Clinical Value of Blood Markers to Assess the Severity of Coronavirus Disease 2019

Liuniu Xiao (✉ 754203048@qq.com)
Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology
https://orcid.org/0000-0003-4233-5186

Xiao Ran
Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology

Yan-Xia Zhong
Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology

Shu-Sheng Li
Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology

Research Article

Keywords: COVID-19, IL-6, IL-8, CRP, platelet

Posted Date: October 14th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-89388/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Version of Record: A version of this preprint was published at BMC Infectious Diseases on September 6th, 2021. See the published version at https://doi.org/10.1186/s12879-021-06623-5.
Abstract

Background: Severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) is threatening the world with the symptoms of seasonal influenza. This study was conducted to investigate the patient characteristics and clinical value of blood markers to assess the severity of coronavirus disease 2019 (COVID-19).

Methods: 187 patients, diagnosed with COVID-19 (non-severe and severe cases) and admitted to hospital between January 27th and March 8th of 2020, were enrolled in the present study.

Results: A higher proportion of clinical symptoms, including cough, expectoration, myalgia and fatigue were observed in non-severe group. Significant increased level of WBC count, neutrophils, CRP, IL-6 and IL-8 were noted in severe group. The level of neutrophils, CRP, IL-6 and IL-8 were significantly increased, while platelet was remarkably decreased in the severe group. The risk model based on lymphocyte, IL-6, IL-8, CRP and platelet had the highest area under the receiver operator characteristic curve (AUROC). The baseline of IL-6, IL-8 and CRP was positively correlated with other parameters except lymphocyte, hemoglobin and platelet. While the baseline of platelet was negatively correlated with other parameters except lymphocyte and hemoglobin. Additionally, there was no connection between severity of COVID-19 and cultures of blood, sputum and catheter secretion.

Conclusions: The present study suggested that IL-6, IL-8, CRP and platelet played a critical role in deterioration of COVID-19 with potential value for monitoring the severity of COVID-19.

Background

The coronavirus disease in China, that began in December 2019 (COVID-19), is an emerging lethal respiratory disease and has threatened global health[1,2]. Full-genome sequencing has labeled the pathogen as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is a novel coronavirus declared by the World Health Organization (WHO)[3]. SARS-CoV-2 is a type of single-strand positive RNA virus, its genetic characteristics are significantly different from those of SARS-CoV or MERS-CoV[4]. It is reported that the spread from person-to-person in the hospital and community settings has been accumulating all over the world[5]. Since the transmission routes include droplet, contact, and aerosol, quarantine has been an effective method to protect susceptible people and reduce transmission[6].

Based on current epidemiological investigation, over 2 million people worldwide have been affected by COVID-19[3], which has shown to exceed the outbreak of severe acute respiratory syndrome (SARS) in 2002 and Middle East respiratory syndrome (MERS) in 2012[7], undermining the global economy, and destabilizing societies across the world. A total of 90,877 cases and 4,744 deaths have been reported in China, moreover, 68,139 cases and 4,512 deaths have been reported in the most serious region of Wuhan by May 20, 2020. The 28-day mortality of the critical patients was reported to be approximately 61.5%, which was considerable [8]. It is well known that most patients with COVID-19 have fever along with nonspecific respiratory symptoms, such as cough, expectoration dyspnea. The prevalence of
extropulmonary symptoms often arise from digestive system, many patients initially present with diarrhea, nausea and vomiting[9]. Unfortunately, no specific therapeutic interventions exist so far, thus, supportive care remains the cornerstone for managing the patients. A novel vaccine of COVID-19 is being conducted for clinical trails, which might make a great progress for the further treatments.

Most cases of COVID-19 are mild, however, patients with severe disease can quickly progress to multiple organ dysfunction syndrome (MODS) and even death. Approximately 5% develop critical illness requiring an intensive care unit (ICU) admission[10]. Those admitted to the Intensive Care Unit (ICU) were found to have higher concentrations of pro-inflammatory cytokines as well as T-helper-2 (Th2) cytokines suppressing inflammatory responses[11]. Aberrant immune-inflammatory storm and anti-inflammatory syndrome may play an important role in the disease progression[12]. Recently investigator have found the rising of neutrophils as well as the decreasing of lymphocyte can be used as a indicator of disease progress. Other immune-inflammatory biomarkers, such as higher levels of Interleukin-6 (IL-6), C-reaction protein (CRP) and Procalcitonin (PCT) were independent risk factors for assessing the severity of COVID-19[12, 13]. However, how to predict the disease severity through potential risk factors at early stage is still an unsolved problem, especially for the most serious area of Wuhan in China. Therefore, we performed a retrospective study to evaluate the diagnostic value of clinical features and laboratory markers to discriminate between severe and non-severe COVID-19 disease among adults presenting to hospital.

