The Expression of Serum Ferritin and Its Clinical Predictive Value in Patients With Hemorrhagic Fever With Renal Syndrome

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Abstract

As a natural focus disease characterized by systemic immunopathological injury, there is a considerable diversity and heterogeneity in clinical course and prognosis of the patients with hemorrhagic fever with renal syndrome (HFRS). Therefore, the importance of early prediction and prognostic evaluation of severe patients in reducing mortality is self-evident. In this study, we detected the expression of serum ferritin and investigated its role on disease severity assessment and prognosis prediction in HFRS patients. The levels of serum ferritin in acute phase were higher than that of healthy controls and in convalescent phase of the same type, and gradually increased with the aggravation of the disease, which showed highest expression in the critical-type patients. Serum ferritin demonstrated significant correlations with conventional laboratory parameters (WBC, PLT, AST, ALB, APTT, Fib) and the length of hospital stay. High levels of ferritin were significantly associated with the death of HFRS, with a hazard ratio of 4.12. Serum ferritin showed a comparable predictive efficacy for the prognosis (death) of HFRS with that of conventional laboratory parameters, which with the AUC of 0.860 (95%*CI*: 0.782 ~ 0.938, P < 0.001). These results indicated that the detection of serum ferritin might be beneficial to early prediction and prognosis evaluation in severe HFRS patients and which is worthy of clinical application.

Introduction

Ferritin is a kind of soluble macromolecular protein complex formed by apoferritin and Fe^{3+}, which mainly exists in reticuloendothelial cells of the liver, spleen and bone marrow. Under normal circumstances, ferritin can provide iron for the synthesis of hemoglobin in bone marrow, and which can also been released into the serum according to the needs of the body [1]. The level of serum ferritin can be considered as the indicator of iron deficiency or excess and which is frequently used in clinical settings, nevertheless, the mechanism of secretion as well as the function of serum ferritin is unclear.

It is currently believed that serum ferritin levels are not only affected by iron metabolism, but also by infection, tissue necrosis and other inflammatory activities. Ferritin regulates immune activities through different mechanisms, including pro-apoptotic and anti-apoptotic pathways and inducing the synthesis of pro-inflammatory cytokines [2]. In recent years, an increasing number of researches have shown that serum ferritin is closely related with coronary heart disease, atherosclerosis, acute cerebral infarction, myelodysplastic syndrome, non-alcoholic fatty liver, rheumatoid arthritis, anticardiolipin syndrome, hemophagocytic syndrome, type 2 diabetes and other diseases [3–6]. In addition, more and more studies have shown that serum ferritin can serve as a new biomarker to assess the body's inflammatory state and oxidative stress [7]. Increased serum ferritin may also be related to the inflammation and infection, which is helpful to evaluate the disease severity and patient's prognosis of sepsis [8].

In terms of viral infections, studies have also shown that serum ferritin is associated with viremia, inflammatory response and the death in patients with COVID-19, Ebola viral diseases and Crimean-Congo hemorrhagic fever [9–11], while with few studies on the dynamic changes of serum ferritin in patients with hemorrhagic fever with renal syndrome (HFRS), and the role of which in HFRS is still unclear. In this study, we observed the changes of serum ferritin in 102 HFRS patients with different clinical types and different stages, and investigated its predictive value on disease severity and prognosis (death) of HFRS patients.

Results

Demographic characteristics

A total of 102 patients with an average age of 42.15 ± 14.73 years were enrolled in this study, including 81 (79.4%) males and 21 (20.6%) females. 17 cases were classified as mild-type, 19 cases were classified as moderate-type, 27 cases were classified as severe-type, and 39 cases were classified as critical-type. Of all the enrolled patients, 18 (17.6%) critical patients were died and the rest were recovered and discharged (Table 1). There was no significant difference in age, gender distribution and the timing of blood collection during the acute phase between all types of patients and healthy controls ($P > 0.05$). With the aggravation of the disease, the lengths of hospital stay increased gradually, and showed longest in the critical-type patients (Table 1).

Table 1
Demographic, clinical and laboratory parameters of the study population

	Mild (*n* = 17)	Moderate (*n* = 19)	Severe (*n* = 27)	Critical (*n* = 39)	[a] HC (*n* = 28)	$\chi^2/F/H$	*P*	Alive (*n* = 84)	Dead (*n* = 18)	$\chi^2/t/Z$	*P*
Gender											
Female	6 (35.3%)	5 (26.3%)	3 (11.1%)	7 (17.9%)	6 (21.4%)	4.279	0.233	17 (20.2%)	4 (22.2%)	0.036	0.850
Male	11 (64.7%)	14 (73.7%)	24 (88.9%)	32 (82.1%)	22 (78.6%)			67 (79.8%)	14 (77.8%)		
Age, years	35.88 ± 17.48	40.89 ± 13.34	43.11 ± 12.99	44.82 ± 14.90	38.65 ± 13.26	1.564	0.203	41.34 ± 14.46	46.80 ± 15.95	-1.330	0.187
[b] Timing, days	7.0 (5.0-7.5)	6.0 (5.0–7.0)	6.0 (5.0–7.0)	6.0 (5.0–8.0)	—	1.036	0.793	7.0 (5.0–8.0)	5.0 (4.0–6.0)	-2.866	0.004
Hospital stay, days	9.0 (8.0–11.0)	13.0 (11.0–15.0)	18.0 (16.0–23.0)	19.0 (7.0–25.0)	—	24.177	< 0.001	16.0 (12.0–23.0)	6.0 (2.0–10.0)	-5.159	< 0.001
Ferritin, ng/mL											
Acute phase	6795 (735-14392)	8958 (4316–12227)	10329 (4104–17769)	19289 (6392–27440)	101 (31–133)	71.734	< 0.001	10199 (3001–17870)	25954 (12828–31363)	-3.576	< 0.001
Convalescent phase	371 (134–501)	574 (135–1085)	676 (491–1352)	219 (76-1259)		33.074	< 0.001	—	—	—	—
WBC, $\times10^9$/L	9.56 (7.58–12.90)	8.94 (4.95–14.36)	13.94 (9.15–19.46)	14.95 (9.78–34.43)	—	15.326	0.002	11.28 (8.16–16.56)	31.67 (12.31–49.29)	-3.179	0.001
PLT, $\times10^9$/L	79.0 (53.5-108.5)	36.0 (24.0–59.0)	35.0 (23.0–56.0)	25.0 (13.0–49.0)	—	21.518	< 0.001	39.0 (23.0–63.0)	21.0 (11.0–39.0)	-3.015	0.003
ALB, g/L	31.31 ± 4.26	29.12 ± 3.82	27.64 ± 5.92	27.02 ± 6.72	—	2.487	0.065	29.06 ± 5.04	23.79 ± 7.91	3.405	0.001
AST, U/L	69.5 (43.8-102.8)	75.0 (50.5-114.8)	85.0 (61.0-126.0)	142.0 (94.0-241.5)	—	25.000	< 0.001	89.0 (59.0-126.0)	234.0 (120.0-823.0)	-4.349	< 0.001
BUN, mmol/L	6.95 (4.49–11.73)	12.51 (6.50–21.70)	19.80 (11.77–27.45)	14.97 (8.85-25.00)	—	14.280	0.003	14.18 (6.88–23.11)	12.49 (7.80-24.45)	-0.005	0.996
Cr, μmol/L	115.1 (74.0-214.6)	184.1 (77.1-520.5)	301.4 (114.5–523.0)	250.4 (129.6-473.9)	—	10.136	0.017	212.7 (105.0-480.8)	218.8 (99.8-292.4)	-0.364	0.716
APTT, sec	32.6 (29.0-36.2)	35.5 (31.5–43.2)	38.9 (33.0-44.3)	39.3 (33.6–62.7)	—	10.494	0.015	35.9 (31.9–42.9)	64.8 (42.8–74.2)	-4.613	< 0.001
Fib, g/L	3.03 ± 0.86	2.55 ± 0.77	2.69 ± 1.02	2.02 ± 0.94	—	5.407	0.002	2.63 ± 0.94	1.57 ± 0.72	4.178	< 0.001

