Conference Paper

Procalcitonin, but not D-dimer, is an Inapplicable Biomarker for Severe COVID-19

H. Muflihah*[1], S. B. Rahiman[1], H. S. Sastramihardja[1] and F. A. Yulianto[2]

[1]*Department of Pharmacology, Faculty of Medicine Universitas Islam Bandung, Bandung, Indonesia*
[2]*Department of Public Health, Faculty of Medicine Universitas Islam Bandung, Indonesia*

Abstract.
Pro-calcitonin and D-dimer are among predictive biomarkers for the severity and mortality of COVID-19. The application of these parameters in the clinical setting of Indonesian hospitals is less documented. This study aims to evaluate the association between procalcitonin and D-dimer with COVID-19. This research is part of a retrospective study evaluating 249 hospitalized COVID-19 patients in Bandung, Indonesia. Patients who were positive for procalcitonin or D-dimer were selected. Clinical data of age, sex, comorbid condition, peripheral oxygen saturation (SpO2), and death were assessed. There were 39 and 28 patients tested for procalcitonin and D-dimer respectively. The level of procalcitonin was not associate with the severity of COVID-19 (p=0.442), death (p=0.506), comorbid condition (p=0.601) or the use of the antibiotics. However, the level of D-dimer in patients with severe COVID-19 was significantly higher than those with non-severe COVID-19 (p=0.0468). Our study shows that procalcitonin levels are not associated with COVID-19. However, D-dimer is associated with the severity of COVID-19.

Keywords: COVID-19, D-dimer, procalcitonin, severity

Corresponding Author: H. Muflihah; email: henimuflihah@gmail.com

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1. INTRODUCTION

Over a year pandemic of coronavirus disease 2019 (COVID-19) has caused at least 195 million cases of coronavirus disease 2019 (COVID-19) and more than 4 million of death worldwide [1]. The unpredictable progression on the severity of the disease has led to exploration for potential biomarkers associated with severe COVID-19 or the death. Among evaluated laboratory parameters, pro-calcitonin and D-dimer are considered as potential biomarkers associated with the severity of COVID-19 [2,3].

Procalcitonin is a glycoprotein consisting of 116 amino acid without hormonal activity and pre-cursor of calcitonin hormone [4]. It is synthesized by parafollicular cells in thyroid under physiological condition and the normal range of serum procalcitonin was 0-0.05 ng/ml [5]. Procitonin is increased in bacteremia, septic shock, and infection in lower respiratory tract infection [4,6]. A retrospective study in China showed that procalcitonin

could be used as an independent predictive factor for severe COVID-19, but further study was suggested to validate the finding, due to the very low number of patients [5].

D-dimer is a fibrin degradation product consisting of covalently linked two D fragments of the fibrin [7]. Increased plasma D-dimer indicates breaks down of fibrin in the bloodstream that could represent the activation of coagulation and fibrinolysis [7]. D-dimer has been shown to indicate severe COVID-19 since early time of pandemic [8,9]. The peak of D-dimer was also shown as prognostic marker for the death in COVID-19 patients [3]. However, misinformation on studies reporting prognostic value of D-dimer is the non-standardized units and the specific cut-off

value for D-dimer [10]. The Indonesian guideline for COVID-19 management recommends measuring D-dimer for initiation anticoagulant therapy, however, the cut-off value as well as the unit was not clearly defined.

Studies evaluating the prognostic role of serum procalcitonin primarily conducted in China and used cut-off point of 0.05 ng/mL [2]. A recent Indonesian study showed the increased procalcitonin level on the severe and critical illness compared to the moderate COVID-19 [11]. However, this study evaluated patients in the setting of intensive care unit and high care unit where only few COVID-19 patients admitted. The Indonesian study evaluating D-dimer levels in association with the severity of COVID-19 is limited. Therefore, a study to evaluate procalcitonin and D-dimer in association with the severity of COVID-19 in local setting of Bandung would provide valuable evidence for reliable application of these biomarkers in Indonesia.

2. METHODS

2.1. Study Design and Clinical Data

This study is part of a retrospective study investigating clinical use of antiviral, antibiotic, and immunomodulatory drugs in hospitalized COVID-19 patients in Bandung, Mrs. Muflihah in 2021. This study used medical records of 251 COVID-19 patients hospitalized in two main hospitals affiliated with Faculty of Medicine Universitas Islam Bandung. The subjects of this study were adults (≥ 18 years old), confirmed COVID-19 had initial peripheral oxygen saturation (SpO_2), and had laboratory result for procalcitonin or D-dimer. The medical record reported both procalcitonin and D-dimer using the unit ng/mL. The manufacturer for D-dimer (Roche CARDIAC) mentioned that 1 µg/mL corresponds to 1 µg FEU/mL. Patients were defined confirmed COVID-19 based on the positive result of reverse transcription-quantitative polymerase chain reaction (RT-qPCR) assay

detecting nucleic acid of SARS-CoV-2 from nasopharyngeal and oropharyngeal sample. Patients were categorized as non-Severe COVID-19 if the initial SpO_2 was ≥ 90% and severe COVID-19 if the SpO_2 below than 90%. Data of death and discharged alive were collected at the and hospitalization for COVID-19.