**Methods**

**Data collection**

The Tongji Hospital Affiliated to Tongji Medical College of Huazhong University of Science and Technology in Wuhan was designated to treat COVID-19. Nasal swab or pharyngeal swab specimens were collected from all suspected SARS-CoV-2 infection patients either in the emergency room or during hospitalization. A confirmed case of COVID-19 was defined as positive result by real-time reverse transcription polymerase chain reaction (RT-PCR) method, carried out according to a validated protocol. A consecutive sample of 187 hospitalized patients, with confirmed COVID-19 from January 27th to March 8th, 2020 were enrolled. Study participants were classified as severe or non-severe COVID-19 disease, based on criteria from the seventh edition of the Chinese National Health Commission disease classification system. Severe disease was based on the presence of the following criteria: First, shortness of breath, respiratory rate (RR) ≥ 30 times/min; Second, oxygen saturation ≤ 93% on room air at rest; Third, partial pressure of arterial oxygen (PaO₂) / oxygen concentration (FiO₂) ≤ 300 mmHg; Fourth, pulmonary imaging recognized obvious lesion progression > 50% within 24–48 hours[12, 13].

This study was approved by the Human Research Ethics Committee of the Tongji Hospital (TJ-IRB20200225), written informed consents were obtained from all participants.
Clinical Symptoms And Laboratory Examinations

Demographic characteristics, symptoms, underlying comorbidities and laboratory results were obtained from electronic medical records. The clinical symptoms included fever, cough, expectoration, chest distress, myalgia, fatigue, diarrhea and nausea. Venous blood samples were collected on admission and were analyzed within 24-hours. The results of blood, sputum and catheter cultures were included when available.

Routine blood tests were detected by HF-3000 hematology analyzer (HLIFE, China). Plasma inflammatory parameters, such as C-reaction protein (CRP) and Procalcitonin, were tested by the Dry Fluorescent Immunoassay (SKY-300, China). Serum cytokines were measured by enzyme linked immunosorbent assay (ELASA) kit. The baseline laboratory examinations were performed within three days after admission.

Statistical analysis

Continuous data were presented as a mean ± standard deviation (mean ± SD), categorical data were presented as frequencies and percentages. Differences in values between the severe and non-severe groups were compared using Student’s T-tests, or a Chi-squared test, or Fisher's exact, where appropriate. A multivariate logistic regression model was used to identify the best predictors of severe COVID-19. A liberal $p$-value of $<0.2$ was used to include all potential predictors identified from univariate analysis. Receiver operator characteristics (ROC) curves and area under curve (AUC) for univariate comparison of predictors of severe COVID-19 disease are also presented. Correlations between different variables were determined by Spearman rank correlation analysis. All levels of statistical significance were set at 0.05. All data management and analysis were performed using SPSS 22.0 and Graphpad Prism 6.0.

Results

Demographic Characteristics of patients with COVID-19

Characteristics of study participants, based on severity of COVID-19, are presented in Table 1. Among the 187 patients, 144 cases (77%) were classified as severe COVID-19 disease, and 111 (59%) were male. The severe group were older age (62.6 ± 1.10 vs 57.7 ± 2.28, $p$ = 0.04). The most common symptoms of all patients were fever (90%), cough (83%) and expectoration (54%). A higher ratio of cough (98% vs 78%, $p$ = 0.001) and expectoration (87% vs 44%, $p < 0.001$) was observed in the non-severe group, compared to the severe group. Other common symptoms such as myalgia (37% vs 15%, $p = 0.002$), and fatigue (42% vs 22%, $p = 0.018$), were also more common in the non-severe group. No difference in the rates of underlying disease or past history were observed between the severe and non-severe COVID-19 groups (Table 1).
Table 1
Demographic Characteristics of patients with COVID-19