[a] HC: Healthy Controls.

[b] Timing: Timing of blood collection during the acute phase, duration from illness onset to the day of blood collection.

Serum ferritin and conventional laboratory parameters

The levels of serum ferritin among the subgroups were comparatively analyzed to investigate the clinical correlation of ferritin with disease severity. In all types of patients, the levels of serum ferritin in acute phase were significantly higher than that of healthy controls and convalescent phase of the same type ($P < 0.05$). The levels of serum ferritin in acute phase had an increasing tendency with the aggravation of the disease, and showed the highest expression in critical-type patients ($P < 0.05$) (Fig. 1). As shown in Table 1, more severe patients and the dead got higher levels of ferritin and worse expressions of the conventional laboratory parameters. For the renal function markers (BUN and Cr), nevertheless, there was no difference between the alive and the dead ($P > 0.05$). During the convalescent phase, serum ferritin of HFRS patients was still higher than that of healthy controls, while demonstrated no significant difference among the four types (Table 1 and Fig. 1).

The correlations of serum ferritin with conventional laboratory parameters and hospital stay

Spearman correlation analysis showed that serum ferritin during the acute phase was closely correlated with routine laboratory parameters such as WBC, PLT, ALB, AST, APTT and Fib ($|r_s|>0.500$, $P < 0.001$), and the correlation with PLT was the highest (r_s=-0.792, $P < 0.001$) (Table 2 and Fig. 2). For the reason that the death usually occurred soon after admission in the non-survivors, we used only the length of hospital stay of the survivors for correlation analysis. The result showed that serum ferritin during the acute phase was also significant correlated with the length of hospital stay (Table 2).

Table 2

[a]Correlations of serum ferritin with hospital stay and laboratory parameters

Hospital stay and laboratory parameters	Ferritin, ng/mL	
	r_s	P value
[b] Hospital stay, days	0.257	0.026
WBC, $\times 10^9$/L	0.589	< 0.001
PLT, $\times 10^9$/L	-0.792	< 0.001
HGB, g/L	0.430	< 0.001
ALB, g/L	-0.517	< 0.001
ALT, U/L	0.282	< 0.001
AST, U/L	0.678	< 0.001
PT, sec	0.168	0.032
APTT, sec	0.552	< 0.001
Fib, g/L	-0.567	< 0.001
BUN, mmol/L	0.234	0.001
Cr, μmol/L	-0.030	0.686
K^+, mmol/L	0.027	0.719
Na^+, mmol/L	-0.494	< 0.001
Ca^{2+}, mmol/L	-0.481	< 0.001
[a] The correlations were calculated by Spearman rank correlation analysis.		
[b] The data of hospital stay was derived from the survivors.		

The construction of prognosis risk model for HFRS and the evaluation of predictive efficacy

Ferritin and the conventional laboratory parameters (WBC, PLT, ALB, AST, APTT and Fib) with statistical significance between survivors and non-survivors were incorporated into the logistic regression analysis to construct the prognosis (death) risk model. Considering the relatively small sample size and the possibility of over-fitting in the multivariate logistic regression model, we adopted a forward stepwise method (probability for stepwise: entry $P < 0.05$, removal $P > 0.1$) for logistic regression analysis. Finally, WBC, AST and APTT were enrolled in the prognosis (death) risk model for HFRS which was constructed as following: logit(P)=−9.273 + 0.078×WBC (10^9/L) + 0.009×AST (U/L) + 0.088×APTT (sec) (Table 3).

Table 3
Logistic regression analysis to construct the prognostic risk model in patients with HFRS

Variables	*b*	*P* value	S_b	*Wald*χ^2	*OR* (95% *CI*)
WBC	0.078	0.009	0.030	6.741	1.081 (1.019–1.147)
AST	0.009	0.004	0.003	8.310	1.009 (1.003–1.015)
APTT	0.088	0.002	0.029	9.179	1.091 (1.031–1.155)
β	-9.273	0.000	1.975	22.033	-
Ferritin, WBC, PLT, ALB, AST, APTT and Fib were incorporated into the logistic regression analysis, and finally WBC, AST, APTT were enrolled in the prognosis (death) risk model: logit(P)=-9.273 + 0.078×WBC (10^9/L) + 0.009×AST (U/L) + 0.088×APTT (sec).					
Modeling methods: forward stepwise method, probability for stepwise: entry $P < 0.05$, removal $P > 0.1$. All variables were derived from the values in acute phase.					

