cases of CCHF, and the same parameter was documented as a prognostic factor of fatality [4]. In multivariate analysis, an increase in ALT was associated with fatality (OR 1.003; 95% CI 1.001–1.005; p 0.007). An increase in ALT as the disease progresses could be an indicator of liver failure.

No serum IgM or IgG was detected for three of the fatal cases, although the virus was detected by PCR (Table 2). The absence of a significant antibody response in fatalities has been reported previously [7]. Twenty-one patients (including all the fatal cases) became stuporous, despite vigorous supportive therapy, as reported previously in another study from Turkey [6]. However, in contrast to Swanepoel *et al*. [7], no renal failure was observed.

There was an intention to treat three of the four fatal cases with oral ribavirin (there is no parenteral form of ribavarin available currently in Turkey). All the fatal cases had haematemesis and melaena, and, except for one patient, stayed at the clinic for only 12, 36 and 48 h, respectively. The bleeding sites and the limited total stay period hampered the effectiveness of the drug. Evaluation studies for ribavarin should take into account the severity of the patients, the period from the onset of disease, gastrointestinal symptoms such as haematemesis, and the duration of treatment. No complications following use of ribavirin were noted.

In conclusion, higher AST (≥ 700 U/L) and ALT (≥ 900 U/L) levels are suggested for inclusion in a revision of the severity criteria for CCHF. Oral ribavirin is not effective for patients with haematemesis, and intravenous ribavirin is necessary for the treatment of CCHFV infection.

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