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Original article

An evaluation of the different biomarkers to discriminate bleeding in Crimean-Congo Hemorrhagic Fever

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ABSTRACT

Crimean-Congo Hemorrhagic Fever (CCHF) is an acute viral hemorrhagic disease. In this study, an evaluation was made of the potential use of iron metabolism and liver function biomarkers to estimate the bleeding status in CCHF patients. This prospective study was conducted in Cumhuriyet University, Turkey. Only patients with confirmed CCHF were enrolled in the study. The study subjects comprised 40 CCHF patients and 37 healthy control subjects. Serum iron, unsaturated iron binding capacity (UIBC), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) levels were determined using the colorimetric method. Serum ferritin levels were measured using the electrochemiluminescent method. The serum ferritin ($p = 0.037$), AST ($p = 0.0002$), ALT ($p = 0.002$), LDH ($p = 0.0005$) and aPTT ($p = 0.001$) values were higher in patients with bleeding than in patients without bleeding. Receiving operating characteristic analyses were applied for the area under the curve (AUC) values for ferritin, aPTT, and AST to discriminate the bleeding status in patients, an these were determined as 0.717, 0.819, and 0.882, respectively. A cut-off value of 149 U/L for AST was obtained to discriminate the bleeding condition in CCHF patients. Higher ferritin ($p < 0.0001$) levels were determined in patients compared to the control group. The iron ($p = 0.180$) and UIBC ($p = 0.0017$) values were lower in patients than in the control group. Cytokine storm due to an increase in ferritin levels may contribute to the increased inflammation and coagulation abnormalities in CCHF patients. It was concluded that routine screening of the AST level would be helpful to estimate the bleeding status in addition to screening liver damage in CCHF patients.

1. Introduction

Crimean-Congo Hemorrhagic Fever (CCHF) is an acute viral hemorrhagic disease with a 3–30% case fatality rate. The virus that causes CCHF belongs to the genus *Orthonairovirus* of the family *Nairoviridae* virus (Adams et al., 2017). The CCHF virus is transmitted through tick bites or direct contact with blood, body fluids or other infected tissues of an infected animal or human (Cevik et al., 2008). CCHF virus infections are important for public health because of the nature of disease transmission, the serious effects on patients and spread within communities, and the absence of any antiviral drug that can be used in treatment (Dowall et al., 2017). CCHF is characterized by vascular endothelial damage, disseminated intravascular coagulation, thrombocytopenia, liver damage, coagulation abnormalities and hemorrhagia including ecchymosis, gingival bleeding, epistaxis and gastrointestinal bleeding, which are prominent findings for the estimation of the case fatality rate (Ergonul et al., 2006).

Significant histopathological findings are seen in the liver in CCHF patients. In addition, increased serum concentrations of liver damage biomarkers such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) have been reported in patients with CCHF. The liver has an important role in the coagulation system with the production of different coagulation factors such as fibrinogen (factor I), thrombin (factor II), and V, VII, IX, X (Peck-Radosavljevic, 2007). Liver diseases may affect the synthesis and post-translational modification of the aforementioned coagulation factors (Hansson and Stenflo, 2005). In previous studies, coagulation problems have been shown in CCHF patients through the mechanism of decreased liver function (Cevik et al., 2008; Rodrigues et al., 2012; Wallace, 2016). The liver also has an important role in the regulation of iron storage and homeostasis with the synthesis of ferritin, which is an iron-binding positive acute phase protein. (Anderson and Shah, 2013; Peng and Uprichard, 2017). Serum

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Table 1
Detailed information on patients bleeding over the course of hospitalization.

