Admission C-reactive protein, D-dimer, IL-6 and XCL1/lymphotactin levels as predictors of 28-day mortality in COVID-19 patients: a prospective cohort study

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INTRODUCTION

Coronavirus disease 2019 (COVID-19) is induced by SARS-CoV-2, a new coronavirus variant. The first case was reported in Wuhan, Hubei Province, People’s Republic of China (PRC) in December 2019, causing severe pneumonia and even mortality.1 The World Health Organization (WHO) declared COVID-19 a pandemic at the beginning of March 2020, and more than 200 million cases and more than 4 million deaths have since occurred worldwide.2 Moreover, more than 4 million individuals in Indonesia have tested positive, and more than 140,000 patients have died.3 Previously, the case fatality rate in Indonesia was 2.7% higher than the global rate. Recently, Indonesia reported the second-highest fatality rate in Southeast Asia.5 COVID-19 symptoms range from moderate to severe.6 Zou et al.7 discovered that the amount of SARS-CoV-2 in the respiratory tracts of asymptomatic patients was comparable to that in the respiratory tracts of symptomatic patients, indicating that both groups had the same potential to transmit the virus.

To evaluate the severity of COVID-19, CRP and D-dimer levels are frequently used in clinical settings. Moreover, interleukin-6 (IL-6) is used in more specific situations if the patient is experiencing a cytokine storm. Additionally, XCL1/lymphotactin is a chemokine that influences lymphocyte trafficking.8 Severe COVID-19 is characterized by excessive cytokine secretion and coagulation hyperactivity, as indicated by elevated levels of fibrin degradation products and coagulation activity.9

Background: Predictors of mortality in COVID-19 patients, which are essential factors in guiding patient management, are poorly understood. In Indonesia, several inflammatory markers have been utilized to evaluate the severity of COVID-19. This study aimed to determine the role of C-reactive protein (CRP), D-dimer, interleukin-6 (IL-6), and XCL1/lymphotactin levels in predicting 28-day mortality in patients with COVID-19.

Methods: A prospective cohort study was conducted with COVID-19 patients admitted to the emergency department at Cipto Mangunkusumo Hospital and Medistra Hospital, Jakarta, Indonesia, from June 2020 to February 2021. The predictors of 28-day mortality in COVID-19 patients in this study included CRP, D-dimer, IL-6, and XCL1 levels at admission. Cox proportional hazard regression analysis was used to determine the independent predictors of 28-day mortality in the study population.

Results: A total of 120 patients with COVID-19 were enrolled in the study; 21 (17.5%) died within 28 days after admission. According to our multivariate analysis, a CRP level (HR, 8.55; 95% CI, 3.310-22.088) ≥ 110 mg/L and a D-dimer level (HR, 20.642; 95% CI, 6.909-61.667) ≥ 4640 ng/mL were identified as independent predictors of 28-day mortality in COVID-19 patients.

Conclusion: A C-reactive protein level ≥ 110 mg/L and a D-dimer level ≥ 4640 ng/mL can be used to predict 28-day mortality in COVID-19 patients.

Keywords: C-reactive protein, COVID-19, D-dimer, IL-6, predictor of mortality, XCL1.

correlation between increased levels of IL-6 and both viral replication and the persistence of viral infection. Likewise, CRP levels can be utilized to detect severe pulmonary infections in the early stages of pregnancy. D-dimer is produced during the formation and cleavage of cross-linked fibrin, which signifies that coagulation and fibrinolysis have been activated. There have been reports indicating that COVID-19 is linked to hemostatic abnormalities, with nonsurvivors exhibiting significantly elevated D-dimer levels. The binding of XCL1 to the XCR1 receptor, which is extensively expressed on dendritic cells, induces chemotactic activity against CD8+ and CD4+ T cells, NK cells, B cells, and neutrophils. Viral infections in both animals and humans have been found to increase XCL1 expression in NK cells and CD8+ T cells. Numerous inflammatory markers have been utilized to evaluate the severity of COVID-19, including C-reactive protein (CRP) levels, ferritin levels, lactate dehydrogenase (LDH) levels, the neutrophil-to-lymphocyte ratio (NLR), the erythrocyte sedimentation rate (ESR), and D-dimer levels. Analysis of CRP, D-dimer, or IL-6 markers has been shown in several different studies to evaluate COVID-19 mortality. In light of the investigation conducted by Lei et al. concerning the influence of XCL1 chemotactic activity during viral infection, we sought to ascertain whether the XCL1 marker could serve as a predictor for mortality among patients affected with COVID-19. As far as our knowledge extends, no study has examined the predictive value of a combination of CRP, D-dimer, IL-6, and XCL1 about 28-day mortality in patients with COVID-19. To further our understanding of the pathophysiology of the SARS-CoV-2 infection process, the purpose of this study was to determine the role of CRP, D-dimer, IL-6, and XCL1 levels in predicting 28-day mortality in patients with COVID-19.

