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Combining Serum Interleukin-6 and other Inflammatory Biomarkers to Improve the Prediction of COVID-19 Severity

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ABSTRACT

Objectives: The hyperinflammatory response of (COVID-19) disease 2019 patients impact disease progression. Inflammatory markers could help detect disease early, predict severity and guide treatment.This study was conducted to evaluate if combining baseline inflammatory biomarkers could improve COVID-19 severity prediction. **Methods:** This study was directed in patients who confirmed COVID-19. Clinical data and laboratory investigations were composed from the hospital's accounts on admission. The level of interleukin (IL)-6 in serum samples was estimated by enzyme-linked immunosorbent assay (ELISA) in all participants. **Results:** Our findings revealed that patients in the severe group had significantly higher absolute neutrophil count, ferritin, IL-6, CRP, and procalcitonin (PCT) levels paralleled to non-severe patients. Also, the absolute lymphocyte count was significantly lower. Furthermore, it was found that the area under the curve (AUC) increased to 0.998 and 0.962, respectively, when IL-6 with ferritin or IL-6 with CRP combinations were used to identify non-severe and severe COVID-19 individuals. **Conclusion:** IL-6 and CRP were autonomous variables for predicting the severity of patients with COVID-19, as shown by the multivariate regression analysis. In contrast, ferritin, IL-6, and CRP were the greatest predictors of COVID-19 severity, with a diagnostic sensitivity of 90.24%, 97.56%, and 92.68%, and a diagnostic specificity of 100 %, 84.09%, and 79.5%, respectively by ROC curve analysis. So, combining IL-6 and CRP on admission levels, or other inflammatory indicators like ferritin, can improve COVID-19 severity prediction and allow for early intervention.

Key Words: COVID-19; Biomarkers; Combination; Interleukin-6; C-reactive protein (CRP); Ferritin; Severity

1. INTRODUCTION

Coronavirus (COVID-19) is a greatly infectious disease caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and was first identified in Wuhan, China, in early December 2019. Subsequently then, the disease has quickly grown into a pandemic that has impacted innumerable countries worldwide [1]. In February 2020, the first incidence of COVID-19 was recorded in Egypt, and the number of patients has been steadily rising since then. Based on data from Egypt's Ministry of Health

(MoH), Egypt ranked tenth in the death rates beyond the total number of COVID-19 infected subjects by (5.7%), as of April 28, 2022 [2]. Symptoms of COVID-19 range from mild (fever, cough, and diarrhea) to severe symptoms (acute respiratory distress syndrome, shock, multiple organ failure, and even death), which puts a strain on hospital resources such as mechanical ventilators and ICU beds, mainly in stumpy resource countries [3,4]. It has yet to be successfully treated with a specific antiviral drug; Hence the clinical evaluation depends primarily on symptom control [5].The pathologic hyper-inflammatory response in patients with COVID-19 is important for disease progression [6]. Several inflammatory biomarkers are increased with patients in ICU or patients via severe diseases when compared to individuals with lower-severity disorders [7-9]. As a result, these biomarkers could be employed as indicators for early disease detection and better severity prediction, guiding early treatment decisions and lowering patients with COVID-19 mortality rates [10].In this context, our study hypothesized that simultaneous interpretation of combined baseline inflammatory biomarkers on admission to the hospital could provide a better and more accurate prediction of disease severity that may help in risk stratification and hospital resource management.

2. Methodology

2.1. Patients

This cross-section study was performed in accordance with the provisions of the Declaration of Helsinki and the patients with COVID-19 admitted to COVID-19 isolation hospitals from January to February 2021. COVID-19 Patients were diagnosed accompanied by the guidance of the WHO interim following the Egyptian Ministry of Health and Population (MOH) definitions [11,12] and confirmed by a positive reaction by a real-time reverse transcription–polymerase chain reaction (RT-PCR) assay for SARS-CoV-2 from the respiratory specimens. Patients with acute trauma, autoimmune disorders, other infectious diseases of the respiratory tract, hematological diseases treated with radiotherapy, and patients who had undergone chemotherapy were not included. Before the study began, all participants or else their first-degree relatives provided their written informed consent.

