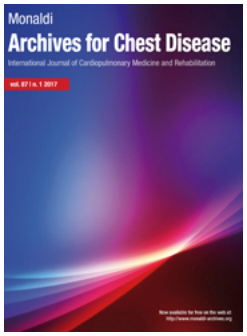

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An in-depth investigation of serum Krebs von den Lungen-6 and other biomarkers in COVID-19 severity and mortality

Andro Pramana Witarto,[1] Alfian Nur Rosyid,[2] Bendix Samarta Witarto,[1]
Shidi Laras Pramudito,[1] Achmad Januar Er Putra[1]

[1]Medical Program, Faculty of Medicine, Universitas Airlangga, Surabaya; [2]Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Airlangga Hospital, Universitas Airlangga, Surabaya, Indonesia

Correspondence: Alfian Nur Rosyid, Department of Pulmonology and Respiratory Medicine, Universitas Airlangga Hospital, Faculty of Medicine, Universitas Airlangga, Jl. Dharmahusada Permai 60115, Surabaya, East Java, Indonesia.
E-mail: alfian-n-r-10@fk.unair.ac.id

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Abstract

Krebs von den Lungen-6 (KL-6) is a glycoprotein mainly expressed by type II pneumocytes and recently known as a lung injury biomarker. However, the number of studies is still limited, especially in Indonesian COVID-19 populations. Therefore, we aim to provide correlation, sensitivity, and specificity analyses of KL-6 and other biomarkers in Indonesian COVID-19 severity and mortality. We conducted a cross-sectional study involving adult COVID-19 patients at Universitas Airlangga Hospital, Surabaya, East Java, Indonesia, between March 26, 2021, and August 25, 2021. KL-6 and other biomarker levels were compared according to severity (severe versus non-severe) and mortality (non-survivor versus survivor). We also included the receiver operating characteristic analysis to define the optimal cut-off, sensitivity, and specificity of KL-6 to determine COVID-19 severity and mortality. We enrolled 78 COVID-19 patients (23 non-survivors), including 39 non-severe and 39 severe patients. There was no significant difference in serum KL-6 levels, neither in severity nor mortality groups. KL-6 had the strongest positive correlations with ferritin in severe patients (r=0.313) and non-survivors (r=0.467). We observed that the best sensitivity was KL-6 combined with platelet-to-lymphocyte ratio (PLR) (0.818) in severe patients and with neutrophil-to-lymphocyte ratio (NLR)/PLR/ferritin/C-reactive protein (0.867) in non-survivors. In contrast, the best specificity was found when KL-6 was combined with NLR/D-dimer (0.750) in severe patients and with D-dimer (0.889) in non-survivors. Serum KL-6 is a useful auxiliary laboratory evaluation index for COVID-19 lung injury to depict its severity and mortality.

Keywords: KL-6, COVID-19, mortality, severity, biomarkers.

Introduction

The Coronavirus disease 2019 (COVID-19) pandemic has affected around 520 million people worldwide with 6,286,057 confirmed cases and 156,586 fatalities reported in Indonesia from January 3, 2020 to May 30, 2022 according to the World Health Organization (WHO) reports [1]. COVID-19 is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which infiltrates cells through the angiotensin-converting enzyme-2 (ACE2) receptor [2,3]. This receptor is expressed in various organs, especially in the apical part of the lung [4,5]. Once infiltrating the alveolar cells, it will trigger the systemic inflammatory response, which

manifests in varying clinical presentations from asymptomatic to severe complications, such as acute respiratory distress syndrome (ARDS) and multi-organ dysfunction syndrome (MODS) [5].

Krebs von den Lungen-6 (KL-6) is a mucin-like glycoprotein that belongs to the *MUC1* gene family. It is mainly expressed in type II pneumocytes, bronchial epithelial cells, pancreas, and mammary ducts [6-8]. The damage and proliferation of pneumocytes may cause the 'leak' of KL-6 into the systemic bloodstream, thus allowing its serum measurement [9,10]. It was first suggested as a tumor marker in the lung, breast, and pancreatic cancers. However, some studies recently suggests KL-6 as a biomarker to evaluate interstitial lung diseases (ILDs), including hypersensitivity pneumonitis and COVID-19-related pulmonary fibrosis [11-13]. Serum KL-6 has a moderately high sensitivity and specificity on the COVID-19 severity assessment, which may be beneficial as an additional assessment [14]. Furthermore, a different study reports that KL-6 >1000 U/mL is associated with COVID-19 mortality [15].

Some inflammatory biomarkers and laboratory parameters are found to reflect the COVID-19 disease severity and prognosis [16,17]. Several studies have attempted to correlate serum KL-6 with those markers and reported various positive or negative correlations [13,18]. The correlation studies between KL-6, other serum biomarkers, and the COVID-19 severity and mortality may provide novel information on the pathophysiology and disease activity at the molecular level. However, this study area is still not widely researched, especially in Indonesian populations. Therefore, we aim to provide more insights into the correlation between KL-6 and other biomarkers in Indonesian COVID-19 patients, including its sensitivity and specificity in the severity and mortality.

Materials and Methods

Study design and setting

We conducted a cross-sectional study involving 78 adult COVID-19 inpatients at Universitas Airlangga Hospital, Surabaya, East Java, Indonesia, between March 26, 2021, and August 25, 2021. The patients were treated in the generic ward, high care unit, or intensive care unit based on their COVID-19 severity. All patients have given their written informed consent. The serum samples were analyzed at the laboratory of Universitas Airlangga Hospital. All data were collected from the patient's medical records and the laboratory computerized data. This study was approved by the ethics committee of Universitas Airlangga Hospital (ethics number 121/KEP/2021).

