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C-type natriuretic peptide is associated with the severity of Crimean-Congo hemorrhagic fever

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SUMMARY

Background: Crimean-Congo hemorrhagic fever (CCHF) is characterized by vascular dysfunction, indicating the involvement of endothelial cells. C-type natriuretic peptide (CNP) plays a critical role in the coordination of vascular tone and is associated with the prognosis in critically ill patients such as those with sepsis and septic shock. We investigated whether CNP is related to the severity of CCHF.
Methods: Forty-eight consecutive patients with a laboratory confirmed diagnosis of CCHF and 40 age–sex-matched healthy volunteers as the control group were prospectively enrolled into the study. CCHF patients were classified according to the disease severity into a non-severe group ($n = 28$) and a severe group ($n = 20$).
Results: The CNP levels were detected to be 0.43 (0.4–0.7) ng/ml in the control group, 0.87 (0.7–1.0) ng/ml in the non-severe CCFH group, and 1.27 (0.8–1.7) ng/ml in the severe CCHF group. According to the receiver operating characteristics curve analysis, the optimal cut-off value of CNP to predict disease severity was >1.22 ng/ml, with 89.3% specificity and 55% sensitivity. CNP >1.22 ng/ml, lactate dehydrogenase >480 IU/l, and aspartate aminotransferase >202 IU/l were found to have prognostic significance in the univariate analysis. In the multivariate logistic regression analysis by forward stepwise method, CNP >1.22 ng/ml (odds ratio 8.336, $p = 0.016$) and lactate dehydrogenase >480 IU/l (odds ratio 16.206, $p = 0.002$) remained associated with disease severity after adjustment for confounding variables.
Conclusions: CNP measurement could help in the risk stratification of patients with CCHF.

1. Introduction

Crimean-Congo hemorrhagic fever (CCHF) is a zoonotic viral disease caused by a tick-borne virus of the genus *Nairovirus*.[1] CCHF has a fatality rate of 3–30% of cases,[2] and the pathogenesis of the disease is not well understood. Endothelial damage has an important role in the pathogenesis of CCHF.[3] In severe cases, death occurs as a result of disseminated intravascular coagulation, multiorgan failure, and circulatory shock. Systemic inflammatory reactions occur during hemorrhagic manifestations.[4,5]

C-type natriuretic peptide (CNP) is a member of the family of natriuretic peptides.[6] It is mostly produced by endothelial cells,[7] and it plays a critical role in the coordination of vascular tone.[8] CNP release in response to proinflammatory cytokines suggests an interaction of macrophageal cytokine synthesis and vascular endothelium.[9] Recent studies have found that CNP and the N-terminal pro-peptide of CNP (NT-proCNP) are increased in critically ill patients such as those with sepsis and septic shock.[10,11]

The potential use of CNP in the risk stratification of patients with CCHF has not been previously studied. We investigated whether CNP is related to the severity of CCHF.

2. Methods

Forty-eight consecutive patients with a laboratory confirmed diagnosis of CCHF and 40 age–sex-matched healthy volunteers as the control group were prospectively enrolled in the study at Cumhuriyet University School of Medicine between January and October 2011. Informed consent was obtained from all study participants. The diagnosis of CCHF was confirmed by positive qualitative PCR test in the Virology Laboratory of the Refik Saydam National Hygiene Center of the Turkish Ministry of Health. The TaqMan-based one-step reverse transcriptase PCR assay was used to detect CCHF viral RNA.[12]

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All confirmed CCHF patients were classified into two groups based on disease severity (severe (n = 20) and non-severe (n = 28)), as previously reported by Cevik et al.[13] Patients with at least one of the following were considered severe cases: somnolence, melena, activated partial thromboplastin time (APTT) ≥60 s, and thrombocyte count ≤20 × 10^9 cells/l. Data collected, per protocol, included age, gender, duration of symptoms, history of hypertension, diabetes mellitus, and smoking, admission CNP, biochemical and coagulation parameters, and complete blood count. Of note, only admission parameters were considered in this study (first ever obtained parameter, within 15 min of admission). History of hypertension was defined as a previous blood pressure >140/90 mmHg on more than two occasions during office measurements, or having received antihypertensive treatment. Diabetes mellitus was defined as a previous fasting blood glucose ≥126 mg/dl, or having received antidiabetic treatment.