ROC curves were used to assess the predictive efficacy of the prognosis (death) risk model, serum ferritin and conventional laboratory parameters. According to the order of area under ROC curve from large to small, these early predictors were the prognosis (death) risk model (0.936), AST (0.921), APTT (0.865), Ferritin (0.860), PLT (0.840), WBC (0.827), Fib (0.825) and ALB (0.801), successively (Table 4). Consequently, the prognosis (death) risk model showed the highest predictive efficacy, and serum ferritin also demonstrated a comparable predictive value with WBC, PLT, AST, APTT and other conventional laboratory parameters, which with the AUC of 0.860 (95% *CI*: 0.782 ~ 0.938, $P < 0.001$) and achieved a high sensitivity of up to 94.4% (Table 4 and Fig. 3).

Table 4
Predictive efficacy of prognosis risk model and laboratory parameters

	AUC (95% *CI*)	*P* value	Cut-off value	Sensitivity	Specificity	[a] Comparison of AUC (risk model)		[b] Comparison of AUC (ferritin)	
						Z value	P value	Z value	P value
Prognosis risk model	0.936 (0.867 – 1.000)	< 0.001	-4.953	94.4%	59.0%	—	—	1.877	0.0605
Ferritin, ng/mL	0.860 (0.782 – 0.938)	< 0.001	5793.8	94.4%	62.0%	1.877	0.0605	—	—
WBC, $\times 10^9$/L	0.827 (0.709 – 0.944)	< 0.001	10.58	88.9%	62.7%	3.248	0.0012	0.537	0.5916
PLT, $\times 10^9$/L	0.840 (0.771 – 0.910)	< 0.001	41.5	70.5%	88.9%	2.690	0.0071	0.532	0.5948
HGB, g/L	0.607 (0.455 – 0.760)	0.135	—	—	—	—	—	—	—
ALB, g/L	0.801 (0.677 – 0.925)	< 0.001	27.75	74.7%	83.3%	3.439	0.0006	0.877	0.3805
AST, U/L	0.921 (0.868 – 0.974)	< 0.001	115	88.9%	80.6%	0.394	0.6935	1.380	0.1676
APTT, sec	0.865 (0.760 – 0.969)	< 0.001	39.15	88.9%	78.8%	1.748	0.0805	0.345	0.7304
Fib, g/L	0.825 (0.724 – 0.927)	< 0.001	1.84	89.0%	66.7%	2.241	0.0250	0.498	0.6183
Cr, μmol/L	0.509 (0.388 – 0.630)	0.902	—	—	—	—	—	—	—
BUN, mmol/L	0.595 (0.459 – 0.732)	0.184	—	—	—	—	—	—	—

[a] The AUC value of each laboratory parameter was compared with that of the prognosis risk model.

[b] The AUC value of each parameter was compared with that of the ferritin.

Taking death/survival as the terminal event, lengths of hospital stay as the survival time, and grouping based upon the cut-off values of ROC curves, the results of Kaplan-Meier survival analysis with log-rank test showed that high levels of ferritin (> 5793.8 ng/mL) during the acute phase were significantly associated with the death in HFRS patients, with a HR of 4.12 (1.34 ~ 12.65) in comparison with the low ferritin (< 5793.8 ng/mL) (Fig. 4).

Discussion

As an acute natural focus epidemic disease which caused by Hantavirus infection, HFRS is characterized by fever, shock, hemorrhage and acute kidney injury [12]. China is the main epidemic area of HFRS, with more than 90% of the total cases in the world [13]. As a severely afflicted area, the HFRS incidence of Shaanxi province has an increasing tendency, also with more severe clinical course and higher fatality rate infected by Hantaan virus [14]. HFRS has pathophysiological characteristics of systemic inflammatory response syndrome (SIRS), and increased vascular endothelial permeability is the pathological basis for the clinical manifestations [12, 15, 16]. Typical cases usually present with fever, hypotensive shock, oliguria, polyuria, and convalescent stages. Fever, hypotensive shock and oliguria may overlap in some critically ill patients, which may result in refractory shock, acute respiratory distress syndrome (ARDS), encephalopathy, severe coagulation dysfunction and even multiple organ dysfunction syndrome (MODS) [12, 17].

Although the domestic clinical classification standard plays an important role in the prevention and treatment of HFRS in China [18], while most parameters of which are based upon the subjective symptoms and the doctor's physical examination. Therefore, the disease severity assessment and prognosis prediction are difficult and limited by the non-quantifiable parameters of the classification standard. In recent years, several studies have explored some early warning indicators which can reflect the severity and predict the prognosis of

HFRS, such as interleukin-34, adiponectin, pentraxin-3, interleukin-6, tumour necrosis factor-α (TNF-α), sCD163, growth arrest-specific 6 protein, urinary neutrophil gelatinase-associated lipocalin (NGAL), etc. [19–25]. For the reasons that the high cost of detection, complicated operation methods and limited predictive effectiveness, however, the above indicators are not extensively used in clinical practice. Therefore, it is necessary to explore novel biomarkers for clinical application.

In this study, we detected the levels of serum ferritin in patients with HFRS and found that the concentrations of ferritin during the acute phase were significantly higher than that of the healthy controls, and increased with the exacerbation of the disease. This result reflected the so-called "cytokine storm" caused by super immune response after Hantavirus infection [26] and also indicated that ferritin can be used as a biomarker to evaluate the inflammatory state and oxidative stress, which with similar results in sepsis, COVID-19, Ebola viral disease and Crimean-Congo haemorrhagic fever [8–11]. The invasion of Hantavirus can cause excessive inflammation and massive inflammatory factors release, which may induce hepatocytes to synthesize and release ferritin. In addition, the greatly increased neutrophils and mononuclear macrophages of HFRS can also synthesize and release ferritin during the anti-inflammatory phagocytosis process, which may in turn causes the increasing of serum ferritin. Furthermore, the hepatocyte damage itself can cause a large amount of ferritin to be released into blood [5], which may also be one of the mechanisms for the increase of serum ferritin during the acute phase.