| Variables               | All patients (n = 187) | Non-severe group (n = 43) | Severe group (n = 144) | P value |
|-------------------------|------------------------|---------------------------|------------------------|---------|
| Age (years)             | 61.53 ± 1.675          | 57.67 ± 2.275             | 62.55 ± 1.098          | 0.0404  |
| Gender                  |                        |                           |                        |         |
| Male                    | 111 (59.36)            | 28 (65.12)                | 83 (57.64)             | 0.4795  |
| Female                  | 76 (40.64)             | 15 (34.88)                | 61 (42.36)             |         |
| Symptoms                |                        |                           |                        |         |
| Fever                   | 169 (90.37)            | 42 (97.67)                | 127 (88.19)            | 0.0784  |
| Cough                   | 154 (82.35)            | 42 (97.67)                | 112 (77.78)            | 0.0012  |
| Expectoration           | 101 (54.01)            | 37 (86.05)                | 64 (44.44)             | <0.0001 |
| Chest Distress          | 77 (41.18)             | 13 (30.23)                | 64 (44.44)             | 0.1134  |
| Myalgia                 | 37 (19.79)             | 16 (37.21)                | 21 (14.58)             | 0.0020  |
| Fatigue                 | 50 (26.74)             | 18 (41.86)                | 32 (22.22)             | 0.0175  |
| Diarrhea                | 48 (25.67)             | 9 (20.93)                 | 39 (27.08)             | 0.5510  |
| Nausea                  | 18 (9.63)              | 6 (13.95)                 | 12 (8.33)              | 0.3746  |
| Underlying Diseases     |                        |                           |                        |         |
| Hypertension            | 89 (47.59)             | 18 (41.86)                | 71 (49.31)             | 0.4868  |
| Diabetes                | 33 (17.65)             | 5 (11.63)                 | 28 (19.44)             | 0.3611  |
| Cardiovascular Disease  | 21 (11.23)             | 6 (13.95)                 | 15 (10.42)             | 0.5825  |
| Chronic Lung Disease    | 12 (6.42)              | 2 (4.65)                  | 10 (6.94)              | 0.7365  |
| Chronic Hepatic Disease | 3 (1.60)               | 1 (2.33)                  | 2 (1.39)               | 0.5456  |
| Chronic Kidney Disease  | 3 (1.60)               | 1 (2.33)                  | 2 (1.39)               | 0.5456  |
| Past History            |                        |                           |                        |         |
| Smoking                 | 8 (4.28)               | 1 (2.33)                  | 7 (4.86)               | 0.6842  |
| Drinking                | 5 (2.67)               | 2 (4.65)                  | 3 (2.08)               | 0.3245  |
Baseline laboratory results for severe and non-severe patients are presented in Table 2. Higher levels of inflammatory markers were associated with severe disease: leukocyte, \(p = 0.001\); neutrophils, \(p < 0.001\); C-reactive protein (CRP), \(p = 0.021\); interleukin-6 (IL-6), \(p = 0.031\); and, interleukin-8 (IL-8), \(p = 0.007\). Platelet counts were lower in the severe group compared to the non-severe (\(p < 0.001\)). Other inflammatory cytokines including interleukin-10 (IL-10) and Tumor Necrosis Factor-a (TNF-a) were no different, beyond chance, between the two groups (all \(p\)-values > 0.05).

### Table 2

| Variables                   | All patients (n = 187) | Non-severe group (n = 43) | Severe group (n = 144) | \(P\) value |
|-----------------------------|------------------------|---------------------------|------------------------|-------------|
| WBC count (\(\times 10^9\))| 8.62                   | 5.59                      | 9.50                   | 0.001       |
| Neutrophils (\(\times 10^9\))| 6.45                   | 3.47                      | 7.32                   | < 0.001     |
| Lymphocyte (\(\times 10^9\))| 1.55                   | 2.43                      | 1.30                   | 0.157       |
| Hemoglobin (g/L)            | 118                    | 123                       | 117                    | 0.096       |
| Platelet (\(\times 10^9\)) | 180                    | 240                       | 163                    | < 0.001     |
| C-reaction protein (mg/L)   | 66.4                   | 22.4                      | 73.6                   | 0.021       |
| Procalcitonin (ng/mL)       | 1.45                   | 1.40                      | 1.46                   | 0.961       |
| Interleukin-6 (pg/mL)       | 66.02                  | 6.62                      | 78.52                  | 0.031       |
| Interleukin-8 (pg/mL)       | 37.84                  | 8.96                      | 43.98                  | 0.007       |
| Interleukin-10 (pg/mL)      | 12.2                   | 5.8                       | 13.5                   | 0.056       |
| TNF-a (pg/mL)               | 13.0                   | 8.3                       | 13.9                   | 0.148       |

### Logistic Regression Analysis For The Severe Covid-19

A multivariate logistic regression analysis was performed to identify predictors of severe COVID-19. Using a \(p\)-value < 0.2, as the cut-off for inclusion, age, hemoglobin, platelets and inflammatory associated markers were included into the final analysis. The results demonstrated that only neutrophils (Odd Ratio (OR) = 1.541, Confidence Interval (CI) 1.049–2.262) and platelets (\(p\)-values = 0.004, OR = 0.890, Confidence Interval (CI) 0.884–0.997) were independent risk factors for identifying severe COVID-19 (Table 3).
Table 3
Logistic Regression analysis of variables for the severe COVID-19 severity

| Variables          | Univariate Analysis | Multivariate Analysis |
|--------------------|---------------------|-----------------------|
|                    | P value             | HR                    | P value             | HR                    |
| Age                | 0.0404              | 1.031                 | 0.793               | 0.994                 |
| WBC count          | 0.001               | 1.220                 | 0.890               | 1.009                 |
| Neutrophils        | < 0.001             | 1.325                 | 0.027               | 1.541                 |
| Lymphocyte         | 0.157               | 0.940                 | 0.123               | 0.992                 |
| Hemoglobin         | 0.096               | 0.987                 | 0.766               | 0.996                 |
| Platelet           | < 0.001             | 0.993                 | 0.004               | 0.890                 |
| C-reaction protein | 0.021               | 1.014                 | 0.791               | 0.998                 |
| Interleukin-6      | 0.031               | 1.040                 | 0.846               | 1.006                 |
| Interleukin-8      | 0.007               | 1.105                 | 0.153               | 1.180                 |
| Interleukin-10     | 0.056               | 1.210                 | 0.090               | 0.741                 |
| TNF-a              | 0.148               | 1.059                 | 0.723               | 1.062                 |