2.2. Ethical Approval

The protocol of this study was approved by the Health Research Ethics Committee Al Islam Hospital No.001/KEPPIN-RSAI/02/2021.

2.3. Statistical Analysis

The value of Procalcitonin or D-Dimer was tested for normality data using Saphiro Wilk-test. Descriptive analysis for non-normally distributed data used median and interquartile range (IQR) (25% and 75% percentile) and the association of procalcitonin or D-dimer with other variables of clinical condition (death, severity, comorbid) was analyzed using Mann-Whitney. Statistically significance was considered if the p-value was less than 0.05. The statistical analysis and data display was performed using GraphPad Prism V.8 software (La Jolla, CA).

3. RESULTS

3.1. Clinical Characteristics of Patients with Procalcitonin and D-dimer Result

Out of 249 hospitalized COVID-19 patients, 59 had laboratory result of procalcitonin and D-dimer. The clinical characteristic of these patients was shown in Table 1. More than half of the patients was male (64.4%) and had no comorbid condition (55.9%). The patients had mean of age 53.2 years old, median of procalcitonin 0.23 ng/mL and median of D-dimer 505 ng/mL. Out of 59, 5 patients (8.5%) were dead.

3.2. The Association of Procalcitonin Level with the Characteristic Associated with Severe COVID-19

To evaluate the role of procalcitonin as biomarker for the severity of COVID-19, we assessed its association with severe COVID-19 defined as SpO_2 <90%, the death and the presence of comorbid. **Figure 1** showed that the procalcitonin level was not

Table 1: Characteristics of hospitalized patients having procalcitonin and D-dimer result.

Characteristics		Total N=59
Sex		
Male n (%)	38	64.4 %
Female n (%)	21	35.6 %
Age in years mean (95% CI)	53.2	(49.6-56.7)
Comorbid condition		
With comorbid	26	44.1 %
No comorbid	33	55.9%
Procalcitonin in ng/mL (n=39) median (IQR)	0.23	(0.1-0.79)
D-dimer in ng/mL (n=28) median (IQR)	505	(229.5-1295)
Death n (%)	5	8.5 (%)

significantly associated with the severity (p=0.442, Fig. 1A), death (p=0.506, Fig. 1B), nor comorbid condition (p=0.601, Fig. 1C). The scattered plot showed that there were 2 patients with high level of procalcitonin (above 21 and 25 ng/ml) in the group of non-severe (Figure 1A) and discharged alive (Figure 1B). Thus, our study found that the level procalcitonin was failed to show as biomarker of the severity of COVID-19.

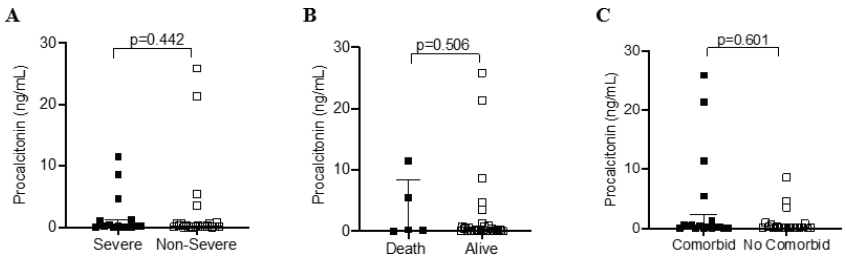

Figure 1: The level of procalcitonin based on clinical characteristics of disease severity, death, and comorbid condition**.** A. Procalcitonin level in patients with severe or non-severe COVID-19. B. Procalcitonin in COVID-19 patients discharge alive and death at the end of hospitalization. C. Procalcitonin level in COVID-19 patients with comorbid or no comorbid condition. Data are median with interquartile range (IQR). Statistical significances were analyzed using Mann-Whitney test.

3.3. The Level of D-dimer was Associated with the Severity of COVID-19

The role of D-dimer as biomarker for severe COVID-19 and guidance for anticoagulant therapy were evaluated. Figure 2A showed that the level of D-dimer was associated with severe COVID-19 (p=0.0468). There was a trend of higher level of D-dimer in the male patients (Figure 2B) and patients treated with heparin (Figure 2C), but these were not statistically significant (p=0.0829). This result showed the role of D-dimer as biomarker for severe COVID-19 but was not used as guidance for the initiation of heparin therapy.