In accordance with the Egyptian MOH protocol (version 1.4, 30 May) [13]. COVID-19 was classified as having the following clinical features (a) mild type: asymptomatic or symptomatic with leukopenia or lymphopenia and without radiological symptoms of pneumonia; (b) moderate type: patients with radiological symptoms of pneumonia and/or leukopenia or lymphocytopenia; (c) severe type: breathing rate more than 30 breaths/min and Oxygen saturation (SpO2) less than 92% on room air. Also, patients with severe type exhibit PaO2/FiO2 ratio less than 300 or higher than 50% lesion on CT or progressive lesion within 24–48 hours; (d) critical type: breathing failure that needs mechanical ventilation, shock, or ICU admission for combined organ failure. On the base of the previous classification, we distributed all patients into two groups: (1) non-severe patients with mild and moderate symptoms (2) severe, and critical types.

2.2. Biochemical and Molecular parameters

Demographic characteristics, including sex and age, underlying comorbidities as well as clinical features including cough, fever, dyspnea, fatigue, bony aches, sore throat, diarrhea, chest pain and decreased appetite were reported. In addition, routine laboratory data, such as CBC with differential counts, estimation of CRP level, PCT, ferritin level, and D-dimer, were obtained from hospital records upon admission.

2.3. Laboratory assessment of IL-6

On admission, 3 mL of venous blood from each individual was drawn into a separate tube to be used for the IL-6 test in serum. For 10 minutes, samples were centrifuged at 4000 rpm and at room temperature. Then, the sera were separated and stored until analysis of IL-6 with a commercially available

human IL-6 ELISA kit { Cusabio Technology LLC, USA, Cat. No. CSB-E04638h}, based on the instructions and guidance of the manufacturer. Results of IL-6 were reported in pg/ml.

2.4. Statistical analysis

With the aid of IBM SPSS Statistics 21.0, the data was analyzed, then presented for non-parametric data, expressed as medians with interquartile ranges (IQR), and mean ± standard deviations (SD) for parametric data. Qualitative variables were expressed as numbers and percentages. The Mann-Whitney test, independent t test, Fischer exact test, and Chi-square test, were used to compare data.

Additionally, using the receiver operating characteristic (ROC) curve, the combined detection of laboratory markers was examined, also risk factors were assessed using logistic regression analysis. Association between variables was made by Spearmen correlation analysis. A p-value of 0.05 or less was regarded as statistically significant.

2.5. Institutional Review Board (IRB)- Ethical Approval

The Research Ethical Committee of the Faculty of Pharmacy, Port Said University (REC.PHARM.PSU) approved this research study under approval number REC.PHARM.PSU.22-8.

3. Results

3.1. Baseline features of COVID-19 patients

The study incorporated 85 hospitalized individuals positive for COVID-19. On admission, 44 (51.8%) patients were classified as having mild or moderate symptoms, and 41 (48.2%) patients had severe or critical symptoms. The mean (±SD) age of all patients that were included was 58.85 ± 11.65 years and ranged between 24 and 83 years, with no significant difference between non-severe and severe patients. Among the 85 patients, 43 (50.6%) were females while 42 (49.4%) were males. Males were more affected by severe disease (29 males (70.7%) vs. 12 females (29.3%)). Of all associated co-morbidities, diabetes mellitus 51.8% and hypertension 41.2% were the most common and only diabetes mellitus was associated with severe disease (28 severe patients (63.6%) vs. 16 non-severe patients (39.0%); $p = 0.023$). The most prevalent presenting symptoms at the time of hospital admission were cough (74.1%), dyspnea (71.8%) and fever (45.9%), as shown in **(Table 1).**

Table 1: Demographic data, co-morbidities, and presenting symptoms of the studied COVID-19 patients, compared between severe and non-severe groups