Roc Curves For Predicting The Severe Covid-19

ROC curves and associated AUC are presented in Fig. 1. AUC values were 0.722 (CRP), 0.697 (PCT), 0.706 (IL-6), 0.828 (IL-8), 0.689 (IL-10) and 0.704 (TNF-a). This suggested a reasonable prediction for the COVID-19 severity by the above immune-inflammatory biomarkers. What’s more, lower levels of lymphocytes and platelets could also indicate severe disease, since AUC 0.761 and 0.764, respectively (Table 4).
### Table 4
ROC curve of risk model and laboratory parameters for the severity of COVID-19

| Variables          | AUROC | Std.Error | P value | Asymptotic 95% CI |
|--------------------|-------|-----------|---------|-------------------|
|                    |       |           |         | Lower Bound | Upper Bound       |
| WBC                | 0.618 | 0.057     | 0.169   | 0.506       | 0.729             |
| Neutrophils        | 0.681 | 0.056     | 0.034   | 0.572       | 0.790             |
| Lymphocyte         | 0.761 | 0.052     | 0.002   | 0.138       | 0.341             |
| Hemoglobin         | 0.408 | 0.079     | 0.281   | 0.252       | 0.563             |
| Platelet           | 0.764 | 0.055     | 0.002   | 0.129       | 0.344             |
| C-reaction protein | 0.722 | 0.071     | 0.010   | 0.584       | 0.860             |
| Procalcitonin      | 0.697 | 0.072     | 0.022   | 0.555       | 0.838             |
| Interleukin-6      | 0.706 | 0.061     | 0.016   | 0.587       | 0.825             |
| Interleukin-8      | 0.828 | 0.052     | < 0.001 | 0.725       | 0.930             |
| Interleukin-10     | 0.689 | 0.069     | 0.027   | 0.553       | 0.825             |
| TNF-a              | 0.704 | 0.069     | 0.017   | 0.569       | 0.839             |

**Note:** ROC, Receiver Operator Characteristics; AUROC, area under receivers operator characteristic curve; CI, confidence interval

### Correlations Between Clinical Serum Markers

Since the markers of IL-6, IL-8, CRP and platelet were significant predictors for COVID-19 severity, we further analyzed their correlations with other variables. There was a positive correlation between the baseline IL-6 and neutrophils ($r = 0.5410, p < 0.0001$), IL-8 ($r = 0.6371, p < 0.0001$), IL-10 ($r = 0.6755, p < 0.0001$), TNF-a ($r = 0.5134, p < 0.0001$), CRP ($r = 0.5965, p < 0.0001$) and PCT ($r = 0.6602, p < 0.0001$). Meanwhile, it was negatively correlated with lymphocyte ($r = -0.4528, p < 0.0001$), hemoglobin ($r = -0.2246, p = 0.0096$) and platelet ($r = -0.5155, p < 0.0001$). (Fig. 2). The similar result also appeared to the other two immune-inflammatory biomarkers (IL-8 and CRP). The IL-8 was found to be positively related to neutrophils ($r = 0.4277, p < 0.0001$), IL-6 ($r = 0.6371, p < 0.0001$), IL-10 ($r = 0.6756, p < 0.0001$), TNF-a ($r = 0.6182, p < 0.0001$), CRP ($r = 0.4294, p < 0.0001$) and PCT ($r = 0.4722, p < 0.0001$), while negatively related to lymphocyte ($r = -0.4359, p < 0.0001$), hemoglobin ($r = -0.1822, p = 0.0331$) and platelet ($r = -0.4142, p < 0.0001$). (Fig. 3). Additionally, the baseline of CRP was positively correlated with neutrophils ($r = 0.5191, p < 0.0001$), IL-6 ($r = 0.6082, p < 0.0001$), IL-8 ($r = 0.4294, p < 0.0001$), IL-10 ($r = 0.5662, p < 0.0001$), TNF-a ($r = 0.4145, p < 0.0001$) and PCT ($r = 0.7013, p < 0.0001$), whereas negatively correlated with lymphocyte ($r = -0.5885, p < 0.0001$), hemoglobin ($r = -0.1996, p = 0.0198$) and platelet ($r = -0.4799, p < 0.0001$). (Fig. 4). Besides, it is interesting to note a common negative relationship between platelet and other parameters,
including neutrophils \( (r = -0.4067, p < 0.0001) \), IL-6 \( (r = -0.5155, p < 0.0001) \), IL-8 \( (r = -0.4142, p < 0.0001) \), IL-10 \( (r = -0.5510, p < 0.0001) \), TNF-a \( (r = -0.3399, p < 0.0001) \), CRP \( (r = -0.4799, p < 0.0001) \) and PCT \( (r = -0.5332, p < 0.0001) \). Meanwhile, platelet was positively related to lymphocyte \( (r = 0.4145, p < 0.0001) \) and hemoglobin \( (r = 0.2396, p = 0.0010) \) (Fig. 5).