Eligibility criteria

The patients were enrolled according to the Indonesian COVID-19 national guideline of diagnosis and treatment [19]. The patients were eligible if meeting inclusion criteria as follows: (1) adult COVID-19 patients ≥18 years confirmed by reverse transcription polymerase chain reaction (RT-PCR) of nasopharyngeal swab; (2) patients undergoing chest X-ray (CXR) examination; (3) patients with KL-6 test results; and (4) participation willingness by signing the informed consent form. While, the exclusion criteria were patients with pre-existing or history of lung tuberculosis (TB), ILDs, and/or chronic obstructive pulmonary disease (COPD), pregnant women, and patients with human immunodeficiency virus (HIV) infection.

Clinical data and laboratory assessment

We extracted age, gender, length of hospital stay (LoS), and laboratory data from the electronic medical records. The collected laboratory data were complete blood count (CBC), neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), liver function test, renal function test, serum electrolyte, D-dimer, ferritin, interleukin-6 (IL-6), procalcitonin (PCT), C-reactive protein (CRP), and KL-6. Serum KL-6 was measured using the Bioassay Technology Laboratory KL-6 ELISA reagent kit (BT-Lab, Cat.No E1980Hu) and interpreted using iMark™ Microplate Absorbance Reader (Bio-Rad Laboratories Inc., Hercules, CA, USA). The patients were then categorized into four severity levels based on the 3rd edition of the Indonesian COVID-19 management guideline: (1) mild, patients who had any signs and/or symptoms of COVID-19 without any proof of pneumonia or hypoxia; (2) moderate, patients who had pneumonia proven by clinical assessment or imaging with SpO_2 ≥93% on room air at sea level; (3) severe, patients who had severe pneumonia indicated by respiratory rate >30 breaths/min, severe respiratory distress, or SpO_2 <93% on room air at sea level; and (4) critical, patients who had ARDS, sepsis, and/or, septic shock [19,20]. We further simplified the severity classification for statistical analysis into non-severe (mild and moderate) and severe (severe and critical). The patients were also classified into survivor and non-survivor.

Statistical analysis

The results were presented as mean ± standard deviation (SD) if the data were normally distributed based on Shapiro-Wilk normality test (p ≥0.05), or median (interquartile range [IQR]) if otherwise. Comparison between groups, severe vs. non-severe or non-survivor vs. survivor, was performed using the Chi-square test for nominal data (gender), Mann-Whitney

U test for ordinal data (PCT), and independent T-test or Mann-Whitney U test for continuous data. Shapiro-Wilk normality test was also carried out before comparing continuous variables between groups to determine the appropriate statistical test. An independent T-test was selected if the *p*-value of the normality test was ≥0.05; otherwise, the Mann-Whitney U test was chosen. The correlation between KL-6 and other COVID-19 biomarkers, including NLR, PLR, D-dimer, ferritin, IL-6, and CRP, was analyzed using Pearson's correlation coefficient. The positive or negative correlations were then classified as very weak (r = 0.00–0.19), weak (r = 0.20–0.39), moderate (r = 0.40–0.59), strong (r = 0.60–0.79), and very strong (r = 0.80–1.00). For KL-6, an additional binary logistic regression analysis and its 95% confidence interval (CI) was performed to depict its relationship with COVID-19 severity and mortality. Furthermore, we analyzed the receiver operating characteristics (ROC) curve and its components, including area under the ROC curve (AUC) and its 95% CI, sensitivity, specificity, and optimal cut-off values based on Youden's index, to assess the KL-6 and other serum biomarkers in determining COVID-19 severity and mortality. The Youden's index analysis on the optimal sensitivity and specificity was calculated by using the following formula: J = Sensitivity + Specificity − 1. We also performed logistic regression analysis where severe patients and non-survivors were tested as the dependent variables against KL-6, NLR, PLR, D-dimer, ferritin, IL-6, and CRP as the independent variables. All the statistical analyses were conducted using IBM SPSS Statistics version 25.0 (SPSS Inc., Chicago, IL, USA). *P*-value <0.05 was considered statistically significant. Missing values were imputed in the aforementioned statistical application by aggregating five iterations of multiple imputations by chained equations imputation method into their mean values [21]. Multiple imputations were only conducted if the missing values on a variable were <5% to avoid result bias [22].

Results

Patients' characteristics

A total of 78 COVID-19 patients were enrolled in this study, including 39 patients in the non-severe group (12 mild, 27 moderate) and 39 patients in the severe group (18 severe, 21 critical) (Table 1 and *Supplementary Table 1*). Based on the mortality group, the patients were divided into two groups: 53 survivors (12 mild, 23 moderate, 10 severe, 8 critical) and 23 non-survivors (4 moderate, 8 severe, 13 critical) (Table 2 and *Supplementary Table 2*). The mean age and LoS were 50.29 ± 12.16 years and 11.61 ± 7.89 days, respectively. Of 78 patients, 41 patients (52.60%) were male. There was no significant difference in age, LoS, and the number of male

patients between severe and non-severe patients, likewise the non-survivors and survivors. Missing values were present for D-dimer, ferritin, IL-6, PCT, and CRP (*Supplementary Table 3*). In the laboratory examination (Table 1-2 and *Supplementary Table 1-2*), we found that white blood cells (WBCs), neutrophils, NLR, serum glutamic oxaloacetic transaminase (SGOT), blood urea nitrogen (BUN), D-dimer, ferritin, and CRP were significantly different in the severe and non-survivor groups compared to non-severe and survivor groups, respectively (p <0.05). However, platelets and PLR were only significantly different in the severe patients compared to the non-severe patients (290.28 ± 123.38 vs. 227.21 ± 64.09 [p = 0.006] and 331.73 ± 258.38 vs. 215.17 ± 156.14 [p = 0.013], respectively), but not in the mortality groups. On the contrary, significantly different potassium and IL-6 levels were observed in the non-survivor group compared to the survivors (4.32 ± 0.58 vs. 3.75 ± 0.45 mmol/L [p = 0.000] and 866.15 ± 2553.07 vs. 52.56 ± 90.59 pg/mL [p = 0.002], respectively).