Our previous studies have shown that HFRS patients usually manifest a high degree of inflammatory reactions, significant coagulation abnormalities and vascular endothelial injury, which accompanied by high WBC levels, long APTT, reduced PLT and Fib decrease [27–29]. Furthermore, long-temporal hypoperfusion and hypoxia on the liver and heart during the clinical course usually manifest by increased cell membrane permeability, massive release of AST from the cytoplasm into the blood, and also accompanied with synthesis dysfunction of ALB and extravascular leakage [27–29]. In this study, the levels of serum ferritin were significant correlated with WBC, PLT, ALB, AST, APTT, Fib and the length of hospital stay. In addition, high levels of ferritin (> 5793.8 ng/mL) during the acute phase were significantly associated with the death in HFRS patients, with a HR of 4.12 in comparison with the low ferritin (< 5793.8 ng/mL). The above results collectively suggest that serum ferritin might be benefit to reflecting the severity of HFRS. Furthermore, ROC curve analysis showed that ferritin got a considerable predictive value accompanied by high sensitivity in predicting the prognosis of HFRS, which is equivalent to conventional laboratory parameters such as WBC, PLT, AST and APTT. Together, all these results demonstrate that serum ferritin might be a commendable biomarker for disease severity assessment and prognosis prediction in HFRS.

For its simple method, easy operation, and low price of detection, serum ferritin has been carried out in most hospitals in China. Nevertheless, many hospitals set the detection threshold of 2000μg/L and only take ferritin as a tumor marker for therioma screening. Due to the considerable diversity and heterogeneity of the ferritin levels in different diseases or even in different clinical stages of the same disease, therefore, the clinical application of ferritin is limited in reflecting the inflammatory state and oxidative-stress degree of infectious diseases. According to the results of this study, we believed that ferritin could be benefit to early warning and prognosis assessment of HFRS, and serum ferritin detection worthy be further popularized and recommended as a routine test item for HFRS. Of course, there is an urgent need to break through the detection threshold limit. Considering the different degree of inflammation in different types and stages of HFRS, it is necessary to reasonably dilute the blood samples to obtain more accurate results.

The prognosis risk model constructed by logistic regression analysis in this study showed that the predictive power for HFRS prognosis of the multiple indicators coalition was significantly higher than that of a single laboratory parameter. However, the three indicators finally enrolled in the model were WBC, AST and APTT, and ferritin failed to enter the model. This study was conducted in a single center for infectious diseases and the results might be limited by the relatively small sample size. In addition, the results of this study should be interpreted conservatively due to the relatively broad timing of blood collection and the inevitable experimental errors. Therefore, it is essential to conduct a prospective, large sample, multicenter cohort study to further confirm the predictive efficacy and clinical application value of ferritin. Finally, we hope to establish a more perfect prognostic risk model or even formulate a scoring system and new clinical classification standards for HFRS by effectively integrating imaging examinations, clinical data, laboratory parameters and novel biomarkers, which will be great significance for clinicians to adjust the treatment in time and improve the prognosis of the critically ill patients.

Conclusions

Serum ferritin might be beneficial to early evaluate the disease severity and predict the prognosis in patients with HFRS, which is worthy of clinical application. The detection of serum ferritin could help clinicians quickly identify the severe patients at an early stage and timely take optimal therapeutic schedule for them, so as to improve the therapeutic effect of HFRS.

Methods

Patients and clinical data

This study was approved by the ethics committee of the Second Affiliated Hospital of Air Force Medical University and was performed in accordance with the Helsinki Declaration. From October 2011 to December 2013, 102 HFRS patients admitted by the Second Affiliated Hospital of Air Force Medical University were prospectively enrolled in this study after signing the informed consent form. All enrolled patients met the following inclusion criteria: (1) Confirmed by serological examination (HFRS-specific IgM and IgG positive) and consistent with the typical clinical manifestations of HFRS; (2) 18–70 years old; (3) During the acute phase of HFRS at the time of hospital admission. Besides, patients with pregnancy, chronic kidney diseases, diabetes, cardiovascular diseases, autoimmune diseases, viral hepatitis, bacterial infection and other infectious diseases were excluded. In addition, we recruited 28 healthy volunteers as controls. All of them were informed about the objectives of this study, and they agreed and signed the informed consent form.

According to the domestic clinical classification criteria of HFRS [18], all enrolled patients were divided into mild-type group, moderate-type group, severe-type group and critical-type group. Based upon the typical clinical characteristics, we further divided the clinical course into the acute phase (including the febrile, hypotensive and oliguric stages) and the convalescent phase (including the diuretic and convalescent stages) [27]. Prognosis was defined as death or survival during hospitalization.

The detection of serum ferritin

Venous blood samples were collected (desiccant tube without anticoagulant, 5ml) once each during the acute phase and the convalescent phase, and were centrifuged by low-temperature high-speed centrifuge (4℃, 1200g, 10 min) within 30 minutes after drawing. Ferritin levels of the supernatant serum were detected with double antibody sandwich enzyme-linked immunosorbent assay (ELISA) kits (Quantikine, XiTang Inc., Shanghai, China). The experimental operation was carried out strictly in accordance with the instruction of the kit. A standard curve was drawn with standard concentration as abscissa and optical density (OD) value as ordinate. The corresponding concentration of ferritin was calculated by the OD value. Each sample was set with multiple holes, and the average value was taken as the level of serum ferritin.

Clinical data collection

Conventional laboratory parameters including blood routine test (WBC, PLT, HGB), biochemical test (ALB, AST, ALT, BUN, Cr, K^+, Na^+, Ca^{2+}) and coagulation test (PT, APTT, Fib) were detected in the clinical laboratory as ordered by attending physicians, also with the same sampling time of ferritin. The demographic information, prognosis (death or discharge) and the duration of hospital days were also collected and summarized from the electronic medical records.

Statistical analysis

Shapiro-Wilk test was used to calculate the normality of the continuous variables. For the normally distributed variables, they were presented as mean ± standard deviation and were compared by one-way analysis of variance or Student's t-test. By contrast, the non-normally distributed data were presented as median (25th percentile, 75th percentile), which were compared by Mann-Whitney U test or Kruskal-Wallis H test, and further with Nemenyi rank sum test for pairwise comparisons between groups. Wilcoxon matched-pairs signed-ranks test was used to compare the levels of serum ferritin between acute phase and convalescent phase. The categorical variables were presented as numbers (percentage) and were compared by chi-square test. Spearman non-parametric test was used to evaluate the correlations of ferritin with conventional laboratory parameters and the length of hospital stay [21]. Logistic regression analysis was used to construct a prognosis (death) risk model of HFRS based upon the ferritin and conventional laboratory parameters during acute phase. The predictive efficacy of ferritin, conventional laboratory parameters and risk model for the prognosis (death) was evaluated by the receiver operating characteristic (ROC) curve analysis, which was quantified by the area under ROC curve (AUC) and compared by Mann-Whitney U test. Kaplan-Meier survival curves with log-rank test were used to assess the association of laboratory markers with mortality and calculate the hazard ratio (HR) of death [21]. A two-sided $P < 0.05$ was considered statistically significant. All statistical analyses and graphing were performed using SPSS Statistics 23.0 software (IBM Inc, Chicago IL, USA) and GraphPad Prism 8 (GraphPad Software, San Diego CA, USA), respectively.