Blood sampling was performed as soon as a venous and/or arterial line was placed in all patients. The 10-ml blood samples were centrifuged in gel-containing vacuumed biochemistry tubes at 400 rpm for 4 min and the serum was stored in Eppendorf tubes at −23 °C. Serum CNP levels were measured by CNP-22 (human, rat, mouse, porcine) enzyme immunoassay (EIA) kit, based on standard sandwich ELISA technology.

2.1. Statistical analysis

Continuous variables were expressed as the mean ± standard deviation, or median (interquartile range) in the presence of an abnormal distribution, and categorical variables as percentages. SPSS 14.0 (SPSS, Inc., Chicago, IL, USA) was used to perform the statistical procedures. Receiver operating characteristic (ROC) curve analysis was performed to identify the optimal cut-off point of CNP, lactate dehydrogenase (LDH), and aspartate aminotransferase (AST) (at which sensitivity and specificity would be maximal) for the prediction of disease severity. Areas under the curve (AUC) were calculated as measures of the accuracy of the tests. We compared the AUC with the use of the Z-test. Comparisons between groups were performed using one-way analysis of variance (ANOVA) with post-hoc analysis by Tukey's HSD, or an independent samples t-test, and the Kruskal–Wallis test or Mann–Whitney U-test for normally and non-normally distributed data, respectively. Categorical variables were analyzed between groups using the Chi-square test. We used univariate analysis to quantify the association of variables with disease severity. Variables found to be statistically significant in the univariate analysis, as well as potential confounders (age, gender, and duration of symptoms), were used in a multivariate logistic regression model with forward stepwise method in order to determine the independent prognostic factors of disease severity. A p-value of 0.05 was considered statistically significant.

3. Results

The CNP levels were detected to be 0.43 (0.4–0.7) ng/ml in the control group, 0.87 (0.7–1.0) ng/ml in the non-severe CCHF group, and 1.27 (0.8–1.7) ng/ml in the severe CCHF group. CNP levels were observed to be significantly higher in patients with severe CCHF compared to those with non-severe CCHF and the control group (p = 0.007 and $p < 0.001$, respectively). In addition, those with non-severe CCHF were also found to have a significantly higher CNP level relative to the control group ($p < 0.001$) (Table 1, Figure 1).

A comparison of the baseline characteristics of patients in the non-severe and severe CCHF groups is presented in Table 1. Serum levels of AST and LDH were significantly higher in the severe cases than in the non-severe cases. There was no significant difference between the two groups in terms of other clinical and laboratory parameters (Table 1).

According to the ROC curve analysis, the optimal cut-off value of CNP to predict disease severity was >1.22 ng/ml, with 89.3% specificity and 55% sensitivity (AUC 0.732, 95% confidence interval 0.585–0.850; Figure 2). In addition, the optimal cut-off value of LDH to predict disease severity was >480 IU/l, with 85% sensitivity and 67.9% specificity, and of AST was >202 IU/l, with 70% sensitivity and 75% specificity.

Results of the univariate and multivariate logistic regression analyses for the prediction of severe CCHF are listed in Table 2. CNP >1.22 ng/ml, LDH >480 IU/l, and AST >202 IU/l were found to have prognostic significance. In the multivariate logistic regression model with forward stepwise method, increased CNP >1.22 ng/ml

Table 1
Baseline characteristics of study groups

	Control group (n = 40)	Non-severe CCHF (n = 28)	Severe CCHF (n = 20)	p-Value
Study marker				
C-type natriuretic peptide (ng/ml)	0.43 (0.4–0.7)	0.87 (0.7–1.0)	1.27 (0.8–1.7)	0.007
Baseline characteristics				
Age (years)	54 ± 14	51 ± 10	54 ± 9	0.592
Male/female	19/21	15/13	12/8	0.650
Hypertension		6 (21%)	8 (40%)	0.165
Diabetes mellitus		4 (14%)	3 (15%)	1.000
Smoking		7 (25%)	9 (45%)	0.148
Duration of symptoms (days)		2.4 ± 1.3	2.8 ± 1.9	0.436
Biochemical tests				
BUN (mg/dl)		17 ± 7	17.4 ± 10	0.859
Creatinine (mg/dl)		0.9 ± 0.2	1.07 ± 0.8	0.891
ALT (IU/l)		62 (32–111)	73 (37–109)	0.565
AST (IU/l)		149 (69–211)	251 (139–381)	0.011
LDH (IU/l)		390 (306–577)	1004 (512–1574)	<0.001
Coagulation tests				
Prothrombin time (s)		13.5 ± 3	13.8 ± 3.7	0.721
APTT (s)		40 (37–52)	62 (55–89)	<0.001
INR		1.2 ± 0.2	1.3 ± 0.3	0.639
Complete blood count				
WBC (× 10^9 cells/l)		3.1 ± 2.9	3.6 ± 2.9	0.573
Hemoglobin (g/dl)		14.1 ± 1.7	14.4 ± 2.1	0.624
Thrombocyte count (× 10^9 cells/l)		69 (45–102)	24 (18–46)	<0.001

