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Expressions of SAA, CRP, and FERR in different severities of COVID-19

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Abstract. – OBJECTIVE: To explore the expression and significance of SAA, CRP and FERR in patients diagnosed with COVID-19.

PATIENTS AND METHODS: A total of 225 patients diagnosed with COVID-19 who were admitted to the North Hospital of First Hospital in Changsha, China, from 9th February 2020 to 7th March 2020 were enrolled. Their general data, laboratory test results and levels of SAA, CRP and FERR were extracted from electronic medical records.

RESULTS: Age was an important risk factor for the severity of COVID-19 in the patients. Compared with the non-severe group, the severe group showed statistical significance in the levels of total protein, albumin, ALT and AST in liver function, UA in renal function, myocardial enzyme CK-MB and LDH, and immunoglobulin IgG and IgM. The levels of SAA, CRP, and FERR were significantly increased in patients with severe COVID-19. ROC curve analysis results showed that the AUC, from small to large, was as follows: SAA+CRP+FERR, CRP + FERR, SAA + CRP, SAA + FERR, SAA, FERR, and CRP, which indicated the benefit of the combination of the three indicators. The sensitivity and specificity of the combined detection of the three indicators were higher than those of the detection of any single indicator or two combined indicators. A Spearman correlation analysis of the data showed that the initial CRP/SAA, SAA/FERR, and CRP/FERR were positively correlated. The continuous results of SAA, CRP and FERR throughout the study period showed that the values of the severe group on a given day were higher than those of the non-severe group; the values of the two groups peaked on the 5th or 7th day and then decreased, and the decreasing trend of the severe group was more evident.

CONCLUSIONS: SAA, CRP and FERR are sensitive serological indicators used to evaluate the severity of COVID-19. The combined detection of serum SAA, FERR, and CRP, which are positively related to COVID-19 infection, offers guiding significance for the occurrence of COVID-19 infection and the severity of the disease. Such detection provides effective detection indicators for the progress and prognosis of COVID-19; these indicators will enable effective intervention measures to be implemented in time and the rates of severe illness and mortality to be reduced.

Key Words:
Coronavirus disease 2019, Serum amyloid A, C-reactive protein, Ferritin, Disease severity.

Introduction

The continuous outbreak of the novel coronavirus disease in 2019 (COVID-19) has caused tremendous pressure on the international medical system and economy. In China, the first batch of patients with COVID-19 was reported by Wuhan, Hubei Province, after which the disease erupted rapidly[1]. The epidemic in China has been controlled, with more than 80000 confirmed cases, more than 4000 deaths and a mortality rate of 5.55%. By contrast, the current

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international situation is serious[2]. COVID-19 is the seventh known member of the coronavirus family that infects humans[3]. The coronavirus causing COVID-19, which is called severe acute respiratory syndrome (SARS) coronavirus 2 (SARS-CoV-2), belongs to the β genus of the coronavirus family; it has a capsule and round or oval particles with a diameter of 60-140 nm[4]. It may enter the host cell through the binding of the RBD domain of the spike protein and the host cell receptor angiotensin-converting enzyme 2 (ACE2)[5,6]. The disease develops rapidly and can be transmitted from person to person[3,7,8]. It mainly manifests in patients as fever, fatigue, dry cough and myalgia. Patients with severe disease may experience dyspnea and/or hypoxemia. Severe disease may rapidly progress to acute respiratory distress syndrome (ARDS), septic shock or even death[9,10]. Therefore, early description of severe disease will help allocating limited medical resources to patients who need urgent treatment. At present, the diagnosis method of COVID-19 is in a stage of continuous improvement. CT scanning and nucleic acid quantitative analysis play important roles in the diagnosis of the disease[11,12]. However, CT scanning incurs high costs and requires complex operation; thus, it cannot be used for high-frequency monitoring. Quantitative analysis of nucleic acids can only reflect whether a patient's nucleic acid is positive, but it cannot assess the condition in a timely manner. Previous clinical studies on COVID-19 focus on the epidemiological and clinical characteristics of patients with definite diagnoses[8,13,14].

