Original Article

TANAFFOS

Interleukin-6 in COVID-19 Severity Stratification

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Background: The increase in the number of patients with COVID-19 on a global scale made the early recognition of severe forms of the disease essential. Considering that IL-6 acts as a pro-inflammatory mediator, mediating acute phase responses, the objective of this study was to assess its value in the early severity stratification of SARS-CoV2 infection.

Materials and Methods: It was a prospective study included IL-6 measurement in patients with SARS-CoV2 infection upon admission to the emergency department. Two groups were considered (Group I: patients without hospitalization criteria; Group II: patients with hospitalization criteria). Analyzed variables were serum levels of IL-6, C-reactive protein, ferritin, d-dimers, sociodemographics, ventilator support, ICU admission, mortality, dates of diagnosis, hospitalization, and discharge. For the statistical analyses, Mann-Whitney test, Pearson's chi-square test, area under the receiver operating characteristic curve, Youden index, and Spearman correlation were applied.

Results: A total number of 117 patients were included. Mean age was significantly higher for group II (72,35±15,39 years; p<0,001). No statistically significant difference was seen between the groups regarding gender (p=0,111). The IL-6 values showed an excellent power of discrimination for the need for hospitalization (AUC=0,888; p<0,001) and the need for ICU admission (AUC=0,897; p=7.9 x 10-5). Also, its cut-off value of 12,4pg/mL for the need for hospitalization and 42,95 pg/mL for the need for ICU admission was determined. Positive correlation was seen between IL-6 value and length of stay [r(35)=0,380; p=0,020]. Three deaths were observed among patients with hospitalization criteria (8,1%).

Conclusion: The value of IL-6 at admission seems to independently influence the probability of hospitalization (general ward or ICU) and its duration.

Keywords: COVID-19; Interleukin-6; SARS-CoV-2

INTRODUCTION

At the end of 2019, reports of pneumonia cases of unknown origin appeared in a Chinese province caused by a new virus - Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) - which, due to the ease of transmission, was classified by the World Health Organization as a Public Health Emergency at a global level (1, 2).

This pathology was named COVID-19 and, since its appearance, several forms of presentation have been described: from the absence of symptoms or mild symptoms to severe and eventually fatal forms with systemic manifestations such as sepsis, septic shock, and syndromes of multiorgan dysfunction (1-3).

Although it is not known with precision why some individuals develop a more severe disease, it is thought

that the cytokine storm may play an important role in the pathogenesis of severe cases (3). Some retrospective studies and meta-analyses have shown how increased levels of IL-6 and C-reactive protein (CRP) correlates with mortality and disease severity compared to moderate disease. These studies have also established a correlation between sustained IL-6 secretion and serum viral load in critically ill patients (4). In addition to these markers, there is also evidence that serum levels of ferritin and d-dimers increase with the worsening of the disease, functioning as mortality risk indicators (5).

Given the increase in the number of patients with COVID-19 on a global scale, early recognition of severe forms of the disease has become essential for the timely triage of these patients (3). Considering that IL-6 acts as a pro-inflammatory mediator and plays a key role in mediating acute phase responses (3), it seems pertinent to try to establish the value of IL-6 in the early severity stratification of lung involvement by COVID-19.

Accordingly, the objective of this study was to assess the value of IL-6 in the early severity stratification of SARS-CoV-2 infection.

MATERIALS AND METHODS

In this prospective study, the measurement of IL-6 (in addition to commonly measured severity markers, i.e., CRP, ferritin, and d-dimers) was performed as part of the initial study of patients with SARS-CoV2 infection upon admission to the emergency department in the period between 20/10/2021 and 31/01/2022. As a case definition, patients over 18 years of age and with SARS-CoV2 infection confirmed by RT-PCR were enrolled. The IL-6 assay was performed by chemiluminescence.

Participants were divided into two groups according to the disease severity: Group I: patients without hospitalization criteria; Group II: patients with hospitalization criteria. The Portuguese Directorate General of Health hospitalization criteria were considered for this classification. Accordingly, severe disease was diagnosed at the presence of pneumonia and at least one of

the following criteria: tachypnea greater than 30 cycles per minute, respiratory difficulty, peripheral oxygen saturation less than 90% on room air, hemodynamic instability, persistent fever for more than 48 hours or reappearance after apyrexia, change of consciousness state, hemoptysis, persistent vomiting, severe diarrhea or severe dehydration, leukopenia, lymphopenia or thrombocytopenia in the absence of another cause, and the existence of decompensated chronic diseases or conditions associated with severe COVID-19 or high mortality (decompensated chronic disease, chronic kidney disease on dialysis, active malignant neoplasm, and severe immunosuppression) (6).