Bleeding type	Lowest Platelet Value (mm^3/mcL)	Lowest PT value (s)	Highest PT value (s)	Lowest aPTT values (s)	Highest aPTT values (s)	Lowest INR values	Highest INR values	Status
Ecchymosis	11.000	12.4	13.9	43.3	68.7	1.08	1.19	Death
Epistaxis	38.000	9.6	11.1	35	68.3	0.84	0.97	Recovery
Petechia	41.000	9.9	12.9	30	50.2	0.86	1.12	Recovery
Vaginal bleeding	36.000	10.7	11.4	22.4	44	0.91	1.02	Recovery
Ecchymosis	7.000	10.3	12.3	31.4	51.7	0.9	1.07	Recovery
Epistaxis	19.000	10	10.8	23.4	43.7	0.88	0.94	Recovery
Petechia	12.000	9.7	10.1	25.9	43.1	0.85	0.88	Recovery
Petechia	59.000	9	9.1	29.7	36.7	0.79	0.80	Recovery
Gastrointestinal bleeding	15.000	10.7	20.9	34.2	59.4	0.93	1.82	Death

Table 2
Comparisons of laboratory parameters between the CCHF patients with and without bleeding.

Laboratory Parameters	Bleeding		P
	Yes (n = 9)	No (n = 31)	
RBC (x10^{12}/L)	4.54 ± 0.09	4.89 ± 0.07	0.020
Hb (g/dL)	13.31 ± 0.29	14.27 ± 0.2	0.073
Hct (%)	38.24 ± 0.89	41.71 ± 0.66	0.013
MCV fL/red cell	84.23 ± 1.32	85.27 ± 0.7	0.498
MCH (pg/cell)	29.31 ± 0.5	29.13 ± 0.27	0.763
MCHC (g/dL)	34.8 ± 0.36	34.16 ± 0.17	0.107
RDW-CV (%)	13.81 ± 0.27	13.66 ± 0.12	0.590
MPV (fL)	10.43 ± 0.26	10.55 ± 0.20	0.768
¥PLT (10^9)	32 ± 5	89 ± 8	0.001
¥Fe (µg/dL)	99.44 ± 44.62	74.13 ± 9.82	0.930
UIBC (µg/dL)	230 ± 33 ± 19	251 ± 15.26	0.534
Ferritin (ng/mL)	1691 ± 616	1085 ± 821	0.037
AST (U/L)	492.00 (186.50 – 623.00)	103.00 (52.00 – 163.00)	0.0002
ALT (U/L)	117.00 (81.70 – 211.00)	59.00 (32.00 – 94.00)	0.002
LDH (U/L)	543.00 (741.00 – 1288.00)	348.00 (286.00 – 585.00)	0.0005
Total bilirubin (mg/dL)	0.61 (0.37 – 1.58)	0.46 (0.35 – 0.60)	0.068
Direct bilirubin (mg/dL)	0.13 (0.07 – 0.70)	0.11 (0.08 – 0.11)	0.250
PT (s)	11.10 (10.00 -13.15)	11.10 (10.70 – 12.80)	0.955
aPTT (s)	46.39 ± 12.64	35.6 ± 6.85	0.001
INR	0.99 ± 0.2	1.01 ± 0.18	0.80
Fibrinogen (mg/dL)	223.8 ± 23.54	254.6 ± 49.82	0.08

aPTT: activated partial thromboplastin time, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, Fe: Iron, Hct: Hematocrit, RBC: Red blood cell, Hb: Hemoglobin, INR: International normalized ratio, LDH: Lactate dehydrogenase, PT: Prothrombin time, MCV: Mean corpuscular volume, MPV: Mean platelet volume, MCH: Mean corpuscular hemoglobin, PLT: Platelet, RDW-CV: Red cell distribution volume-coefficient of variation, UIBC: Unsaturated iron binding capacity.*Results were expressed as mean ± SD, ¥Results were expressed as median (1^{st}-3^{rd} quartile).