Inflammatory factors, such as serum amyloid A (SAA), C-reactive protein (CRP) and ferritin (FERR), are often used to diagnose and evaluate many inflammatory diseases. SAA is a non-specific acute-phase protein. It is composed of 104 amino acids, and its synthesis is mainly influenced by the cytokines IL-1β, IL-6, and TNF-α[15]. The main function of SAA is to be activated rapidly when inflammation occurs in the carrier, to combine with high-density lipoproteins (HDLs), transport lipids, influence the metabolism of cholesterol, and then affect the inflammatory response *in vivo*[16]. SAA can directly or indirectly promote the inflammatory response through various ways. It can chemotactically activate a variety of inflammatory cells through HDL binding and promote the release of inflammatory factors[17]. It can also increase the survival period of neutrophils via the P2X7 receptor, a ligand-gated ion channel that can be permeable to potassium (K), sodium (Na) and calcium (Ca), to interfere with the signal transduction of lipoxygen and then resist inflammatory regression[18]. CRP is an important acute-phase protein synthesized by the liver when the body is stimulated by inflammation, such as microbial invasion or tissue damage[19]. It plays an important role in a host's resistance to pathogen invasion and inflammatory response[20]. FERR represents iron storage and is a biomarker of inflammation. An increase in the FERR level can not only reflect the degree of hepatocyte destruction and inflammation but also serve as a functional index of liver metabolism[21-23]. In bacterial or viral infection, an increase in FERR is related to the release of iron in the endoplasmic reticulum system and to a decrease in FERR transport capacity due to liver and spleen damage[24,25]. The killing of bacteria or regulation of inflammatory reaction can accelerate the synthesis and release of intracellular FERR and increase the serum FERR of patients[26,27]. Although some studies report changes in SAA and CRP in the peripheral blood of patients with COVID-19[28,29], but no report has been published on FERR. Moreover, the relationship between the three indicators and the severity of the disease is poorly understood.

Therefore, it is important to identify sensitive indices that can reflect illness severity, to conduct real-time monitoring, and to adjust treatment strategies in time to reduce the rates of severe illness and mortality. In clinical practice, the SAA, CRP, and FERR of most patients show significant changes. Therefore, in this study, we systematically studied the differences and dynamic changes in the above indicators between groups of patients with severe and non-severe COVID-19 infection to evaluate their clinical value in predicting the severity and prognosis of COVID-19.

Patients and Methods

Data Sources

We conducted a retrospective study on the expression and significance of SAA, CRP and FERR for evaluating the different severities of COVID-19. A total of 225 patients diagnosed with COVID-19 who were admitted to the North Hospital of First Hospital of Changsha, China, from 9 February 2020 to 7 March 2020 were enrolled in this study. The North Hospital of First Hospital in Changsha, located in Hunan, is one of

the top three teaching hospitals in the city, and it is responsible for the receiving and treatment of COVID-19 patients. All these 225 patients were tested positive for SARS-CoV-2 via RT-PCR on samples from respiratory tract. According to the COVID-19 diagnosis and treatment plan issued by the National Health Commission, these patients were divided into non-severe and severe groups for comparison. All cases received antiviral medication, supportive oxygen therapy and other supportive treatments. Their general data, laboratory test results and levels of SAA, CRP and FERR were extracted from electronic medical records and studied. This work was approved by the First Hospital of Changsha Ethics Committee. This is a retrospective case series study; no patients participated in the study design, no study questions were established, and no results were directly measured. No patients were asked to advise on the interpretation or recording of the results.

Statistical Analysis

The data were statistically analyzed using SPSS 19 (IBM Corp., Armonk, NY, USA). The continuous variables were presented as median with interquartile range (IQR), and the groups were compared through the Mann-Whitney U test. The categorical variables were expressed as frequencies and percentages, and the data were compared between groups via a chi-square test. Receiver operator characteristic (ROC) curves were used for predictive analysis by calculating the area under the ROC curve (AUC), sensitivity and specificity. Linear correlation analysis was performed on the continuous variables between the two groups using the Spearman's rank correlation coefficient. A line chart was used to describe the continuous changes in the SAA, CRP and FERR in the patients. A p-value below 0.05 was considered statistically significant.