Results are n (%), mean ± standard deviation, or median (interquartile range).
CCHF, Crimean-Congo hemorrhagic fever; BUN, blood urea nitrogen; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; APTT, activated partial thromboplastin time; INR, international normalized ratio; WBC, white blood cell.

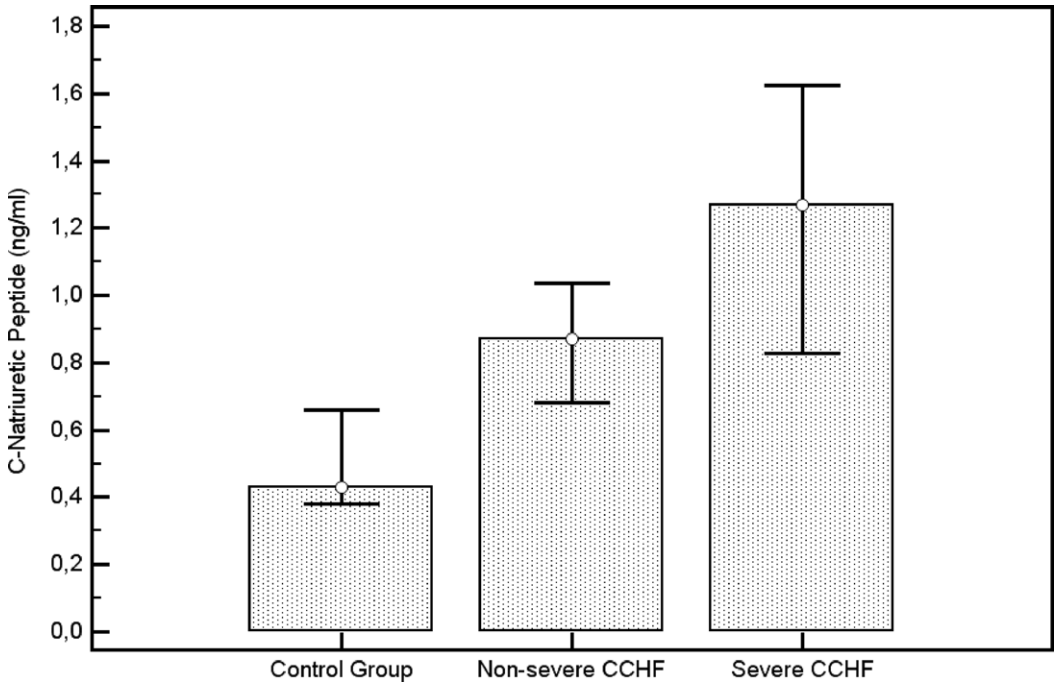

Figure 1. Comparison of C-type natriuretic peptide levels (median (interquartile range)) between the three groups.