	All Cases	**Severity**		**P-value**
		Non-severe	**Severe**	
	(n=85)	**(n=44)**	**(n=41)**	
Age (years)				
Mean ± SD	58.85 ± 11.65	59.34 ± 9.17	58.32 ± 13.93	0.688•
Range	24 – 83	38 – 75	24 – 83	
Sex n., %				
Female	43 (50.6%)	31 (70.5%)	12 (29.3%)	**< 0.001***
Male	42 (49.4%)	13 (29.5%)	29 (70.7%)	
Co-morbidities n., %				
DM	44 (51.8%)	16 (39.0%)	28 (63.6%)	**0.023***
HTN	35 (41.2%)	18 (40.9%)	17 (41.5%)	0.959*
CKD	5 (5.9%)	4 (9.1%)	1 (2.4%)	0.361**
IHD	12 (14.1%)	7 (15.9%)	5 (12.2%)	0.623*
CLD	1 (1.2%)	0 (0.0%)	1 (2.4%)	0.482**
Symptoms n., %				
Cough	63 (74.1%)	29 (65.9%)	34 (82.9%)	0.073*
Fever	39 (45.9%)	13 (29.5%)	26 (63.4%)	**0.002***
Dyspnea	61 (71.8%)	38 (86.4%)	23 (56.1%)	**0.002***
Fatigue	8 (9.4%)	8 (18.2%)	0 (0.0%)	**0.006****
Bony aches	17 (20.0%)	8 (18.2%)	9 (22.0%)	0.664*
Sore throat	4 (4.7%)	0 (0.0%)	4 (9.8%)	**0.050****
Diarrhea	9 (10.6%)	4 (9.1%)	5 (12.2%)	0.642*
Chest pain	1 (1.2%)	0 (0.0%)	1 (2.4%)	0.482**
Decreased appetite	1 (1.2%)	0 (0.0%)	1 (2.4%)	0.482**

CKD: chronic kidney disease; CLD, chronic liver disease; DM: Diabetes mellitus; HTN, hypertension; IHD: ischemic heart disease. P-value >0.05: Non-significant; P-value <0.05: Significant; P-value< 0.01: highly significant
**: Fischer exact test; *: Chi-square test; •: Independent t-test

3.2. Laboratory markers for COVID-19 patients

Accompanied by the outcomes, patients in the severe group had a considerably higher absolute neutrophil count than those in the non-severe group (6.19 $\times 10^3$/µl (4.9 – 8.7) vs. 3.35 $\times 10^3$/µl (2.51 – 6.09); p <0.001) as well as, ferritin (1020 ng/ml (645 – 1380) vs. 150 ng/ml (111 – 272); p <0.001), IL-6 (78 pg/ml (60 – 123) vs. 18 pg/ml (9 – 23); p <0.001), CRP(101 mg/L (56 – 138) vs. 29.3 mg/L (26 – 33); p <0.001) and PCT levels (0.16 ng/ml (0.09 – 0.35) vs. 0.08 ng/ml (0.04 – 0.11); p <0.001). On the other hand, the absolute lymphocyte count was significantly lower (1.06 ± 0.65 $\times 10^3$/µl vs. 2.00 ± 0.63 $\times 10^3$/µl; p <0.001) (**Table 2).**

Table 2: The laboratory findings of the studied patients at the time of diagnosis, compared between severe and non-severe groups

		All Cases	Severity		P-value
			Non-Severe	Severe	
		(n=85)	(n=44)	(n=41)	
TLC ($\times10^3$/µl)	Mean ± SD Range	7.12 ± 2.00 2.6 – 12.5	6.75 ± 1.85 4.14 – 9.9	7.52 ± 2.10 2.60 – 12.5	0.074•
Hemoglobin (gm/dl)	Mean ± SD Range	12.81 ± 1.79 9.44 – 16.4	12.43 ± 1.85 9.44 – 15.6	13.00 ± 1.65 9.7 – 16.4	0.136•
Platelets ($\times10^3$/µl)	Mean ± SD Range	262.21 ± 106.61 111 – 651	268.80 ± 113.32 111 – 479	255.15 ± 99.83 117 – 651	0.558•
Neutrophils ($\times10^3$/µl)	Median (IQR) Range	5.4 (3 – 6.69) 0.88 – 21	3.35 (2.51 – 6.09) 1.9 – 6.69	6.19 (4.9 – 8.7) 0.88 – 21	***<0.001*‡**
lymphocytes($\times10^3$/µl)	Mean ± SD Range	1.55 ± 0.79 0.26 – 3.3	2.00 ± 0.63 0.8 – 3.3	1.06 ± 0.65 0.26 – 3	***<0.001*•**
D-dimer (mg/L)	Median (IQR) Range	0.65 (0.5 – 0.89) 0.2 – 12.7	0.6 (0.47 – 0.77) 0.27 – 1.36	0.65 (0.49 – 1.70) 0.2 – 12.0	0.207‡
Ferritin (ng/ml)	Median (IQR) Range	375 (143 – 1000) 50 – 2305	150 (111 – 272) 50 – 400	1020 (645 – 1380) 50 – 2305	***<0.001*‡**
IL-6 (pg/ml)	Median (IQR) Range	45 (16 – 80) 6 – 600	18 (9 – 23) 6 – 120	78 (60 – 123) 13 – 600	***<0.001*‡**
CRP (mg/L)	Median (IQR) Range	45 (29 – 101) 1.96 – 498	29.3 (26 – 33) 1.96 – 107	101 (56 – 138) 5.4 – 498	***<0.001*‡**
Procalcitonin (ng/ml)	Median (IQR) Range	0.11 (0.06 – 0.16) 0.03 – 6.8	0.08 (0.04 – 0.11) 0.04 – 0.23	0.16 (0.09 – 0.35) 0.03 – 6.8	***<0.001*‡**