Exclusion criteria were the presence of chronic lung disease, rheumatologic pathology, and/or previous inflammatory bowel disease.

Data were collected regarding sociodemographic characteristics, comorbidities, laboratory data (IL-6, CRP, ferritin, and d-dimers), dates of diagnosis, hospitalization and hospital discharge, necessary ventilator support (when applicable), need for ICU admission, and mortality.

The collected data were stored in a database created in the statistical program IBM SPSS Statistics version 28®, guaranteeing the information confidentiality and anonymization.

The variables were organized according to Table 1, and a descriptive analysis was performed. The comparison between continuous and categorical variables was established using the Mann-Whitney test and the comparison between categorical variables was established using Pearson's chi-square test. The predictive value of IL-6, CRP, ferritin, and d-dimers in relation to the need for hospitalization was evaluated using the area under the receiver operating characteristic curve (AUROC), determining a cut-off value for hospitalization using the Youden index.

AUROC's power of discrimination was defined according to the following criteria (7):
- AUROC = 0.5: no discriminating power
- $0.5 < AUROC < 0.7$: weak discrimination
- $0.7 \leq AUROC < 0.8$: acceptable discrimination
- $0.8 \leq AUROC < 0.9$: excellent discrimination
- $AUROC \geq 0.9$: exceptional discrimination

Spearman's correlation was used to establish the correlation between the aforementioned analytical variables and the length of hospital stay. Also, a significance level of 5% (p < 0.05) was considered.

The study was duly authorized by the Ethics Committee for Health of the institution where it took place, and the procedures were followed according to the regulations established by the Helsinki Declaration of the World Medical Association.

RESULTS

Sociodemographic Data

A total number of 117 patients were included in the study, 10 met the exclusion criteria.

Participants included 80 patients in group I and 37 in group II. The mean age was 54.61 ± 21.50 years (18-96 years) with a significantly higher value for individuals in group II (p < 0.001). There was no statistically significant difference between groups regarding gender (p=0.111).

Predictive value of serum levels of IL-6, CRP, ferritin, and d-dimers regarding the need for hospitalization

Regarding the analytical values (IL-6, CRP, ferritin, and d-dimers), a significantly higher value was found for all of them for patients with hospitalization criteria (p < 0.001; Table 1)(Figure 1).

The AUROC values (Figure 2) of these parameters concerning the need for hospitalization were 0.888 for IL-6, 0.812 for ferritin, 0.827 for d-dimers, and 0.897 for CRP, all with a statistically significant value and excellent discrimination power (Figure 2).

For our sample, we obtained an optimal IL-6 cut-off value of 12.4 pg/mL to establish the need for hospitalization, with a sensitivity of 97% and specificity of 69%, corresponding to a Youden index of 0.66. The remaining variables' cut-off values were 2.85 mg/dL, 299.5 ng/dL, and 511.5 ng/mL for the isolated values of CRP, ferritin, and d-dimers, respectively (Table 2).

Table 1. Sociodemographic and clinical variables - Descriptive and comparative analysis between groups

		Total (n=117)	Group I (n=80)	Group II (n=37)	p
Age (mean± SD)		54.61 ± 21.50 (18-96)	46.40 ± 18.85 (18-88)	72.35 ± 15.39 (29-96)	<0.001△
Gender	Female	55 (47.0%)	42 (52.5%)	13 (35.1%)	0.111*
	Male	62 (53.0%)	38 (47.5%)	24 (64.9%)	
IL-6 (pg/mL)		45.34 ± 113.00	26.33 ± 113.79	88.45 ± 106.08	<0.001△
CRP (mg/dL)		4.95 ± 7.13	2.05 ± 2.87	11.23 ± 9.00	<0.001△
Ferritin (ng/dL)		495.66 ± 830.46	231.00 ± 234.58	1082.48 ± 1276.02	<0.001△
D-dimers (ng/mL)		2100.63 ± 7839.00	619.51 ± 812.17	5303.05 ± 13459.29	<0.001△
Length of stay (days; mean± SD)				19.49 ± 17.02 (2-75)	
Need for ICU admission		9 (7.7%)		9 (24.3%)	
Need for IMV		9 (7.7%)		9 (4.3%)	
Number of deaths		3 (2.6%)		3 (8.1%)	