**Different Cultures And The Severity Of Covid-19**

In this study, positive blood cultures and sputum cultures were not associated with severe disease \( (p = 0.461 \text{ and } 0.198, \text{ respectively}) \). Catheter secretion cultures from the hydrothorax, or ascites were also not associated with more severe COVID-19 disease \( (p = 0.461) \) (Fig. 6).

**Discussion**

This study has been able to identify characteristics associated with severe COVID-19 disease among adults presenting to hospital. Importantly, higher levels of inflammatory markers and lower platelet levels have been shown to be strongly associated with more severe disease. Besides, none of the blood, sputum or catheter secretion culture shows any prediction for disease severity.

The association between increasing age and more severe COVID-19 found in our study, is consistent with previous reports by Chaochao Tan et. al[14] and Bo Xu et.al[15]. On average patients who did not survive to hospital discharge were twenty years older than those surviving to discharge [15]. The overall prognosis of elder was worse than that of young or middle-aged patients might be attribute to their poor state of malnutrition or underling complications[16]. In our study, symptoms of cough, expectoration, myalgia and fatigue were significantly higher in the non-severe group. As severe patients may have already passed this symptomatic process into a multiple organ dysfunction stage, could explain the lower incidence of overt symptoms in this group compared to the non-severe group. In contrast to other reports, this study failed to show any difference in the rates of underlying diseases or past medical history between the severe and non-severe COVID-19 groups. Conflicting with a systematic review by Zhaohai Zheng et. al [17] that reported higher rates of underlying diseases such as hypertension, diabetes, cardiovascular disease and respiratory disease among critically ill patients.

In terms of laboratory results, several studies have reported increased neutrophils and decreased lymphocytes as a strong predictor of severe COVID-19 disease. For example, Sunxin Wan et.al reported the leukocyte counts of most patients with COVID-19 were in a normal range, but the classified neutrophil counts were significantly higher in the severe group [2]. Jun Zhang et.al reported lymphocyte counts were lower among deteriorating patients compared to those in being discharged [1]. In our study, both neutrophils and lymphocytes were shown to have excellent predictive ability to identify severe COVID-19 disease, which was in agreement with the typical progress of virus infection. Another important finding in our study was related to platelet levels, a lower level of platelet was identified as an independent risk factor for severe COVID-19, which was in consistent with the study published by Zhe Zhu et.al[8]. As we know, platelets are small (2–4 µm in diameter) anucleated cells derived from megakaryocytes in bone
marrow, and are responsible for maintaining the integrity of the vasculature. In COVID-19 subjects, platelets were hyperactivated, with aggregation occurring at suboptimal thrombin concentrations, so the circulation level were significantly decreased[18]. What's more, the severe cases experienced higher viral infection and mechanical ventilation, which led to endothelial injury, platelet aggregation and megakaryocyte reduction, as a result, the platelet production decreased and the consumption increased[19]. In turn, a common negative correlation was found between platelet and other parameters increasing along with the severity of disease.

With the disease progress, vigorous pro-inflammatory/anti-inflammatory response to the virus induces apoptosis in lung epithelial and endothelial cells. Inflammatory mediators play an important role in the pathogenesis of severe disease. CRP as an acute-phase protein that stimulated by the release of apoptosis cells, could indicate the severity of inflammation. Prior studies have revealed that CRP on admission could be a predictive factor for progressive respiratory failure in SARS-CoV as well as MERS-CoV infected patients[20]. A recent study by Saurabh Aggarwal et.al demonstrated that the CRP level showed no obvious difference between critically ill COVID-19 patients and those with mild disease [21]. However, L. Wang published their research about CRP that early stage of COVID-19 CRP levels were positively correlated with lung lesions and could reflect disease severity[22]. Our study showed a similar result with the later, confirmed its role in predicting the severity COVID-19. As a quality improvement project, PCT, a kind of procalcitonin without hormone activity and the precursor of calcitonin[13], was introduced as an antibiotic stewardship tool, also reflecting the inflammatory response. A meta-analysis concluded that PCT > 0.5 ng/mL is associated with increased risk of COVID-19 critical illness especially when the leukocyte is initially normal or reduced[23]. In our study, the AUC of PCT was 0.697, providing a reasonable discrimination between individuals with severe and non-severe COVID-19. In addition, these two inflammatory association factors (CRP and PCT) was significantly correlated with other cytokines, indicating the fact that excess inflammatory responses deteriorate the normal physiological function in viral infection, making the disease worse in progress.