Results

Analysis of General Data and Laboratory Test Results of Patients With COVID-19

The general data and laboratory test results of patients with COVID-19 who were admitted to the North Hospital of First Hospital of Changsha, China, from 9 February 2020 to 7 March 2020 were collected from the hospital. As shown in Table I, these consisted of 194 non-severe cases (86.22%) and 31 severe cases (13.78%). Of the non-severe group, there were 91 males (46.91%) and 103 females (53.09%). Of the severe group, there were 17 males (54.84%) and 14 females (45.16%). There was no statistical difference between the two groups ($p > 0.05$). The median age of the non-severe group was 43 years (IQR 33–57), and that of the severe group was 64 years (IQR 45-66). The data showed that age was an important risk factor for the severity of COVID-19 in these patients (Table I).

As shown in Table I, organ damage outside the lungs was commonly seen in the liver, heart and kidney. Compared with the non-severe group, the severe group had higher levels of serum alanine transaminase (ALT), aspartate aminotransferase (AST), uric acid (UA), creatine kinase (CK) isoenzyme (CK-MB) and lactate dehydrogenase (LDH) but lower levels of serum total protein (Tpro), albumin (ALB) and ratio of albumin to globulin (A/G). There was statistical difference between the two groups ($p < 0.05$). Other common serum biochemical items, such as total bile acid (TBA), blood urea nitrogen (BUN), creatinine (CREA), CK and electrolytes (including K, Na, chloride [Cl] and total Ca [T_Ca]), did not statistically differ between the two groups ($p > 0.05$).

When a virus invades the human body, the immune system will be activated, antibodies will be produced, and the serum immunoglobulins will change accordingly. Compared with the severe group, the non-severe group had higher levels of serum immunoglobulin G (IgG) and immunoglobulin M (IgM) ($p < 0.05$), whereas the levels of immunoglobulin E (IgE), immunoglobulin A (IgA), complement 3 (C3) and complement 4 (C4) showed no differences ($p > 0.05$). These data indicate that the production of IgG and IgM may help inhibit the progress of the disease (Table I).

Specific Biomarker Indicating Different Severities of COVID-19

In this retrospective study on the biochemical indicators of patients, we found that the levels of serum SAA, CRP and FERR of many patients increased sharply at the time of admission. We speculated whether these three factors were related to the severity of the disease. Therefore, we observed the levels of these factors in the two groups of patients. Table II shows the levels of serum SAA (median = 3.91 mg/L, IQR [1.00, 18.79]), CRP (median = 3.90 mg/L, IQR [1.43, 11.35]) and FERR (median = 257.31 ng/mL, IQR [121.95,

Table I. Analysis of general data and laboratory test results of patients with COVID-19 .