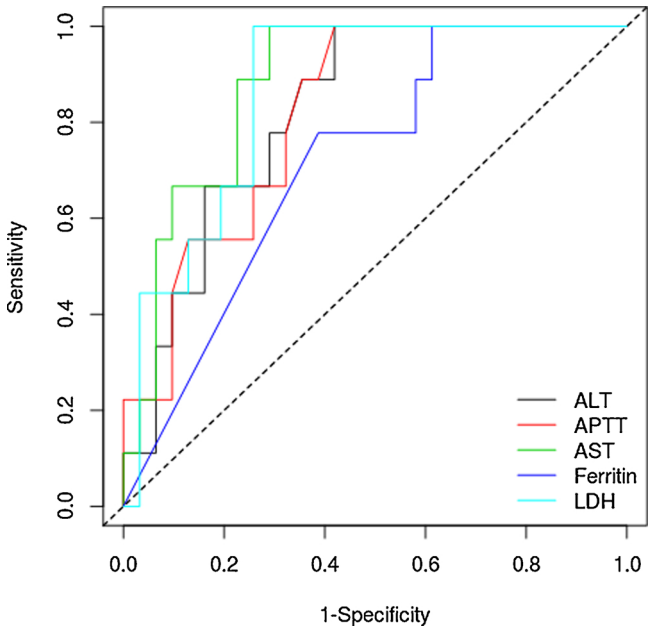

Fig. 1. Evaluation of the diagnostic performance of ferritin, AST, ALT, LDH and aPTT variables predicting the bleeding condition in CCHF patients.

ferritin concentrations are not only affected by iron metabolism but also by inflammatory activity including infections, increased blood cell turnover and tissue necrosis affecting the ferritin concentrations (Oguz et al., 2013; Wang et al., 2010). Ferritin modulates immune activity via different mechanisms, including the effects on pro-apoptotic and anti-apoptotic pathways and the capability of iron restriction during inflammation. An association between induced pro-inflammatory cytokine synthesis and ferritin production has also been reported (Cozzi et al., 2003; Pham et al., 2004; Weinberg, 2009). Increased serum ferritin levels have been reported in acute viral hepatitis and CCHF (Metanat et al., 2013; Milman and Graudal, 1984; Wu et al., 2014). However, much uncertainty still exists about the relationship between ferritin and CCHF.

In the present study, it was hypothesized that iron metabolism and liver function biomarkers could be helpful in the estimation of bleeding status in CCHF patients with the use of well-known coagulation tests including activated partial thromboplastin time (aPTT) and prothrombin time (PT). Therefore, serum concentrations of ferritin, iron (Fe), unsaturated iron binding capacity (UIBC), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were measured in patients with CCHF and the power of these biomarkers to discriminate bleeding in patients with CCHF was determined. To the best of our knowledge, there has been no previous study that has evaluated the potential role of serum iron metabolism and liver damage biomarkers to discriminate the bleeding status in CCHF patients.

2. Methods

This prospective study was conducted in Cumhuriyet University, Turkey. Clinical samples were collected from March to September 2018. Only patients with CCHF confirmed by the national reference Virology Laboratory of Refik Saydam National Hygiene Center in Ankara, Turkey, were enrolled in the study. CCHF was confirmed by the presence of CCHFV RNA with the reverse transcriptase-polymerase chain reaction and/or detection of specific IgM in the blood using enzyme-linked immunosorbent assay (ELISA). The study subjects comprised 40 CCHF patients [20 males and 20 females; 19–74 years old (mean age: 53 ± 15 years)] and 37 healthy control subjects [17 males and 20 females; 18–75 years old (mean age: 45 ± 15 years)].

Table 3
Statistical diagnostic measurements of ferritin, aPTT, ALT, AST and LDH levels in discriminating bleeding condition.

Variables	Statistical diagnostic measurements				ROC curve statistics	
	SEN(95%CI)	SPE(95%CI)	PPV(95%CI)	NPV(95%CI)	AUC	*p*
Ferritin (≥2000 ng/mL)	0.78(0.40-0.97)	0.61(0.42-0.78)	0.37(0.16-0.62)	0.90(0.70-0.99)	0.717	0.005
aPTT (≥36.7 s)	1.00(0.66-1.00)	0.58(0.39-0.75)	0.41(0.21-0.64)	1.00(0.81-1.00)	0.819	< 0.001
ALT (≥62 U/L)	1.00(0.66-1.00)	0.58(0.39-0.75)	0.41(0.21-0.64)	1.00(0.81-1.00)	0.823	< 0.001
AST (≥149 U/L)	1.00(0.66-1.00)	0.71(0.52-0.86)	0.50(0.26-0.74)	1.00(0.85-1.00)	0.882	< 0.001
LDH (≥480 U/L)	1.00(0.66-1.00)	0.74(0.55-0.88)	0.53(0.28-0.77)	1.00(0.85-1.00)	0.864	< 0.001

SEN: Sensitivity; SPE: Specificity; PPV: Positive predictive value; NPV: Negative predictive value; AUC: Area under curve, ROC: Receiver operating characteristic, CI: Confidence interval.

