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Increased serum ferritin levels in patients with Crimean-Congo hemorrhagic fever: can it be a new severity criterion?

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Summary
Objectives: Serum ferritin is one of the markers indicating hemophagocytosis that may have a role in the pathogenesis of Crimean-Congo hemorrhagic fever (CCHF). This study was designed to determine any correlation between serum ferritin and routine diagnostic laboratory markers of CCHF, and to investigate the relationship between serum ferritin levels and disease severity.
Methods: Sixty-six patients with CCHF admitted to the hospital during the spring and summer months of 2006 and 2007 were included in the study. Serum ferritin levels were measured in sera obtained during the initial days of hospitalization. Data from 53 patients showing decreasing platelet counts over the first three days were used for further analysis and these patients were divided into two groups according to disease severity: group A included severe cases with lowest platelet counts $\leq 20 \times 10^9$/l and group B included mild cases with lowest platelet counts $>20 \times 10^9$/l.
Results: Forty patients (60.6%) were male (mean age 43 $\pm$ 17 years). Three patients died, thus the fatality rate was 4.5%. Fifty-one patients (77.3%) had abnormal serum ferritin levels, with levels above 500 ng/ml in 62.1%. There was a significant negative correlation between ferritin levels and concordant platelet counts ($p < 0.001$; $r = -0.416$) and ferritin was also found to be positively correlated with aspartate aminotransferase ($p < 0.001$; $r = 0.625$), alanine aminotransferase ($p < 0.001$; $r = 0.479$), and lactate dehydrogenase ($p < 0.001$; $r = 0.684$). Group A had higher ferritin levels than group B ($p < 0.001$). Receiver operating characteristic analysis

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revealed that a ferritin level of ≥1862 ng/ml had a sensitivity of 87.5% and a specificity of 83.8% in differentiating severe cases from mild ones.
Conclusions: Increased serum ferritin levels may suggest a significant role of hemophagocytosis in the pathogenesis of CCHF and may be a useful marker for diagnosis, disease activity, and prognosis.

Introduction

Crimean-Congo hemorrhagic fever (CCHF) is a potentially fatal viral disease caused by a *Nairovirus* belonging the family *Bunyaviridae*. It has been described in parts of Africa, Asia, Eastern Europe, and the Middle East and has a case—fatality of 3—80%. Humans are infected by Hyalomma ticks or by handling the blood or secretions of infected people or domestic animals. CCHF has been reported to be endemic in some regions of Turkey, especially in the Middle Black Sea Region during the summer months over the last five years, and is one of the important public health issues in Turkey because of its high fatality rate.[1—4]

Ferritin is a positive acute phase protein whose levels increase in the presence of various acute or chronic disease conditions. Extremely elevated serum ferritin levels have been found in histiocytic malignancies and adult-onset Still's disease as well as hemophagocytic lymphohistiocytosis (HLH).[5—7] HLH is a rare and serious clinical form of CCHF,[2,8] and is characterized by fever, hepatosplenomegaly, cytopenia, elevated triglyceride, lactate dehydrogenase (LDH) and ferritin levels, and hemophagocytosis in bone marrow, liver and lymph nodes, associated with excessive production of various cytokines by hyper-activated T helper lymphocytes and macrophages.[9—11] Serum ferritin is an important diagnostic laboratory marker of HLH, and is frequently higher than 500 ng/ml in patients with HLH.[7,12] To date, there has been only one study[2] that has reported the elevation of serum ferritin levels in CCHF.

In the present study, serum ferritin levels, a diagnostic marker of HLH, were investigated in CCHF patients. We aimed to determine whether serum ferritin levels were more increased in severe cases than in milder cases, and to determine any correlation between ferritin and other important laboratory markers in clinical practice, such as platelet count or biochemical enzyme levels.