	Non-Severe (n=194)	Severe (n=31)	t/Z	p
Gender				
Male	91 (46.91%)	17 (54.84%)	0.72	0.40
Female	103 (53.09%)	14 (45.16%)		
Age	43.00 (33.00-57.00)	64.00 (45.00-66.00)	3.90	0.00
Laboratory parameters				
K (mmol/L)	4.40 (4.20-4.60)	4.70 (4.30-4.95)	1.25	0.21
Na (mmol/L)	139.00 (138.00-140.00)	137.00 (136.00-139.00)	1.47	0.14
Cl (mmol/L)	104.00 (102.00-105.00)	104.00 (101.50-106.00)	0.69	0.49
T_Ca (mmol/L)	2.16 (2.12-2.22)	2.12 (1.96-2.23)	1.57	0.12
TBIL (μmol/L)	8.60 (6.83-11.58)	9.05 (6.57-13.50)	0.35	0.73
DBIL (μmol/L)	2.80 (2.20-3.60)	3.45 (2.10-6.03)	1.26	0.21
IBIL (μmol/L)	5.70 (4.53-7.98)	5.90 (4.40-7.33)	0.32	0.75
Tpro (g/L)	65.65 (62.80-69.63)	60.40 (56.53-69.45)	2.57	0.01
ALB (g/L)	40.90 (38.33-44.40)	31.90 (30.90-41.25)	3.08	0.00
GLB (g/L)	24.25 (21.93-28.58)	28.00 (21.23-30.45)	0.78	0.44
A/G	1.73 (1.44-2.00)	1.22 (1.10-1.89)	2.11	0.04
ALT (U/L)	22.20 (16.13-35.58)	39.00 (20.57-80.05)	2.06	0.04
AST (U/L)	26.00 (20.47-34.00)	33.70 (28.68-46.93)	2.08	0.04
TBA (μmol/L)	4.00 (3.25-8.90)	4.50 (3.68-7.88)	0.73	0.47
BUN (mmol/L)	5.18 (4.29-6.18)	4.60 (3.58-7.61)	0.26	0.80
CREA (μmol/L)	59.80 (49.10-71.30)	52.75 (37.15-65.98)	1.45	0.15
UA (μmol/L)	162.45 (133.35-229.53)	288.50 (254.20-347.10)	3.77	0.00
CK (IU/L)	51.50 (35.38-64.53)	41.15 (29.35-64.63)	0.77	0.44
CK-MB (U/L)	9.05 (7.10-11.68)	11.55 (9.78-19.75)	2.08	0.04
LDH (U/L)	140.55 (125.05-175.13)	210.95 (186.85-255.23)	3.72	0.00
IgE (IU/mL)	22.24 (11.70-52.56)	23.45 (11.46-57.32)	0.31	0.80
IgG (g/L)	11.40 (9.50-13.13)	7.95 (6.70-9.58)	2.14	0.01
IgM (g/L)	1.15 (1.00-1.43)	0.70 (0.50-0.90)	1.99	0.04
IgA (g/L)	1.85 (1.55-2.35)	1.45 (1.51-2.38)	0.47	0.65
C4 (g/L)	0.24 (0.21-0.26)	0.17 (0.15-0.23)	1.63	0.12
C3 (g/L)	1.12 (1.00-1.38)	1.06 (1.01-1.33)	0.58	0.59

K: serum potassium; Na: serum sodium; Cl: serum chloride; T_Ca: serum total calcium; TBIL: total bilirubin; DBIL: direct bilirubin; IBIL: indirect bilirubin; Tpro: total protein; ALB: albumin; GLB: globulin; A/G: albumin/ globulin; ALT: alanine aminotransferase; AST: aspartate aminotransferase; TBA: total bile acids; BUN: urea nitrogen; CREA: creatinine; UA: uric acid; CK: creatine kinase; CK-MB: creatine kinase isoenzyme; LDH: lactate dehydrogenase; IgE: immunoglobulin E; IgG: immunoglobulin G; IgM: immunoglobulin M; IgA: immunoglobulin A; C4: complement 4; C3: complement 3.

479.76]) of the non-severe group. The figure also shows the concentrations of serum SAA (median = 48.57 mg/L, IQR [9.30, 469.16]), CRP (median = 14.00 mg/L, IQR [6.31, 57.70]) and FERR (median = 1383.51 ng/mL, IQR [700.09, 2000.00]) of the severe group. There were significant increases in these factors between the two groups (Table II).

Diagnostic Value of SAA, CRP and FERR and Joint Indices for COVID-19

To test whether SAA, CRP and FERR could diagnose and predict COVID-19, we used ROC curve analysis and calculated the AUC. The levels of SAA, CRP and FERR of all the patients in non-severe group in the recovery period were set as negative, whereas the levels at admission were selected as positive. The AUC, from high to low, was as follows: SAA + CRP + FERR > CRP + FERR > SAA + CRP > SAA + FERR > SAA = FERR > CRP; the specific values were 0.90, 0.88, 0.86, 0.85, 0.83, 0.83 and 0.77, respectively. In addition, we used the Jordan index method to calculate the critical value. Findings showed that the combination of SAA + CRP + FERR had the highest predictive value for disease diagnosis, which was higher than that of any single index or two combined indices. At this time, the sensitivity was 78.34%, and the specificity was 86.29% (Figure 1 and Table III).

