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Interleukin-6 and C-reactive Protein on Admission as Predictor of Mortality in Severe COVID-19 Patients: A Retrospective Cohort Study

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Abstract

BACKGROUND: Predictors of mortality, an important factor to guide management in COVID-19 patients, have not been fully understood. Several inflammatory markers have been used to assess severity in COVID-19 patients in Indonesia. Few studies, however, have shown these markers as predictor of mortality. The common laboratory test for assessing severity in COVID-19 patients includes interleukin-6 (IL-6) and C-reactive protein (CRP).

AIM: The aims of this study were determine the relationship between the two inflammatory biomarkers and mortality as well as their cutoff values in severe COVID-19 patients.

METHODS: We retrospectively analyzed 80 confirmed patients with severe COVID-19 admitted to an intensive care unit of a secondary hospital in Indonesia between August and December 2020. They were analyzed for baseline clinical and laboratory findings at admission and during the disease. The primary outcome was mean level IL-6 and CRP of severe COVID-19 patients on admission. The secondary outcome was cutoff of IL-6 and CRP to predict mortality during the first 14 days of hospitalization.

RESULTS: CRP and IL-6 levels were evaluated as prognostic factors for outcome using the ROC curve. A total of 80 confirmed patients consisting of 53 (71.25%) survivors and 23 (28.75%) non-survivors. Mortality was weakly correlated with levels of IL-6 (r = 0.249) and CRP (r = 0.247). The IL-6 cutoff was 101.64 pg/ml (AUROC 0.658 (95% CI 0.529 – 0.787); p = 0.028). The CRP cutoff was 46.45 mg/L (AUROC 0.659 (95% CI 0.532–0.786); p = 0.027).

CONCLUSIONS: Levels of IL-6 and CRP at the first day of admission were weak predictors of mortality in severe COVID-19 patients.

Introduction

Coronavirus disease 2019 (COVID-19) has been reported for the 1st time in Wuhan, China in December 2019 [1], which has been spread worldwide rapidly. Since March 11, Coronavirus disease was declared as pandemic by the World Health Organization (WHO) [2]. Severe acute respiratory syndrome coronavirus 2 (Sars-Cov-2) is the main cause of COVID-19 [3], [4]. In Indonesia, a total of 377,541 cases out of 2,647,094 people tested were reported with 12,959 deaths [5]. However, the predictor for risk mortality in COVID-19 patients has not been understood.

Several inflammatory markers have been used to assess severity in COVID-19 patients in Indonesia. These markers include increased neutrophil lymphocyte ratio (NLR), ferritin, lactate dehydrogenase (LDH), erythrocyte sedimentation rate (ESR), procalsitonin, C-reactive protein (CRP), and decreased absolute lymphocyte count (ALC). Few studies, however, have shown these markers as predictor of mortality.

The common laboratory test for assessing severity in COVID-19 patients includes interleukin-6 (IL-6) and C-reactive protein (CRP). IL-6 has been shown to be a key mediator of the progression of cytokine storm in COVID-19 infection. Increased levels of IL-6 were positively correlated with increased viral replication and persistence of viral infection [6]. Similarly, patients with severe pulmonary infection can be early diagnosed with CRP [7].

This study aims to analyze whether IL-6 and CRPon admission can be used to predict mortality in severe COVID-19 patients.

Methods

Design

This was a single-center retrospective data analysis of confirmed cases of severe COVID-19

diagnosed between August and December 2020 treated in an isolation room of the secondary hospital in Semarang, Indonesia. The study protocol was approved by Local Ethics Committee (No.89/EC/KEPK/2021). Informed consent was released because of the retrospective nature of the study.

Study population

The sample size was calculated using categorical output prognostic studies. All patients aged ≥18 years who had a confirmed diagnosis of COVID-19 with real-time reverse transcription of polymerase chain reaction assay (rRT-PCR) were involved in this study. The patient had at least one symptom of pneumonia such as fever, cough, shortness of breath, and plus one of the following: Respiratory distress, breathing rate >30 times/min or the oxygen saturation at room air (SpO_2) <93%, treatment with oxygen therapy, antiviral drugs, dexamethasone, and anticoagulat. Exclusion criteria include autoimmune disorders, immunodeficiency diseases, malignancy, burn, referrals from other hospitals and having received therapy, and incomplete medical records. The variables studied were the levels of IL-6 and levels of CRP on admission and the outcome was the mortality during the first 14 days of hospitalization.