Methods

We retrospectively evaluated patients with acute febrile syndrome, characterized by malaise, bleeding, leukopenia, and thrombocytopenia, who were admitted to the infectious diseases clinics of Tokat Cevdet Aykan State Hospital or Gaziosmanpasa University School of Medicine Training Hospital during the spring and summer of 2006 and 2007, and diagnosed with CCHF. Because CCHF is endemic in Tokat Province, all patients with symptoms or signs compatible with CCHF were considered probable cases. Three probable cases and 63 cases that were confirmed by positive serology or by detection of CCHF virus nucleic acid were included in the study. None of the patients received specific or supportive treatment other than intravenous fluids and/or antipyretics.

Patient serum samples obtained within three days of admission to the hospital represented the acute phase sample. Part of each sample was kept at −40 °C for further analysis, while the rest was immediately sent to the Refik Saydam Hygiene Center in Ankara for ELISA and PCR testing.[13,14] Patients who had positive IgM and/or positive PCR results for CCHF virus were included in the study. Complete blood counts of patients were measured on a daily basis following admission to the hospital, and biochemical parameters (aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine phosphokinase (CK), and LDH) were tested within 48 hours of hospitalization.

Ferritin was measured using a commercial electrochemiluminescence kit (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's guidelines. The serum AST/ALT ratio was calculated for all patients. We investigated whether ferritin level was related to other concordant laboratory parameters (platelet count, AST, ALT, CK, LDH, and AST/ALT ratio).

To make a more homogeneous group, cases admitted to the hospital late were excluded and the remaining 53 patients showing a decreasing platelet count over the first three days of hospitalization were included in further analysis. These patients were divided into two groups according to disease severity. Swanepoel et al. have described the fatality criteria in CCHF.[15] According to these criteria, patients are defined as having severe disease if they have a leukocyte count $\geq 10 \times 10^9$/l, a platelet count $\leq 20 \times 10^9$/l, an AST level >200 U/l, an ALT level >150 U/l, an activated partial thromboplastin time (aPTT) >60 seconds, and/or a fibrinogen level <110 mg/dl during the first five days after onset of illness. None of our patients showed an increase in aPTT in the first three days and fibrinogen was not examined. The levels of AST and ALT that predict fatality are controversial. In a recent report by Cevik et al.,[16] a platelet count $\leq 20 \times 10^9$/l has been found to be independently associated with mortality, whereas AST or ALT have not been independent predictors of fatality. Thus we used the criterion of a platelet count $\leq 20 \times 10^9$/l to differentiate severe cases from mild cases. Patients whose platelet counts decreased to $\leq 20 \times 10^9$/l any time during hospitalization were considered as severe cases. After excluding late admission cases, the patients with lowest platelet counts $\leq 20 \times 10^9$/l were assigned to group A, while those with lowest platelet counts $>20 \times 10^9$/l to group B. Serum ferritin levels, AST, ALT, CK, LDH activities, and AST/ALT ratios in acute phase sera of the patients (within the first three days of hospitalization, mostly pre-hemorrhagic) were compared between these two groups.

Statistical analysis

The Mann—Whitney U-test was used to compare the continuous parameters between group A and group B. Spearman correlation analysis was performed for correlation between

Table 1 Demographic and laboratory parameters of patients who died

Parameter	Patient 1	Patient 2	Patient 3
Age (years)	64	69	45
Gender (M/F)	M	M	F
Lowest platelet count ($\times 10^9$/l)	2.000	17.000	3.000
Ferritin (ng/ml)	12 126	8694	6426
AST/ALT ratio	3.45	2.02	3.46
AST (U/ml)	207	168	267
ALT (U/ml)	60	83	77
CK (U/ml)	1387	108	636

M, male; F, female; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CK, creatine phosphokinase.

serum ferritin level and other laboratory findings. A receiver operating characteristics (ROC) curve was used to determine the performance of ferritin level as a severity criterion according to platelet count. All variables are presented as mean ± standard deviation (SD) and range. A p-value of <0.05 was considered significant. Analyses were performed using commercial software SPSS 16.0 (demo version; SPSS, Inc., Chicago, IL, USA).