CRP: C-reactive protein; IL-6: Interleukin-6; TLC: Total leucocytic count. P-value >0.05: Non-significant; P-value <0.05: Significant; P-value< 0.01: highly significant

‡: Mann Whitney test; •: Independent t-test

In the 85 included patients, IL-6 showed significantly positive correlations with total leukocytic count (r= 0.267; p = 0.014), absolute neutrophil count (r=0.318; p =0.003) as well as ferritin (r= 0.580; p <0.001), CRP (r= 0.704; p < 0.001) and PCT levels (r= 0.346; p = 0.001). while a strong negative correlation with a count of absolute lymphocytes was found (r=-0.492; p <0.001). There were no significant correlations between IL-6 level and hemoglobin level, platelet count and D-dimer level (**Table 3).**

Table 3: Correlation of interleukin-6 with the other studied COVID-19 patients

	Interleukin-6	
	r	P-value
Total leucocytic count	**0.267***	**0.014**
Neutrophils	**0.318****	**0.003**
Lymphocytes	**-0.492****	**<0.001**
Hemoglobin	0.110	0.318
Platelets	-0.028	0.797
D-dimer	0.163	0.136
Ferritin	**0.580****	**<0.001**
CRP	**0.704****	**<0.001**
Procalcitonin	**0.346****	**0.001**

P-value >0.05: Non significant (NS); P-value <0.05: Significant (S); P-value< 0.01: highly significant (HS)

CRP: c-reactive protein; r: Spearman rank correlation coefficient

3.3. Risk factors for COVID-19 severity

A multivariate logistic regression study demonstrated that with one unit rise in IL-6>23 pg/ml and CRP >33 mg/L, the risk of COVID-19 non-severe patients developing severe symptoms rose dramatically (IL-6: OR:44.715, p =0.013; CRP: OR= 41.482, p = 0.007), proving that the severity of COVID-19 was influenced by IL-6 and CRP independently (**Table 4).**

Table 4: Logistic regression analysis of significant risk factors associated with severity of COVID-19

	Univariate analysis				**Multivariate analysis**			
	P-value	**Odds ratio (OR)**	**95% C.I. for OR**		**P-value**	**Odds ratio (OR)**	**95% C.I. for OR**	
			Lower	**Upper**			**Lower**	**Upper**
IL6 >23 pg/ml	**<0.001**	211.429	24.815	1801.435	**0.013**	44.715	2.244	891.162
CRP >33 mglL	**<0.001**	49.259	12.331	196.778	**0.007**	41.482	2.834	607.221

CI: Confidence interval; CRP: C-reactive protein; IL-6: Interleukin-6; OR: Odds ratio

3.4.Correlation of IL-6 with inflammatory markers

Along with the ROC curve study, ferritin, IL-6, and CRP (AUC: 0.965, 0.933, and 0.907 at cutoff points of >400 ng/ml, >23 pg/ml, and >33 mg/L, respectively) were the most effective indicators of COVID-19 severity with diagnostic sensitivity of 90.24%, 97.56% and 92.68% and diagnostic specificity of 100 %, 84.09% and 79.5%, respectively (**Table 5 and Figure 1).**

Table 5: Receiver operating characteristic (ROC) analysis for the significant parameters between non-severe and severe COVID-19 groups