Abbreviations

HFRS

Hemorrhagic fever with renal syndrome; ELISA:Enzyme linked immunosorbent assay; ROC curve:Receiver operating characteristic curve; AUC:Area under the ROC curve; COVID-19:Corona virus disease 2019; OD:Optical density; HR:Hazard ratio; WBC:White blood cells; PLT:Platelet; HGB:Hemoglobin; ALB:Albumin; ALT:Alanine aminotransferase; AST:Aspartate aminotransferase; PT:Prothrombin time; APTT:Activated partial thromboplastin time; Fib:Fibrinogen; BUN:Blood urea nitrogen; Cr:Creatinine; SIRS:Systemic inflammatory response syndrome; ARDS:Acute respiratory distress syndrome; MODS:Multiple organ dysfunction syndrome; NGAL:urinary neutrophil gelatinase-associated lipocalin.

Declarations

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Author contributions

DH and LJQ conceived and designed the study. HHF and DH conducted the statistical analysis and manuscript drafting. DH, WXY and LJ collected the venous blood samples and detected the serum ferritin using the ELISA kits. HHF and ZJY contributed to clinical data collection. WPZ and LJQ reviewed the manuscript and made critical revision. All authors have made substantial contributions to this study, and all of them read and approved the final manuscript.

Competing Interests

The authors declare no competing interests.

Data availability

The datasets used and/or analyzed in this study are available from the corresponding author on reasonable request.

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References

1. Wallace, D. F. The regulation of iron absorption and homeostasis. *Clin. Biochem. Rev*, **37**, 51–62 (2016).
2. Lynch, S. *et al.* Biomarkers of Nutrition for Development (BOND)-Iron Review. *J. Nutr.* **148**(**suppl_1**), 1001S-1067S. https://doi.org/10.1093/jn/nxx036 (2018).
3. Silvestre, O. M. *et al.* Ferritin levels and risk of heart failure-the Atherosclerosis Risk in Communities Study. *Eur. J. Heart Fail*, **19**, 340–347 https://doi.org/10.1002/ejhf.701 (2017).
4. Kawabata, H. *et al.* Serum ferritin levels at diagnosis predict prognosis in patients with low blast count myelodysplastic syndromes. *Int. J. Hematol*, **110**, 533–542 https://doi.org/10.1007/s12185-019-02710-1 (2019).
5. Buzzetti, E. *et al.* Evaluating the association of serum ferritin and hepatic iron with disease severity in non-alcoholic fatty liver disease. *Liver Int*, **39**, 1325–1334 https://doi.org/10.1111/liv.14096 (2019).
6. Son, N. E. Influence of ferritin levels and inflammatory markers on HbA1c in the Type 2 Diabetes mellitus patients. *Pak. J. Med. Sci*, **35**, 1030–1035 https://doi.org/10.12669/pjms.35.4.1003 (2019).
7. Salman, Z., Yılmaz, T. & Mehmetçik, G. The relationship between ferritin levels and oxidative stress parameters in serum of β-thalassemia major patients. *Arch. Biochem. Biophys*, **659**, 42–46 https://doi.org/10.1016/j.abb.2018.09.020 (2018).
8. Kelly, B. J. *et al.* Combined Biomarkers Predict Acute Mortality Among Critically â…ç Patients With Suspected Sepsis. *Crit. Care Med*, **46**, 1106–1113 https://doi.org/10.1097/CCM.0000000000003137 (2018).

