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RESEARCH ARTICLE

Immature platelet fraction as a useful marker in Crimean-Congo hemorrhagic fever

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ABSTRACT

The aim of this study is to evaluate the clinical significance and diagnostic performance of the immature platelet fraction (%IPF) in Crimean-Congo hemorrhagic fever (CCHF). Samples obtained from 32 healthy control subjects and 40 CCHF patients (9 positive and 31 negative radiological findings) were evaluated in the study. The samples obtained from CT-positive subjects demonstrated higher IPF% values which also exhibited a positive correlation with mean platelet volume (MPV) and platelet size deviation width (PDW) values.The patient group IPF% values were positively correlated with the duration of hospital stay. The ROC analysis also suggested the potential importance of IPF values higher than 10.5% in diagnosing CCHF patients with positive radiological findings.The results of our study showed that % IPF can be considered as a useful parameter in the follow-up of the disease course in patients with CCHF.

Keyworld: Crimean-congo hemorrhagic fever; ommature platelet fraction; mean platelet volume; computed tomography.

INTRODUCTION

Crimean-Congo hemorrhagic fever (CCHF) is a tick-borne acute viral hemorrhagic disease characterized by fever and/or bleeding with a case fatality rate of 5-30% (Tekin & Engin, 2020). CCHF virus belongs to the Nairovirus genus in the Bunyaviridae family and is endemic in Africa, Asia, Eastern Europe, and the Middle East (Şensoy *et al*., 2011). CCHF is characterized by vascular endothelial damage, disseminated intravascular coagulation, plateletopenia, liver damage, coagulation abnormalities, and hemorrhage, including ecchymosis, gingival bleeding, epistaxis, and gastrointestinal bleeding (Büyüktuna *et al*., 2019). Plateletopenia is a common laboratory parameter of this disease, and other findings are leukopenia, coagulopathy, and elevated liver enzymes (Hatami *et al*., 2019).

Platelets newly released from the bone marrow are larger, more reactive, and contain more RNA than mature ones. Because of their similarity with reticulocytes, they are called reticulated (RP) or immature platelets (IP) (Buttarello *et al*., 2020). The immature platelet fraction (IPF%) is analyzed as part of the complete blood count, and IPF% is fast, inexpensive, and feasible (Briggs *et al*., 2004). There are studies showing the clinical importance of IPF, such as differential diagnosis of thrombocytopenia, idiopathic thrombocytopenia (ITP), early diagnosis of infectious diseases, and prediction of sepsis in critically ill patients (Buttarello *et al*., 2020; Ekiz *et al*., 2013; Li *et al*., 2020; Strati *et al*., 2017). When thrombocytopenia is caused by platelet destruction, the bone marrow compensates by releasing immature platelets into the blood, causing an increase in IPF (Strati *et al*., 2017). Thus, the number of immature platelets in the circulation reflects the rate of thrombopoiesis and bone marrow function. For this reason, IPF% may be a useful diagnostic parameter in diagnosis and follow-up in patients who develop thrombocytopenia due to peripheral destruction of platelets or platelet production defect (Ali *et al*., 2019; Buoro *et al*., 2017; Jeon *et al*., 2017; Li *et al*., 2020).

Radiological findings may be positive before the development of hemorrhage in patients with CCHF, and in this case, information about the progression of the disease can be obtained (Aktas *et al*., 2017). As with many other viral diseases, CCHF causes pulmonary symptoms such as ARDS, hemoptysis, cough, dyspnea, chest pain, pleural effusion, pneumonia, consolidation, alveolar hemorrhage, and atelectasis (Aktas *et al*., 2019; Bilgin *et al*., 2014), and abdominal findings such as intra-abdominal free fluid, hepatomegaly, gallbladder wall thickening, splenomegaly, and GI bleeding. In addition, it has been reported that radiological findings and platelet count and platelet sub-parameters such as MPV are closely related (Aktas *et al*., 2017; Özmen *et al*., 2016). The aim of this research is to evaluate the clinical significance and diagnostic performance of IPF% in CCHF patients.