Outcomes and definitions

The primary outcome was mean level IL-6 and CRP of severe COVID-19 patients on admission. The secondary outcome was cutoff of IL-6 and CRP to predict mortality during the first 14 days of hospitalization.

Procedure

Demographics, medical history, clinical data, radiological finding, and biological finding on admission were collected from medical record.

Serum CRP concentrations were measured using latex agglutination turbidimetric immunoassays (Kit C-reactive Protein NANOPIA) and the lower reference limit was 0.3 mg/dL. The remaining serum was transferred in 1.5 mL sterile microcentrifuge tubes and stored at −20°C for IL-6 analysis. IL-6 concentrations were measured using ELISA (Human IL-6 ELISA kit Sigma-Aldrich-RAB0306). All tests were performed according to the manufactory's instructions.

Statistical analysis

Data analysis using Spearman's correlation, a non-parametrically distribution was found. To find out cutoff points of CRP and IL-6 for severe COVID-19 patients, receiver operating characteristic (ROC) analyzes were applied. Data were analyzed using SPSS 24.0 (SPSSInc., Chicago, IL, USA).

Results

Study population

A total of 80 COVID-19 patients were involved in this study. Patients were splitted into two groups: 57 survivors and 23 non-survivors. The demographic and clinical characteristics of patients according to the outcome are presented in Table 1. The mean age of the survivor and non-survivor group was 50.74 ± 11.2 and 54.74 ± 9.6, respectively. Most of the patients were 18 to ≤60 years (80%). There was no significant difference in the number of female and male patients (56.2% vs. 43.8%, p = 0.304). There was no significant difference in the comorbid patients between the survivor and non-survivor group (p = 0.754). The onset of symptoms between the survivors and non-survivor group was not significantly different. The most common symptoms in severe COVID-19 were fever (80 %), cough (57.5%), shortness of breath (41.2%), and weakness (32.5%). In this study, several symptoms related to the gastrointestinal system also appeared, namely, nausea (35%), vomiting (23.75%), and diarrhea (8.75%). Anosmia only occurred in 3.75% of all patients.

Table 1: Characteristics of the study population

Description	Survivors (n = 57)	Non-survivors (n = 23)	p value
Age (years)	50.74 ± 11.2	54.74 ± 9.6	p = 0.138^
>60 years (n = 16; 20%)	9 (56.25%)	7 (43.75)	
18–60 years (n = 64; 80%)	48 (75%)	16 (25%)	
Gender			p = 0.304*
Male (n = 35; 43.8%)	27 (77.1%)	8 (22.9%)	
Female (n = 45; 56.2%)	30 (66.7%)	15 (33.3%)	
Comorbid#			p = 0.754**
Yes (n = 65; 81.2%)	47 (72.3%)	18 (27.7%)	
1 comorbid (n = 42; 64.61%)	33 (78.6%)	9 (21.4%)	
2 comorbid (n = 20; 30.76%)	13 (65%)	7 (35%)	
3 comorbid (n = 3; 4.61%)	1 (33.3%)	2 (66.77%)	
No (n = 15; 18.8%)	10 (66.6%)	5 (33.3%)	
Symptom onset (days)	6 ± 2.4	7 ± 3.7	p = 0.727***
Radiological finding			
Bilateral Bronchopneumonia (n = 71; 88.75%)	50 (70.4%)	21 (29.6%)	
Right bronchopneumonia (n = 8; 10%)	6 (75%)	2 (25%)	
Left bronchopneumonia (n = 1; 1.25%)	1 (100%)	0 (0%)	
Length of hospitalization (days)	10 ± 1.7	7 ± 4.3	p = 0.001***
Leukocytes (normal 3.6–11×10^3/µL)	6.67 ± 3.57	7.9 ± 15.5	p = 0.234***
Lymphocytes (normal 25–40%)	19.6 ± 8.95	14.1 ± 7.5	p = 0.011^
Neutrophils (normal 50–70%)	71.7 ± 10.82	77.3 ± 7.49	p = 0.033***
Neutrophil lymphocyte ratio (NLR)	3.6 ± 4.75	6.2 ± 4.8	p = 0.01***
Absolute lymphocyte count (ALC)	1313 ± 537.5	1147 ± 564.8	p = 0.221***
C-reactive protein (CRP)	34.6 ± 45.5	57.8 ± 58.98	p = 0.027***
Interleukin-6 (IL-6)	76.7 ± 155.27	130.2 ± 356.9	p = 0.028***