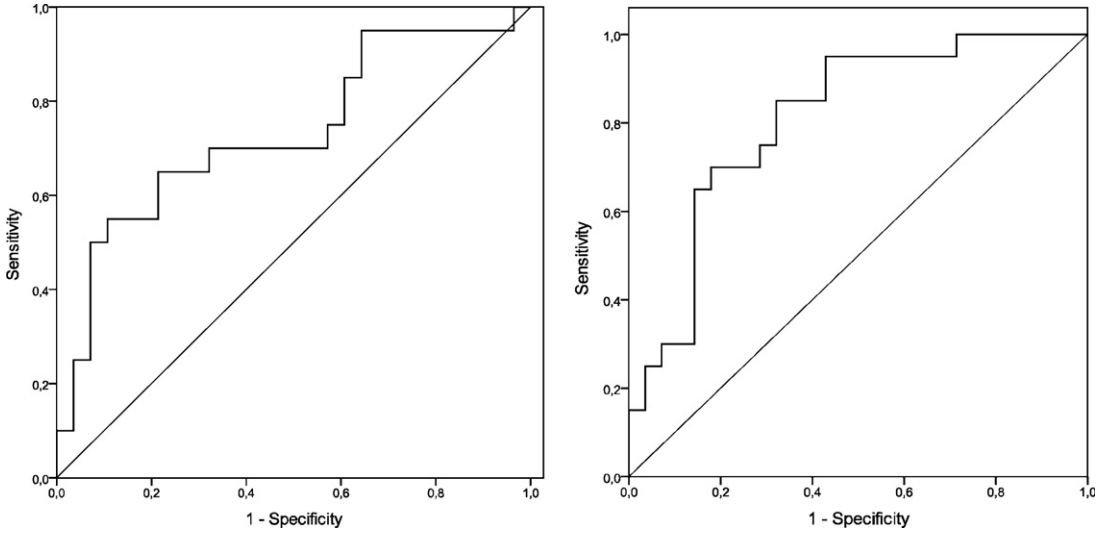

Figure 2. ROC curve analysis for C-type natriuretic peptide (AUC 0.732, 95% CI 0.585–0.850) and lactate dehydrogenase (AUC 0.809, 95% CI 0.670–0.908).

and LDH >480 IU/l remained associated with an increased risk of the development of severe CCHF after adjustment for other potential confounders (age, gender, and duration of symptoms) and variables found to be statistically significant in the univariate analysis (Table 2).

4. Discussion

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

Previous studies have reported that decreased levels of thrombocytes and fibrinogen, and increased levels of white blood cells, AST, alanine aminotransferase (ALT), creatine kinase, LDH, and soluble urokinase plasminogen activator receptor, as well as a prolonged APTT, are associated with a poor outcome in CCHF patients.[3,14–16] Ozturk et al. and Bodur et al. have shown that the level of hyaluronic acid, sICAM-1 (soluble intercellular adhesion molecule 1), sVCAM-1 (soluble vascular cell adhesion molecule 1), and VEGF-A (vascular endothelial growth factor A) can be used as a prognostic marker in CCHF.[17,18] Furthermore, the severe form of CCHF is characterized by hemorrhage, disseminated intravascular coagulation, vascular dysfunction, and shock.[15,19]

Recent studies have shown that serum CNP and NT-proCNP levels are increased in sepsis, septic shock, and any critically ill patients, conditions characterized by inadequate tissue perfusion.[10,11,20] Vlachopoulos et al. found that NT-proCNP is associated with arterial stiffness, endothelial function, and early atherosclerosis.[21] In a global arterial approach, their study demonstrated a relationship

Table 2
Univariate and multivariate analyses of severe CCHF

	Univariate analysis			Multivariate analysis[a]		
	p-Value	OR	95% CI	*p*-Value	OR	95% CI
C-type natriuretic peptide >1.22 ng/ml	0.010	5.622	1.520–20.799	0.016	8.336	1.495–46.496
LDH >480 IU/l	0.001	11.963	2.775–51.580	0.002	16.206	2.871–91.477
AST >202 IU/l	0.003	7.000	1.940–25.255			
Age (years)	0.320	1.032	0.970–1.099			
Gender	0.658	0.769	0.240–2.460			
Duration of symptoms (days)	1.157	0.429	0.806–1.662			
Hypertension	0.168	2.444	0.686–8.712			
Diabetes mellitus	0.945	1.059	0.209–5.534			
Smoking	0.152	2.455	0.719–8.380			
BUN (mg/dl)	0.855	1.006	0.940–1.077			
Creatinine (mg/dl)	0.366	1.821	0.497–6.675			
ALT (IU/l)	0.339	1.003	0.997–1.010			
Prothrombin time (s)	0.714	1.033	0.867–1.232			
INR	0.631	1.657	0.211–13.003			
WBC ($\times 10^9$ cells/l)	0.567	1.060	0.867–1.296			
Hemoglobin (g/dl)	0.616	1.085	0.788–1.495			

CCHF, Crimean-Congo hemorrhagic fever; OR, odds ratio; 95% CI, 95% confidence interval; LDH, lactate dehydrogenase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; ALT, alanine aminotransferase; INR, international normalized ratio; WBC, white blood cell.