IMV: invasive mechanical ventilation; △Mann-Whitney Test; *Pearson's chi-square test

Table 2. Predictive value of serum levels of IL-6, CRP, ferritin, and d-dimers regarding the need for hospitalization (AUROC and cut-off values)

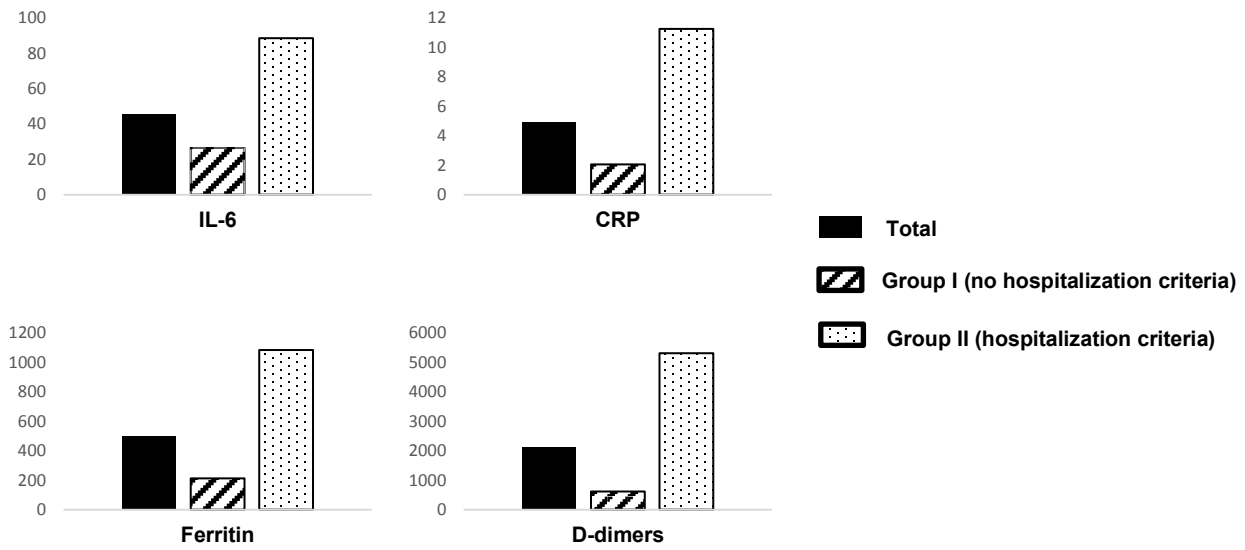|	The cut-off value for hospitalization	Sensitivity	Specificity	Youden Index	AUC	p
IL-6 (pg/mL)	12.4	97%	69%	0.66	0.888	1.74×10^{-11}
CRP (mg/dL)	2.85	92%	81.2%	0.73	0.897	5.35×10^{-12}
Ferritin (ng/dL)	299.5	75%	75%	0.50	0.812	8.37×10^{-8}
D-dimers (ng/mL)	511.5	86.5%	67.5%	0.54	0.827	1.35×10^{-8}