Diagnostic value of KL-6 and other biomarkers in COVID-19 severity and mortality

The mean of KL-6 levels in this study was 50.25 ± 28.61 U/mL. There was no significant difference in the mean of KL-6 levels, neither in the severity group (p = 0.261) (Table 1) nor the mortality group (p = 0.524) (Table 2). In both severity (Figure 1) and mortality groups (Figure 2), KL-6 was weakly correlated with NLR, PLR, D-dimer, and IL-6. The strongest positive correlation was observed between KL-6 and ferritin in severe patients (r = 0.313) and non-survivors (r = 0.467), while the strongest negative correlation was also observed between KL-6 and ferritin in non-severe patients (r = –0.325) and survivors (r = –0.329). The odds ratios of KL-6 in the binary logistic analysis were 0.989 (95% CI = 0.972–1.005; p = 0.179) for severe vs. non-severe patients and 0.994 (95% CI = 0.976–1.012; p = 0.490) for non-survivors vs. survivors.

ROC analyses were performed to assess the diagnostic value of KL-6 in COVID-19 severity (Figure 3 and Table 3). KL-6 had the lowest AUC among COVID-19 biomarkers in severe patients (0.458; 95% CI = 0.281–0.635) with an optimal cut-off of ≥29.901 (Sn/Sp = 0.909/0.150). However, KL-6 might be beneficial to increase the diagnostic value of NLR and PLR in severe COVID-19 patients, indicated by the AUC increase (0.723 to 0.736 and 0.634 to 0.673, respectively). There was a noticeable increase in the specificity of NLR after including KL-6 in the analysis (0.600 to 0.750), and also in the sensitivity of PLR (0.636 to 0.818). D-dimer, ferritin, IL-6, and CRP showed no improvements in severity diagnostic value after including KL-6 in their analyses. Our analyses showed that in the COVID-19 severity, the best

sensitivity was found when KL-6 was combined with PLR (0.818), and the best specificity was found when KL-6 was combined with NLR (0.750) and D-dimer (0.750).

The lowest AUC in the mortality analysis was also observed in KL-6 (0.442; 95% CI = 0.253–0.631) (Figure 3 and Table 4). The optimal cut-off for KL-6 was ≥39.555 (Sn/Sp = 0.533/0.556). Yet, an increased AUC of NLR, PLR, D-dimer, ferritin, IL-6, and CRP was observed after combining their analyses with KL-6. KL-6 might also increase the sensitivity of ferritin and IL-6 and the specificity of NLR, PLR, and D-dimer in the non-survivors. Moreover, the best sensitivity was 0.867 in KL-6 when combined with NLR, PLR, ferritin, and CRP. In contrast, the best specificity was only seen in the combination of KL-6 and D-dimer (0.889). We further found an interesting finding in the ROC analysis of COVID-19 mortality related to the CRP. Before including KL-6 in the analysis, CRP had a high specificity (0.926) and a low sensitivity (0.400). On the contrary, after including KL-6, CRP shifted to have a higher sensitivity (0.867) and a lower specificity (0.556).

Discussion

To the best of our knowledge, this is the first study in Indonesia that thoroughly analyzed the KL-6 importance, its correlation to other biomarkers, including NLR, PLR, D-dimer, ferritin, IL-6, and CRP, and also their ROC analyses in the COVID-19 severity and mortality. We find no significant difference in serum KL-6 in the severity and mortality groups. However, in our study, KL-6 and ferritin have the strongest negative correlations among all other biomarkers. Interestingly, despite the insignificant difference between KL-6 and its lowest AUC in both COVID-19 severity and mortality, KL-6 is still beneficial in increasing the diagnostic value of NLR and PLR in severe patients and non-survivors, and also the diagnostic value of D-dimer, ferritin, IL-6, and CRP in non-survivors. These findings imply that KL-6 may still be a valuable biomarker to depict the COVID-19 severity and mortality.

As previously stated, our results show no significant difference in serum KL-6, neither in the severity nor mortality groups. Several studies show that KL-6 is dependently affected by the ethnicity and the genotype variation of the study population [11,23,24]. Moreover, KL-6 tends to fluctuate over some duration; thus, the serum sampling timing and the disease duration before sampling may affect the KL-6 levels [25]. KL-6 also tends not to rise during mild lung injury compared to other transiently-increasing biomarkers, such as surfactant protein A (SP-A) and D (SP-D). This further suggests that KL-6 may not increase during an early inflammation process, but rather when there has already been severe alveolar damage and increased alveolar

permeability [26]. In addition, this finding may be explained by using different reagent kits to measure KL-6, which yields lower or higher levels regardless of the COVID-19 severity and mortality, as described by Suryananda and Yudhawati [27].