	AUC	**Cut off Point**	**Sensitivity (%)**	**Specificity (%)**	**PPV (%)**	**NPV (%)**
Neutrophils ($\times10^3$/µl)	0.761	>3.8	85.37	61.36	67.3	81.8
Lymphocytes ($\times10^3$/µl)	0.865	<1.41	75.61	88.64	86.1	79.6
Procalcitonin (ng/ml)	0.752	>0.14	51.22	97.73	95.5	68.3
Ferritin (ng/ml)	0.965	>400	90.24	100	100	91.7
IL-6 (pg/ml)	0.933	>23	97.56	84.09	85.1	97.4
CRP (mg/L)	0.907	>33	92.68	79.55	80.9	92.1

CRP: C-reactive protein; IL-6: Interleukin-6; NPV: Negative predictive value; PPV: Positive predictive value.

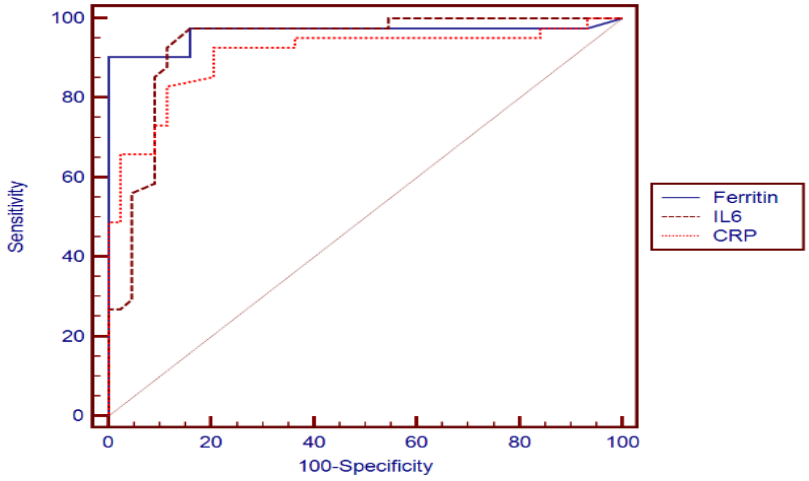

Figure 1: Receiver operating characteristic (ROC) curve for CRP, IL-6 and ferritin between non-severe and severe groups.

The combined prediction of COVID-19 severity was superior to each marker's individual prediction. The AUC increased to 0.998 and 0.962, respectively, when IL-6 with ferritin or IL-6 with CRP combinations were used to identify non-severe and severe individuals with COVID-19 (**Table 6)**.

Table 6: Receiver operating characteristic (ROC) curve for combination of IL6 with the other studied parameters

	AUC	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
IL-6 +Neutrophils	0.958	92.68	90.91	90.5	93.0
IL-6 + Lymphocytes	0.953	97.56	88.64	88.9	97.5
IL-6 + Procalcitonin	0.940	97.56	88.64	88.9	97.5
IL-6 + Ferritin	**0.998**	97.56	100.0	100.0	97.8
IL-6 + CRP	**0.962**	95.12	90.91	90.7	95.2

CRP: C-reactive protein; IL-6: Interleukin-6; NPV: Negative predictive value; PPV: Positive predictive value.

4. DISCUSION

The COVID-19 pandemic is characterized by inflammation, immunological dysregulation, and cytokine storms [14]. There have been several studies undertaken on the condition, including biomarkers for diagnosis, prognosis, prevention strategies and prospective treatments [15].In the current study, a comprehensive analysis of data from 44 non-severe and 41 severe confirmed Patients with COVID-19 revealed that males were more significantly affected by the severe disease, and diabetes mellitus was also significantly linked to the severity of the disease. Furthermore, the most prevalent presenting symptoms during the hospital admission were cough, dyspnea, and fever. The previous findings could be explained by what "Manavi" et al. [16,18] have indicated that females may have better immune responses than males, increasing their resistance to illnesses. Also, "Marhl" et al. [17] referred to the greater risk of severe COVID-19 in diabetics as the abnormal regulation of angiotensin-converting enzyme-2 (a

functional receptor on cell surfaces through which SARS-CoV-2 enters the host cells), liver dysfunction, and chronic inflammation.