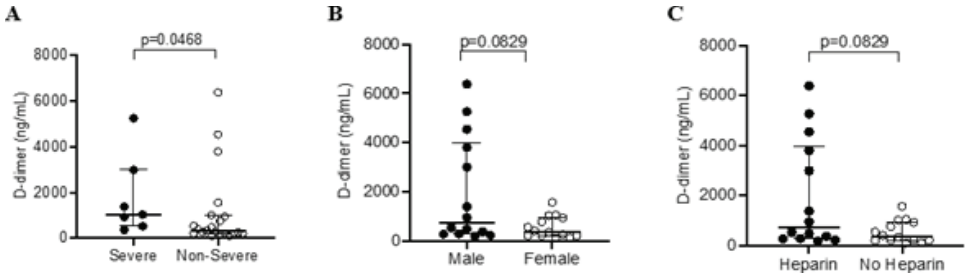

Figure 2: Plasma level of D-dimer in association with the severity of COVID-19, sex, and anticoagulant therapy. A. The level of D-dimer in patients with severe COVID-19 compared to the non-severe. B. Plasma level of D-dimer in male and female. C. The level of D-dimer in patients receiving anti-coagulant heparin and those who were untreated. Data were presented as median with interquartile range (IQR). Statistically significant difference was determined by Mann-Whitney test.

4. DISCUSSION

Our study evaluated procalcitonin and D-dimer as the laboratory biomarkers for the severity of COVID-19. We found that procalcitonin was failed to show association with the severity of COVID-19. However, the level of D-dimer was associated with severe COVID-19.

The failure of procalcitonin being a predictive factor for severe COVID-19 in our study was in line with a systematic review showing the controversy results of procaclitonin in 14 studies [9]. A half of the studies found insignificant difference in the level of procalcitonin between the severe and non-severe COVID-19 patients[9]. However, when the cut-off 0.05 ng/mL was used, elevated procalcitonin (≥0.05 ng/mL) found to be a promising prognostic biomarker for progression to be more severe COVID-19 disease from two recent systematic review [2,12]. Interestingly, in our study high levels of procalcitonin were found in the non-severe cases.

Studies evaluated prognostic value of D-dimer in COVID-19 had considerable variation on the sampling time and the reporting units for the D-dimer. For example, a study found that the peak D-dimer was more valuable prognostic biomarker of death than the initial one in COVID-19 [3]. In contrast, another study found that the initial D-dimer measured on admission could predict mortality of hospitalized COVID-19 patients [13]. Although D-dimer was associated with the severity of COVID-19 in our study, we did not record the sampling time for D-dimer. Further validating study should record the sampling time for D-dimer on the laboratory result. Various manufacturers prefer different units for D-dimer. Most of studies reported D-dimer in mg/L or µg/ml without mentioning the value as D- dimer units (DDU) or fibrinogen equivalent units (FEU) which is approximately two times of DDU [10]. A meta- analysis study showed that the severe COVID-19 was associated with higher concentration of D-dimer using mg/L unit [14].

Another systematic review used µg/ml FEU for the unit and found that the mean D-dimer in severe and mild COVID-19 patients were 3.55 and 0.58 µg/ml FEU respectively [7]. Using the unit µg/ml FEU, most of the normal range of D-dimer is <0.5 µg/ml [3,15]. The critical value for prognostic biomarker of intubation was 0.75 mg/L and

12.75 mg/L for initial and peak D-dimer respectively [3]. Our laboratory site reported D-dimer in the unit ng/ml, despite the manufacturer unit µg/ml FEU. The referred normal range was <100 ng/ml or 0.1 µg/ml FEU. The cut-off value for the referred normal range in our study was five times lower than that referred by most of studies either in the same unit (< 550 ng/mL FEU)[10] or converted into µg/ml FEU (<0.5 µg/ml) [3,15]. We found the converted median (0.505 µg/ml) and IQR (0.2295-1.2995) was above the referred normal range. However, we reported three values of D-dimer that were above the measuring range (0.1-4 µg/ml) from the manufacturer. Indeed, standardization of the unit D-dimer is a critical issue to avoid misinformation.

Although the pathogenesis for increased procalcitonin and D-dimer in COVID-19 disease is unclear, several mechanisms have been proposed. Inflammatory cascades, cytokine storm, and activation of coagulation cascades are known pathogenesis of COVID-19. These could trigger sepsis and dissemination intravascular coagulation (DIC). Increased D-dimer in COVID-19 is part of clinical manifestation of coagulation dysfunction [15] that was a risk factor for development of acute respiratory distress syndrome (ARDS) and progression from ARDS to death [16]. Increased level of serum procalcitonin was a result of massive secretion of procalcitonin form extra-thyroid organs triggered by inflammatory cytokine [2]. The up-surge gamma interferon inhibits the release of procalcitonin causing its level remains lower than 0.05 ng/mL in non-severe COVID-19 [17]. However, as increased procalcitonin is common in

bacteremia and septic shock [6], instead of biomarker for severe COVID-19, increased procalcitonin may rather indicate bacterial infection in COVID-19 infection. Our result found that procalcitonin and D-dimer is not a routine laboratory examination in hospitalized COVID-19 patients. The cost and the availability of the facility are very likely main reason for examining these parameters in certain hospitalized COVID-19 patients. In our hand, D-dimer was found to be more applicable prognostic biomarker for the severity of COVID-19 patients than the procalcitonin.

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