Table 4
Logistic regression analysis results of the evaluation of risk factors for bleeding condition.

Variables	Bleeding Condition			
	Univariate		Multivariate	
	Beta(95% CI)	*p*	Beta(95% CI)	*p*
Ferritin (ng/mL)	1.001(1.000-1.002)	0.064		
aPTT (s)	1.147(1.020-1.289)	0.022		
ALT (U/L)	1.010(1.001-1.019)	0.031		
AST (U/L)	1.006(1.002-1.010)	0.003	1.005(1.001-1.010)	0.022
LDH (U/L)	1.003(1.001-1.006)	0.010		

CI: Confidence interval.

Table 5
Comparisons of laboratory parameters between patients and control groups.

Laboratory Parameters	Patients (n = 40)	Controls (n = 37)	P
RBC (x10^{12}/L)	4.8 ± 0.40	4.97 ± 0.41	0.09
Hb (g/dL)	14.05 ± 1.41	14.95 ± 1.25	0.004
Hct (%)	40.93 ± 3.76	44.05 ± 3.51	0.0003
MCV fL/red cell	85.04 ± 3.97	88.67 ± 3.07	< 0.0001
MCH (pg/cell)	29.17 ± 1.53	30.08 ± 1.18	0.0051
MCHC (g/dL)	34.31 ± 1.04	33.92 ± 0.43	0.041
RDW-CV (%)	13.7 ± 0.72	13.17 ± 0.47	0.0004
MPV (fL)	10.53 ± 1.06	9.76 ± 1.18	0.0039
AST (U/L)	139.0 (61.00 – 306.50)	17.5(13.3-24.5)	0.001
ALT (U/L)	73.0 (37.25 – 115.80)	18.0(16.0-21.0)	0.001
aPTT (s)	38.03 ± 9.47	30.00 (26.30- 32.60)	0.001
LDH (U/L)	417.50 (311.30 – 685.00)	210.00 (175.50-223.00)	0.001
¥PLT (10^{9})	68.00 (7.00 – 36.50)	252.00 (213.00 – 275.50)	< 0.0001
¥Fe (µg/dL)	61.00 (26.75 – 106.30)	80.00 (47.50 – 94.50)	0.180
UIBC	246.30 ± 87.55	301.20 ± 56.03	0.0017
Ferritin (ng/mL)	1457.00 (330.70 - 2000)	31.26 (18.72 – 65.55)	< 0.0001

Fe: Iron, Hct: Hematocrit, RBC: Red blood cell, Hb: Hemoglobin, MCV: Mean corpuscular volume, MPV: Mean platelet volume, MCH: Mean corpuscular hemoglobin, PLT: Platelet, RDW-CV: Red cell distribution volume-coefficient of variation, UIBC: Unsaturated iron binding capacity.*Results were expressed as mean ± SD, ¥Results were expressed as median (1st-3rd quartile).

Overnight fasting blood samples were collected from all participants into red top tubes (Becton Dickinson, UK). The serum sample tubes were allowed to clot before centrifugation. After centrifugation at 4 °C for 15 min at 3500 rpm, the serum was aliquoted and immediately frozen at −80 °C (WiseCryo, South Korea). Patient samples were taken on the first day after admission. The mean admission time of the patients to hospital was the 3rd day (range 1–8 days) after the onset of symptoms. Serum ferritin levels were measured using the electrochemiluminescent method (Roche Cobas e170, Germany). Serum iron and unsaturated iron binding capacity (UIBC) were determined using the colorimetric method (Mindray, BS 2000, China). Records were obtained from Cumhuriyet University Hospital laboratory information system to determine age, gender, and the values of alanine aminotransferase (ALT), total bilirubin, direct bilirubin, lactate dehydrogenase (LDH), international normalization ratio (INR), aspartate aminotransferase (AST), prothrombin time (PT), platelet, activated partial thromboplastin time (aPTT), and Complete Blood Count (CBC) parameters. The control group subjects were selected from healthy volunteers. Exclusion criteria for the control group included clinical suspicion of infection and the presence of liver disease, kidney disease, diabetes mellitus, alcohol consumption, rheumatic disease, malignancy, pregnancy or smoking. The protocol was approved by the Ethics Committee of Cumhuriyet University (2018-06/11). Informed consent was obtained from all study subjects. The study was conducted in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