[a] C-type natriuretic peptide >1.22 ng/ml, LDH >480 IU/l, AST >202 IU/l, age, gender, and duration of symptoms were entered into the multivariate logistic regression model.

between NT-proCNP and functional and early structural arterial changes.

The specific mechanisms underlying the pathogenesis of CCHF infection have not been clearly explained. Endothelial cells and mononuclear phagocytes are major targets for the CCHF virus.[22] In particular, infection of the endothelium has an important role 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.[23,24] Furthermore, it is probable that the endothelium plays a role in the pathogenesis of CCHF through the secretion of cytokines and other inflammatory mediators.[22] Recent studies have demonstrated that serum levels of tumor necrosis factor-alpha, interleukin-1, and interleukin-6 are higher in fatal than non-fatal CCHF cases.[25,26] On the other hand, in CCHF patients, toll-like receptors can potentially be related to the natural course and/or severity of the disease, as described in a recent paper.[27] As a result of this pathophysiological pathway, it appears that CNP is released from the damaged endothelium in association with the severity of disease.

Our study findings of higher ALT, AST, and LDH levels in patients with more severe disease are in accordance with those of previous studies.[3,14,15] However, only LDH levels along with CNP were identified as independent predictors for the severity of the disease.

There are some limitations of the current study. First this was a single-center study and it represents data from a tertiary care center. Furthermore, viral load measurements would have provided more insight with regard to the level of CNP. However, the central laboratory does not provide quantitative results with the confirmation of CCHF in our country.

Increased levels of CNP, one of the most important indicators of endothelial function, is not surprising in CCHF, which is characterized by endothelial damage. Within the light of our study, we think that low CNP levels could potentially identify those with mild progression of the disease. In addition, we believe that the evaluation of CNP levels could be helpful for the selection of patients for aggressive treatment and intensive care unit admission.

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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

1. Whitehouse CA. Crimean-Congo hemorrhagic fever. *Antiviral Res* 2004;**64**:145–60.
2. Ergonul O, Celikbas A, Dokuzoguz B, Eren S, Baykam N, Esener H. The characteristics of Crimean-Congo hemorrhagic fever in a recent outbreak in Turkey and the impact of oral ribavirin therapy. *Clin Infect Dis* 2004;**39**:285–9.
3. Schnittler HJ, Feldman H. Viral hemorrhagic fever—a vascular disease? *Thromb Haemost* 2003;**89**:967–72.
4. Sannikova IV, Pacechnikov VD, Maleev VV. Respiratory lesions in Crimean-Congo hemorrhagic fever. *Ter Arkh* 2007;**79**:20–3.
5. Doganci L, Ceyhan M, Tasdeler NF, Sarikayalar H, Tulek N. Crimean-Congo hemorrhagic fever and diffuse alveolar haemorrhage. *Trop Doct* 2008;**38**:252–4.
6. Rubattu S, Sciarretta S, Valenti V, Stanzione R, Volpe M. Natriuretic peptides: an update on bioactivity, potential therapeutic use, and implication in cardiovascular diseases. *Am J Hypertens* 2008;**21**:733–41.
7. Garbers DL, Chrisman TD, Wiegn P, Katafuchi T, Albanesi JP, Bielinski V, et al. Membrane guanylyl cyclase receptors: an update. *Trends Endocrinol Metab* 2006;**17**:251–8.
8. Heublein DM, Clavell AL, Stingo AJ, Lerman A, Wold L, Burnett Jr JC. C-type natriuretic peptide immunoreactivity in human breast vascular endothelial cells. *Peptides* 1992;**13**:1017–9.
9. Barr CS, Rhodes P, Struthers AD. C-type natriuretic peptide. *Peptides* 1996;**17**:1243–51.
10. Hama N, Itoh H, Shirakami G, Suga S, Komatsu Y, Yoshimasa T, et al. Detection of C-type natriuretic peptide in human circulation and marked increase of plasma CNP level in septic shock patients. *Biochem Biophys Res Commun* 1994;**198**:1177–82.
11. Bahrami S, Pelinka L, Khadem A, Maitzen S, Hawa G, van Griensven M, et al. Circulating NT-proCNP predicts sepsis in multiple-traumatized patients without traumatic brain injury. *Crit Care Med* 2010;**38**:161–6.
12. Yapar M, Aydogan H, Pahsa A, Besirbellioglu BA, Bodur H, Basustaoglu AC, et al. Rapid and quantitative detection of Crimean-Congo hemorrhagic fever virus by one-step real-time reverse transcriptase-PCR. *Jpn J Infect Dis* 2005;**58**:358–62.
13. Cevik MA, Erbay A, Bodur H, Gulderen E, Bastug A, Kubar A, 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, Leman PA, Mynhardt JH, Harvey S. The clinical pathology of Crimean-Congo hemorrhagic fever. *Rev Infect Dis* 1989;**11**:794–800.
15. Ergonul O, Celikbas A, Baykam N, Eren S, Dokuzoguz B. Analysis of risk-factors among patients with Crimean-Congo haemorrhagic fever virus infection: severity criteria revisited. *Clin Microbiol Infect* 2006;**12**:551–4.
16. Yilmaz G, Mentese A, Kaya S, Uzun A, Karahan SC, Koksal I. The diagnostic and prognostic significance of soluble urokinase plasminogen activator receptor in Crimean-Congo hemorrhagic fever. *J Clin Virol* 2011;**50**:209–11.