Cytokines are small protein molecules that play an immunomodulating function to maintain the body homeostasis. The autoamplifying of pro-inflammatory cytokines, such as IL-6, IL-8, and TNF-a, can result in a dramatic deterioration for a host, often referred to a “cytokine storm”. This is in contrast to an excessive secretion of anti-inflammatory cytokines (IL-10), which might lead to the persistent immunoparalysis and high risk of secondary infection. Several studies have attempted to explore the relationship between inflammatory cytokines and COVID-19. Zhe Zhu et.al found that high levels of IL-6 was an independent risk factor for severe COVID-19, and it had a potential value in the monitoring the course of disease in severe cases [12]. Jun Zhang et.al investigated a number of factors related to disease progression among hospitalized patients with COVID-19, also discovering that IL-6 and IL-10 were elevated among those with worse outcomes [1]. Consistent with previous studies, our study also showed that IL-6 and IL-8 were higher among severe versus non-severe patients. Overall, in our study high levels of immune-inflammatory markers (IL-6, IL-8, IL-10 and TNF-a) were all shown to be strong predictors of severe COVID-19 using ROC analysis. IL-6 is a multifunctional cytokin that transmits cell signaling and regulates immune cells. It not only plays a key role in cytokin storm, but induces a variety
of acute-phase protein, such as CRP, complement components and so on. A recent study confirmed the role of IL-6 in severe COVID-19 cases by immune analysis, suggesting that Th1 cells and monocytes could express high level of IL-6, resulting in deteriorating the tracheobronchial epithelial cells and aggravating the disease [12]. IL-8 has a potential function in recruitment and activation of neutrophils, and combines with the special surface antibody releasing large numbers of reactive oxygen radicals to aggravate the lung injury. So IL-8 commonly elevates in patients with critical disease, presenting a state of inflammation and organ damage[24]. Consequently, there is no doubt that IL-6 and IL-8 have a positive relationship with other immune-inflammatory markers. TNF-α, another important inflammatory cytokine, acts as an early and sensitive mediator in cytokin storm. Once the viral infection induces the inflammatory defense response, TNF-α could be stimulated by activated neutrophils and lymphocyte and exerted a synergistic effect in the secretion of other cytokines[25]. In contrast to the mentioned pro-inflammatory cytokines, IL-10, as a classical anti-inflammatory cytokine, takes part in maintaining the immune system balance. Since critical patients present a higher level of inflammatory response, regulatory T cells (Tregs) are induced to inhibit the over activation immune response through producing anti-inflammatory cytokines, such as IL-10, transcription growth factor-β (TGF-β) [26]. As a result, IL-10 also shows a positive correlation with other immune-inflammatory parameters. And lastly, few researchers have drawn on any structured research into the association between various cultures and severity of COVID-19. Our study included the analysis of a large number of various blood, sputum and catheter cultures, which were not associated with more severe COVID-19 disease. The reason might be the lower incidence of positive secretion cultures in our hospital.

Nowadays, a number of researchers have reported some newly indicators for severe COVID-19, which might be our research target next step. For example, Yayun Yang et.al showed serum apolipoprotein A 1 serve as an indicator to reflect the severity of COVID-19[26]. Jenifer Gómez-Pastora et. al examined the level of ferritin in COVID-19 patients, confirmed the hyperferritinemia in critically ill, but failed to reveal whether it was the product of inflammation or a pathogenic mediator[28]. Critical patients often experience the respiratory failure in the late stage, so mechanical ventilation is a necessary method to relieve symptoms. At this point, Erika Poggiali et.al presented that Lactate dehydrogenase (LDH) was a predictor of respiratory failure in severe cases[28]. Lastly, adaptive immune response is thought to protect from acquiring SARS-CoV-2 infection, of which neutralizing antibodies (NtAb) seemingly play a major role, such as NtAb50. However, Roberto Gozalbo-Rovira et. al found very weak correlations between NtAb50 and COVID-19 severity[30].

The results of our study need to be considered in the context of potential limitations. Firstly, this is a single-center and retrospective study. Large prospective studies, over an extended period of time, is needed to verify our results. Secondly, the inflammatory associated cytokines IL-10 and TNF-α, although showing promise in ROC AUC analysis to predict severe disease, they did not reach statistical significance, an may represent Type II error. Lastly, another potential limitation is that we failed to show any relationship between various cultures and COVID-19 severity, which may be due to our low positive detection rate, and needs to be confirmed by larger studies.
Conclusions

A retrospective study was conducted to evaluate the diagnostic value of clinical features and laboratory markers to discriminate between severe and non-severe COVID-19 disease. Higher serum levels of inflammatory markers appear to be strong predictors of severe COVID-19, various serum biomarkers coordinate with each other to promote the disease progress. The present study suggested that IL-6, IL-8, CRP and platelet played a critical role in deterioration of COVID-19 with potential value for monitoring the severity of COVID-19.