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MATERIALS AND METHODS

In this study, 32 subjects in the healthy control group and 40 patients among ages of 22 and 67 who applied to Tokat Gaziosmanpaşa University Health Research and Application Center were evaluated (Power analysis; 1-β=80, α=0.05 and Effect size= 0.321). All clinical laboratory measurements were made in TOGU Health Research and Application Center Central Laboratory. To confirm CCHF in patients clinically diagnosed as CCHF, the viral genome was detected using enzyme-linked immunosorbent analysis (ELISA) or polymerase chain reaction (PCR). Blood samples from these patients were sent to the TR Ministry of Health Refik Saydam Hıfzısıhha Central Laboratory. No statistical significance was observed between the groups in terms of gender or age (P>0.05). In the patient group, those with a history of chronic inflammatory disease, cancer, hematological disease, kidney, liver disease, anticoagulant and biological drug use were excluded from the study. Demographic, laboratory, and computed tomography (CT) data of the patients were used. The patient group was divided into two groups according to the presence of CT findings. There were 9 (22.5%) patients in the radiological finding positive group and 31 (77.5%) patients in the radiological finding negative group. For the CT findings in the positive group patients, gastrointestinal system bleeding was detected in two patients, pleural effusion was detected in seven patients, and free fluid was detected in the abdomen in addition to pleural effusion in four patients. Other CT findings were consolidation, hepatomegaly, ground glass densities, intestinal wall thickening, and edema. The study was approved by the ethical committee of our institute (Tokat GaziosmanpasaUniversity Clinical Research Ethical Committee; protocol number: 20-KAEK-172) and was planned and conducted according to the provisions of the Helsinki Declaration. In addition, an informed written consent form was obtained from the participants.

For the evaluation of the study parameter, blood samples were collected, centrifuged, and stored at -80°C. Laboratory evaluation of the patients and the control groups included IPF%. Hematological parameters and platelet count, IPF% and EDTA K2 anticoagulant whole blood samples containing other platelet indices were measured with a complete blood count (CBC), automatic hematology analyzer Sysmex-XN 1000 (Sysmex, Kobe, Japan). This analyzer uses a specific channel (PLT-F) for IPF% measurement, as it is determined with a fluorescent method (CV% in low-normal values: 4.3%–5.7%, CV% in high-normal values: 5.3%). Clinical biochemical parameters were measured by spectrophotometric methods with the Cobas c501 (Roche Diagnostics) device.

Statistical analysis was performed using SPSS 20.0 software for Windows (SPSS, Chicago, IL). Whether the variables show normal distribution or not was analyzed by the Test of Homogeneity of Variances. Since the variables did not show normal distribution, nonparametric tests, which were more suitable than statistical tests, were used. Mann – Whitney U test was used for non-homogenously distributed data and Student T test was used for homogeneously distributed data. Mann-Whitney U test was used in comparison of patients with healthy volunteers as well as in binary group comparisons. The categorical variables were compared using the chi-squared test. Kruskal-Wallis H test was used to compare more than two groups. Spearman and pearson correlation analysis was used to determine the relationship between numerical variables. ROC curves were used for the comparison of sensitivity and specificity. Differences of p<0.05 were considered to be statistically significant.

RESULTS

Forty patients were included in the study, 29 (72.5%) male and 11 (27.5%) female, with a mean age mean (standard deviation) of 54.6 (17.1) years. Eleven (34.4%) of the 32 subjects in the healthy control group were women and 21 (65.6%) were men, with a mean age of 52.3 (13.6). No statistically significant difference was observed between the groups in terms of gender or age (P>0.05).

Patients' serum biochemical and hematological markers on the first day of admission to the hospital were compared with those of the healthy control group (Table 1). Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT)creatine kinase (CK), and lactate dehydrogenase (LDH)values in the patient group were significantly higher than in the control group (p<0.05). White blood cells (WBC), hemoglobin, and thrombocyte levels in the patient group were significantly lower in than the control group

Table 1. Comparison of CCHF group and healthy control group

	CCHF (n= 40)	Healhty Control (n= 32)	p-Value
Age (years)	54.6±17.1	52.3±13.6	>0.05
Gender (Female/Male)	11/29	11/21	>0.05
AST (IU/L)	206.1 (25.5-628.0)	19.1 (11.5-27.0)	<0.001**
ALT (IU/L)	96.9 (14.6-514.9)	15.2 (7.8-41.9)	<0.001**
CK (IU/L)	212 (13-5968)	114 (52-605)	<0.05**
LDH (IU/L)	475 (193-1073)	193 (144-332)	<0.001**
BUN (mg/dL)	14.7 (6.8-48.3)	12.2 (6.7-22.7)	<0.05**
Creatinine (mg/dL)	0.9 (0.5-1.6)	0.6 (0.5-1.0)	<0.05**
WBC (x10^9cells/L)	3.5±3.0	8.9±2.2	<0.001*
Hemoglobin (g/dL)	13.2±1.8	14.1±4.0	<0.005*
Thrombocyte count (x10^3cells/µL)	33 (8-117)	272 (191-432)	<0.001**
IPF%	9.6±4.7	3.6±2.8	<0.001*
MPV fL	11.8±1.0	9.8±0.9	<0.001*
PCT (%)	0.06±0.03	0.26±0.05	<0.001*
PDW (fL)	14.0±2.5	10.9±2.3	<0.001*
PLCR (%)	48.7±59.4	23.9±6.9	0.022*
PT (s)	17.0±9.1	14.2±0.8	>0.05
APTT (s)	43.9 (26.9-91.0)	30.3 (0.8-4.2)	<0.001**
INR	1.12 (0.84-4.25)	1.10 (0.96-1.25)	>0.05**

*Student T testi, p<0.05.
**Man Whitney U Testi, p<0.05.

Table 2. Comparison of the radiological finding–positive CCHF and radiological finding–negative CCHF groups

	Radiological finding positive group (n= 9)	Radiological finding negative group (n= 31)	p-Value
Age (years)	64.7±13.9	51.6±17.0	>0.05
Gender (Female/Male)			
Duration of symptoms (days)	18.8±7.9	6.1±2.4	<0.001*
AST (IU/L)	229.4±212.4	257.7±415.	>0.05
ALT (IU/L)	151.1 ±119.1	160.8 (200.5)	>0.05
CK (IU/L)	97.5 (13-5981)	239.0 (42.0-862.0)	>0.05
LDH (IU/L)	588.1±386.2	612.9±493.0	>0.05
BUN (mg/dL)	26.3±26.8	17.9±10.8	>0.05
Creatinine (mg/dL)	0.82 (0.56-1.60)	0.85 (0.52-1.52)	<0.05
WBC (x10^9cells/L)	5.8 (2.0-18.4)	2.2 (0.9-5.6)	<0.05
Hemoglobin (g/dL)	12.3±2.6	13.5±1.4	>0.05
Thrombocyte count (x10^3cells/µL)	35±28.5	45.6±26.4	>0.05
IPF (%)	12.7 (8.8-25.4)	8.4 (3.4-21.6)	<0.001**
MPV (fL)	12.26±1.15	11.65±1.01	>0.05
PCT (%)	0.05±0.03	0.06±0.03	>0.05
PDW (fL)	15.14±2.68	13.73±2.44	>0.05
PLCR (%)	45.6 (40.1-413.0)	38.7 (25.7-56.9)	0.015**
PT (s)	15.6 (13.4-26.3)	13.8 (11.2-47.1)	<0.05**
APTT (s)	50.25±19.21	45.3±12.4	>0.05
INR	1.7±0.9	1.2±0.6	<0.05*

*Student T testi, p<0.05.
**Man Whitney U Testi, p<0.05.

(p<0.05). IPF% levels were 9.6 (4.7) in the patient group and 3.6 (2.8) in the healthy control group (p<0.001). Mean platelet volume (MPV), platelet distribution width (PDW), and platelet larger cell ratio (P-LCR) values were significantly higher in the patient group than in the control group (p<0.05). However, plateletcrit (PCT) values were found to be significantly lower in the patient group than in the control group (p<0.05). Among the coagulation parameters, only activated partial thromboplastin time (aPTT) values were found to be significantly higher in the patient group than in the control group (p<0.001).

In comparing the duration of symptomps between the radiological finding–positive and radiological finding–negative groups, the duration of symptomps was 18.8 (7.9) days in the radiological finding–positive CCHF group and 6.1 (2.4) days in the radiological finding–negative group (p<0.001). In comparing the laboratory parameters between the radiological finding–positive and radiological finding–negative groups, IPF% values were 12.7% (8.8–25.4) in the radiological finding–positive CCHF group and 8.4% (3.4–21.6) in the radiological finding–negative group (p<0.001). P-LCR values were significantly higher in the radiological finding–positive CCHF group than in the radiological finding–negative CCHF group (p<0.05). However, no significant difference was found between the other biochemical and hematological parameters (p>0.05) (Table 2).

Corelation between the IPF% values of the CCHF patient group and the study parameters were evaluated using correlation analysis, there was a strong positive correlation between IPF% and MPV and PDW values. As well as a moderate positive correlation between IPF% and duration of symptoms, ALT values, and P-LCR values founded. There was a weak positive correlation between IPF% and age, AST, CK, LDH, and aPTT. A moderate negative correlation was found between IPF% values and thrombocyte count and PCT values (Table 3).

The performance of thrombocyte count and related parameters in diagnosing patients with radiological finding–positive CCHF was evaluated by ROC analysis (Table 4, Figure 1). An IPF% value

Table 3. Relationship of IPF% with study parameters in the CCHF patient group

	IPF%	
	r- Value	p-Value
Duration of symptoms	0.614	<0.001
Age	0.243	0.040
ALT	0.415	<0.001
AST	0.292	0.013
CK	0.337	0.006
LDH	0.340	0.005
Thrombocyte count	-0.609	<0.001
MPV	0.835	<0.001
PCT	-0.531	<0.001
PDW	0.745	<0.001
PLCR	0.417	<0.001
APTT	0.361	0.002

Table 4. ROC analysis results of PLT, IPF% and other PLT subunits in the diagnosis of positive radiological findings in CCHF patients

Test Result Variable(s)	AUC	p-Value	Asymtotic 95% Confidence Interval	
			Lower Bound	Upper Bound
PLT	0,342	0,154	0.135	0.550
IPF%	0,894	<0,0001	0.775	1.000
PT	0,754	0,021	0.584	0.925
PLCR	0,769	0,015	0.602	0.936
PDW	0,647	0,184	0.428	0.865
MPV	0.665	0.136	0.483	0.846

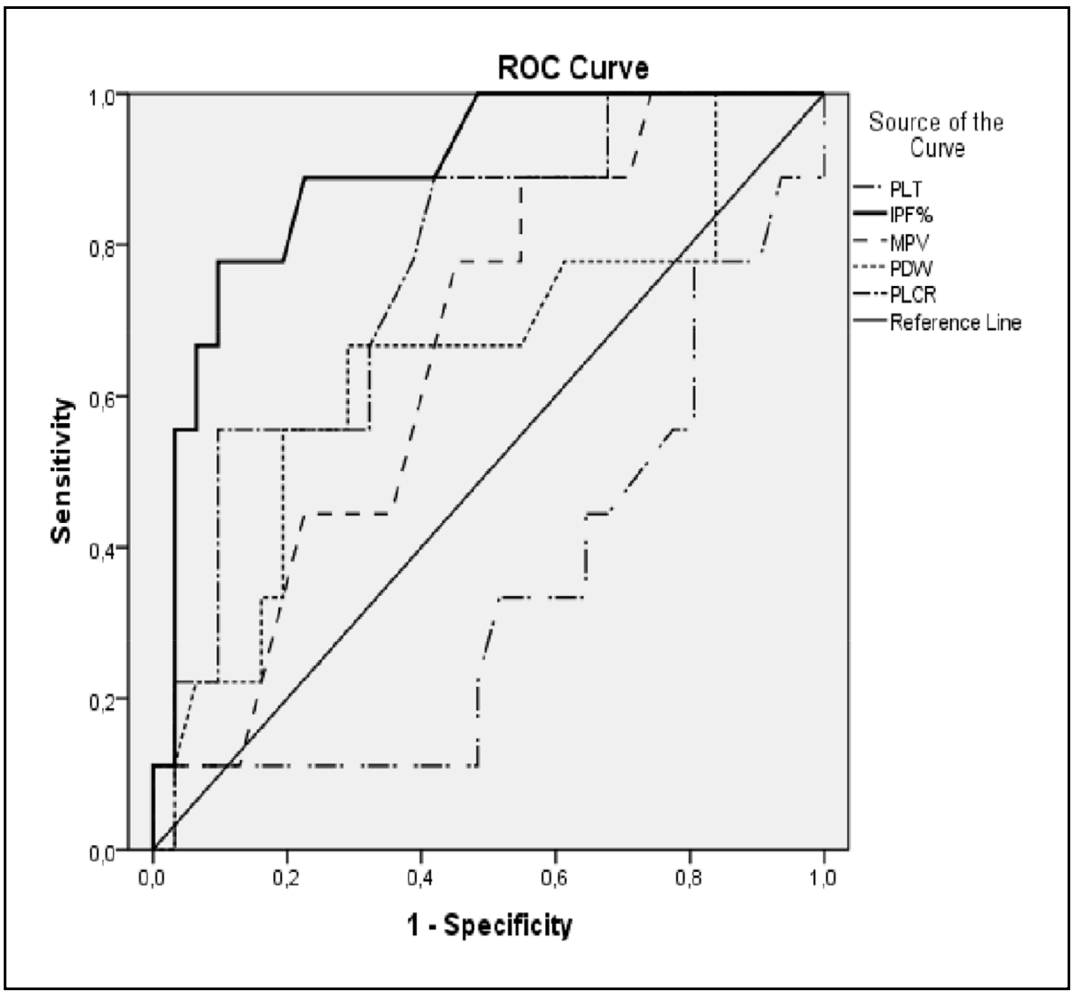

Figure 1. ROC curves of IPF% and other hematological parameters in the diagnosis of positive radiological findings in CCHF patients.

below 9.8 differentiated radiological finding–positive CCHF from the radiological finding–negative CCHF with 88.9% sensitivity and 77.4% specificity (p<0.001). An IPF% value below 10.5 differentiated radiological finding–positive CCHF from the radiological finding–negative CCHF with 77.8% sensitivity and 99.9% specificity (p<0.001). This performance of IPF% in diagnosing patients with radiological finding–positive CCHF was higher than the diagnostic performance of PLT, PDW, P-LCR, MPV, PT, INR, and aPTT.Except of IPF%, only AUC values of P-LCR and PT were significant in the diagnosis of radiological finding-positive CCHF (P<0.05) (Table 4).

DISCUSSION

CCHF is a tick-borne viral zoonosis widely distributed in Africa, Asia, and Eastern Europe within the ranges of ticks belonging to the genus Hyalomma. The case fatality rate is approximately 40%, but it can range from 20% to 80% (Goodman, 2005). CCHF were not widely reported until early 1999. The prevalence of the disease has eleveted since 2000 and this has brought new research and studies (Chinikar *et al*., 2020).The first CCHF symptomatic case in Turkey was reported in the Kelkit Valley of Tokat province in 2002 (Leblebicioglu *et al*., 2016). Although the area where the disease is seen has expanded in recent years, few cases have been published from Western Anatolia, where the disease is seen sporadically (Ertugrul *et al*., 2009). At the time of our study, the incidence of CCHF was reported as 8.1% in our region. In the same period, a total of 343 CCHF cases and 13 CCHF-related deaths were reported across the country (Sönmez, 2022). CCHF is seen commonly in western China, across southern Asia to the Middle East, Spain, the Balkans, and most of Africa. Approximately 1000 CCHF cases of infection are reported in the Middle East and Eastern European countries annually. The notified rates of mortality of CCHF epidemics and outbreaks differ greatly; nevertheless, the average mortality rate is reported at 5-50% (Monsalve-Arteaga *et al*., 2017). The mean mortality rate from CCHF is around 5% in Turkey (Guven *et al*., 2017). During our study, 40 patients diagnosed with CCHF in our region were evaluated with computerized tomography findings. Nine CCHF patients with positive CT findings constituted the risk group. The clinical and diagnostic importance of IPF% values, a hematological parameter, in the early diagnosis of this patient group was evaluated.

In CCHF, in addition to non-specific symptoms such as fever, fatigue, myalgia, serious spontaneous bleeding and hypovolemic shock may occur due to a significant decrease in platelet levels. In CCHF patients, some laboratory parameters such as aPTT, INR, ALT, AST, CK, and LDH increase, while leukocytes and platelets decrease. In addition, prothrombin time and activated partial thromboplastin time are prolonged in CCHF (Gürbüz *et al*., 2021; Kilinc *et al*., 2016; Mostafavi *et al*., 2014; Yeşilbağ *et al*., 2020). In our study, ALT, AST, CK, LDH, BUN, creatinine, MPV, PDW, P-LCR, and aPTT levels were increased and WBC, Hb, PLT, and PCT levels were decreased in the CCHF patient group compared to the control group. These results were compatible with the literature (Bakır *et al*., 2015; Yilmaz *et al*., 2010; Yilmaz *et al*., 2016).

Recent advances in automated blood cell analyzers can be very useful for laboratory measurements of thrombocytopenia if platelet indices such as mean platelet volume (MPV), platelet size deviation width (PDW), and platelet large cell ratio (P-LCR) and IPF% can provide information on platelet kinetics. There is increasing interest

in the use of platelet markers to distinguish between different forms of thrombocytopenia (Niethammer & Forman, 1999; Park *et al*., 2002; Threatte, 1993). Bleeding, which is one of the most important causes of mortality in CCHF cases, is related to platelet count and function. The most common cause of CT positive findings in these cases is bleeding. Early diagnosis of CT positive cases can reduce mortality. There are studies in the literature showing that PLT sub-parameters such as PCT, PDW, and MPV may be useful parameters in predicting bleeding and mortality in CCHF patients. Duygu *et al*. (2018) reported in their study that PDW values decreased even more in patients with decreased platelet values in CCHF cases and that a decrease in PDW values in parallel with the worsening of the disease may be associated with hemorrhage and mortality. Yılmaz *et al*. (2016) reported that PDW may be a marker at least as useful as platelet count in determining risk of hemorrhage in patients with CCHF. Ekiz *et al*. (2013) showed that CCHF patients had significantly higher MPV levels than controls, but the MPV levels were found to be normal range in this study. In our study, MPV and PDW values were higher in CCHF patients compared to the control group. However, there was no significant changes in the radiological finding–positive group. The fact that these parameters, which are thought to be useful in estimating hemorrhage and mortality, do not change in such a critical situation may be due to the insufficient number of our cases.

However, little is known about P-LCR, IPF%, and thrombocytopenia, and whether platelet indices are satisfactory laboratory tests for thrombocytopenia has not been fully discussed. In our assessment, not only MPV and PDW, but also P-LCR and IPF% were significantly higher in CCHF than in healthy controls. P-LCR and IPF% radiological findings were significantly higher in the positive CCHF group than in the negative CCHF group. While IPF% values were moderately positively correlated with duration of symptoms and PDW, they were moderately weakly correlated with platelet count. In various studies investigating the benefits of IPF in infectious diseases, it has been stated that it may be useful in early diagnosis and control of the disease, although the results are somewhat contradictory (Buttarello *et al*., 2020). Two recent studies have suggested that IPF may help identify patients very early in the course of sepsis and be used as a screening parameter for bacterial infection, allowing treatment initiation before clinical initiation (Di Mario *et al*., 2009; De Blasi *et al*., 2013). Oehadian *et al*. (2015) reported that IPF% increased in dengue, leptospirosis, and enteric fever, which are three diseases with thrombocytopenia, and the IPF% was highest in dengue fever. In these events with platelet destruction/consumption, the increase in IPF% is correlated with the decrease in platelets. It has been proven in the literature that the IPF% is effective in the differential diagnosis of thrombocytopenia due to accelerated platelet destruction (Li *et al*., 2020). CCHF is a viral disease with accelerated platelet destruction and thrombocytopenia. The diagnostic value of IPF% is one of the interesting issues in the differential diagnosis of thrombocytopenia in patients with thrombocytopenia and bleeding. Park *et al*. (2016) showed that IPF has high sensitivity and accuracy in distinguishing septic patients from non-septic patients. In studies on the diagnostic value of IPF% in the follow-up of Dengue fever progression, it has been reported that IPF% >10% predicts platelet recovery within 24-72 hours (Looi *et al*., 2021). According to the results of our study, IPF % had adequate diagnostic performance in identifying patients with positive radiological findings in CCHF. While the patient is being followed, an IPF >10.5% has the potential to diagnose lung involvement with 77.8% sensitivity and 99.9% specificity. An important point to be investigated here is the diagnostic value of IPF% in predicting lung involvement in CCHF patients. Prospective studies with larger numbers of patients are needed on this subject.

When the patient group IPF% values were compared with the control group, we detected a significant increase in IPF% values in CCHF patients. At the same time, a strong positive correlation was found between IPF% and MPV and PDW values, and a moderate positive correlation was found between duration of symptoms, ALT, and P-LCR values. In CCHF patients, IPF% values increased even more in those with positive radiological findings. This was also valid for P-LCR and PT results. However, IPF% was an interesting parameter that increased in the CCHF patient group and increased even more in the presence of radiological findings. IPF% was moderately positively correlated with the duration of the disease. When we evaluated its diagnostic adequacy in CCHF patients with positive radiological findings, we found that it had an adequate performance. The relationship between IPF% and duration of symptoms indicated that IPF% was clinically important in the follow-up of the disease course. Organ involvement positive patients in CCHF patients show a poor prognostic course. In our study, positivity of radiological findings such as organ involvement or bleeding in CCHF patients was evaluated as an indicator of disease severity. The reasons for the higher IPF% values in the radiological positive group compared to the negative group perhaps due to the lower platelet counts of the positive group and the longer duration of the patient group.

Hospitalized patients with CCHF demonstrated that progressive thrombocytopenia in CCHF infection was accompanied by a concomitant increase in IPF%. The relationship between IPF% and duration of symptoms indicated that IPF% was clinically important in the follow-up of the disease course. IPF% was significantly higher in patients with positive radiological findings compared with patients with negative radiological findings in CCHF. This difference in IPF% between patients with radiological finding–positive CCHF and patients with radiological finding–negative CCHF had diagnostic value for it to be a potential tool for diagnosis of organ involvement in CCHF. The ROC analysis suggested the potential importance of IPF values higher than 10.5% in diagnosing CCHF patient with positive radiologic findings. Further studies required to investigate IPF% diagnostic value in the organ involvement of CCHF and the association with progression from the febrile stage to the defervescent stage of the disease.

IPF% values in CCHF patients with a severe course of CCHF with positive radiological findings were higher levels than in patients with negative findings. IPF% may be a potential routine laboratory biomarker to be used in the diagnosis and follow-up of CCHF.

Limitations of this study

This study had several limitations. The sample size was relatively small, and the study was conducted at a single center that may not be representative of CCHF patients from other centers and countries. Since the disease was seen as sporadic cases in the region during the study period, the patient sample group was insufficient. Accordingly, the number of groups with positive radiological findings was insufficient. The failure of IPF% to work and radiological follow-up during the disease follow-up also led to insufficient results regarding the use of IPF% in follow-up.

The main strength of the manuscript

The idea of using IPF values is of high importance as it provides an inexpensive and rapid approach to estimating the severity of CCHF infection. Moreover, a comparison of IPF values between CT-positive CCHF, CT-negative CCHF, and control groups, to evaluate the relationship with other parameters (such as MPV, PDW, ALT, AST, and duration of symptoms) the increase depend on the severity of infection, and ROC analysis enables for in-depth research. Our investigation is the first research that we could detect from literature search in which IPF levels were evaluated in CCHF patients.

Conflict of Interest

The authors, Zeliha Cansel Özmen, Köksal Deveci, Umut Safiye S Coşkun, Zafer Özmen, Leyla Aydoğan and Hüseyin Ş Barut declare that there is no conflict of interest.

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