*Chi square test; **Fisher exact test; ^Unpaired T test, t***Mann–Whitney test, #Diabetes mellitus; Hipertension; Coronary heart disease; Chronic Kidney Disease; Congestive Heart Failure

The mean number of leukocytes in survivor and non-survivor group was still within normal limits (p = 0.234). Similarly, the number of lymphocytes in both groups decreased.

The number of neutrophils in both groups increased equally, but in the non-survivor group, it was much higher than those who survived (p = 0.033).

The survivor group had a significantly lower neutrophil-lymphocyte ratio compared to non-survivor (3.6 ± 4.75 vs. 6.2 ± 4.8, p = 0.01).

There was no significant difference in absolute lymphocyte count (ALC) between the two groups (p = 0.221).

C-reactive protein (CRP)

The mean levels of C-reactive protein (CRP) increased above normal in both groups. The non-survivor group had a lower mean CRP than the survivor group (34.6 ± 45.5 vs. 57.8 ± 58.98, p = 0.027). The CRP levels were weakly correlated with mortality (0.249).

Interleukin-6 (IL-6)

The mean level of interleukin-6 (IL-6) also increased in the survivor and non-survivor group (76.7 ± 155.27 vs. 130.2 ± 356.9, p = 0.028). The level of IL-6 was weakly correlated with mortality (0.247).

ROC curve

The CRP cutoff was 46.45 mg/L (AUROC 0.659 (95% CI 0.532–0.786); p = 0.027).The IL-6 cutoff was 101.64 pg/ml (AUROC 0.658 (95% CI 0.529–0.787); p = 0.028). CRP and IL-6 are weak mortality predictors in severe COVID-19 patients (Figure 1).

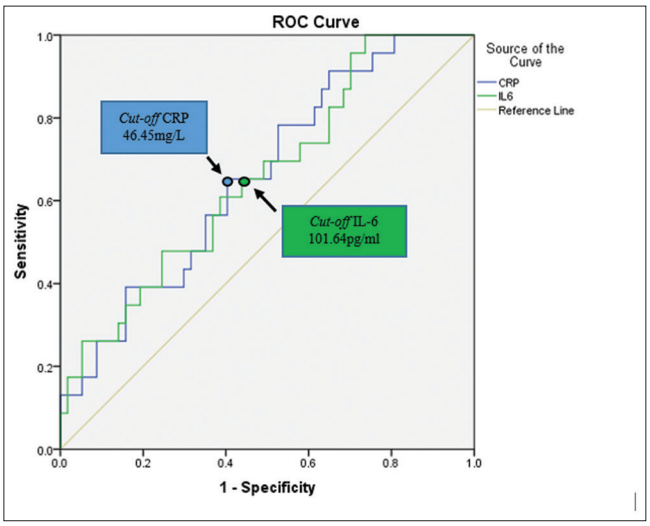

Figure 1: ROC curve and optimal cutoff CRP and IL-6

Discussion

This study focused on IL-6 and CRP values in severe COVID-19 patients at the first admission. The main finding was that although IL-6 and CRP were the predictors for severity, both inflammatory biomarkers were not robust predictors of the mortality.

In this study, there were more confirmed COVID-19 patients aged 18–60 years than that of aged >60 years. However, there was no significant difference in outcome between survival and non-survival groups with respect to age. Old age is a risk factor for infection compared to young and middle age related to physiological changes including decreased immune system function and the occurrence of multimorbidity due to chronic disease is higher in the elderly population [8], [9]. The increase in disease progression in the elderly is associated with decreased function of T and B cells as well as the presence of excess production of cytokines which can lead to deficient viral replication control and a prolonged proinflammatory response that has the potential to lead to poor outcomes [10]. It has been mentioned that elderly patients with at least one previous comorbid have poor outcome [11]. In view of our results, using age to predict mortality seems to be unreliable.