Declarations

Ethics approval and consent to participate

This study was approved by the Human Research Ethics Committee of the Tongji Hospital, written informed consent was obtained from all participants.

Consent for publication

Not consent is required.

Availability of data and materials

All data are available in the manuscript.

Competing interests

None of the authors declare that there were any competing interests.

Funding

This research was supported by the Fundamental Research Funds for Central Universities: 2019kfyRCPY098.

Authors’ contributions

LNX, XR, YXZ, and SSL participated in the study design, data analysis, and writing of the paper. All authors read and approved the final paper.

Acknowledgements

The authors would like to thank the authors of all the published research that contributed to the data used in this study.

References
[1] Zhang J, Yu M, Tong S, Liu L, Tang L. Predictive factors for disease progression in hospitalized patients with coronavirus disease 2019 in Wuhan, China. J CLIN VIROL 2020;127:104392.

[2] Wan S, Xiang Y, Fang W, Zheng Y, Li B, Hu Y, Lang C, Huang D, Sun Q, Xiong Y, Huang X, Lv J, Luo Y, Shen L, Yang H, Huang G, Yang R. Clinical features and treatment of COVID-19 patients in northeast Chongqing. J MED VIROL 2020;92:797.

[3] Juan Chen, Al ZZE. The clinical and immunological features of pediatric COVID-19 patients in China. Genes & Diseases 2020.

[4] Peng L, Liu K, Xue F, Miao Y, Tu P, Zhou C. Improved Early Recognition of Coronavirus Disease-2019 (COVID-19): Single-Center Data from a Shanghai Screening Hospital. ARCH IRAN MED 2020;23:272.

[5] Li X, Wang L, Yan S, Yang F, Xiang L, Zhu J, Shen B, Gong Z. Clinical characteristics of 25 death cases with COVID-19: A retrospective review of medical records in a single medical center, Wuhan, China. INT J INFECT DIS 2020;94:128.

[6] Ferrari D, Motta A, Strollo M, Banfi G, Locatelli M. Routine blood tests as a potential diagnostic tool for COVID-19. Clinical Chemistry and Laboratory Medicine (CCLM) 2020;58:1095.

[7] Zhang L, Liu Y. Potential interventions for novel coronavirus in China: A systematic review. J MED VIROL 2020;92:479.

[8] Zou X, Li S, Fang M, Hu M, Bian Y, Ling J, Yu S, Jing L, Li D, Huang J. Acute Physiology and Chronic Health Evaluation II Score as a Predictor of Hospital Mortality in Patients of Coronavirus Disease 2019. CRIT CARE MED 2020;48:e657.

[9] Pan L, Mu M, Yang P, Sun Y, Wang R, Yan J, Li P, Hu B, Wang J, Hu C, Jin Y, Niu X, Ping R, Du Y, Li T, Xu G, Hu Q, Tu L. Clinical Characteristics of COVID-19 Patients With Digestive Symptoms in Hubei, China: A Descriptive, Cross-Sectional, Multicenter Study. The American journal of gastroenterology 2020;115:766.

[10] Martindale R, Patel JJ, Taylor B, Arabi YM, Warren M, McClave SA. Nutrition Therapy in Critically Ill Patients With Coronavirus Disease 2019. JPEN-PARENTER ENTER 2020;44:1174.

[11] Velavan TP, Meyer CG. Mild versus severe COVID-19: Laboratory markers. INT J INFECT DIS 2020;95:304.

[12] Zhu Z, Cai T, Fan L, Lou K, Hua X, Huang Z, Gao G. Clinical value of immune-inflammatory parameters to assess the severity of coronavirus disease 2019. INT J INFECT DIS 2020;95:332.

[13] Liu F, Li L, Xu M, Wu J, Luo D, Zhu Y, Li B, Song X, Zhou X. Prognostic value of interleukin-6, C-reactive protein, and procalcitonin in patients with COVID-19. J CLIN VIROL 2020;127:104370.
[14] Tan C, Huang Y, Shi F, Tan K, Ma Q, Chen Y, Jiang X, Li X. C-reactive protein correlates with computed tomographic findings and predicts severe COVID-19 early. J MED VIROL 2020;92:856.

[15] XU B, FAN C, WANG A, ZOU Y, YU Y, HE C, XIA W, ZHANG J, MIAO Q. Suppressed T cell-mediated immunity in patients with COVID-19: A clinical retrospective study in Wuhan, China. J INFECTION 2020;81:e51.

[16] Li T, Zhang Y, Gong C, Wang J, Liu B, Shi L, Duan J. Prevalence of malnutrition and analysis of related factors in elderly patients with COVID-19 in Wuhan, China. EUR J CLIN NUTR 2020;74:871.