2.1. Statistical analysis

Histogram, q-q plots and the Shapiro-Wilk test were applied to assess the conformity of the data to normal distribution. The Levene test was used to assess the variance homogeneity. To compare the differences between groups, the Independent Samples t-test, and the Mann Whitney U test were used for parametric and non-parametric variables, respectively. Chi-square analysis was applied to categorical variables. Spearman correlations were used in the univariate analysis and multivariate (backward stepwise procedure) binary logistic regression analyses were used to examine the risk factors influencing the bleeding condition. Receiver operating characteristic (ROC) curves were plotted for the ferritin, aPTT, ALT, AST and LDH values to detect the predictive performance of the bleeding condition. The area under the curve (AUC) values and cut-off values were determined for each variable. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and AUC values were calculated. Analyses were conducted using Turcosa Cloud (Turcosa Ltd Co, Turkey) statistical software. A value of $p < 0.05$ was considered statistically significant.

3. Results

Bleeding was determined in 9 patients. Detailed information of patients' bleeding over the course of hospitalization is given in Table 1. Serum ferritin, AST, ALT, LDH and aPTT values were determined to be higher in patients with bleeding. The comparisons of the laboratory parameters between the CCHF patients in which bleeding was present or absent are shown in Table 2. ROC analyses were applied for ferritin, aPTT, ALT, AST and LDH variables to discriminate the bleeding status in patients, and the AUC values were found to be 0.717, 0.819, 0.823, 0.882 and 0.864, respectively as shown in Fig. 1. The AUC values for AST were higher than those for ferritin, ALT, aPTT and LDH. In the ROC analysis, a cutoff value of 149 U/L for AST was obtained with sensitivity of 100% and specificity of 71% in the discrimination of the bleeding

Table 6
Spearman correlations between laboratory parameters.

Laboratory Parameters	RBC	HB	HCT	MCV	MCH	MCHC	RDW	MPV	PLT	Fe	UIBC	Ferritin	ALT	AST	LDH	Total bilirubin	Direct bilirubin	PT	APTT	INR
HB	0.853																			
HCT	0.857	0.95***																		
MCV	−0.084	0.33**	0.41*																	
MCH	0.019	0.47**	0.39	0.80																
MCHC	0.261	0.43**	0.16	−0.09	0.46**															
RDW	0.019	−0.28	−0.23	−0.41**	−0.51**	−0.24														
MPV	0.057	0.17	0.21	0.35*	0.22	−0.05	−0.16													
PLT	−0.241	−0.14	−0.11	0.17	0.05	−0.26	−0.15	−0.09												
Fe	0.482*	0.51***	0.49**	0.11	0.24	0.34	−0.03	0.22	−0.47											
UIBC	−0.187	−0.29	−0.20	−0.06	−0.28	−0.41**	0.08	0.09	0.28**	−0.73										
Ferritin	0.179	0.24	0.15	0.05	0.16	0.38*	−0.05	0.17**	−0.49**	0.43**	−0.51**									
ALT	0.206	0.07	0.00	−0.28	−0.16	0.31*	0.28	0.08	−0.78	0.41	−0.18	0.58								
AST	0.192	0.03	−0.02	−0.33	−0.24	0.22	0.23	0.09	−0.84	0.33	−0.21	0.59	0.92***							
LDH	0.236	0.11	0.05	−0.25	−0.14	0.27	0.18	0.08	−0.83	0.44	−0.28	0.58	0.84	0.93*						
Total bilirubin	0.182	0.01	−0.01	−0.41**	−0.28	0.12	0.24	−0.04	−0.44**	0.22**	0.02	0.06	0.35	0.31	0.20					
Direct bilirubin	0.206	0.00	−0.03	−0.49*	−0.32	0.19	0.31	−0.05	−0.34*	0.27	−0.07	0.11	0.33	0.30	0.24	0.86				
PT	0.069	0.10	0.10	0.11	0.13	0.03	0.03	−0.09	0.18	−0.07	0.02	0.08	−0.15	−0.10	−0.09	0.02	0.22			
APTT	0.238	0.14	0.09	−0.23	−0.12	0.20	0.12	0.03	−0.57	0.19	−0.13	0.31*	0.48*	0.61	0.61	0.24	0.29	0.20		
INR	0.03	0.06	0.08	0.13	0.09	−0.05	0.09	−0.08	0.22	−0.08	0.06	0.03	−0.16	−0.14	−0.14	−0.02	0.16	0.95***	0.08	
Fibrinogen	−0.27	−0.18	−0.19	0.11	0.04	−0.22	−0.03	−0.03	0.50**	−0.27	0.06	−0.24	−0.48	−0.48**	−0.42**	−0.44**	−0.41*	−0.05	−0.42**	−0.02