Serum KL-6 has the strongest correlation with ferritin, but not with the other biomarkers in this study. The differences in their production sources – WBCs and platelets in the bone marrow [28], CRP in the liver [29], KL-6 in the damaged type II pneumocytes [14], and ferritin in several damaged cells, including the pulmonary cells [30] – might explain our findings. Ferritin has a similar function as KL-6; thus, acting as an acute-phase reactant in metabolic stress and inflammatory response [31]. In a study by Lee *et al.* [30], a decrease in lung function may also be a strong mortality predictor. The lung function deterioration further carries several consequences, including the surge of serum ferritin released from damaged cells. Hence, ferritin can be considered a substantial factor related to patients' mortality [32].

We also observe no notable correlations between KL-6 and other biomarkers. One of them is its correlation with D-dimer. As an explanation, even though D-dimer might still be useful for determining the COVID-19 severity and mortality [33], a longitudinal study by Fogarty *et al.* [34] shows no association between D-dimer and lung function, meaning that D-dimer is not suitable to represent lung function. Furthermore, no correlation between KL-6 and the rest biomarkers, including NLR, PLR, IL-6, and CRP, may be caused by several underlying comorbidities in the patients. These comorbidities probably affect those biomarkers and their correlations: (1) NLR can be affected by myocardial infarction (MI), asthma, and COPD [35]; (2) PLR can be affected by heart failure and cardiogenic pulmonary oedema [36,37]; and (3) IL-6 and CRP can be affected by diabetes mellitus, chronic kidney disease (CKD), and cardiovascular disease [38-41]. Aside from comorbidities, the genotypic characteristics of SARS-CoV-2 may also affect these biomarkers due to the difference in clinical presentations [42].

Contrary to our findings, previous studies conducted in China, Japan, and Italy show that serum KL-6 has great discriminatory power for evaluating disease severity and predicting the risk of mortality of COVID-19 patients with AUCs ranging from 0.793 to 0.850. The reported optimal cut-off values also vary from 303 to 642 U/ml, which are higher than our study [15,43-46]. We again speculate that the differences in study location and population characteristics may influence these findings. In this context, our results may warrant further validation in more extensive studies involving various racial ethnicities in Indonesia. Additionally, to the best of our knowledge, our study is the first to evaluate KL-6 combined with each NLR, PLR, D-dimer,

and ferritin to predict the COVID-19 severity and mortality. Although KL-6 shows the lowest AUC, its combination with specific biomarkers may be beneficial in increasing their diagnostic performance in COVID-19 patients. Another study by Bergantini *et al*. [46] also shows that KL-6 combined with IL-6 and CRP has an exceptional AUC of 0.95, which is higher than their initial AUCs when used alone in evaluating disease severity. These findings highlight the significance of an integrative approach using currently available biomarkers to aid in COVID-19 patient prognosis.

Despite the promising findings, our study carried several limitations. First, this study was a single-centred cross-sectional study with a limited number of patients. Its nature may affect the interpretation since it is difficult to determine the causality and temporal relation between factors and outcomes. However, a cross-sectional study still carries an important strength in creating an opening for more in-depth studies and filling the knowledge gaps [47], including KL-6 in COVID-19. Second, there were some missing values on several variables in our study. However, we have already performed multiple imputations to replace them. Since this method might cause statistical bias, we have already created multiple imputed data sets prior to processing the data and only performed the imputations to the variables with missing values <5% to avoid any potential bias [22,48]. Third, the time interval between the hospital admission and the blood sampling in each patient was not similar. At this interval, the patients received different therapeutic approaches with varying lengths of time to maintain their best clinical conditions. Some treatments are known to affect the KL-6 levels in several diseases, including interstitial pneumonia with autoimmune features (IPAF), idiopathic pulmonary fibrosis (IPF), and psoriasis [49-51]. Therefore, any treatments in COVID-19 may also affect the KL-6 levels and its statistical analysis.

Conclusions

In summary, this study demonstrated that serum KL-6 was helpful as an auxiliary laboratory index of COVID-19 lung injury to depict its severity and mortality. This was proven by the improvements of sensitivity and specificity, either in severity or mortality groups, among the combination of KL-6 with other analyzed biomarkers, such as NLR, PLR, D-dimer, ferritin, IL-6, and CRP. The strongest correlation between KL-6 and ferritin further strengthened the importance of evaluating KL-6 in COVID-19 lung injury. Furthermore, we found that serum KL-6 levels in the Indonesian COVID-19 population were low due to the genetics and the reagent kits used to evaluate KL-6. Based on the current findings, KL-6 may be included in the

integrative approach of COVID-19 diagnosis in addition to other previously-available biomarkers, especially in confirming the COVID-19 severity and prognosis. Nevertheless, when using KL-6, clinicians should consider factors such as the type of reagent kits, study location, and population characteristics, including ethnicities. However, several future studies analyzing and discussing serum KL-6, either on a larger scale of the Indonesian population or worldwide population, are still needed to confirm our findings and further extend the knowledge of KL-6 in COVID-19 severity and mortality. The future studies are also recommended to conduct a longitudinal approach to obtain more insight regarding the temporal dynamics of KL-6 levels.

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Online supplementary material
Supplementary Table 1. Other characteristics of COVID-19 patients based on the severity groups.
Supplementary Table 2. Other characteristics of COVID-19 patients based on the mortality groups.
Supplementary Table 3. Details of missing data and multiple imputation status.