Figure 1. Mean serum levels of IL-6, CRP, Ferritin and D-dimers at admission
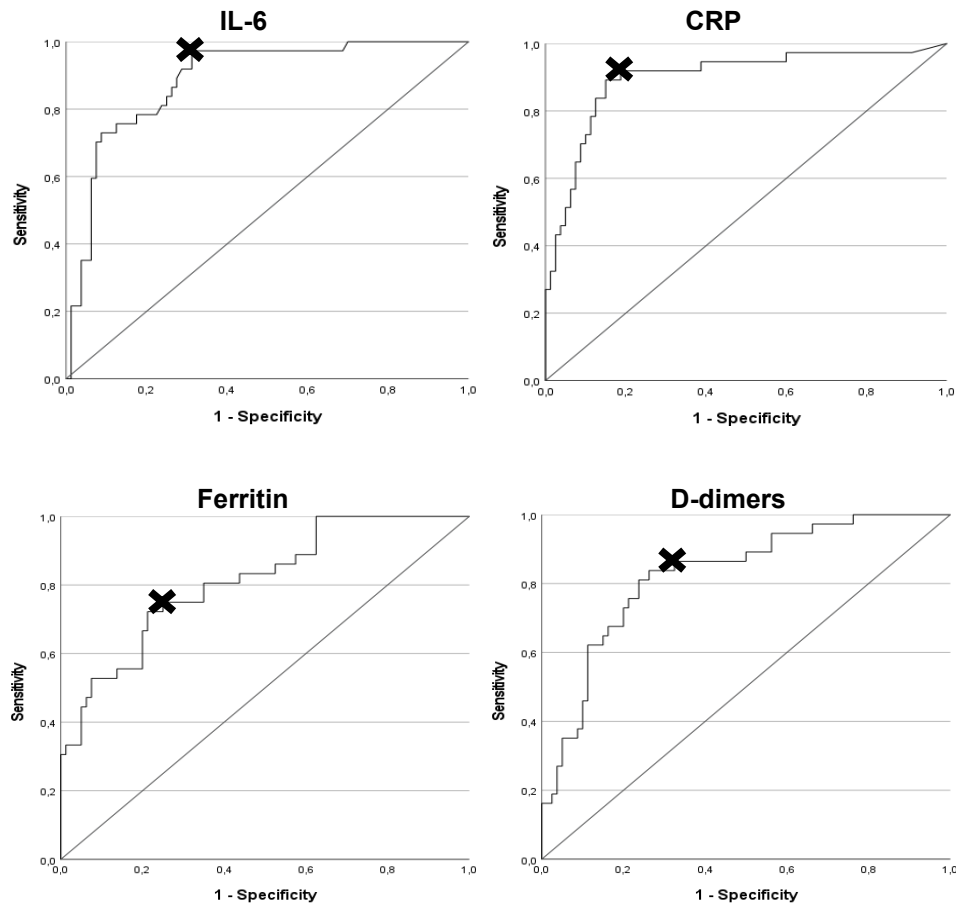

Figure 2. Predictive value of serum levels of IL-6, CRP, ferritin and d-dimers regarding the need for hospitalization (ROC curves)

Correlation between IL-6, CRP, ferritin, and d-dimers serum levels and length of hospital stay

The mean length of hospital stay for the individuals in our study was 19.49±17.02 days (2-75 days).

Among the considered analytical parameters, there was a statistically significant positive correlation between the serum level of IL-6 and the length of hospital stay, r(35)=0.380; p=0.020. For the remaining clinical variables, there was a positive correlation with the length of hospital stay, with no statistical significance (Table 3).

Table 3. Relationship between IL-6, CRP, ferritin, d-dimers, and length of hospital stay (Spearman's correlation)

		Length of Stay
IL-6 (pg/mL)	Correlation coefficient	0.380
	p	0.020
CRP (mg/dL)	Correlation coefficient	0.167
	p	0.322
Ferritin (ng/dL)	Correlation coefficient	0.156
	p	0.364
D-dimers (ng/mL)	Correlation coefficient	0.119
	p	0.485

Predictive value of serum IL-6 level regarding the need for ICU admission

It was found that 24.3% (n=9) of patients requiring hospitalization were admitted to the ICU.

By analyzing the ROC curve, it was found that the IL-6 serum levels showed an excellent power of discrimination, with statistical significance, to predict the need for ICU admission (AUC=0.897; p=7.9 x 10^{-5}; Figure 3).

IL-6: 42,95 pg/mL
Sensitivity: 100%
Specificity: 83,3%
Youden Index: 0,83
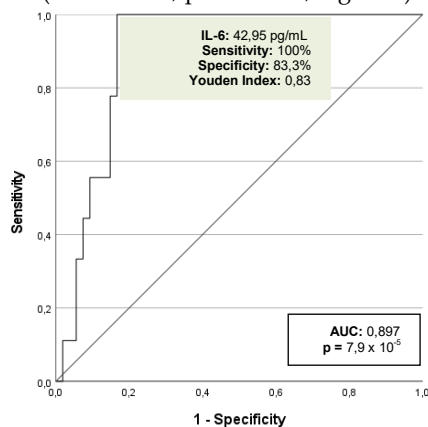
AUC: 0,897
p = 7,9 x 10^{-5}

Figure 3. Predictive value of serum IL-6 level regarding the need for ICU admission (ROC curve)

We obtained an optimal IL-6 cut-off value of 42,95 pg/mL to establish the need for ICU admission with a sensitivity of 100% and specificity of 83,3%, corresponding to a Youden index of 0,83 (Figure 3).