* **p < 0.05, **: p < 0.01, *** : p < 0.001.**

Table 7
Comparisons of laboratory parameters between the CCHF patients with and without FFP treatment.

Laboratory Parameters	Fresh frozen plasma		P
	Yes (n = 17)	No (n = 23)	
RBC (x10^{12}/L)	4.87 ± 0.3	4.77 ± 0.45	0.476
Hb (g/dL)	14.39 ± 1.3	13.8 ± 1.56	0.191
Hct (%)	41.71 ± 3.23	40.35 ± 4.08	0.262
MCV fL/red cell	85.69 ± 3.53	84.55 ± 4.27	0.373
MCH (pg/cell)	29.57 ± 1.52	28.88 ± 1.51	< 0.0001
MCHC (g/dL)	34.49 ± 0.81	34.17 ± 1.18	0.331
RDW-CV (%)	13.66 ± 0.74	13.72 ± 0.71	0.789
MPV (fL)	10.66 ± 1.0	10.43 ± 1.12	0.492
¥PLT (10^{9})	56 ± 39	91 ± 54	0.03
¥Fe (µg/dL)	67.50 (36.50 – 122.50)	54.00 (23.00 – 99.00)	0.331
UIBC (µg/dL)	233.5 ± 80.03	255.7 + 93.35	0.436
Ferritin (ng/mL)	2000 (867.3 – 2000)	747.3 (211.50 - 2000)	0.012
AST (U/L)	184.00 (135.50 – 516.50)	76.00 (37.00 – 237.00)	0.027
ALT (U/L)	85.00 (60.50 – 119.50)	56.00 (32.00- 107.00)	0.142
LDH (U/L)	585.00 (402.00 – 834.00)	344.00 (286.00 – 480.00)	0.005
Total bilirubin (mg/dL)	0.43 (0.32 – 0.67)	0.52 (0.35 – 0.65)	0.630
Direct bilirubin (mg/dL)	0.12 (0.08 – 0.145)	0.11 (0.07 – 0.16)	0.794
PT (s)	12.81 ± 2.6	11.02 ± 1.98	0.006
aPTT (s)	43.82 ± 11.13	33.67 ± 4.72	0.0003
INR	1.09 ± 0.22	0.94 ± 0.11	0.007
Fibrinogen (mg/dL)	238.2 ± 47.56	254.70 ± 46.07	0.287

aPTT: activated partial thromboplastin time, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, Fe: Iron, Hct: Hematocrit, RBC: Red blood cell, Hb: Hemoglobin, INR: International normalized ratio, LDH: Lactate dehydrogenase, PT: Prothrombin time, MCV: Mean corpuscular volume, MPV: Mean platelet volume, MCH: Mean corpuscular hemoglobin, PLT: Platelet, RDW-CV: Red cell distribution volume-coefficient of variation, UIBC: Unsaturated iron binding capacity.*Results were expressed as mean ± SD, ¥Results were expressed as median (1st-3rd quartile).

condition of CCHF patients (Table 3). The logistic regression analysis results of the evaluation of the risk factors for bleeding are shown in Table 4.