**MATERIAL AND METHODS**

**Study design**
We conducted a prospective cohort study at Cipto Mangunkusumo and Medistra Hospital, Jakarta. Patients with SARS-CoV-2 infection who were aged >18 years and admitted to the hospital between June 2020 and February 2021 were included in this study. Patients were diagnosed with SARS-CoV-2 infection based on a positive result for at least two genes (ORF1ab, nucleocapsid, or envelope), as confirmed by reverse-transcriptase polymerase chain reaction (RT-PCR). Pregnant women and individuals afflicted with acquired immunodeficiency syndrome (AIDS) were explicitly excluded from the study.

**Identification of predictors and outcomes**
Biomarkers were measured in duplicate using the following commercially available enzyme-linked immunosorbent assays (ELISAs), performed according to the manufacturer’s recommendations: the human XCL1 ELISA (MyBioSource) and human IL-6 ELISA (MyBioSource). According to the manufacturer’s instructions, the serum samples were diluted to determine the optimal dilution factor for generating an optical density within the detection range of the ELISA reader. The measurements were conducted with a microplate reader (Tecan Sunrise) set to a wavelength of 450 nm. Turbidimetric immunoassays were employed to determine the serum CRP level in conjunction with the Cobas C311 (Roche Diagnostic, USA). The D-dimer level was ascertained utilizing an immunoturbidimetry assay in conjunction with a CS5100 automatic coagulation analyzer (Sysmex, Kobe, Japan).

Patient characteristics, comorbidities, complete blood count, the NLR, and CT data were recorded for each individual upon arrival at the emergency department. We defined mortality as death occurring ≤28 days after the initial diagnosis.

**Statistical analysis**
The sample size was calculated based on the assumption that 13.0% of the patients would experience death. With α = 0.05 and β = 0.20 and assuming a relative risk of 1.75, the investigation needed a minimum of 112 patients. To identify variables that might be significantly associated with mortality, a univariate analysis was conducted to analyze the serum CRP, D-dimer, IL-6, and XCL1 levels and the outcome of COVID-19 mortality within twenty-eight days. To determine the appropriate cutoff values for each significant variable (p<0.05), receiving operating characteristic (ROC) curve analysis was used to determine the values with the best sensitivity and specificity for mortality. According to the univariate analysis, all variables with p values less than 0.25 were included in the Cox proportional hazard regression model. The adjusted hazard ratios (HRs) for mortality were computed using a backward selection algorithm. A Kaplan-Meier curve is displayed for each significant predictor. The statistical analyses were performed using STATA statistical software version 14 (Stata Corp., College Station, TX, USA).

**RESULTS**
A total of 120 patients with COVID-19 were enrolled in the study. The patients were divided into two groups: 99 in the survivor group and 21 in the nonsurvivor group. Table 1 presents the demographic and clinical characteristics of the patients by the outcomes. There was no significant difference in the number of female and male patients (p = 0.913). There were no statistically significant differences between patients aged ≤60 years old and those aged >60 years in the survivor and nonsurvivor groups (p = 0.378). The proportion of patients in the survivor group who did not have any comorbidities was greater than that in the nonsurvivor group (p < 0.001). The hematocrit and hemoglobin (Hb) levels were significantly lower in the nonsurvivor group than in the survivor group (p = 0.002). A greater leukocyte count was observed in the nonsurvivor group than in the survivor group (p < 0.001). There was a significant difference in the percentage of lymphocytes between the survivor and nonsurvivor groups (p < 0.001). The NLR was significantly greater in the nonsurvivor group than in the survivor group (p < 0.001).

Of the 120 patients, 21 (17.5%) died within 28 days. The serum CRP level in the nonsurvivor group (median: 198 mg/L, range 102 to 279.5 mg/L) was significantly greater than that in the survivor group (median: 36.6 mg/L, range 8.08 to 92 mg/L). Additionally, a substantial increase in the D-dimer level was observed in the
The study compared COVID-19 characteristics between survivors and nonsurvivors. The Table 1 shows that:

- Sex: There were no significant differences in sex between survivors and nonsurvivors.
- Age: There were no significant differences in age between survivors and nonsurvivors.
- Comorbidity: Nonsurvivors had a significantly higher rate of comorbidity compared to survivors.
- Laboratory parameters: Nonsurvivors had lower hemoglobin (Hb), hematocrit (Ht), leucocyte count, and lymphocyte percentage, and higher neutrophil-lymphocyte ratio (NLR), C-reactive protein (CRP), and D-dimer levels compared to survivors.