Results

Sixty-six subjects with CCHF were included in the study. Forty patients (60.6%) were male. The mean age was 43 ± 18 (range 12–83) years. Three patients in the study group, who were referred to another treatment center due to severe thrombocytopenia and hemorrhagic complications, died; thus the fatality rate was 4.5%. The lowest serum ferritin level of fatal cases was 6426 ng/ml. Demographic and laboratory parameters of fatal cases are summarized in Table 1.

Serum ferritin levels of all patients varied between 59 and 34 660 with a mean value of 5370 ng/ml. Fifty-one patients (77.3%) had abnormal serum ferritin levels. In forty-one patients (62.1%), serum ferritin levels were above 500 ng/ml. A significant negative correlation was observed between ferritin level and platelet count, which were the mean values of the first three days of hospitalization ($p < 0.001$; $r = -0.416$). Ferritin was found to be positively correlated with AST ($p < 0.001$; $r = 0.625$), ALT ($p < 0.001$; $r = 0.479$), and LDH ($p < 0.001$; $r = 0.684$) (Table 2).

Thirteen cases were excluded due to late admission and the remaining 53 cases were divided into two groups: group A (severe) included 16 cases and group B (mild) included 37 cases. There was no difference between the groups regarding time to reach the lowest platelet count (the mean time was 4.13 days for group A and 4.097 days for group B). However, serum ferritin levels were significantly higher in severe cases (group A) compared to mild cases (group B) ($p < 0.001$; Table 3). The serum AST/ALT ratio was an important prognostic factor in our patient population. We found a significantly higher AST/ALT ratio in group A compared to group B ($p < 0.001$) (Table 3).

To determine ferritin threshold as a severity criterion, a ROC analysis was carried out. The area under the curve was 0.875 ($p < 0.001$) and a ferritin level of ≥1862 ng/ml was found to have a sensitivity of 87.5% and a specificity of 83.8% in differentiating severe cases from mild ones (Figure 1).

Discussion

In this study, we retrospectively analyzed 66 cases diagnosed with CCHF. CCHF was first recognized in Turkey in 2002. Most of the cases have been reported in the Middle Black Sea and Northern Central Anatolia regions, indicating that CCHF is endemic to those regions of Turkey.[2,3,8,17] Tokat Province, in which our patients reside, is located within the endemic region and is in fact the province where the first human CCHF cases appeared in Turkey. During the last five years, an increasing number of human CCHF virus infections has been reported from various parts of Turkey.[18]

Although the pathogenesis of CCHF infection is still not clear, hemophagocytosis, a symptom rarely reported in viral hemorrhagic fevers, was initially observed in patients with CCHF by Karti et al.[8] Recently, two new reports of hemophagocytic syndrome associated with CCHF in Turkey have been published.[2,19] HLH is an uncommon syndrome characterized by a reactive, systemic proliferation of benign histiocytes throughout the reticuloendothelial system. HLH comprises two different conditions that may be difficult to distinguish from one another: a primary and a secondary

Table 2 Demographic characteristics and laboratory findings of patients with Crimean-Congo hemorrhagic fever and results of correlation analysis between ferritin and other laboratory parameters ($N = 66$)

	n	Mean ± SD	Range	r^a	p-Value
Ferritin (ng/ml)	66	5370 ± 8088	59–34 660		
Age (years)	66	43.36 ± 17.87	12–83	0.170	0.172
Platelet count ($\times 10^9$/l)	66	98.659 ± 43.821	26.000–239.670	−0.416	<0.001
AST (U/ml)	64	120.45 ± 86.67	19–350	0.625	<0.001
ALT (U/ml)	64	59.55 ± 52.61	12–309	0.479	<0.001
AST/ALT ratio	64	2.16 ± 0.78	1.05–4.31	0.495	<0.001
CK (U/ml)	46	426.09 ± 540.13	38–2726	0.201	0.179
LDH (U/ml)	27	370.81 ± 184.88	175–752	0.684	<0.001

SD, standard deviation; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CK, creatine phosphokinase; LDH, lactate dehydrogenase.