9. McElroy, A. K. *et al.* Ebola hemorrhagic Fever: novel biomarker correlates of clinical outcome. *J. Infect. Dis*, **210**, 558–566 https://doi.org/10.1093/infdis/jiu088 (2014).
10. Büyüktuna, S. A., Doğan, H. O., Unlusavuran, M. & Bakir, M. An evaluation of the different biomarkers to discriminate bleeding in Crimean-Congo Hemorrhagic Fever. *Ticks Tick Borne Dis*, **10**, 997–1002 https://doi.org/10.1016/j.ttbdis.2019.05.008 (2019).
11. Tjendra, Y. *et al.* Predicting Disease Severity and Outcome in COVID-19 Patients: A Review of Multiple Biomarkers. *Arch. Pathol. Lab. Med*, **144**, 1465–1474 https://doi.org/10.5858/arpa.2020-0471-SA (2020).
12. Jiang, H., Du, H., Wang, L. M., Wang, P. Z. & Bai, X. F. Hemorrhagic Fever with Renal Syndrome: Pathogenesis and Clinical Picture. *Front. Cell Infect. Microbiol*, **6**, 1 https://doi.org/10.3389/fcimb.2016.00001 (2016).
13. Jiang, H. *et al.* Hantavirus infection: a global zoonotic challenge. *Virol. Sin*, **32**, 32–43 https://doi.org/10.1007/s12250-016-3899-x (2017).
14. Tian, H. Y. *et al.* Changes in rodent abundance and weather conditions potentially drive hemorrhagic fever with renal syndrome outbreaks in Xi'an, China, 2005–2012. *PLoS Negl. Trop Dis*, **9**, e0003530 https://doi.org/10.1371/journal.pntd.0003530 (2015).
15. Mackow, E. R. & Gavrilovskaya, I. N. Hantavirus regulation of endothelial cell functions. *Thromb. Haemost*, **102**, 1030–1041 https://doi.org/10.1160/TH09-09-0640 (2009).
16. Vaheri, A. *et al.* Uncovering the mysteries of hantavirus infections. *Nat. Rev. Microbiol*, **11**, 539–550 https://doi.org/10.1038/nrmicro3066 (2013).
17. Avšič-Županc, T., Saksida, A. & Korva, M. Hantavirus infections. *Clin. Microbiol. Infect*, **21S**, e6–e16 https://doi.org/10.1111/1469-0691.12291 (2019).
18. China society for preventive medicine prevention and control of infectious diseases & Infectious diseases branch of Chinese Medical Association. Expert consensus on prevention and treatment of hemorrhagic fever with renal syndrome. *Chinese Journal of Infectious Diseases*, **39**, 257–265 https://doi.org/10.3760/cma.j.cn311365-20210224-00067 (2021).
19. Tang, K. *et al.* Elevated plasma interleukin 34 levels correlated with disease severity-reflecting parameters of patients with haemorrhagic fever with renal syndrome. *Infec. Dis. (Lond)*, **51**, 847–853 https://doi.org/10.1080/23744235.2019.1672887 (2019).
20. Du, H. *et al.* Changes in Plasma Adiponectin Concentrations in Patients With Hemorrhagic Fever With Renal Syndrome: An Observational Prospective Study. *Med. (Baltim)*, **95**, e2700 https://doi.org/10.1097/MD.0000000000002700 (2016).
21. Du, H. *et al.* Predictive value of pentraxin-3 on disease severity and mortality risk in patients with hemorrhagic fever with renal syndrome. *BMC Infect. Dis*, **21**, 445 https://doi.org/10.1186/s12879-021-06145-0 (2021).
22. Wang, P. Z. *et al.* Elevated serum concentrations of inflammatory cytokines and chemokines in patients with haemorrhagic fever with renal syndrome. *J. Int. Med. Res*, **40**, 648–656 https://doi.org/10.1177/147323001204000227 (2012).
23. Wang, J. *et al.* Elevated soluble CD163 plasma levels are associated with disease severity in patients with hemorrhagic fever with renal syndrome. *PLoS ONE*, **9**, e112127 https://doi.org/10.1371/journal.pone.0112127 (2014).
24. Zhang, C. *et al.* Elevated Plasma Growth Arrest-Specific 6 Protein Levels Are Associated with the Severity of Disease During Hantaan Virus Infection in Humans. *Viral Immunol*, **30**, 330–335 https://doi.org/10.1089/vim.2016.0137 (2017).
25. Bunz, H. *et al.* Urinary Neutrophil Gelatinase-Associated Lipocalin (NGAL) and proteinuria predict severity of acute kidney injury in Puumala virus infection. *BMC Infect. Dis*, **15**, 464 https://doi.org/10.1186/s12879-015-1180-9 (2015).
26. Strandin, T., Mäkelä, S., Mustonen, J. & Vaheri, A. Neutrophil Activation in Acute Hemorrhagic Fever With Renal Syndrome Is Mediated by Hantavirus-Infected Microvascular Endothelial Cells. Front. Immunol. 9 2098. https://doi.org/10.3389/fimmu.2018.02098 (2018).
27. Du, H. *et al.* Early indicators of severity and construction of a risk model for prognosis based upon laboratory parameters in patients with hemorrhagic fever with renal syndrome. *Clin. Chem. Lab. Med*, **52**, 1667–1675 https://doi.org/10.1515/cclm-2014-0016 (2014).
28. Du, H. *et al.* Clinical characteristics and outcomes in critical patients with hemorrhagic fever with renal syndrome. *BMC Infect. Dis*, **14**, 191 https://doi.org/10.1186/1471-2334-14-191 (2014).
29. Du, H. *et al.* Clinical study of critical patients with hemorrhagic fever with renal syndrome complicated by acute respiratory distress syndrome. *PLoS ONE*, **9**, e89740 https://doi.org/10.1371/journal.pone.0089740 (2014).