Mortality

In our study, there were 3 deaths among patients with hospitalization criteria (8.1%; Table 1).

The mean age was 80.33±7.64 years (72-87 years). The mean serum levels of IL-6, CRP, ferritin, and d-dimers were, respectively, 61.90±32.05 pg/mL, 9.45±4.53 mg/dL, 347.60±383.24 ng/dL, and 7610.33±10668.57 ng/mL.

DISCUSSION

As a pro-inflammatory mediator, IL-6 is key in mediating acute phase responses (3). Some studies show the correlation between increased levels of IL-6, CRP, ferritin, and d-dimers and the severity of SARS-CoV-2 infection (4,5,9). Given the maintenance of a high number of patients infected with the virus worldwide (10), the need to identify the individuals most likely to have a worse clinical outcome remains critical (11), even though there is a possibility that COVID-19 may become a seasonal pathology (12, 13). IL-6 may be important, having been identified as a possible independent factor of poor prognosis (8,14).

In our study, IL-6 serum levels besides other markers such as CRP, ferritin, and d-dimers, were significantly higher for the group of patients with hospitalization criteria. This finding is in line with what has been described by other studies (1, 3-5, 9, 14), reinforcing the importance of these markers in the identification of more severe disease cases.

All ROC curves used to predict the need for hospitalization according to the values of the different clinical variables showed an excellent power of discrimination. Based on the Youden index, we consider a cut-off value of 12.4pg/mL (Sensitivity=94%; Specificity=69%) to determine the need for hospitalization, despite some discrepancies in reported values. In a study, in which the serum IL-6 levels were evaluated as a

potential marker to distinguish severe and critical illness using ROC curves (3), a value of 9.16 pg/mL was obtained (Sensitivity=82.4%; Specificity=78.5%). In another study (14), a cut-off value of 32,1 pg/mL (Sensitivity=85.19%; Specificity=66.67%) was established for the probability of developing severe disease, with AUROC values similar to those obtained in our study for IL-6 (AUROC=0.808; p<0.001) and CRP (AUROC=0.858; p<0,001) serum levels. These differences may be explained by the different methodologies applied and measurement timing. Nonetheless, they reinforce the importance of IL-6 as a pro-inflammatory mediator responsible for the induction of acute phase responses, capable of leading to a wide spectrum of systemic (fever, recruitment, and activation of leukocytes) and hemodynamic changes (3, 15).

We also verified that the values of all the clinical variables were positively correlated with the length of hospital stay, with a statistically significant value only for IL-6. There is a lack of studies concerning this point, and we can't establish a comparative term with other studies.

Considering the patients with hospitalization criteria, 9 (24.3%) were admitted to the ICU. Analysis of the ROC curve demonstrated that IL-6 was an excellent predictor of ICU admission (AUROC=0.897; p=7.9 x 10^{-5}). The cut-off value obtained was 42.95 pg/mL (Sensitivity=100%, Specificity=83.3%; Youden Index=0.83), which is considerably higher than the obtained value in a previous study (3). Despite having high sensitivity and specificity, this result is limited by the small number of patients admitted to the ICU in our sample.

Regarding mortality, no conclusions can be drawn given the small number of in-hospital deaths observed (n=3; 8.1%). Even so, it is noteworthy that in these cases, the levels of IL-6, CRP, and d-dimers were, in average terms, higher in relation to the mean levels of the total sample.

This study has some limitations. This is a single-center study leading to a small and less representative sample. In addition, some relevant factors such as vaccination and certain comorbidities were not taken into account and may have influenced the number of ICU admissions and deaths.

CONCLUSION

According to our research, the levels of serum IL-6 upon admission appear to have a significant impact on the likelihood of being admitted to the hospital, either in the general ward or in the ICU, as well as the duration of admission. However, further studies are required to establish more precise cut-off values that can provide better guidance for monitoring these patients.

Conflict of Interest

It is declared that all authors, after reading and approving the manuscript, agreed with the authorship and authorized its submission and publication. The authors have no conflict of interest to declare.

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