Higher ferritin levels were determined in the patients than in the control group. Iron and UIBC values were lower in patients than in the control group (Table 5.). A positive correlation was determined between UIBC and platelet values in the patient group ($p < 0.01$, $r = 0.28$). A negative correlation was found between ferritin and platelet values in patients ($p < 0.01$, $r = -0.49$). aPTT values were positively correlated with both ALT ($p < 0.05$, $r = 0.48$) and ferritin values ($p < 0.01$, $r = 0.31$). Spearman correlations between laboratory parameters are shown in Table 6. Thrombocyte suspension and fresh frozen plasma (FFP) were given as supportive care to 11 and 17 patients, respectively. To determine the patients who needed platelet and FFP suspensions, the criteria used were those given in the study by Leblebicioglu et al. (2012). Before the administration of the platelet suspension and FFP, blood samples were taken from those patients. Serum ferritin, AST, LDH and aPTT values were higher in FFP-treated patients than in patients without FFP treatment. The comparisons of laboratory parameters between the CCHF patients with and without FFP treatment are shown in Table 7. Higher ferritin, AST, and LDH values were found in patients with thrombocyte treatment than in patients without thrombocyte treatment. The comparisons of laboratory parameters between CCHF patients with and without thrombocyte treatment are shown in Table 8.

Table 8
Comparisons of laboratory parameters between the CCHF patients with and without thrombocyte suspension treatment.

Laboratory Parameters	Thrombocyte Suspension		*P*
	No (n = 29)	Yes (n = 11)	
RBC (x10^{12}/L)	4.78 ± 0.43	4.88 ± 0.34	0.496
Hb (g/dL)	13.98 ± 1.46	14.25 ± 1.31	0.583
Hct (%)	40.74 ± 3.91	41.43 ± 3.44	0.611
MCV fL/red cell	85.14 ± 3.83	84.76 ± 4.49	0.793
MCH (pg/cell)	29.18 ± 1.48	29.16 ± 1.74	0.982
MCHC (g/dL)	34.27 ± 1.11	34.4 ± 0.87	0.728
RDW-CV (%)	13.7 ± 0.72	13.68 ± 0.75	0.944
MPV (fL)	10.54 ± 1.07	10.5 ± 1.09	0.921
¥PLT (10^{9})	82.00 (51.00 – 124.00)	30.00 (16.00 – 58.00)	0.001
¥Fe (µg/dL)	61.00 (24.00 – 90.00)	67.00 (39.00 – 137.00)	0.682
UIBC (µg/dL)	256 ± 85.12	220.60 ± 92.82	0.259
Ferritin (ng/mL)	985 ± 785.7	1845 ± 513.5	0.002
AST (U/L)	103.00(44.50-213.00)	248.00 (150.00-569.00)	0.006
ALT (U/L)	60.00(27.50-109.50)	85.00 (71.00-193.00)	0.074
LDH (U/L)	448.1 ± 262.9	806.50 ± 429.90	0.01
Total bilirubin (mg/dL)	0.46 (0.33 – 0.6)	0.51 (0.37 – 0.71)	0.648
Direct bilirubin (mg/dL)	0.11 (0.08 – 0.17)	0.1 (0.07 – 0.15)	0.626
PT (s)	11.78 ± 2.28	11.78 ± 1.70	0.692
aPTT (s)	36.59 ± 8.49	41.84 ± 11.23	0.06
INR	1.01 ± 0.2	1.01 ± 0.14	0.614
Fibrinogen (mg/dL)	254 ± 46.26	231 ± 46.21	0.168

aPTT: activated partial thromboplastin time, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, Fe: Iron, Hct: Hematocrit, RBC: Red blood cell, Hb: Hemoglobin, INR: International normalized ratio, LDH: Lactate dehydrogenase, PT: Prothrombin time, MCV: Mean corpuscular volume, MPV: Mean platelet volume, MCH: Mean corpuscular hemoglobin, PLT: Platelet, RDW-CV: Red cell distribution volume-coefficient of variation, UIBC: Unsaturated iron binding capacity.*Results were expressed as mean ± SD, ¥Results were expressed as median (1st-3rd quartile).