Figures

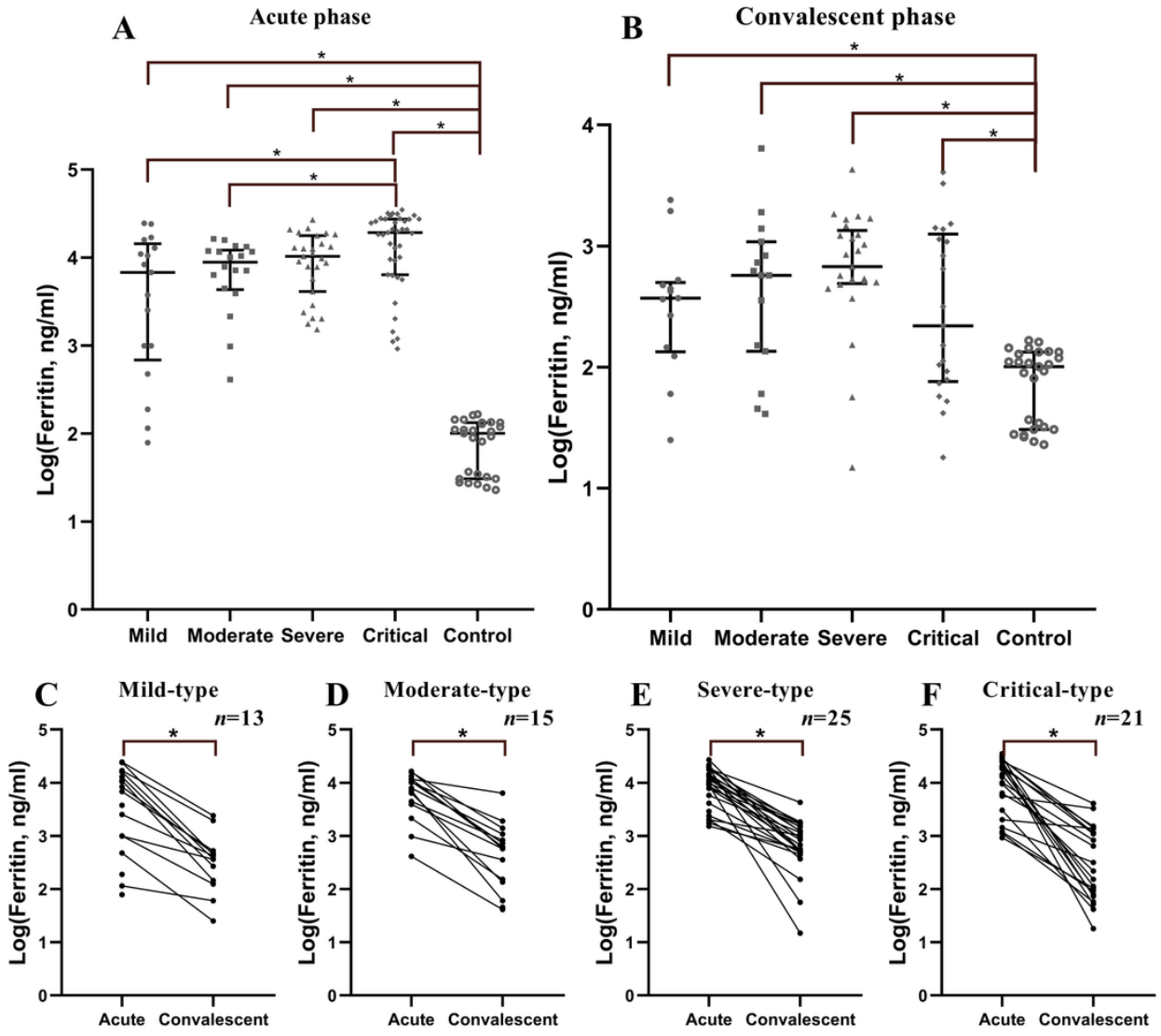

Figure 1

Levels of serum ferritin during the clinical course in patients with HFRS The levels of serum ferritin during the acute phase (A) and the convalescent phase (B) were compared by Kruskal-Wallis H test; pairwise comparisons among the five groups were performed using the Nemenyi rank test. The differences of serum ferritin between acute phase and convalescent phase of the mild-type (C), the moderate-type (D), the severe-type (E), and the critical-type (F) were compared by Wilcoxon matched-pairs signed-ranks test. The logarithmic value of ferritin was used in the figure for the reason of the great differences between the groups.* P<0.05

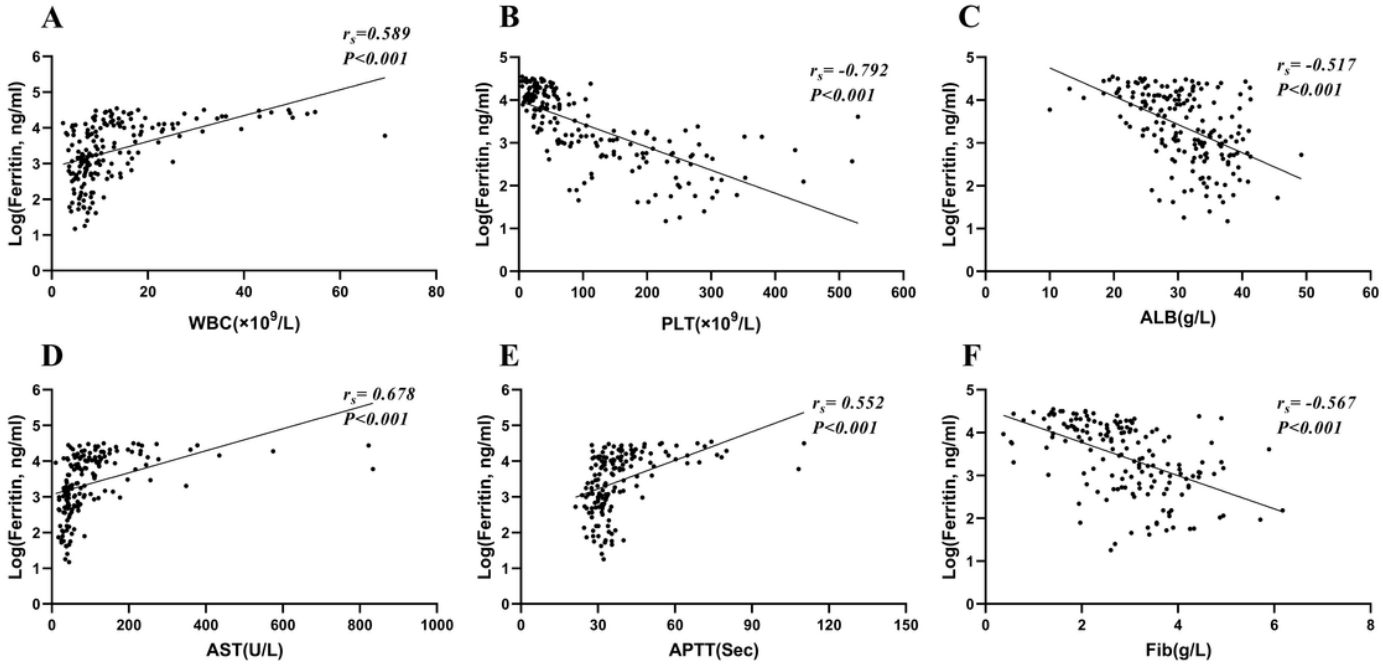

Figure 2

The correlations between serum ferritin and conventional laboratory parameters The correlations between serum ferritin and WBC (A), PLT (B), ALB (C), AST (D), APTT (E), Fib (F) were assessed by Spearman rank correlation analysis. The ordinate showed the logarithmic value of ferritin.