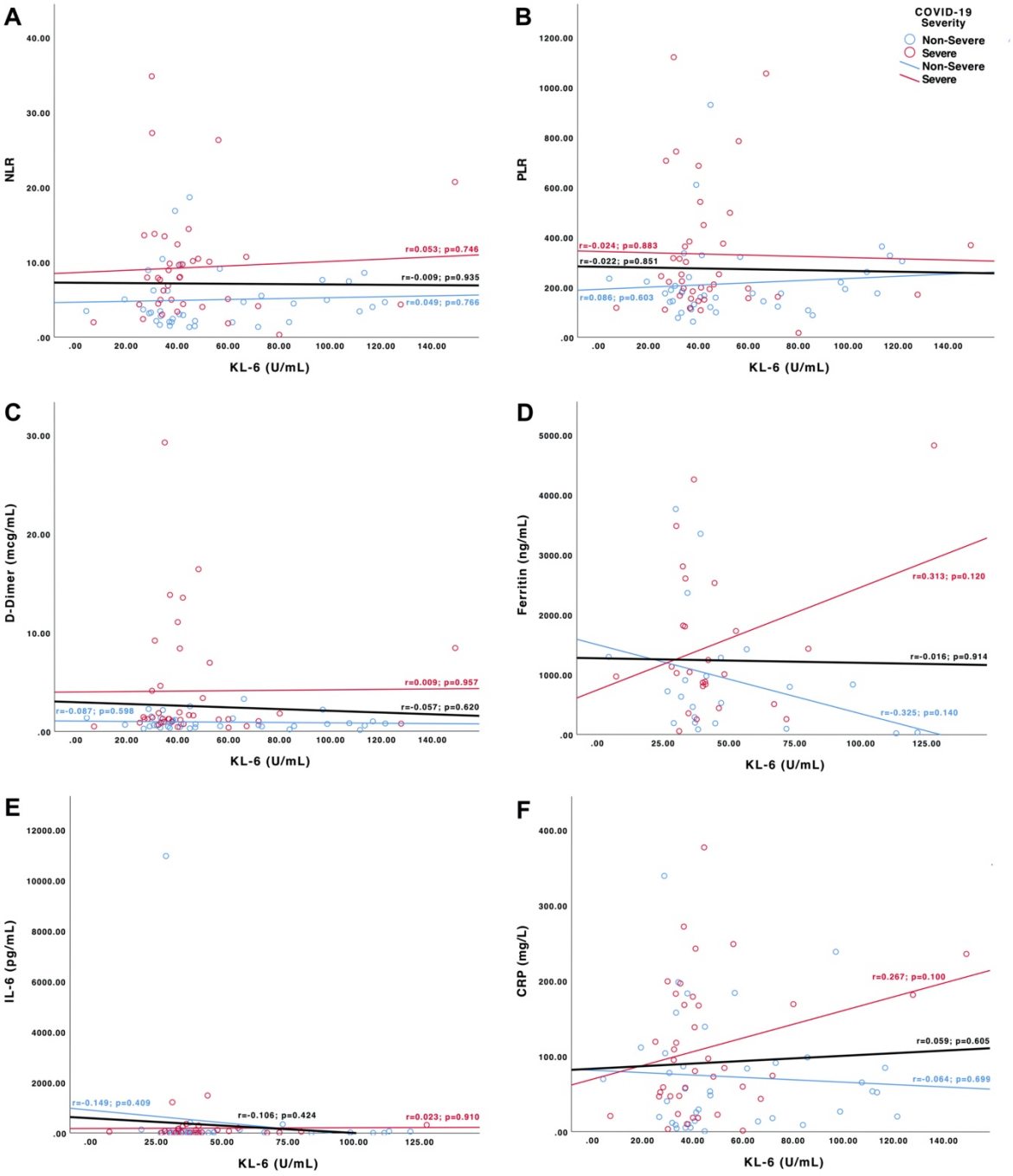

Figure 1. The correlation strengths between KL-6 and other COVID-19 severity biomarkers, including (a) neutrophil-to-lymphocyte ratio (NLR), (b) platelet-to-lymphocyte ratio (PLR), (c) D-dimer, (d) ferritin, (e) interleukin-6 (IL-6), and (f) C-reactive protein (CRP).