The Table 2 provides receiver operator curve variables, cutoff values, sensitivity, and specificity for significant parameters in predicting COVID-19 mortality. Cutoff values for the analyzed parameters were derived by applying the ROC curve. The cutoff values for in-hospital death prediction were as follows:

- Hb: ≤ 11 g/dL for nonsurvivors, compared to survivors with a level of 10.6–9.13 g/dL.
- Ht: ≤ 32.5% for nonsurvivors, compared to survivors with a level of 26.5–36.3%.
- Leucocyte count: ≥ 13,500/µL for nonsurvivors, compared to survivors with a level of 10,900–14,000/µL.
- Lymphocyte percentage: ≤ 7.25% for nonsurvivors, compared to survivors with a level of 18.9–30.1%.
- Neutrophil-lymphocyte ratio (NLR): ≥ 11.86 for nonsurvivors, compared to survivors with a level of 3.89–9.22.
- C-reactive protein (CRP): ≥ 110 mg/L for nonsurvivors, compared to survivors with a level of 36.6–80.8 mg/L.
- D-dimer: ≥ 4640 ng/mL for nonsurvivors, compared to survivors with a level of 1100–2820 ng/mL.

DISCUSSION
This study focused on CRP, D-dimer, IL-6, and XCL1 levels at admission in patients with severe COVID-19. The main finding was that although CRP, D-dimer, and IL-6 levels are increased in non-surviving patients, it is currently unknown which of these levels can be used to predict mortality within 28 days.

In this study, there was no significant difference in outcomes between the nonsurvivor and survivor groups concerning age. Bover et al. reported that age is not the only risk factor that
Table 3. Univariate analysis of the risk factors for COVID-19 mortality

<table>
<thead>
<tr>
<th>Variable</th>
<th>Survivors (n = 99)</th>
<th>Nonsurvivors (n = 21)</th>
<th>HR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb, g/dL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;11</td>
<td>75 (91.5)</td>
<td>7 (8.5)</td>
<td>4.931 (1.988-12.231)</td>
<td>0.001</td>
</tr>
<tr>
<td>≤ 11</td>
<td>24 (63.2)</td>
<td>14 (36.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ht, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;32.5</td>
<td>76 (90.5)</td>
<td>9 (9.5)</td>
<td>4.386 (1.815-10.596)</td>
<td>0.001</td>
</tr>
<tr>
<td>≤ 32.5</td>
<td>23 (63.9)</td>
<td>13 (36.1)</td>
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<tr>
<td>Leucocyte/µL</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>&lt; 13500</td>
<td>81 (91.0)</td>
<td>8 (9.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 13500</td>
<td>18 (58.1)</td>
<td>13 (41.9)</td>
<td>5.537 (2.292-13.378)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lymphocyte, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>&gt;7.25</td>
<td>80 (94.1)</td>
<td>5 (5.9)</td>
<td></td>
<td></td>
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<tr>
<td>≤ 7.25</td>
<td>19 (54.3)</td>
<td>16 (45.7)</td>
<td>10.184 (3.723-27.855)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Neutrophil-Lymphocyte Ratio (NLR)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>&lt; 11.86</td>
<td>79 (94.0)</td>
<td>5 (6.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 11.86</td>
<td>20 (55.6)</td>
<td>16 (44.4)</td>
<td>9.685 (3.542-26.487)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 110</td>
<td>81 (81.8)</td>
<td>6 (28.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 110</td>
<td>18 (18.2)</td>
<td>15 (71.4)</td>
<td>8.550 (3.310-22.088)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>D-dimer (ng/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 4640</td>
<td>88 (88.9)</td>
<td>4 (19.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 4640</td>
<td>11 (11.1)</td>
<td>17 (81.0)</td>
<td>20.642 (6.909-61.667)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Abbreviations: Hb, hemoglobin; Ht, hematocrit; HR, hazard ratio; CI, confidence interval

Table 4. Final Cox proportional hazard regression model for predicting COVID-19 mortality

<table>
<thead>
<tr>
<th>Variable</th>
<th>HR (CI 95%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP</td>
<td>3.463 (1.265-9.484)</td>
<td>0.016</td>
</tr>
<tr>
<td>D-dimer</td>
<td>12.833 (4.025-40.918)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Abbreviations: CRP, C-reactive protein; HR, hazard ratio; CI, confidence interval

Contributes to the critical condition of COVID-19 patients. Age and comorbidities influence the critical phase in COVID-19 patients; however, statistical analysis revealed that the number and severity of comorbidities have a greater impact on critical COVID-19 patients than age. This previous finding is consistent with our findings that patients who had multiple comorbidities had a higher risk of death.