17. Ozturk B, Kuscu F, Tutuncu E, Sencan I, Gurbuz Y, Tuzun H. Evaluation of the association of serum levels of hyaluronic acid, sICAM-1, sVCAM-1, and VEGF-A with mortality and prognosis in patients with Crimean-Congo hemorrhagic fever. *J Clin Virol* 2010;**47**:115–9.
18. Bodur H, Akinci E, Ongürü P, Uyar Y, Baştürk B, Gözel MG, et al. Evidence of vascular endothelial damage in Crimean-Congo hemorrhagic fever. *Int J Infect Dis* 2010;**14**:704–7.
19. Joubert JR, King JB, Rossouw DJ, Cooper R. A nosocomial outbreak of Crimean-Congo haemorrhagic fever at Tygerberg Hospital. Part III. Clinical pathology and pathogenesis. *S Afr Med J* 1985;**68**:722–8.
20. Koch A, Voigt S, Sanson E, Dückers H, Horn A, Zimmermann HW, et al. Prognostic value of circulating amino-terminal pro-C-type natriuretic peptide in critically ill patients. *Crit Care* 2011;**15**:R45.
21. Vlachopoulos C, Ioakeimidis N, Terentes-Printzios D, Aznaouridis K, Baou K, Bratsas A, et al. Amino-terminal pro-C-type natriuretic peptide is associated with arterial stiffness, endothelial function and early atherosclerosis. *Atherosclerosis* 2010;**211**:649–55.
22. Burt FJ, Swanepoel R, Shieh WJ, Smith JF, Leman PA, Greer PW, et al. Immunohistochemical and in situ localization of Crimean-Congo hemorrhagic fever (CCHF) virus in human tissues and implications for CCHF pathogenesis. *Arch Pathol Lab Med* 1997;**121**:839–46.
23. Ergönül O. Crimean-Congo haemorrhagic fever. *Lancet Infect Dis* 2006;**6**: 203–14.
24. Appannanavar SB, Mishra B. An update on Crimean Congo hemorrhagic fever. *J Glob Infect Dis* 2011;**3**:285–92.
25. Ergonul O, Tuncbilek S, Baykam N, Celikbas A, Dokuzoguz B. Evaluation of serum levels of interleukin (IL)-6, IL-10, and tumor necrosis factor-alpha in patients with Crimean-Congo hemorrhagic fever. *J Infect Dis* 2006;**193**:941–4.
26. Papa A, Bino S, Velo E, Harxhi A, Kota M, Antoniadis A. Cytokine levels in Crimean-Congo hemorrhagic fever. *J Clin Virol* 2006;**36**:272–6.
27. Engin A, Arslan S, Kizildag S, Oztürk H, Elaldi N, Dökmetas I, et al. Toll-like receptor 8 and 9 polymorphisms in Crimean-Congo hemorrhagic fever. *Microbes Infect* 2010;**12**:1071–8.