In our study, severe patients had a significantly higher absolute neutrophil count, ferritin, IL-6, CRP, and PCT levels than non-severe patients. In contrast, their absolute lymphocyte count was significantly lower. In line with our results is a study by "Taha" [18] and colleagues in which they described the baseline attributes of 180 COVID-19 Egyptian Patients. They found that patients with severe illnesses and non-survivors were much older. Their neutrophil count, PCT, ESR, CRP, and ferritin levels were all significantly higher than those in the control group, while their lymphocyte count was significantly lower. Several other research demonstrated comparable outcomes [18-22].

Inflammation shows important role in COVID-19 development. An inflammatory cytokine storm, a series of medical events in which the immune system produces an excessive number of inflammatory signals and cytokines that can lead to organ failure and death, exacerbates COVID-19 infection [23]. IL-6 modulates immune and inflammatory responses; it is essential for the advancement of COVID-19 pneumonia. It has been shown that monitoring IL-6 levels can guide therapy options in COVID-19 patients with hyper-inflammatory conditions [24]. Several other studies detected rise IL-6 levels in more severe COVID-19 cases [25-27]. CRP elevations in COVID-19 patients have been utilized to help with triage, diagnosis, and prognosis. The liver produces CRP, which rises in response to cytokines like IL-6 during inflammation or infection [27]. After an inflammatory insult, CRP secretion peaks at 48 hours, with a 19-hour half-life. It can rise before a patient's vital signs or leukocytes [28]. Virus-related inflammatory factors can trigger neutrophils and stimulate them to generate cytotoxic mediators and large amounts of reactive oxygen species to suppress the virus. On the other hand, it can cause lymphocyte exhaustion because viruses can directly destroy target cells and excite immune cells to engage in the anti-viral response, culminating in severe lymphocyte damage and death [29]. Previous studies revealed that the neutrophil-to-lymphocyte ratio increases considerably with COVID-19 severity and could be utilized as a predictive indicator to closely monitor COVID-19 patients. [30,31].Ferritin's ability to bind iron and store it is also linked to immunological and inflammatory responses to viral infection. [32,33]. Some patients with severe COVID-19 infection have a clinical picture compatible with subsequent hemophagocytic lymphohistiocytosis. High ferritin levels generally indicate a bad prognosis in patients with COVID-19 [34]. Thyroid parafollicular C cells ordinarily synthesize and release PCT. It is also produced in several extra-thyroid tissues during infections, driven by elevated levels of cytokines [35]. Recently, PCT has been proposed as a feasible inflammatory prognostic biomarker for recognizing COVID-19 patients at high risk of clinical deterioration. [36]. A meta-analysis by "Lippi and Plebani" [37] linked high PCT values to a 5-fold greater risk of severe COVID-19. Our hypothesis in this study was that the simultaneous interpretation of combined baseline inflammatory biomarkers on admission to the hospital would provide a better and more accurate prediction of COVID-19 severity, which would aid in risk stratification and hospital resource management. Our findings supported this hypothesis.In the current study, among all the studied inflammatory biomarkers, IL-6 and CRP were independent variables for predicting the severity of patients with COVID-19, as shown by the multivariate regression analysis. On the other hand, ferritin, IL-6, and CRP were the best predictors of COVID-19 severity, with a diagnostic sensitivity of 90.24%, 97.56%, and 92.68%, and a diagnostic specificity of 100 %, 84.09%, and 79.5%, respectively by ROC curve analysis. The combined prediction of COVID-19 severity was superior to the individual marker's prediction. The AUC increased to 0.998 and 0.962, respectively, when IL-6 with ferritin or IL-6 with CRP combinations were used to identify non-severe and severe COVID-19 individuals. Several other studies reported that on admission, biomarker- combined detection showed better predictive performance for COVID-19 severity than using a single biomarker alone [38,39].

5. CONCLUSION

The role of laboratory evaluation of inflammation markers in the classification and grading of COVID-19 patients should be explored. In the current study, among all the studied inflammatory biomarkers, IL-6 and CRP were autonomous variables for predicting the severity of patients with COVID-19, as shown by the multivariate regression analysis. In contrast, ferritin, IL-6, and CRP were the greatest predictors of COVID-19 severity, with a diagnostic sensitivity of 90.24%, 97.56%, and 92.68%, and a diagnostic specificity of 100 %, 84.09%, and 79.5%, respectively by ROC curve analysis. So, combining IL-6 and CRP on admission levels, or other inflammatory indicators like ferritin, can improve COVID-19 severity prediction and allow for early intervention.

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