[a] Spearman correlation coefficients between ferritin and other parameters.

Table 3 Comparison of laboratory results between severe (group A) and mild (group B) cases according to the criterion thrombocytopenia of $\leq 20 \times 10^9$/l ($n = 53$)

Parameter	Group A		Group B		*p*-Value
	Mean ± SD	Range	Mean ± SD	Range	
Days to lowest platelet count	4.133	2–7	4.097	2–7	0.990
Ferritin (ng/ml)	12194.81 ± 9618	105–34660	2003.55 ± 3955	59–13250	<0.001[b]
Platelet count (at baseline) ($\times 10^9$/l)	87.479 ± 46.705	48.000–217.330	109.446 ± 38.885	39.000–239.670	0.021[a]
AST (U/ml)	134.88 ± 54.12	32–267	92.72 ± 86.04	19–332	0.005[a]
ALT (U/ml)	49 ± 14.38	27–77	53.42 ± 59.67	12–309	0.57
CK (U/ml)	698.21 ± 778.88	53–2726	364.50 ± 382.77	73–1236	0.263
LDH (U/ml)	457.5 ± 216.71	245–752	280.5 ± 117.68	175–638	0.026[a]
AST/ALT ratio	2.75 ± 0.85	1.19–4.06	1.87 ± 0.46	1.07–2.90	<0.001[b]

SD, standard deviation; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CK, creatine phosphokinase; LDH, lactate dehydrogenase.
[a] Statistically significant.
[b] Highly statistically significant.

form. The primary autosomal recessive form, familial hemophagocytic lymphohistiocytosis (FHL), is a fatal disease with a median survival expressed in months after diagnosis if left untreated, and typically has its onset during infancy or early childhood. Importantly, the onset of FHL and bouts of the disease may be triggered by infections. Secondary HLH (SHLH) may develop as a result of strong immunological activation of the immune system, which may, for example, be caused by a severe infection, particularly systemic viral infections (Epstein–Barr virus, cytomegalovirus, CCHF) and occasionally with bacterial, fungal, or parasitic infections. For most patients with HLH, the outcome is rapid and fatal unless the diagnosis is made early and followed by prompt therapeutic intervention.[9,12,20,21] Recent studies indicate that HLH is seen in nearly half of the patients with CCHF in Turkey. HLH is the most important clinical phenomenon associated with the outcome of the infection.[2,8]

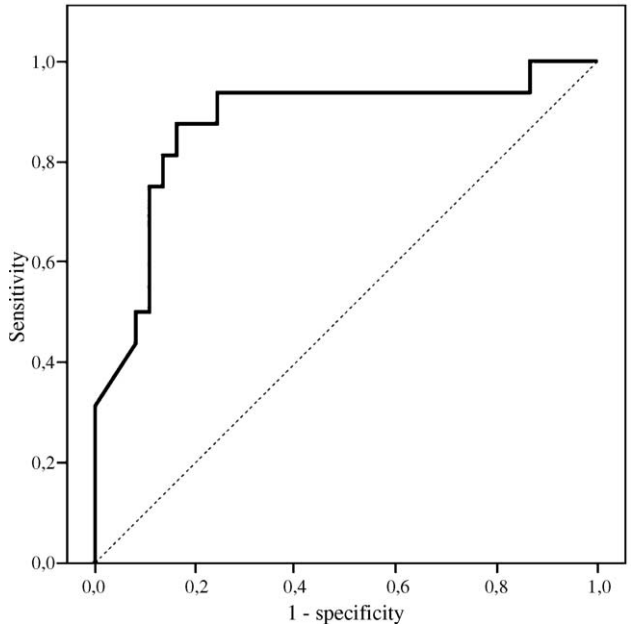

Figure 1 Receiver operating characteristics curve showing the performance of serum ferritin as a predictor of severity (area under curve = 0.875; $p < 0.001$).