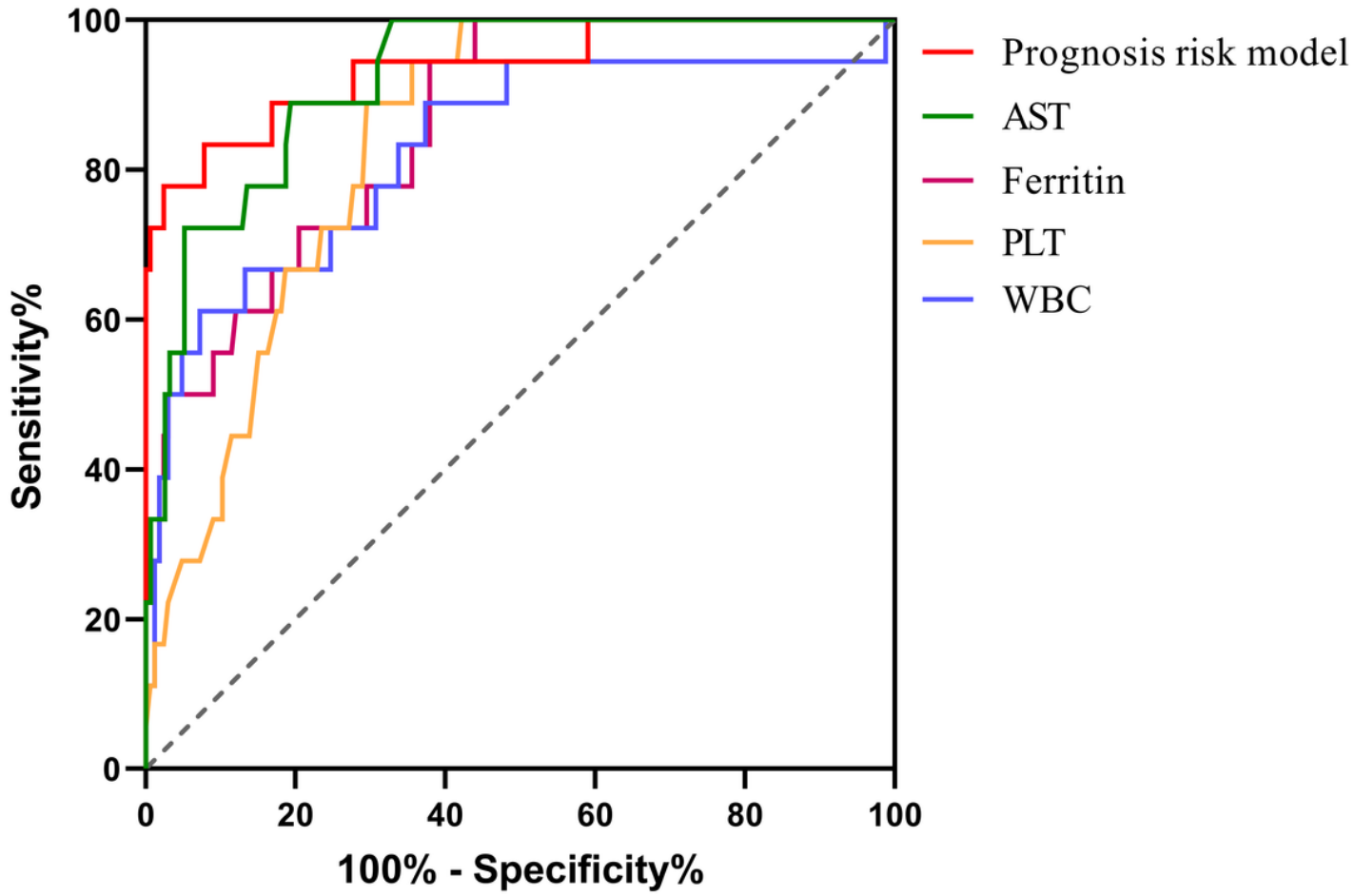

Figure 3

ROC curves of the predictive efficacy on the prognosis (death) of HFRS Figure shows the predictive efficacy of prognosis risk model, ferritin, AST, PLT, WBC for the prognosis (death) in patients with HFRS. The prognosis risk model and ferritin showed significant predictive value, with the AUC of 0.936 and 0.860 (P<0.001), respectively. The prognosis risk model was constructed by multivariate logistic regression analysis.

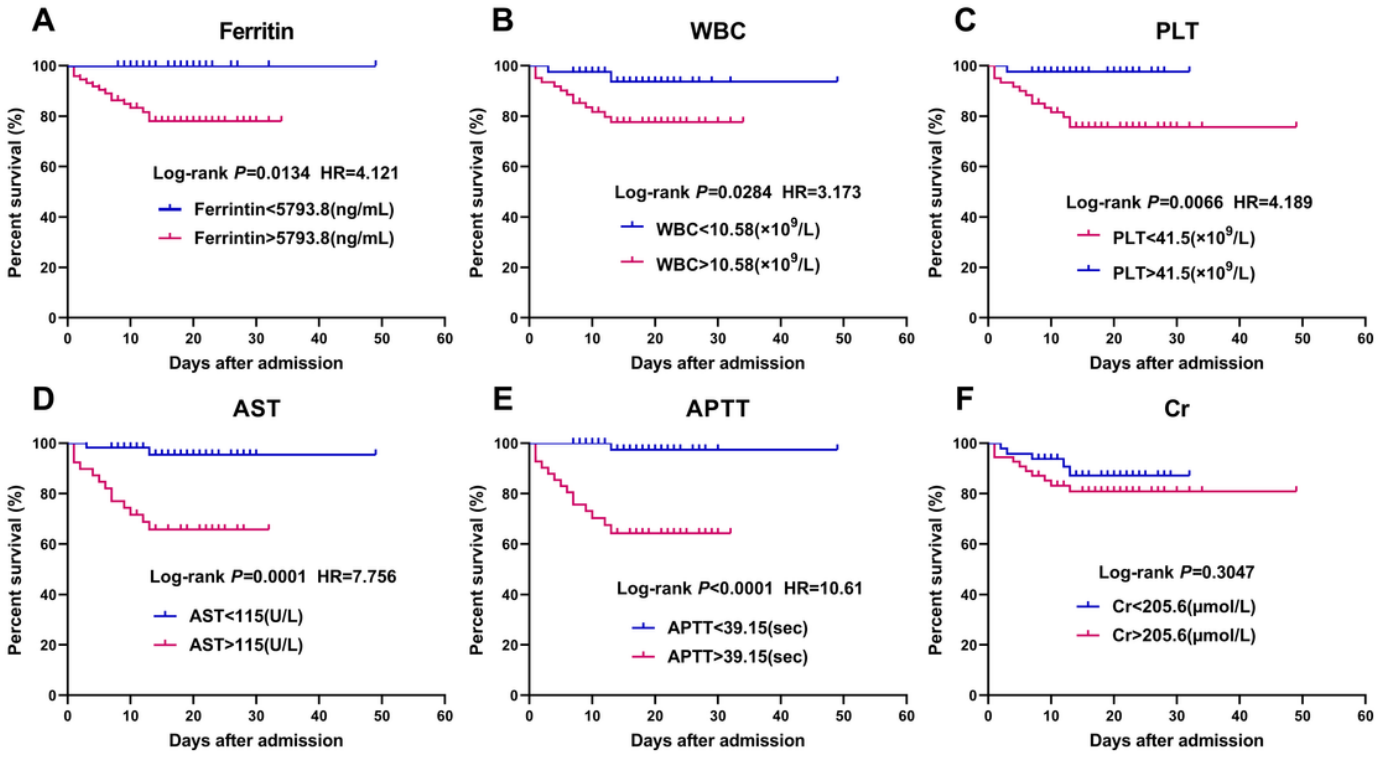

Figure 4

Kaplan-Meier survival curves of ferritin and conventional laboratory parameters Figure shows the association of ferritin (A), WBC (B), PLT (C), AST (D), APTT (E), Cr (F) with the mortality of HFRS, as well as the death hazard ratio (HR) of each parameter.