4. Discussion

Ferritin is an acute phase protein that is synthesised in the liver and peripheral macrophages. In the current study, higher ferritin levels were determined in patients than in the healthy control group. Similarly, in studies by Metanat et al. (2013) and Barut et al. (2010), higher serum ferritin levels were also reported in CCHF patients. It has been indicated that cytokine storm has an important role in the pathogenesis of CCHF (Bente et al., 2010). Increased ferritin levels lead to increased immune activity and thus, pro-inflammatory effects (Kernan and Carcillo, 2017). Cytokines including IL-1 and TNF-alpha have been shown to have the ability to induce ferritin expressions (Torti and Torti, 2002). Previous studies have shown increased levels of TNF-alpha in CCHF disease (Ergonul et al., 2006; Kaya et al., 2014). Therefore, it can be considered that increased ferritin levels might be related to the cytokine storm in CCHF patients. Kernan and Carcillo (2017b) also indicated that an increased ferritin level is associated with decreased iron availability. In the current study, the red blood cell (RBC), hemoglobin (Hb) and hematocrit (Hct) levels were found to be lower in patients than in the control group. Therefore, decreased iron availability might also be related with lower RBC, Hb and Hct levels in patients.

Platelets have a crucial role in the regulation of coagulation by plugging gaps in the endothelium of blood vessels (Slichter, 2004). Previous studies have shown that a low platelet count is associated with a bleeding risk in different conditions including severe sepsis, portal hypertension and cirrhosis (Gauer and Braun, 2012). Low platelet counts and thrombocytopenia have been shown in CCHF disease compared to control groups (Doğan et al., 2018; Leblebicioglu et al., 2016). In this study, the platelet count was determined to be lower in CCHF patients than in the healthy control group, which was in accordance with the findings of previous studies (Doğan et al., 2018; Leblebicioglu et al., 2016).

In a study made by the current author group, no correlation was found between platelet and hsCRP, which is produced in response to inflammation in the liver (Doğan et al., 2018). However, in the present study, a negative correlation was determined between platelet and ferritin levels, and a positive correlation between ferritin and aPTT values. van de Weg et al. (2014a) reported that hyperferritinaemia was associated with coagulation disturbance in dengue fever. The difference between the aforementioned studies may be related to the regulatory effects of ferritin on angiogenesis via inhibition of bradykinin production (Coffman et al., 2009). Bradykinin is a protein mediator that causes vascular dilatation and reduces platelet activation by releasing thromboplastin (Prieto et al., 2002). Therefore, it can be considered that increased ferritin levels may induce coagulation disturbance in CCHF.

Hepatocytes are one of the main targets of CCHFV. Therefore, increased levels of liver damage markers have been reported in patients (Buyuktuna et al., 2017; Ozturk et al., 2012; van de Weg et al., 2014b). In the current study, higher ferritin, aPTT, LDH and AST levels were found in patients than in the control group. Moreover, higher ferritin, LDH and AST levels were found in both patients administered thrombocyte suspension and those who received FFP. Bleeding is one of the most important prognostic factors for CCHF patients(Akinci et al., 2016). In the current study, a cutoff value of 149 U/L for AST was obtained with sensitivity of 100% and specificity of 71% in the discrimination of the bleeding condition in CCHF patients. Previous studies have reported that aPTT can be used for the follow-up of CCHF patients and that prolonged aPTT (> 60 s) is an independent predictor of case fatality rate (Cevik et al., 2008; Onguru et al., 2010). In the current study, the AUC values for AST were higher than for aPTT, LDH and ferritin in the discrimination of the bleeding status in CCHF patients. Therefore, AST could be considered as an alternative biomarker to estimate the bleeding in CCHF patients.

5. Conclusion

Increased ferritin levels may contribute to increased inflammation and coagulation abnormalities in CCHF patients. Therefore, ferritin may be a potential biomarker for the follow-up of CCHF patients. However, due to low correlation coefficient values between ferritin and coagulation-related biomarkers and the lack of additional inflammatory markers more research is needed to confirm these conclusions. Routine screening of AST level can be considered helpful to estimate the bleeding status in addition to screening liver damage in CCHF patients.

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Competing interests

None declared.

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