The most typical findings of HLH are fever, hepatosplenomegaly, and cytopenia (most commonly thrombocytopenia and anemia). Cytopenia affects at least two of three lineages in the peripheral blood. Other common findings include hypertriglyceridemia, coagulopathy with hypofibrinogenemia, liver dysfunction, elevated levels of ferritin (>500 ng/ml) and serum transaminases, and neurological symptoms. Spontaneous partial remissions are observed.[11,12,20,21] Cytopenia and hyperferritinemia in patients with CCHF may result from HLH.[2,8,19] Cytopenia during HLH has been attributed primarily to high concentrations of tumor necrosis factor alpha (TNF-α) and interferon-γ.[11] In the present study, we observed evident thrombocytopenia in 30.3% and increased ferritin levels in 77.3% of all patients.

Ferritin, the major iron storage protein, plays a key role in iron metabolism. Serum ferritin concentration provides an indirect estimate of body iron stores, because it is highly correlated with bone marrow iron. Ferritin is also a positive acute-phase reactant and increases in the presence of various acute or chronic disease conditions. Elevated serum ferritin levels have also been found in liver necrosis, chronic inflammation-related diseases, histiocytic malignancies, and adult-onset Still's disease as well as HLH.[6,22] Serum ferritin levels are above 500 ng/ml in CCHF patients with HLH.[2] Patients with infection-associated HLH usually have persistent unexplained fever, cytopenia, lymphadenopathy, and frequently hepatosplenomegaly and coagulopathy. The possible immunopathologic mechanism of HLH might be excessive production of Th1 cytokines such as interferon-γ, TNF-α, interleukin (IL)-1, or IL-6 from activated lymphocytes or monocytes.[9] Two studies have reported elevations in proinflammatory cytokines in CCHF. Ergonul et al.[23] found that the levels of IL-1, IL-6, and TNF-α were higher among patients who subsequently died compared with those who survived.

Papa et al.[24] reported that TNF-α and IL-6 were the cytokines most often detected during a CCHF viral infection, and that TNF-α was associated with the severe form of CCHF while IL-6 was elevated in both severe and mild cases. Leukopenia, thrombocytopenia, coagulopathy, and hyperferritinemia in patients with CCHF may be caused by hypercytokinemia, as is the case in HLH.

In the current study, we found ferritin levels above 500 ng/ml in 62.1% of all patients with CCHF and in 95% (19/20) of patients with severe case definition. This finding, as well as the negative correlation of ferritin with the platelet count, the positive correlation of ferritin with AST, ALT, and LDH, and the presence of higher levels of ferritin in severe cases, all indicate that ferritin can be used as a severity marker, beyond a diagnostic tool for CCHF. Moreover, we established that a 1862 ng/ml threshold level for ferritin has the most reliable sensitivity and specificity in determining the severe cases. In this study, we used only the thrombocytopenia of $\leq 20 \times 10^9$/l criterion to identify severe cases because the number of fatal cases was not sufficient to compare fatal and nonfatal cases; this may be a limitation of our study. Additionally we could not examine ferritin in the days subsequent to hospitalization.

Ergonul et al.[25] suggested that a high AST/ALT ratio was a prognostic factor for severity among cases of CCHF, and the same parameter was documented to be a prognostic factor for fatality. Our results are concordant with the literature.

Hemophagocytosis is suggested to have a role in the development of cytopenia in CCHF, the mechanism of which still needs to be investigated, probably with cytokine studies. Together with clinical symptoms and patient history, hemophagocytosis may be an indicator of CCHF. In conclusion, our study confirms other reports that have shown elevated serum ferritin levels in patients with CCHF. In addition, serum ferritin levels in CCHF may be a useful marker of disease activity and prognosis. New studies are needed to clarify on which day ferritin levels begin to increase after the onset of symptoms and factors that lead to increases in ferritin levels.

Conflict of interest: No conflict of interest to declare.

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