Clinical, radiological and laboratory characteristics and risk factors for severity and mortality of 289 hospitalized COVID-19 patients

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Abstract

Background Currently, the coronavirus disease 2019 (COVID-19) has become pandemic globally. 10-20% of the cases are severe and more than 397,000 deaths have occurred. The risk factors for the mortality of critically ill COVID-19 patients remain to be elucidated. Conclusions Survived severe and non-survived COVID-19 patients had distinct clinical and laboratory characteristics, which were separated by principle component analysis. Logistic regression revealed several risk factors such as elder age, greater affected lobe numbers and higher level of serum CRP for the mortality of severe COVID-19 patients. Longitudinal changes of laboratory findings indicate the advancement of the disease and may be helpful in predicting the progression of severe patients.

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Abstract

Background

Currently, the coronavirus disease 2019 (COVID-19) has become pandemic globally. 10-20% of the cases are severe and more than 397,000 deaths have occurred. The risk factors for the mortality of critically ill COVID-19 patients remain to be elucidated.

Methods

289 hospitalized laboratory-confirmed COVID-19 patients (119 from Zhongnan Hospital of Wuhan University and 170 from No.7 Hospital of Wuhan) were included in this study. Electronic medical records, including patients' demographics, clinical manifestation, comorbidities, results of laboratory and radiological materials were collected and analyzed. According to the severity and outcomes of patients, they were divided into three groups: non-survived (n=49), survived severe (n=78), and non-severe (n=162) groups. The clinical, laboratory and radiological data were compared among these groups. Principal component analysis (PCA) was applied to reduce the dimensionality and visualize the patients on a low dimensional space. Correlations between clinical, radiological and laboratory parameters were explored. The univariate and multivariate logistic regression methods were used to explore the risk factors associated with mortality in severe patients.

Results

Of the 289 patients in this study, the median age was 57 years (range, 22 - 88) and 155 (53.4%) patients were male. As of the final follow-up date of this study, 240 (83.0%) patients were discharged from the hospital and 49 (17.0%) patients died. Elder age, more underlying comorbidities, and increased laboratory variables such as leucocyte count, neutrophil count, neutrophil-to-lymphocyte ratio (NLR), C-reactive protein (CRP), procalcitonin (PCT), D-dimer, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and blood urea nitrogen (BUN) on admission were found in survived severe cases, compared to non-severe cases. According to the multivariate logistic regression analysis, elder age, a greater number of affected lobe(s), elevated CRP levels on admission and increased prevalence of chest tightness/dyspnea and smoking history were the independent risk factors for the death of severe patients. A trajectory in PCA from "non-severe" towards "non-survived" via "severe & survived" patients was observed. Strong correlations between the age of patients, the affected lobe number(s) and laboratory variables were identified. Dynamic changes of laboratory findings of survived severe cases and non-survived cases during hospital stay showed that continuing increase of leucocytes and neutrophil count, sustained lymphopenia and eosinopenia, progressing decrease in platelet count, as well as high levels of NLR, CRP, PCT, AST, BUN and serum creatinine were associated with in-hospital death.

Conclusions

Survived severe and non-survived COVID-19 patients had distinct clinical and laboratory characteristics, which were separated by principle component analysis. Logistic regression revealed several risk factors such as elder age, greater affected lobe numbers and higher level of serum CRP for the mortality of severe COVID-19 patients. Longitudinal changes of laboratory findings indicate the advancement of the disease and may be helpful in predicting the progression of severe patients.

Key words:

coronavirus disease 2019; severity; clinical characteristics; risk factors; mortality

Introduction

The emerging pandemic of coronavirus disease 2019 (COVID-19), an infectious disease caused by a novel strain of human coronaviruses, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2),[1] has become worldwide the focus of attention. Since its first report in late December 2019 in Wuhan, China,[2] COVID-19 has aggressively spread across the world and dramatically impacted people's health and daily life. As of June 7, 2020, according to the Situation Report issued by the World Health Organization (WHO), the number of confirmed COVID-19 cases were reported in over two hundred countries/areas and exceeded 6.9 million, with about 397,000 reported deaths.[3] The clinical patterns of COVID-19 ranged from asymptomatic cases to critically ill patients.[4] Fever, dry cough, and radiological changes in lungs tend to be the common clinical manifestations in COVID-19 patients. Severe viral pneumonia with respiratory failure and the deterioration of underlying diseases are the main cause of death of severe patients. According to the data provided by the China National Health Commission, the mortality rate of COVID-19 patients was 7.7% in Wuhan,[5] which was higher to that in the world at present (5.8%).[3]

As the number of infected and fatal cases are dramatically increasing throughout the world, it is vital to reveal the clinical, radiological and laboratory characteristics, and more importantly, the risk factors of mortality in severe COVID-19 patients. Our previous study found that higher levels of C-reactive proteins (CRP), D-dimer and procalcitonin (PCT) were associated with severe patients when compared to non-severe patients.[6] However, the risk factors for mortality of the COVID-19 patients have not yet been well described. Elder age, the presence of comorbidities , leukocytosis, high level of D-dimer, lactate dehydratase (LDH) and low platelet counts were reported to be the risk factors associated with in-hospital death of severe patients.[7-11] Due to the distinct criteria used for severe and/or critically ill patients, the prediction value of these risk factors for death in severe patients may be diverse.

The purpose of this study is to compare the clinical, radiological and laboratory characteristics and longitudinal variations in laboratory parameters of the 289 hospitalized patients with COVID-19 with different severity and clinical outcomes. Potential risk factors and clinical findings associated with death in severe COVID-19 patients were analyzed.

Methods2.1 Study design and patients' enrollment

Hospitalized patients admitted to Zhongnan hospital of Wuhan University (n=178) and No.7 hospital of Wuhan (n=241) (admission date between Dec 29th, 2019 and Feb 16th, 2020), who were diagnosed as 'viral pneumonia' according to the clinical symptoms and chest CT images were primarily enrolled in this study. 289 patients with positive real-time reverse transcription–polymerase chain reaction (rRT-PCR) results of SARS-CoV-2 nucleic acid test were diagnosed as COVID-19 and included in the analysis set. According to the disease severity and clinical outcome, these patients were divided into three groups: 1) non-survived cases; 2) survived severe cases; 3) non-severe cases. All the patients were treated following the guidance issued by China National Health Commission (trial version 3-5).[12] In accordance with the criteria stated in the guidance[13] for hospital discharge of a COVID-19 patient, all the following four conditions should be met: 1) normal temperature lasting longer than 3 days; 2) significantly improved respiratory symptoms; 3) substantially improved acute exudative lesions on chest CT images; 4) two consecutive negative nucleic acid test results of respiratory tract samples (at least 24 hours apart). To be noticed, part of the result of these patients had been reported as letter to editor in *Allergy* which only demonstrated the difference of clinical characteristics between patients with initial negative and positive nucleic acid results of SARS-CoV-2.[6] This study was approved by the Zhongnan Hospital of Wuhan University institutional ethics board (No.2020015 and No. 2020028).

2.2 Data Collection

The electronic medical records of each patient were extracted and analyzed by four independent researchers with a standardized data collection form. Patients' demographic and baseline characteristics (including age, sex, exposure history, comorbidities, surgery history and smoking history), symptomatic and radiological characteristics [including signs and symptoms, and chest computed tomography (CT) results], as well as the laboratory findings on admission (including complete blood cells counts and percentages, and biochemical

parameters), and follow-up data of laboratory parameters in severe survived and non-survived patients were obtained and analyzed. The clinical outcome of each patient (i.e. non-survived, discharged, or remained in hospital) as of Mar 28th, 2020 (the final follow-up date) and the intervals of symptoms onset (i.e. the day when the symptoms were noticed) to hospital admission for each patient were also recorded.

Information in regards of the disease severity, occurrence of complications (shock, respiratory failure or acute renal failure), co-infection status with other pathogens during the hospital stay was collected. The available chest CT images of each patient were reviewed by a senior radiologist blinded to the clinical data, in order to confirm detailed abnormality of radiological characteristics of these patients. The presence or absence of the three following features was recorded for each patient: 1) ground-glass opacity (GGO); 2) subpleural lesions and pleural effusion; 3) affected pulmonary lobe numbers.

The presence of exposure history was defined as any close contact with patients diagnosed with COVID-19 (for example, familial cluster occurrence or occupational exposure of healthcare professionals) or has visited Huanan wet market since December 2019. Severe COVID-19 was designated, if the patients met one of the following criteria: (a) respiratory distress with respiratory frequency [?] 30/min; (b) pulse oximeter oxygen saturation [?] 93% at rest; and (c) oxygenation index (artery partial pressure of oxygen/inspired oxygen fraction, PaO_2/FiO_2) [?] 300 mmHg.

2.3 Laboratory tests

The SARS-CoV-2 viral nucleic acid detection on the pharyngeal swab specimens of each patient from the two hospitals were processed by technicians from Zhongnan Hospital of Wuhan University, using rRT-PCR assay.[14] According to the recommendation issued by the National Institute for Viral Disease Control and Prevention (China),[15] a cycle threshold value (Ct-value) less than 37 was defined as a positive test result, whereas a Ct-value of 40 or more was defined as a negative test. All rRT-PCR assays were performed with the same kit.

The complete blood counts, biochemical parameters and variables reflecting hepatic and renal functions on admission and data of follow-up laboratory tests during hospital stay were collected for each patient, including leucocytes and platelets, neutrophils, lymphocytes, monocytes, eosinophils, basophils, C-reactive protein (CRP), serum amyloid A (SAA), procalcitonin (PCT), D-dimer, serum creatine kinase (CK), creatine kinase-MB (CK-MB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN) and serum creatinine.

Serum and respiratory samples, such as pharyngeal swabs were used for detection of co-infected other pathogens, such as Mycoplasma pneumoniae, Chlamydia pneumoniae, Coxsackie virus group B, adenovirus, echovirus, respiratory syncytial virus, Epstein-Barr virus, influenza A virus, influenza B virus, parainfluenza, cytomegalovirus, Gram-positive or Gram-negative bacteria, and fungi.

2.4 Statistical analysis

Categorical variables were expressed as frequencies and percentages (%), and the frequencies of non-survived, survived severe, and non-severe patient groups (total patient number = 289) were compared by partition of *chi-square* test. Continuous variables were described as median and interquartile ranges (IQR) values, and one-way ANOVA test and *Kruskal-Wallis* test were used, as appropriate, to compare the data from three groups. A two-sided α of 0.0167 (after adjustment) was considered statistically significant for partition of*chi-square* test when compare differences between categorical variables among 3 groups. On the other hand, a two-sided α of 0.05 was considered statistically significant for one-way ANOVA test and*Kruskal-Wallis* test when compare differences between continuous variables among 3 groups.

Principal Component Analysis (PCA) was used for dimensionality reduction and visualization of the patients after imputing missing values using the implementation "ppca" in pca Methods package (cite: https://academic.oup.com/bioinformatics/article/23/9/1164/272597). Euclidean distance and *complete* linkage were used for the heatmap between different laboratory parameters in three groups. Statistical

analyses and figures were generated and plotted by GraphPad Prism version 7.00 software (GraphPad Software Inc), SPSS software (version 26.0, IBM) and R software (version 3.4.3, supported by R Foundation for Statistical Computing).

2.5 Logistic regression analysis

Potential risk factors for non-survived COVID-19 patients (n = 49), compared to survived severe patients (n = 78), were analyzed by a multivariate binary logistic model using Forward Stepwise (Wald) model method. Missing values of laboratory data for the logistic regression analysis, including affected lobe number(s), CRP, PCT and D-dimer, were replaced via multiple imputation. Cut-off value of neutrophil-to-lymphocyte ratio (NLR = 7.726) was calculated via ROC analysis, with an AUC of 0.6614, and NLR was analyzed as categorical variables for the logistic regression analysis. All variables were subject to univariate logistic regression, and odds ratios (ORs) were calculated between non-survived and survived severe groups, with 95% confidence intervals generated. Variables were included in binary logistic regression if corresponding p value was less than 0.05. The binary logistic regression analysis was employed to conclude a multivariate model to conclude the risk factors of death among critically ill patients.

Univariate analysis and multivariate regression analysis were performed by SPSS software (version 26.0, IBM), and R software (version 3.4.3, supported by R Foundation for Statistical Computing).

3. Results

3.1 Distinct characteristics of patients with different disease severity and clinical outcomes

A total of 289 patients with COVID-19 were included in this study. Until 28$^{\text{th}}$ Mar. 2020, all 78 (27.0%) severe patients and 162 (56.0%) non-severe patients were discharged from the two hospitals, and 49 (17.0%) patients were non-survived. Demographic, clinical symptomatic and radiological characteristics of these patients on admission were shown in Table 1-3.

Compared with the survived severe patients (group B in Table 1-3), non-survived patients (group A in Table 1-3) were older in age (p =0.029) and had higher prevalence of symptoms including chest tightness/dyspnea ($p = 0.002$), gastrointestinal symptoms manifested as loss of appetite ($p = 0.015$), higher neutrophil counts ($p = 0.004$) and percentages ($p = 0.016$), higher lymphocyte percentages ($p = 0.014$), larger NLR ($p = 0.045$), higher monocyte percentages ($p = 0.006$), higher levels of C-reactive protein ($p < 0.001$), procalcitonin (p= 0.002), D-dimer ($p = 0.005$), ALT ($p = 0.023$), BUN ($p = 0.003$) and serum creatinine ($p = 0.028$). Increased neutrophil percentages ($p = 0.007$), PCT ($p = 0.002$), D-dimer ($p = 0.005$), and BUN ($p = 0.001$), as well as decreased eosinophil percentages ($p = 0.003$) were more commonly observed in non-survived patients (Table 1-3).

In comparison to survived severe patients (group B), non-severe patients (group C in Table 1-3) were relatively younger ($p < 0.001$) and had less exposure history ($p < 0.001$), less underlying comorbidities ($p < 0.001$), less surgery history ($p = 0.004$) and gastrointestinal symptoms ($p = 0.011$). Leucocyte and neutrophil counts, and NLR were lower in non-severe patients than in survived severe patients. Also, lower serum levels of CRP, PCT, D-dimer, CK-MB, ALT, AST and BUN were observed in non-severe patients (Fig. 1, Table 1-3). Lymphopenia, thrombocytopenia and elevated biochemical parameters including liver and renal function-related markers (all p <0.001) were found in significantly low or normal levels in non-severe patients (Table 1-3).

Radiologically, ground-glass opacity [99 (46.3%)] and subpleural lesions [103 (48.1%)] were common CT signs and distributed in different numbers of lobes (Fig. S1). Also, non-severe patients had higher proportion of normal chest CT images or fewer infected pulmonary lobes, compared to survived severe patients (p = 0.01). As expected, dramatic differences of these clinical characteristics, laboratory findings and CT imaging changes between non-survived patients and non-severe patients were found (Table 1-3).

3.2 Predictions of severity

To assess the similarities and differences of patients with different severities, principal component analysis (PCA) was applied to reduce the dimensionality and visualize the patients on a low dimensional space. On the Fig. 2 biplot, a trajectory from "non-severe" towards "non-survived" via "severe & survived" patients was observed. It supports that the blood count parameters and biochemical parameters can potentially indicate the severity of the patient. Interestingly, the heterogeneity within the "non-survived" group is much larger than "non-severe" group, suggesting the existence of various reasons for severity. An heatmap of the overview of changes of laboratory results between three groups is presented in Fig. S2.

3.3 Risk factors of death in severe COVID-19 patients

127 severe patients (49 non-survivors and 78 survivors) were included in the univariate and multivariate logistic regression analysis. In the univariate analysis, odds of in-hospital death were higher in patients with chest tightness/dyspnea and smoking history (Table 4). Also, patients' age, affected lobe number(s), leucocyte counts, levels of CRP, elevated NLR, PCT, BUN and serum creatinine were also associated with death (Table 4). The multivariate analysis revealed that age of patients (OR, 1.04; 95% CI, 1.00-1.08), the presence of smoking history (OR, 5.21; 95% CI, 1.39-19.52), chest tightness/dyspnea (OR, 3.03; 95% CI, 1.18-7.79), number of affected lobes on admission (OR, 1.71; 95% CI, 1.06-2.78) and level of CRP on admission (OR, 1.01; 95% CI, 1.00-1.02) were risk factors associated with death in cases with severe COVID-19 (Table 4, Fig. 1).

3.4 Correlations between radiological and laboratory parameters

In these 289 patients, according to the heatmap of *Spearman*correlation of laboratory results on admission, together with age and affected lobe numbers (as shown in Fig. 3), three clusters of correlation could be found. The first one was moderate correlations among lymphocyte, eosinophils, platelet, monocytes, leucocytes, and neutrophils in all patients. The second one was correlations among biochemical parameters including PCT, BUN, NLR, CRP, serum creatinine, CK, CK-MB, D-dimer, SAA, ALT and AST, as well as age and numbers of affected pulmonary lobes. Many of these markers showed weak and moderate correlations, however some of them showed a strong correlation, such as NLR, neutrophils, ALT and AST. The third one was negative correlations among blood cell components and biochemical parameters, as well as age and affected lobe numbers. Most of them showed weak correlations except a strong correlation between NLR and lymphocyte counts. Increased inflammatory parameters suggesting cytokine storm and multiorgan injury showed a negative correlation with particularly numbers of lymphocytes and eosinophils, but also platelets and basophils. Scatter plots of different correlations between age, affected lobe numbers and other laboratory parameters are shown in Fig. 4, as well as Fig. S3-S5.

3.5 Longitudinal variations of laboratory findings

As disease progressed, differences in longitudinal trends of the laboratory findings were observed between the non-survived patients and survived severe patients. As shown in Fig. 5, leucocyte and neutrophil counts increased in the early stage of hospitalization (3-7 days) and gradually decreased in the late stage of hospitalization (8-14 days) in severe survived patients, but continuously increased in non-survived patients. Even though lymphopenia was observed in both groups during hospitalization, the lymphocyte count was significantly lower in the non-survived group compared to survived severe group. Also, it is noticeable that sustained eosinopenia and progressing thrombocytopenia were seen in non-survived patients, but both blood cell numbers partially relieved in survived severe patients. Sustained high levels of NLR, CRP, PCT, AST, BUN and serum creatinine were associated with the fatal clinical outcome of severe patients.

Discussion

This retrospective cohort study reported the clinical, radiological and laboratory characteristics of 289 hospitalized COVID-19 patients in Wuhan, China, and identified several risk factors for mortality of COVID-19. Of the 289 laboratory-confirmed COVID-19 cases in this study, most of the patients [183 (63.3%)] were more than 50 years old. 53.3% of the patients were male. 58.5% of the patients were associated with underlying comorbidities, including hypertension (28%), diabetes mellitus (9.3%) and coronary heart disease (6.2%),

which were similarly demonstrated in previous studies.[16-19]

The mortality of the 289 hospitalized cases in the present study was 17.0% (49/289), which was lower than that reported by Zhou et al. [54/191 (28.3%)],[18] but much higher than that reported by Guan et al. (1.4%)[19] and that in a large-scale analysis reported by the Chinese Center for Disease Control and Prevention [1023/44672 (2.3%)].[20] The difference may due to the distinct sample sizes and case inclusion criteria of studies. 56.1% (162/289) patients were non-severe and ultimately discharged from the hospitals.

In this study, elder age, more underlying comorbidities (including hypertension), surgery history, higher prevalence of abnormal CT images as well as the increased leucocyte and neutrophil counts, elevated levels of serum CRP, PCT, D-dimer, ALT, AST and BUN on admission were found in survived severe cases, compared to non-severe cases. Significant differences were identified between non-survived patients and survived severe patients, regarding age, clinical symptoms of chest tightness/dyspnea and loss of appetite, neutrophil counts, NLR, and levels of serum CRP, PCT and D-dimer on admission.

It is interesting to find that affected lobe numbers in CT-scans were associated with the severity of the disease. This was not reported previously. In addition, affected lobe numbers have correlated with age, CRP, D-dimer and BUN, which correlated with each other. Continuous surveillance of chest CT is apparently useful for the evaluation of the disease course of COVID-19. However, in critically ill patients, it is not easy to obtain the second chest CT scan during hospitalization, especially in intubated and ventilated patients. In this situation, bedside X-ray will be an alternative. Most of the non-survived patients of this study lacked the second CT scan.

The risk factors of mortality identified in this study include elder age, higher level of CRP, more number of affected lobe(s), chest tightness/dyspnea and smoking history, according to the logistic regression model, which indicate that these parameters can be used for the prediction of the risk of death in severe patients. Although neutrophil counts and biochemical parameters (including D-dimer) were significantly different between the survived severe and non-survived patients in this study, these variables were not found to be an independent risk factor for the mortality of COVID-19 patients.

A previous study has demonstrated that elder age and high level of D-dimer were associated with poor prognosis of hospitalized COVID-19 patients.[18] The present study confirmed that increased age was associated with higher risk of death in COVID-19 patients, this may be caused by the dampened immune function and more underlying comorbidities, which potentially lead to the poor outcome of elder patients.[21]

Several studies have reported that hypertension, hypoxia, leukocytosis, lymphopenia and high serum LDH level were independent predictors for in-hospital death.[7-10] However, in the present study, only dyspnea and leukocytosis, but not other variables, were found to be independent risk predictors of death in critically ill COVID-19 patients, which is different to previous reports. In addition, previous reports identified that lower baseline levels and/or progressively decreasing platelet counts were associated with higher mortality of COVID-19 patients.[11] Although no significant difference in baseline platelet levels was identified between the severe survived and non-survived patients, longitudinal in-hospital follow up data showed progressing thrombocytopenia during hospitalization was more significant in the non-survived patients in the current study.

CRP is a clinically wide used inflammatory marker. High levels of CRP indicate inflammation caused by various conditions including infections. Elevated IL-6, which is the trigger of CRP synthesis in the liver, was also observed in COVID-19 patients. [22] Cytokine storm has been suggested to be an important cause for poor prognosis of critically ill COVID-19 patients. In the current study, higher levels of CRP were found to be associated with the poor clinical outcome of severe patients. Wang et al. identified higher levels of CRP in non-survivors compared with the survivors within 15 days of COVID-19 hospitalization.[10] Additionally, Wu et al. found that elevated high-sensitivity C-reactive protein (hs-CRP) was significantly associated with higher risks of acute respiratory distress syndrome (ARDS) in COVID-19 patients.[7] These data suggested that CRP was one of the indicators of cytokine storm in COVID-19 and associated with the mortality of this disease.

Higher NLR has been suggested to be an independent risk factor of mortality in hospitalized COVID-19 patients in a recent study. In the same study, the levels of serum procalcitonin were positively correlated with the rate of in-hospital death. Although the univariate analysis found that the odd of in-hospital death was higher in patients with higher NLR, it was not an independent risk factor of death for severe patients in the current study.[23]

Interestingly, the prevalence of patients with smoking history is 9.7% in the current study which is lower than that reported by Guan. et al. (current smoker, 12.6%) ,[19] but significantly higher than that reported in a previous study by our team (smokers, 6.4%).[17] We think this difference may reflect the difference in the population of patients and may also be caused by the insufficient data collection of the smoking history, when in consideration of the infectious nature of COVID-19. However, the presence of smoking history was found to be a risk factor of death in critically ill patients, indicating that smoking may be associated with mortality in patients with severe COVID-19. This finding is consistent with previous study by Mehra et al., which concluded that current smokers had higher in-hospital death in COVID-19 patients.[24] However, the limit of number of smokers in the present study may limit the statistical power of the conclusion. Angiotensin-converting enzyme 2 (ACE2) was demonstrated as a receptor for SARS-CoV-2, which was highly expressed in airway epithelial cells and plays an important role in SARS-CoV-2 infection.[25]The higher levels of expression of ACE2 in the lower respiratory tract of current smokers may contribute to the risk of developing severe COVID-19 infection.[26-27]

In concert with previous reports,[16,28] bilateral lung involvement was predominant in patients with abnormal chest CT images, mainly manifested as multiple ground glass opacities and subpleural lesions. In addition, five pulmonary lobes were affected in more than half of the patients with abnormal chest CT images. The strong positive correlations identified between the affected lobe numbers and patients' age, neutrophil counts and lymphocyte counts indicate that more severe pneumonia was associated with the elderly age and higher degree of lymphopenia, which supports that the affected lobe number(s) could be a possible risk factor for severe cases and the in-hospital mortality of severe COVID-19 patients. Leukocytosis, eosinopenia and lymphopenia may be associated with the progression of inflammatory status, and more severe illness was associated with older patients, given the increased level of CRP, SAA, PCT and D-dimer.

Comparison of the dynamic profile of laboratory findings in both groups revealed that sustained increase in leucocyte count, neutrophil count, biological markers, as well as progressing decrease in platelet count, lymphopenia and eosinopenia may be the predictor of death during hospitalization. A previous study has reported an inverse correlation between the serum D-dimer level and the duration of antiviral treatment, which indicates the decreased level of serum D-dimer may represent the status of virus clearance.[29] However, the similar dynamic trend in levels of D-dimer was not observed in the present series of patients during hospitalization, this may due to the limited number of patients with complete laboratory result of biochemical parameters throughout the period of hospitalization.

This study was limited to the relatively small number of patients which may limit the statistic power and the inclusion of hospitalized patients exclusively (non-hospitalized patients were not included in the analysis), these may cause statistical bias and hence the significant difference identified for demographic and symptomatic characteristics, as well as the laboratory findings between the groups. Also, missing data on some variables, such as information of CT images and biochemical parameters, may cause bias in the identification of risk factors for mortality in severe patients.

In summary, this retrospective, bi-center study revealed that elder age, level of CRP, the number of affected pulmonary lobes, the clinical symptoms manifested with chest tightness/dyspnea and the presence of a smoking history were the independent risk factors of mortality for the non-survived patients, in comparison of severe and survived patients. Assessment of these parameters may help to identify severe COVID-19 patients in high risk of death. Earlier medical intervention and support on these patients with high risk may reduce the fatality of this disease.

Figure Legends

Fig 1. Difference in risk factors of death among three group COVID-19 patients with different severity and outcomes. Demographic parameters and variables of laboratory tests (data on admission) with significant differences among the three groups of patients were illustrated, including continuous variables (A-E) and categorical variables (F-K). Elder age (A), a greater number of affected lobe number (B), increased leucocyte count (C), elevated levels of CRP (D) and BUN (E), higher prevalence of patients with smoking history (F), dyspnea (G), a larger proportion of patients with increased NLR (H), PCT (I), BUN (J) and serum creatinine (K) were identified in the non-survived group, compared to survived severe group. Percentages in the bars of F-K represents the percentages of patients with specific demographic/abnormal laboratory findings in each subgroup. Continuous variables of the three groups (A-E) were compared using one-way*ANOVA* test or *Kruskal-Wallis* test, as appropriate. Categorical variables of the non-survived and survived severe groups (F-K) were compared via *chi-square* test or *Fisher's* exact test, as appropriate. * denotes a p value of [?] 0.05, ** denotes$p$ [?] 0.01, *** denotes p [?] 0.001. CRP, C-reactive protein; BUN, blood urea nitrogen; NLR, neutrophil-to-lymphocyte ratio; PCT, procalcitonin.

Fig 2. Principal Component Analysis (PCA). Principal Component Analysis (PCA) was used for dimensionality reduction and visualization of the patients. All patients were included in the analysis; parameters including laboratory results on admission, age and affected lobe number(s) were used in the analysis, results were demonstrated in colored dots separated by three groups of severity. Despite no clear separation between the three groups, there was a clear trajectory from "non-severe" towards "non-survived" via " survived severe".

Fig 3. Heatmap of *Spearman* correlations among laboratory results, as well as with age and affected lobe number.*Spearman* correlation heatmap with correlation coefficient and significance levels based on the laboratory results on admission, as well as patients' age and affected lobe number. Positive correlations are marked in red and negative ones in blue (color scale on the right side). * denotes p [?] 0.05, ** denotes p [?] 0.01, *** denotes p [?] 0.001. CRP, C-reactive protein; NLR, neutrophil-to-lymphocyte ratio; PCT, procalcitonin; D-D, D-dimer; BUN, blood urea nitrogen; AST, aspartate aminotransferase; ALT, alanine aminotransferase; SAA, serum amyloid A; CK, serum creatinine kinase; CK-MB, creatine kinase-MB.

Fig 4. Selected *Spearman* correlations between the number of affected lobe number(s), age of patients and laboratory parameters in COVID-19 patients. Scatter plots described the correlations between affected lobe number(s), age of patients, and laboratory variables (data on admission). Strong positive correlations were observed in all plots. *Spearman's* test was used to evaluate the correlations. CRP, C-reactive protein; D-D, D-dimer; BUN, blood urea nitrogen; NLR, neutrophil-to-lymphocyte ratio; PCT, procalcitonin.

Fig 5. Differences in longitudinal course of laboratory findings between non-survived and survived severe cases. The severe COVID-19 patients were divided into non-survived and survived severe groups according to the clinical outcomes as of Mar 28^{th}, 2020. Data from patients with available laboratory results on admission, 3-7 days after admission and 8-14 days after admission were shown. 'n' represents the number of patients with available follow-up data for each parameter. The red lines represent the values of non-survived patients of each parameter, and the blue lines show the values of survived severe patients. * denotes p [?] 0.05, ** denotes p [?] 0.01, *** denotes p [?] 0.001. NLR, neutrophil-to-lymphocyte ratio; CRP, C-reactive protein; PCT, procalcitonin; AST, aspartate aminotransferase; BUN, blood urea nitrogen.

AUTHOR CONTRIBUTIONS

Jinjin Zhang, Yiyuan Cao, Ge Tan, and Yadong Gao collected, organized the clinical data, and prepared the manuscript. Xiang Dong, and Bin-chen Wang contributed to the collection of patient cases and statistical analysis, and Yiyuan Cao collected and interpreted the radiological materials. Jun Lin, Youqin Yan were involved in the clinical and organization work. Yadong Gao, and Cezmi A. Akdis designed the study and reviewed the manuscript, along with Guang-hui Liu and Mubeccel Akdis.

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