There were more female (56.2%) compared to male (43.8%). However, this difference in number was not statistically significant (p = 0.304). The number of patients who survived was comparable between men (77.1%) and women (66.7%). In this study, there was also no difference between sexes in mortality. Up to now, the mechanisms underlying the susceptibility of different sex to SARS-CoV-2 infection remain unclear [12]. Person's ability to fight SARS-CoV-2 infection between men and woman can be affected by the immune system [13].

The ACE-2 gene is a gene linked to the X chromosome. To avoid duplication, one X chromosome tends to be inactive, but because the ACE2 location escapes inactivation, women have more genetic instructions to activate ACE-2 [12]. In this present study, most of the patients had comorbid. However, it cannot be the predictive factor of mortality (p = 0.754). A person with a comorbid had higher risk to get SARS-CoV-2 infection; however, it was not shown to be correlated with mortality. This finding is consistent with that of a study showing no difference in death rates between patients with and without comorbid [14].

This study found that the most dominant symptoms in severe COVID-19 patients were fever. This finding supports that of a meta-analysis concluding that fever was the most common symptom [15].

NLR of non-survivor group was shown to be higher compared to that of survivor group. NLR is important indicators in severe cases. Increased NLR reflects an imbalance in the inflammatory response. Thus, it can be indicator of disease severity. A meta-analysis of 15 studies concluded that the higher level of severity was associated with increased number of neutrophils or lymphocytes alone [16]. Several studies have reported that the absolute lymphocyte count (ALC) <1.000 cells/μL occurring in COVID-19 patients correlates with disease severity. A similar study by Jason *et al.* 2020 states that the average ALC of severe COVID patients who require intensive care is lower (0.8 ± 0.11 × 10^3 cells/uL) than those who do not need intensive care, namely, (1.4 ± 0.15 × 10^3 cells/uL) (p = 0.01); however, in this study, it was also stated that ALC was not correlated with mortality [17]. Like in our study, there was no significant difference in ALC between survivors and non-survivors group (p = 0.221).

The viral infections, inflammations, and severe trauma can be increased dramatically on CRP levels [18]. Levels of CRP correlate with levels of inflammation and concentrations of CRP levels are not affected by age, sex, and physical condition. The initial diagnosis of pneumonia can use CRP levels. The level of CRP in patients with severe pneumonia is high. The disease severity and lung lesions in the early stages of COVID-19 are having positively correlation [7].

We found that the IL-6 and CRP levels in severe COVID-19 patients on admission have increase above normal range, both those who survivor and those who non-survivor. Based on ROC analysis, IL-6 > 101.64 pg/ml and CRP >46.45 mg/L were more likely to progress toward disease death. However, both inflammatory biomarkers were weak predictors of mortality.

This study had several limitations**.** First, this study was a single-center study, so further studies are required to include multiple centers with a bigger sample size. Second, since this study was designed as cohort study, it lacks a control group. Our finding CRP level and IL-6 level were measured only from the 1st day of admission, so it was still biased whether the increase in CRP and IL-6 is due to the progression of COVID disease or from comorbid disease. Finally, other markers that play a role in the cytokine storm, such as interleukin (IL-1β and IL-10); tumor necrosis factor α; interferon-γ and interferon-β, CXC motif chemokine ligand 10 (CXCL-10), monocyte chemoattractant protein-1 (MCP-1), and macrophage inflammatory protein -1 α (MIP-1 α) which may affect CRP and IL-6 levels. The changes in the levels of the markers over time were not evaluated; further studies to assess their changes are needed to assess the high risk of death. Future studies are suggested to confirm this study's results.

Conclusion

Levels of IL-6 and CRP at the 1st day of admission were weak predictors of mortality in severe COVID-19 patients. A better understanding of the causes of increased IL-6 and CRP in severe COVID-19 patients is needed whether it is related to the development of COVID-19 severity or comorbid disease.

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