[17] Zheng Z, Peng F, Xu B, Zhao J, Liu H, Peng J, Li Q, Jiang C, Zhou Y, Liu S, Ye C, Zhang P, Xing Y, Guo H, Tang W. Risk factors of critical & mortal COVID-19 cases: A systematic literature review and meta-analysis. J INFECTION 2020.

[18] Zaid Y, Puhm F, Allaeyes I, Naya A, Oudghiri M, Khalki L, Limami Y, Zaid N, Sadki K, Ben El Haj R, Mahir W, Belayachi L, Belefquih B, Benouda A, Cheikh A, Langlois M, Cherrah Y, Flamand L, Guessous F, Boillard E. Platelets Can Associate with SARS-Cov-2 RNA and Are Hyperactivated in COVID-19. CIRC RES 2020.

[19] Wang X, Li X, Shang Y, Wang J, Zhang X, Su D, Zhao S, Wang Q, Liu L, Li Y, Chen H. Ratios of neutrophil-to-lymphocyte and platelet-to-lymphocyte predict all-cause mortality in inpatients with coronavirus disease 2019 (COVID-19): a retrospective cohort study in a single medical centre. EPIDEMIOL INFECT 2020;148.

[20] Yamada T, Wakabayashi M, Yamaji T, Chopra N, Mikami T, Miyashita H, Miyashita S. Value of leukocytosis and elevated C-reactive protein in predicting severe coronavirus 2019 (COVID-19): A systematic review and meta-analysis. CLIN CHIM ACTA 2020;509:235.

[21] Aggarwal S, Garcia-Telles N, Aggarwal G, Lavie C, Lippi G, Henry BM. Clinical features, laboratory characteristics, and outcomes of patients hospitalized with coronavirus disease 2019 (COVID-19): Early report from the United States. Diagnosis 2020;7:91.

[22] Wang L. C-reactive protein levels in the early stage of COVID-19. Médecine et Maladies Infectieuses 2020;50:332.

[23] Heesom L, Rehnberg L, Nasim-Mohi M, Jackson AIR, Celinski M, Dushianthan A, Cook P, Rivinberg W, Saeed K. Procalcitonin as an antibiotic stewardship tool in COVID-19 patients in the intensive care unit. J GLOB ANTIMICROB RE 2020;22:782.

[24] Del Valle DM, Kim-Schulze S, Huang H, Beckmann ND, Nirenberg S, Wang B, Lavin Y, Swartz TH, Madduri D, Stock A, Marron TU, Xie H, Patel M, Tuballes K, Van Oekelen O, Rahman A, Kovatch P, Aberg JA, Schadt E, Jagannath S, Mazumdar M, Charney AW, Firpo-Betancourt A, Mendu DR, Jhang J, Reich D, Sigel K, Cordon-Cardo C, Feldmann M, Parekh S, Merad M, Gnjatic S. An inflammatory cytokine signature predicts COVID-19 severity and survival. NAT MED 2020.
[25] Francesco Chiappelli AKGG. CoViD-19 Immunopathology & Immunotherapy. Bioinformation 2020;3:219.

[26] Armin Sadeghi STAM. Th17 and Treg cells function in SARS-CoV2 patients compared with healthy controls. J CELL PHYSIOI 2020:1.

[27] Yayun Yang ZZLF. Low serum apolipoprotein A 1 is an indicator of severity in patients with coronavirus disease 2019. Research Square 2020.

[28] Gómez-Pastora J, Weigand M, Kim J, Wu X, Strayer J, Palmer AF, Zborowski M, Yazer M, Chalmers JJ. Hyperferritinemia in critically ill COVID-19 patients – Is ferritin the product of inflammation or a pathogenic mediator? CLIN CHIM ACTA 2020;509:249.

[29] Erika Poggiali DZPI. Lactate dehydrogenase and C-reactive protein as predictors of respiratory failure in CoVID-19 patients. CLIN CHIM ACTA 2020:135.

[30] Roberto Gozalbo-Rovira EGVL. SARS-CoV-2 antibodies, serum inflammatory biomarkers and clinical severity of hospitalized COVID-19 patients. J CLIN VIROL 2020:104611.

**Figures**
Figure 1

ROC curves for predicting the severe COVID-19
Figure 2

Correlations between IL-6 and neutrophils, lymphocyte, hemoglobin, platelet, CRP, IL-8, IL-10, TNF-a and PCT in patients with COVID-19.
Figure 3

Correlations between IL-8 and neutrophils, lymphocyte, hemoglobin, platelet, CRP, IL-6, IL-10, TNF-a and PCT in patients with COVID-19.
Figure 4

Correlations between CRP and neutrophils, lymphocyte, hemoglobin, platelet, IL-6, IL-8, IL-10, TNF-a and PCT in patients with COVID-19.
Figure 5

Correlations between platelet and neutrophils, lymphocyte, hemoglobin, IL-6, CRP, IL-8, IL-10, TNF-a and PCT in patients with COVID-19.
Figure 6

Different cultures and the severity of COVID-19