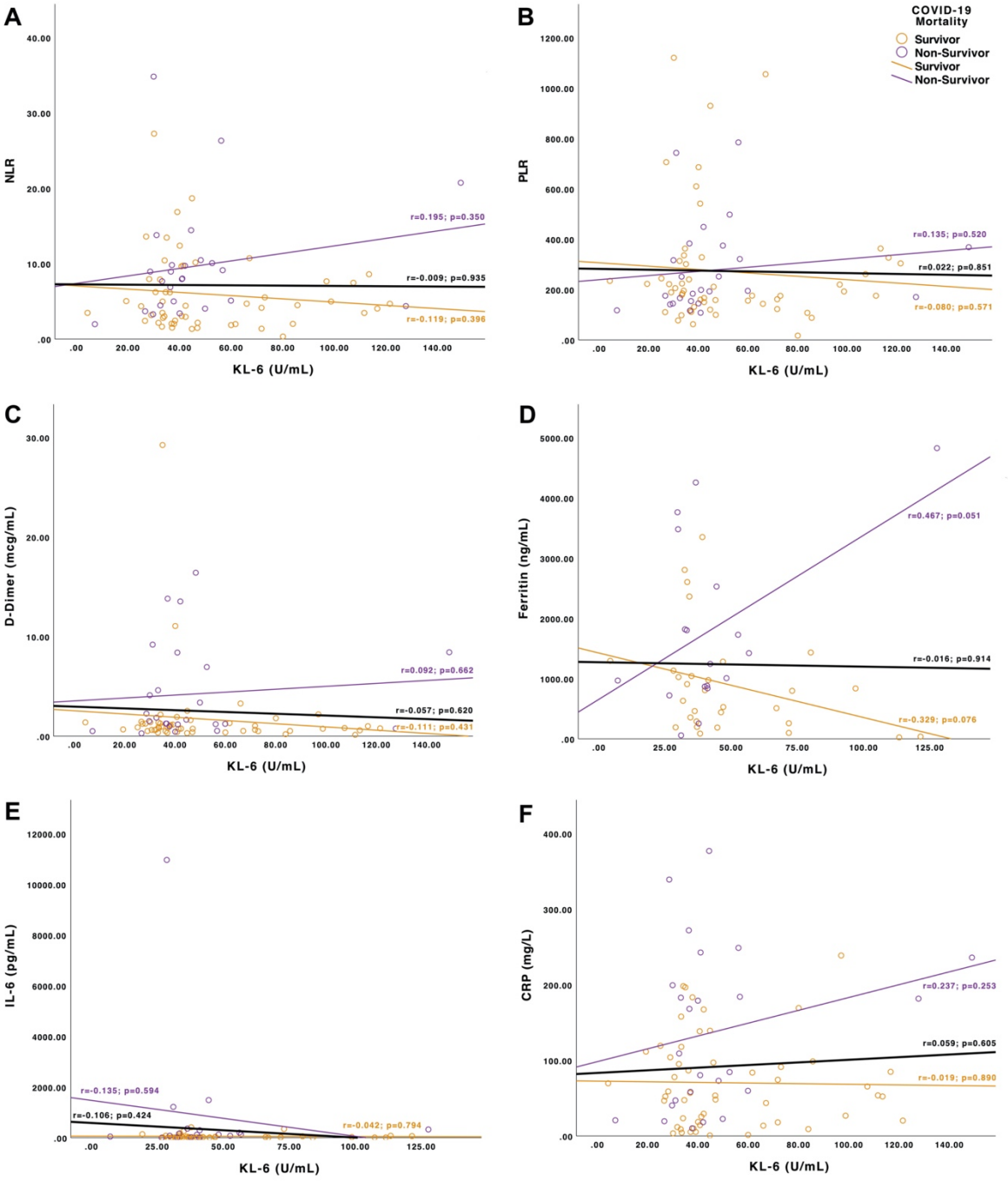

Figure 2. The correlation strengths between KL-6 and other COVID-19 mortality biomarkers, including (a) neutrophil-to-lymphocyte ratio (NLR), (b) platelet-to-lymphocyte ratio (PLR), (c) D-dimer, (d) ferritin, (e) interleukin-6 (IL-6), and (f) C-reactive protein (CRP).