Correlation of SAA, CRP and FERR With Admission of Patients With COVID-19

The data of COVID-19 patients were analysed by Spearman correlation analysis. The results

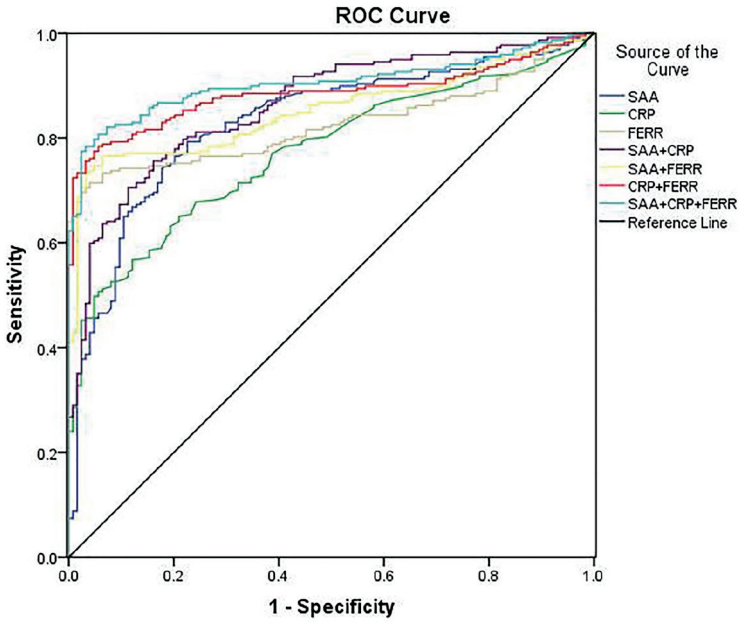

Figure 1. Diagnostic value of SAA, CRP, FERR and joint indexes for COVID-19.

showed a positive correlation between the two variables (CRP/SAA [r = 0.51, p = 0.00], SAA/FERR [r = 0.31, p = 0.01], CRP/FERR [r = 0.48, p = 0.00]).

Changes in SAA, CRP and FERR With Time in Patients With COVID-19

We tracked the serum SAA, CRP and FERR levels of the patients on the 1st, 3rd, 5th, 7th, 9th and 11th day after admission. The results showed that the dynamic changes in SAA, CRP and FERR in the peripheral blood might be consistent with the patients' clinical changes. On the same days, the values of the severe group were higher than those of the non-severe group. The values in both groups peaked on the 5th or 7th day and then decreased, with the decline trend of the severe group being more noticeable (Figure 2).

Discussion

COVID-19, a severe acute infectious disease caused by a novel coronavirus (SARS-CoV-2), has been spreading rapidly worldwide. An increasing number of asymptomatic infections are also being gradually discovered. The initial symptoms/signs of many patients are not specific, and most patients show mild/moderate fever. In some cases, the disease progresses rapidly, leading to multiple organ failure and death[10,30]. The main pathological change in patients with COVID-19 is damage in the lungs and immune system[8]. As a rapid screening method for pulmonary infection, CT can not only identify pulmonary infection but also provide a reference for determining the pathogen type. Therefore, CT scan has a unique diagnostic advantage[11]. The shortcomings of CT are the additional special protection required by medical personnel during the examination of patients with COVID-19, and the risks related to the transportation and examination of patients. The use of CT scan for the dynamic monitoring of lung lesions is limited. Nucleic acid detection can determine whether a patient is infected by COVID-19[12], but it cannot detect the progress and severity of the disease. Therefore, we need simple

Table II. Specific biomarker indicating different severities of COVID-19.

	Non-Severe (n=194)	Severe (n=31)	Z	p
SAA (mg/L)	3.91 (1.00-18.79)	48.57 (9.30-469.16)	2.88	0.00
CRP (mg/L)	3.90 (1.43-11.35)	14.00 (6.31-57.70)	3.86	0.00
FERR (ng/mL)	257.31 (121.95-479.76)	1383.51 (700.89-2000.00)	4.19	0.00