The Hb levels in the nonsurvivor group were found to have decreased significantly. These findings align with previous studies that have shown that SARS-CoV-2 may lead to hypoxia through effects on the respiratory system and the disruption of the inflammatory response, causing anemia. Another potential mechanism of anemia involves iron metabolism disorders in the group of patients with more severe COVID-19.17,18

The nonsurvivor group exhibited a greater quantity of leukocytes than the survivor group. The nonsurvivor group exhibited a higher NLR than the survivor group. This finding is consistent with the findings of Yang et al.19 and Ponti et al.20, which indicated that the severity of symptoms is correlated with an increase in the NLR. In theory, chemokines attract lymphocyte cells into the lung alveoli of COVID-19 patients with severe and critical symptoms, resulting in a reduction in the number of lymphocytes in the blood.20–22

Prior research has demonstrated that increased D-dimer levels are predictive of a poor prognosis in patients with community-acquired pneumonia (CAP).23 Additionally, research has indicated that patients who present with severe cases of COVID-19 have higher blood D-dimer levels upon admission.24–26 D-dimer is a fragment that is generated when plasmin cleaves fibrin during the process of thrombus disruption.27 Hence, an increased D-dimer level at the time of admission may indicate increased fibrinolysis, thrombosis and intravascular coagulation, cytokine storm, tissue damage, or even sepsis, as exemplified by the severe clinical presentation of COVID-19.28,29 Recent studies have also provided evidence that patients with severe COVID-19 have an increased risk of developing deep vein thrombosis and pulmonary embolism throughout the clinical progression of the disease.30–32

Our analysis revealed that COVID-19 patients who presented with increased D-dimer levels at the time of admission had a substantially increased risk of mortality, with a cutoff ≥ 4640 ng/mL or 4.640 μg/mL. According to the findings of Zhang et al.33, a D-dimer level exceeding 2 μg/mL at the time of admission may serve as a prognostic indicator of mortality during hospitalization. Klok et al.34 conducted a study revealing that admission to COVID-19 treatment facilities accompanied by a D-dimer level exceeding 1 μg/mL was associated with an eighteen times greater risk of mortality. Hence, D-dimer levels exceeding ≥ 1 μg/mL during hospitalization can serve as a predictor of mortality when the highest values are considered.

Viral infections, inflammation, and
severe trauma can potentially significantly increase CRP levels.\textsuperscript{35} Inflammation levels are correlated with CRP levels, which are unaffected by age, sex, or physical condition. CRP levels are increased in patients suffering from severe pneumonia. Early-stage lung lesions and disease severity in COVID-19 patients are positively correlated.\textsuperscript{36} Our findings indicate that a cutoff CRP level ≥ 110 mg/L differs significantly between survivors and nonsurvivors. These findings are corroborated by research showing that serum CRP levels exceeding 77.35 mg/L are associated with an increased risk of death in patients with severe COVID-19.\textsuperscript{37,38}

The impact of IL-6 levels on survival and nonsurvival was also examined. Based on our findings, neither the survivor nor the nonsurvivor groups exhibited a statistically significant increase in IL-6. According to research by Montesarchio et al.\textsuperscript{39}, on the first day of admission, there was an increase in CRP levels, and the trend began to decrease by the seventh day of admission, whereas IL-6 levels were not elevated on the first day but exhibited an upward trend until the seventh day. This finding indicates that the increase in IL-6 levels might have occurred after the increase in CRP levels.

The results of the XCL1 level measurements indicated that there was no statistically significant difference in XCL1 levels between the survivor and nonsurvivor groups. According to the research of Zaid et al.\textsuperscript{40} and Schoenberger\textsuperscript{41}, XCL1 levels in the blood are reduced when alveolar XCL1 levels are extremely reduced during infection. This finding explains why the XCL1 levels did not significantly differ between the two groups. Several other chemokines can cause cytokine storms, such as CXCL10, CXCL8, CXCL9, CCL2, CCL3, and CCL5.\textsuperscript{14} Changes in these marker levels over time were not evaluated in this study; however, further research is needed to assess the role of these chemokines in predicting COVID-19 mortality.

CONCLUSION
A C-reactive protein level ≥ 110 mg/L and a D-dimer level ≥ 4640 ng/mL could predict 28-day mortality in COVID-19 patients.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

ETHICS CONSIDERATION
This study was approved by the Faculty of Medicine Universitas Indonesia Ethics Committee, ethics number KET-448/UN2.F1/ETIK/PPM.00.02/2020, and was conducted by the Declaration of Helsinki. All participants gave their informed permission before taking part in the research.

FUNDING
None.

AUTHOR CONTRIBUTION
All authors contributed equally to this research.

ACKNOWLEDGMENTS
The authors thank all the technical teams that participated in the data collection, sample collection, and processing of biological samples. The authors would like to thank all project teams that gave support to the centers participating in this study.

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