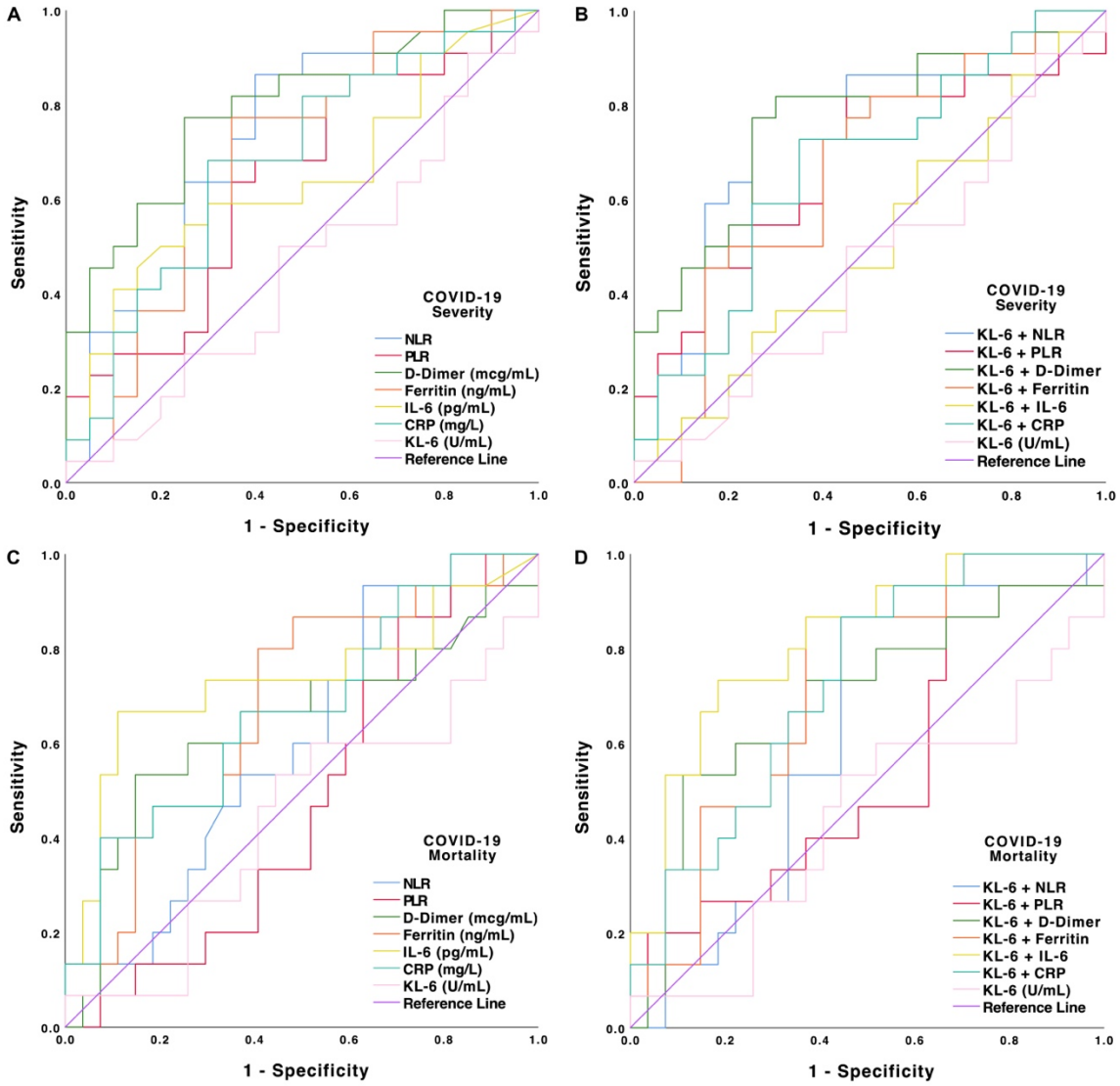

Figure 3. Receiver operating characteristic (ROC) curve of KL-6 and other biomarkers in COVID-19 severity (a, b) and mortality (c, d). CRP, C-reactive protein; IL-6, interleukin-6; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio.

Table 1. COVID-19 patient characteristics based on the severity groups.

Parameters	All Patients (n = 78)	Severe (n = 39) (Severe: n = 18; Critical: n = 21)	Non-Severe (n = 39) (Mild: n = 12; Moderate: n = 27)	*p*-value
Age, years	50.29±12.16 52.00 (43.75, 59.00)	51.49±10.24 53.00 (46.00, 59.00)	49.10±13.85 52.00 (39.00, 58.00)	0.390[a]
Male, n (%)	41 (52.60)	18 (46.15)	23 (58.97)	0.257[b]
Length of stay, days	11.61±7.89 10.00 (7.00, 15.00)	12.03±8.23 10.00 (7.00, 17.00)	11.21±7.61 9.00 (7.00, 12.00)	0.499[c]
NLR	7.14±6.23 4.98 (3.04, 9.65)	9.30±7.31 7.95 (4.37, 10.72)	4.99±3.94 3.47 (2.15, 6.22)	0.001[c]
PLR	273.45±220.04 193.67 (144.73, 322.33)	331.73±258.38 223.85 (161.35, 382.73)	215.17±156.14 175.35 (122.36, 240.06)	0.013[c]
D-dimer, mcg/mL	2.51±4.48 1.05 (0.62, 1.81)	4.09±5.92 1.27 (0.95, 4.61)	0.94±0.70 0.74 (0.50, 1.16)	0.000[c]
Ferritin, ng/mL (N/A = 30)	1237.79±1168.57 894.55 (376.20, 1654.25)	1498.98±1237.17 1034.50 (733.05, 1995.75)	929.11±1024.61 676.10 (189.53, 1285.25)	0.028[c]
Interleukin-6, pg/mL (N/A = 19)	300.77±1434.89 23.90 (10.25, 121.30)	191.79±359.42 65.20 (12.83, 215.30)	386.63±1900.95 18.88 (9.23, 68.55)	0.125[c]
Procalcitonin, ng/mL, n (%)				
<0.05	26 (33.33)	10 (25.64)	16 (41.03)	0.067[c]
0.05 – <0.5	41 (52.56)	21 (53.85)	20 (51.28)	
0.5 – <2	7 (8.97)	5 (12.82)	2 (5.13)	
2 – <10	1 (1.30)	1 (2.56)	0 (0.00)	
≥10	3 (3.84)	2 (5.13)	1 (2.56)	
C-Reactive Protein, mg/L	92.06±83.73 67.50 (22.08, 143.77)	111.28±88.40 84.44 (46.63, 178.86)	72.83±75.05 53.41 (14.30, 98.56)	0.033[c]
Krebs von den Lungen-6, U/mL	50.25±28.61 40.37 (33.20, 59.90)	45.84±25.86 40.00 (32.53, 49.85)	54.66±30.83 41.35 (33.33, 73.00)	0.261[c]

Continuous variables are shown as mean ± SD and median (interquartile range [IQR]). The comparison between two groups, either severe vs non-severe and non-survivor vs survivor, was performed using an independent T-test (a), Chi-square test (b), or Mann-Whitney U test (c).

NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio.

Table 2. COVID-19 patient characteristics based on the mortality groups.