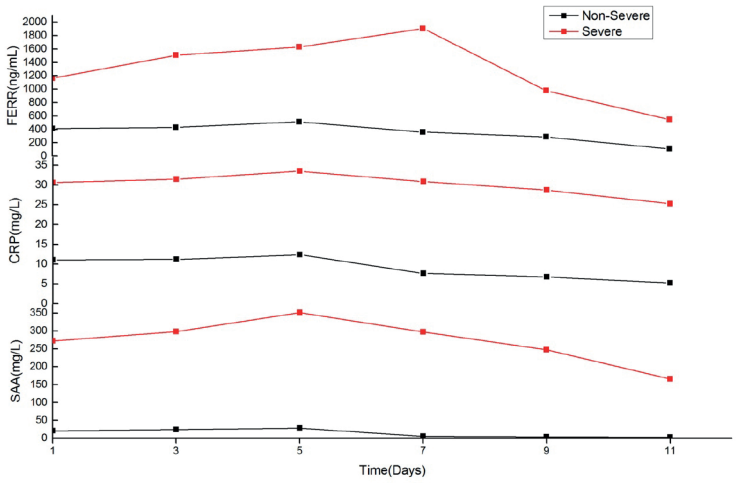

Figure 2. Changes in SAA, CRP and FERR with time in patients with COVID-19.

and sensitive indicators that can respond to the disease, and timely monitoring of the disease in order to take effective interventions.

The results of this study showed that the median age of the non-severe group was 43 years (IQR 33-57), whereas that of the severe group was 64 years (IQR 45-66). The age difference between the two groups was statistically significant, suggesting that the older the person, the greater the probability of severe disease. Therefore, old age may be a risk factor for the severity of the disease, as stated by Zheng et al[31]. Compared with the non-severe group, the severe group had significantly higher levels of ALT, AST, UA, CK-MB, and LDH upon admission. Thus, the patients had multiple organ dysfunction outside the lungs. In addition, liver, kidney and heart injuries in the severe group were more serious. The levels of T_Pro and ALB in the severe group were significantly lower than those in the non-severe group, suggesting that malnutrition was a common symptom in the severe group. In the non-severe group, the levels of IgG and IgM were significantly increased, suggesting that the production of immunoglobulins can effectively inhibit the further damage caused by the virus. These indices are helpful in assessing a patient's condition and are closely related to the acute lung injury caused by COVID-19. A prospective study proposed that the increase in LDH in patients with pneumonia caused by community-acquired viruses is related to death[32]. The clinical value of SAA, a marker of inflammation, has been attracting increasing attention. Some researchers found that the level of SAA in patients with severe respiratory syndrome is significantly high[33], suggesting that SAA may be an important biomarker for monitoring respiratory diseases. When SAA is activated, even in low concentrations, it could promote inflammation by activating chemokines and inducing chemotaxis[34,35]. Patients with respiratory virus infection usually show clinical symptoms 36-48 hours after infection. SAA gradually increases and peaks 3-5 days after infection[36]. A report studied the SAA in the blood of patients with suspected SARS and found that

Table III. ROC curve analysis of cutoff value, sensitivity and specificity of SAA, CRP, FERR and joint indexes.

Variables	Cut-off	AUC (95%CI)	Sig	Sensitivity (%)	Specificity (%)
SAA	3.59	0.83 (0.79-0.88)	0.00	79.26	77.41
CRP	4.65	0.77 (0.72-0.82)	0.00	56.68	87.90
FERR	168.11	0.83 (0.78-0.80)	0.00	74.65	83.06
SAA+CRP	2.06	0.86 (0.82-0.90)	0.00	75.58	83.87
SAA+FERR	2.26	0.85 (0.81-0.89)	0.00	76.96	81.70
CRP+FERR	2.34	0.88 (0.85-0.92)	0.00	68.57	85.32
SAA+CRP+FERR	2.78	0.90 (0.87-0.94)	0.00	78.34	86.29

SAA increased in all kinds of virus and bacterial infections. The increased SAA range reflected the severity of the disease but did not indicate the cause; thus, it cannot distinguish between SARS and non-SARS patients[37]. There is no significant difference between the mean SAA and the physiological changes within individuals in the normal population, and the reference value does not need to be differentiated between men and women. The SAA level slightly increases with age[15]. By contrast, CRP's concentration change is not affected by age, gender, physical condition or similar factors[38]. CRP could activate the complement and enhance the phagocytosis of macrophages and granulocytes *in vivo*; thus, to a certain extent, it could clear the pathogenic microorganisms invading the body and reduce the inflammatory reaction *in vivo*[20]. After infection, the inflammatory reaction is activated, and the CRP level increases rapidly. The increase rate is positively related to the severity of the disease[39]. Efstathiou showed that HIV and EB virus infection could increase the level of FERR[40]. In a study on hyperferritinemia, Sackent et al[41] that the FERR of a group of children with viral infection was higher than that of a bacterial-infection group, whereas the FERR of a bacterial-infection group in adult patients was higher. The mechanism may be as follows: during the inflammatory reaction, the inflammatory factors act on the body to increase the levels of serum FERR. At the same time, the inflammatory factors denature and necrotise the local tissue cells to dissolve and break the cell membranes, causing a leakage of serum FERR from the damaged cells. In addition, FERR in the form of iron has a negative impact on health. It could stimulate further cell damage and thus significantly increase the probability of inflammatory diseases[26,42]. To predict the severity of COVID-19 as early as possible, we analyzed the levels of SAA, CRP, and FERR to explore their clinical value in COVID-19 infection. The results showed that the levels of SAA, CRP, and FERR increased with the aggravation of the disease. Different diagnostic methods are used in clinical practice to diagnose or screen for COVID-19. The ROC curve, which combines sensitivity with specificity, is an effective method of comprehensively and accurately evaluating diagnostic experiments. Thus, it is widely used in assessing medical diagnostic efficacy. The closer the AUC is to 1, the better the diagnosis. The results of this study showed that the AUC, from high to low, was as follows: SAA + CRP + FERR > CRP + FERR > SAA + CRP > SAA + FERR > SAA = FERR > CRP, and the combination of SAA + CRP + FERR had the highest predictive value for the diagnosis of the disease. The sensitivity was 78.34%, and the specificity was 86.29%. Spearman correlation analysis showed that SAA, CRP and FERR were positively correlated, and the data were statistically significant. Then, the serum SAA, CRP and FERR levels were analyzed on the 1st, 3rd, 5th, 7th, 9th, and 11th days after admission. The results showed that the dynamic changes in the SAA, CRP and FERR in the peripheral blood might be consistent with the clinical changes in the patients. On the same days, the serological levels of the three indicators in the severe group were much higher than those in the non-severe group; the levels peaked on the 5th or 7th day after admission and then decreased sharply.

According to the results of this study, the combined detection of serum SAA, FERR and CRP has guiding significance for the occurrence and severity of COVID-19, and they are positively correlated with COVID-19 infection. Therefore, they can serve as effective detection indicators for the diagnosis, treatment, disease progress and prognosis of COVID-19. They will enable effective intervention measures to be implemented in time and reduce the rates of severe illness and mortality. This study has some limitations. This is a single-center retrospective study, which may limit the reliability of our results. However, as the only one in Hunan that currently receives COVID-19 patients, our research results are representative and reliable. As far as we know, this is the first clinical study to show that FERR may be related to the severity of COVID-19. This is also the first report on the clinical value in predicting the severity and prognosis of COVID-19 using the combination of these three indicators.

Conclusions

The combined detection of serum SAA, FERR, and CRP has guiding significance for the occurrence and severity of COVID-19, and they are positively correlated with COVID-19 infection. In addition, COVID-19 may have a severe tendency in older patients and cause multiple organ dysfunction outside the lungs.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Contributors

Yong Chen and Li Wang conceived the idea for and designed the study and had full access to all of the data in the study. Shan-Ling Liu took responsibility for the integrity of the data. Sheying Wang took responsibility ensured the accuracy of the data analysis. Yifan Sun and the other authors drafted the paper and performed the analyses. All the authors critically revised the manuscript for important intellectual content and gave final approval for the version to be published. Shan-Ling Liu and Sheying Wang collected the data. All the authors agree to be accountable for all aspects of this work and guarantee that all questions related to the accuracy or integrity of any part of this paper will be appropriately investigated and resolved.

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