Parameters	All Patients (n = 78)	Non-Survivor (n = 25) (Non-Severe: n = 4; Severe: n = 21)	Survivor (n = 53) (Non-Severe: n = 35; Severe: n = 18)	*p*-value
Age, years	50.29±12.16 52.00 (43.75, 59.00)	52.08±11.19 51.00 (47.50, 59.00)	49.45±12.60 52.00 (41.50, 58.00)	0.377[a]
Male, n (%)	41 (52.60)	13 (52.00)	28 (52.83)	0.945[b]
Length of stay, days	11.61±7.89 10.00 (7.00, 15.00)	9.68±7.29 8.00 (4.50, 15.50)	12.53±8.06 10.00 (8.00, 14.50)	0.067[c]
NLR	7.14±6.23 4.98 (3.04, 9.65)	9.72±7.63 8.02 (4.42, 10.27)	5.93±5.09 4.41 (2.29, 7.93)	0.004[c]
PLR	273.45±220.04 193.67 (144.73, 322.33)	277.77±182.31 194.74 (149.45, 371.20)	271.41±237.37 192.34 (137.15, 308.63)	0.444[c]
D-dimer, mcg/mL	2.51±4.48 1.05 (0.62, 1.81)	4.23±4.76 1.65 (1.07, 7.67)	1.70±4.14 0.81 (0.55, 1.35)	0.001[c]
Ferritin, ng/mL (N/A = 30)	1237.79±1168.57 894.55 (376.20, 1654.25)	1803.63±1401.76 1333.50 (860.33, 2766.75)	898.28±862.16 714.35 (243.25, 1169.50)	0.011[c]
Interleukin-6, pg/mL (N/A = 19)	300.77±1434.89 23.90 (10.25, 121.30)	866.15±2553.07 123.95 (37.10, 332.90)	52.56±90.59 18.80 (8.90, 54.80)	0.002[c]
Procalcitonin, ng/mL, n (%)				
<0.05	26 (33.33)	7 (28.00)	19 (35.85)	0.048[c]
0.05 – <0.5	41 (52.56)	10 (40.00)	31 (58.49)	
0.5 – <2	7 (8.97)	4 (16.00)	3 (5.66)	
2 – <10	1 (1.30)	1 (4.00)	0 (0.00)	
≥10	3 (3.84)	3 (12.00)	0 (0.00)	
C-Reactive Protein, mg/L	92.06±83.73 67.50 (22.08, 143.77)	138.11±106.86 109.60 (43.68, 217.61)	70.33±60.09 53.64 (18.21, 101.27)	0.007[c]
Krebs von den Lungen-6, U/mL	50.25±28.61 40.37 (33.20, 59.90)	46.99±29.88 40.00 (31.79, 51.22)	51.79±28.16 40.70 (33.36, 69.40)	0.524[c]

Continuous variables are shown as mean ± SD and median (interquartile range [IQR]). The comparison between two groups, either severe vs non-severe and non-survivor vs survivor, was performed using an independent T-test (a), Chi-square test (b), or Mann-Whitney U test (c).

NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio.

Table 3. The performance of serum KL-6 levels in determining the severity of COVID-19 patients.

Parameters	AUC (95% CI)	*p*-value	Optimal Cut-Off	Sensitivity	Specificity	Youden's Index
COVID-19 severity (severe patients)						
NLR	0.723 (0.563-0.882)	0.014	≥3.910	0.864	0.600	0.464
PLR	0.634 (0.463-0.805)	0.137	≥190.549	0.636	0.650	0.286
D-dimer	0.794 (0.660-0.929)	0.001	≥0.865	0.773	0.750	0.523
Ferritin	0.684 (0.517-0.851)	0.041	≥802.450	0.773	0.650	0.423
IL-6	0.644 (0.475-0.813)	0.110	≥80.750	0.409	0.900	0.309
CRP	0.682 (0.518-0.845)	0.044	≥57.880	0.682	0.700	0.382
KL-6	0.458 (0.281-0.635)	0.641	≥29.901	0.909	0.150	0.059
KL-6 + NLR	0.736 (0.574-0.898)	0.009	N/A	0.773	0.750	0.523
KL-6 + PLR	0.673 (0.507-0.839)	0.056	N/A	0.818	0.550	0.368
KL-6 + D-dimer	0.764 (0.616-0.911)	0.003	N/A	0.773	0.750	0.523
KL-6 + Ferritin	0.657 (0.486-0.828)	0.082	N/A	0.727	0.600	0.327
KL-6 + IL-6	0.502 (0.325-0.680)	0.980	N/A	0.682	0.400	0.082
KL-6 + CRP	0.668 (0.502-0.834)	0.062	N/A	0.727	0.650	0.377

AUC, area under curve; COVID-19, Coronavirus Disease 2019; CRP, C-reactive protein; IL-6, interleukin-6; KL-6, Krebs von den Lungen-6; N/A, not applicable; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio.

Table 4. The performance of serum KL-6 levels in predicting the mortality of COVID-19 patients.

Parameters	AUC (95% CI)	*p*-value	Optimal Cut-Off	Sensitivity	Specificity	Youden's Index
COVID-19 mortality (non-survivors)						
NLR	0.596 (0.425-0.768)	0.306	≥3.225	0.933	0.370	0.304
PLR	0.474 (0.298-0.651)	0.783	≥137.674	0.867	0.296	0.163
D-dimer	0.643 (0.452-0.834)	0.128	≥1.455	0.533	0.852	0.385
Ferritin	0.679 (0.510-0.848)	0.057	≥823.400	0.800	0.593	0.393
IL-6	0.735 (0.556-0.913)	0.013	≥74.550	0.667	0.889	0.556
CRP	0.672 (0.499-0.844)	0.068	≥174.000	0.400	0.926	0.326
KL-6	0.442 (0.253-0.631)	0.537	≥39.555	0.533	0.556	0.089
KL-6 + NLR	0.625 (0.452-0.798)	0.185	N/A	0.867	0.556	0.422
KL-6 + PLR	0.533 (0.346-0.721)	0.723	N/A	0.867	0.333	0.200
KL-6 + D-dimer	0.694 (0.517-0.870)	0.039	N/A	0.533	0.889	0.422
KL-6 + Ferritin	0.716 (0.560-0.872)	0.022	N/A	0.867	0.630	0.496
KL-6 + IL-6	0.817 (0.686-0.949)	0.001	N/A	0.733	0.815	0.548
KL-6 + CRP	0.726 (0.573-0.879)	0.016	N/A	0.867	0.556	0.422

AUC, area under curve; COVID-19, Coronavirus Disease 2019; CRP, C-reactive protein; IL-6, interleukin-6; KL-6, Krebs von den Lungen-6; N/A, not applicable; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio.