

# Analytical Correlation of D-dimer as Coagulation Biomarker with C-Reactive Protein, Neutrophil Lymphocyte Ratio, and Absolute Lymphocyte Count as Inflammatory Biomarker in COVID-19 Patient: A Retrospective Study

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**Abstract:** *Background and aims:* COVID-19 is an infectious disease cause by SARS-CoV-2 virus. Coagulopathy and Thrombotic complication frequently occur in COVID-19 patient. Both coagulation and inflammatory biomarker were elevated in COVID-19 and associated with severity of disease progression. This article aims to explore the correlation of D-dimer with Inflammatory Biomarker in COVID-19 patient, this inflammatory biomarker include C-reactive protein (CRP), neutrophil lymphocyte ratio (NLR) and absolute lymphocyte count (ALC). *Methods:* This Study is a retrospective observational analytical study with crosssectional design using secondary data of medical record patient conducted at Wangaya Hospital, Denpasar between July 2020 and Desember 2020. Data that meets inclusion criteria were included in this study and retrospectively analysed. *Result:* Kolmogorov Smirnov normality test D-dimer, CRP, NLR and ALC were not normaly distribute ( $p < 0.001$ ;  $p < 0.001$ ;  $p < 0.001$  and  $p = 0.004$  consecutively). Spearman Correlation test we found significantly positive correlation of D-dimer concentration with CRP and NLR ( $r = 0.825$ ,  $p < 0.001$ ; and  $r = 0.735$ ,  $p < 0.001$  consecutively), but D-dimer with ALC were found to be significantly negative correlated ( $r = -0.591$ ,  $p < 0.001$ ). *Conclusion:* Elevated concentration of D-dimer serum were correlate with inflammatory biomarker in COVID-19 patient. This finding may suggest that inflammation is one of the factor that causes activation of coagulation in COVID-19 patient

**Keywords:** COVID-19, SARS-CoV-2, C-reactive protein (CRP), Neutrophil Lymphocyte Ratio (NLR), Absolute Lymphocyte Count (ALC)

## 1. Introduction

On 11 March 2020, World Health Organization declared a pandemic of an infectious disease cause by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), this disease then called Coronavirus Disease 2019 (COVID-19). This virus were transmitted by infected patient through droplet during cough and sneeze. The clinical feature of COVID-19 varied, ranging from asymptomatic and mild state to severe and critical state such as multiorgan dysfunction and acute respiratory distress syndrome.<sup>1</sup>

The rapid progression of COVID-19 necessitates proper and immediate categorization of COVID-19 patient based on disease severity. Biomarker is needed to immediate classify COVID-19 and predict which patient would fall in to severe disease progression or severe complication. Immediate classify COVID-19 severity disease would ensure best management for the patient. An association of biomarker of COVID-19 infection with disease severity progression has been reported in several studies. D-dimer, a coagulation biomarker was reported significantly increased in COVID-19. Non-survivor COVID-19 group patient, tended to have higher D-dimer concentration compared with survivor COVID-19 group.<sup>2</sup>

In COVID-19 patient, inflammatory biomarker also had been reported associated with COVID-19 disease progression. Severe and critical COVID-19 patient tended to have higher C-reactive protein concentration, higher neutrophil lymphocyte ratio and lower of lymphocyte count compared with mild and moderated group.<sup>2,3,4</sup>

Recently, several studies had explored the correlation of D-dimer with other biomarker in any inflammatory disease condition, but only few studies had been reported correlation of D-dimer with other biomarker in COVID-19 condition. This article aims to explore the correlation of D-dimer with Inflammatory Biomarker in COVID-19 patient, this inflammatory biomarker include C-reactive protein (CRP), neutrophil lymphocyte ratio (NLR) and absolute lymphocyte count (ALC), that had been proposed to be associated with COVID-19 disease progression.

## 2. Method

This was retrospective observational study with crosssectional design using secondary data of medical record patient. This Study was conducted at Wangaya Hospital, Denpasar since November 2020 to January 2021. Inclusion criteria for this study was COVID-19 patient 18 year or older, confirmed by RT-PCR who were hospitalized in Wangaya Hospital since July 2020 to December 2020.

Patients with incomplete registered data, were excluded in this study. D-dimer, CRP, NLR, and Absolute lymphocyte count were collected on the first day of admission. All medical laboratory data including D-dimer, CRP, NLR, and Absolute lymphocyte count were generated by laboratory of Wangaya Hospital.

In this study, SPSS 25.0 were used to performed statistical analysis. Data were expressed as mean (minimum-maximum). Correlation analysis will be done by pearson correlation if the data normally distribute. If the data isn't distribute normaly, the analyze will be done by spearman correlation test.

**3. Result**

**Table 1:** Baseline characteristic of COVID-19 Patient

Characteristic	N
Age n (%)	
18-60 years	62 (28)
> 60 years	161 (72)
Gender, n (%)	
Male	125 (56,1)
Female	98 (43,9)
Disease Severity n (%)	
Mild	50 (22.4)
Moderate	120 (53,8)
Severe-Critical	53 (23,8)
Mortalitas n (%)	
Survivor	198 (88,8)
Non Survivor	25 (11,2)
Chest X-Ray laterality n (%)	
Normal	55 (24,7)
Unilateral infiltrate	28 (12,5)
Bilateral infiltrate	140 (62,8)
Comorbidity n (%)	
With Morbidity	60 (26,9)
Without Morbidtiy	123 (73,1)
D-dimer mean (min-max)	2709 (107-16944) ng/mL
CRP mean (min-max)	52.95 (5-200) mg/L
NLR mean (min-max)	6.22 (0.64-31.89)
ALC mean (min-max)	1.4 (0.26-3.60) 10 <sup>3</sup> /μL

Baseline characteristic of COVID-19 patient in this study can be seen in table 1. A total of 223 subject of COVID-19 patient between July 2020 and December 2020 were included in this study. Age majority of patient were more than 60 years (72%). 125 patient (56,1 %) were male, and 98 patient (43,9%) were female. Based on disease severity 50 patient (22.4%) mild, 120 patient (53.8%) were moderate severity, and 53 patient (23.8%) patient were severe-critical severity. Based on mortality profil, 198 patient (88,8%) were survivor, and 25 patient (11,2%) were non-survivor. Base on laterality of affected lung chest x-ray imaging, 55 patient (24,7%) showed normal imaging, 28 patient (12.5%) showed unilateral affected lung, and 140 patient (62.8%) showed bilateral affected lung. Morbidity in this patient were patient who had one or more comorbidities of Diabetes mellitus, cardiovascular disease, respiratory disease, chronic kidney diseases, hypertension and chronic liver disease. 60 patient (26.9%) had comorbidity, and 123 patient (73.1%) didn't had any comorbidity. Mean concentration of D-dimer, CRP, NLR, and Absolute Lymphocyte Count were 2709 (107-16944) ng/mL; 52.95 (5-200) mg/L, 6.22 (0.64-31.89), 1.4 (0.26-3.60) 10<sup>3</sup>/μl consecutively. Mean concentration of

D-dimer, CRP, NLR and ALC based on disease severity in COVID-19 patient can be seen in table 2

**Table 2:** Mean concentration of D-dimer, CRP, NLR and ALC based on disease severity in COVID-19

	COVID-19 Disease Severity Mean (min-max)		
	Mild	Moderate	Severe-Critical
D-dimer (ng/mL)	366 (107-785)	1415 (302-4143)	7851 (1139-16944)
CRP (mg/L)	8.32 (5-21)	35.95 (10-97)	133 (34-200)
NLR	2.44 (0.64-4.94)	4.44 (1.11-14.98)	13.82 (5.52-31.89)
ALC (10 <sup>3</sup> /μL)	2.03 (1.01-3.60)	1.41 (0.36-3.49)	0.76 (0.26-1.94)

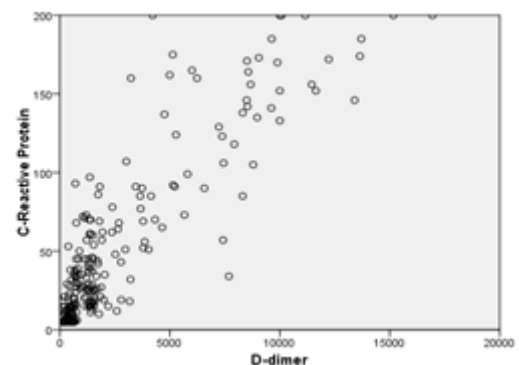
**Table 3:** Spearman Correlation Analysis of D-dimer with CRP, NLR and ALC

Variable	D-Dimer	
	Correlation Coefficient	p value
C Reactive Protein	0.825	p <0.001
Neutrophyl Lymphocyte Ratio	0.735	p <0.001
Absolue Lymphocyte ratio	-0.591	p <0.001

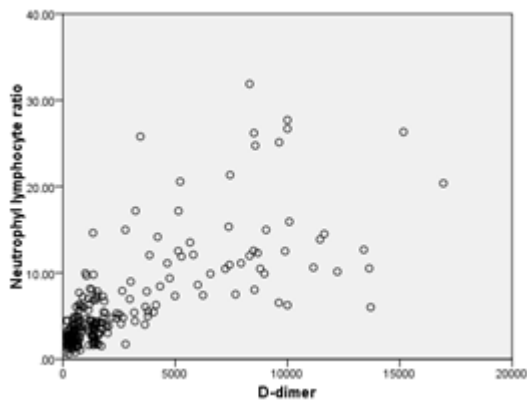
Data distribution of D-dimer, CRP, NLR, and ALC were analysed using Kolmogorov Smirnov normality test. On Kolmogorov Smirnov normality test D-dimer, CRP, NLR and ALC were not normally distribute (p<0.001; p <0.001; p <0.001 and p=0.004 consecutively). Correlation analysed were then performed using Spearman Correlation test. On Spearman Correlation test we found significantly positive correlation of D-dimer concentration with CRP and NLR (r=0,825, p<0.001; and r= 0.735, p <0.001 consecutively), but correlation of D-dimer with ALC were found to be significantly negative(r=-0.591, p<0.001). Spearman correlation test result can be seen on table 3.

**4. Discussion**

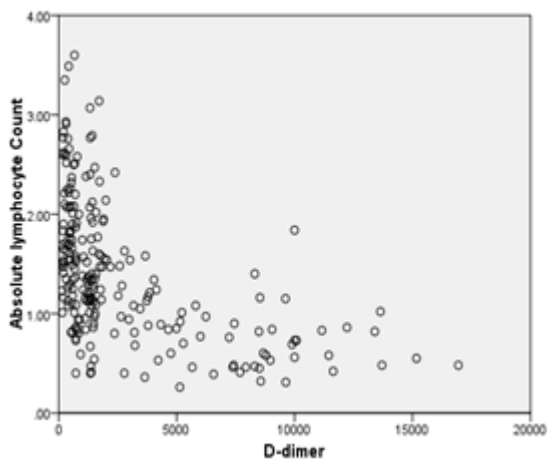
COVID-19 patients frequently have coagulopathy and thrombotic complication. COVID-19 associated coagulopathy has a



**Figure 1:** Scatterplot graph of D-dimer and C-Reactive Protein correlation



**Figure 2:** Scatterplot graph of D-dimer and Neutrophyl lymphocyte ratio correlation



**Figure 3:** Scatterplot graph of D-dimer and absolute lymphocyte count correlation

complex pathophysiology. Several mechanism in COVID-19 that lead in to thrombosis of microvascular and macrovascular including cytokine storm, leukocyte activation, activation of platelet and endothelium resulting in an increased of tissue factor production, coagulation activation and generation of thrombin, and formation of fibrin, imbalance plasminogen activator inhibitor-1 (PAI-1), activated protein C, hypoxic-vaso-occlusion and direct viral effect with cell activation.<sup>5,6,7</sup>

D-dimer is a soluble fibrin degradation product that was produced during degradation of crosslinked fibrin during fibrinolysis. D-dimer was used to be an indirect biomarker of both fibrinolysis and fibrin turnover, hence D-dimer could be used as marker of fibrinolysis and activation of coagulation in multiple clinical scenario.<sup>8</sup>

D-dimer production require three main enzymes: thrombin, activated factor XIIIa, and plasmin. It's started when thrombin generated by the coagulation system cascade that convert fibrinogen in to fibrin monomers. Through noncovalent interaction base on allosteric changes, fibrin monomers then form in to fibrin polymers. Factor XIIIa then strengthen this fibrin polymer. Plasmin then digest this fibrin polymer and produce D-dimer molecule.<sup>8</sup>

D-dimer is quantified and detected in serum, plasma or whole blood using monoclonal antibodies that recognize specific epitope on cross-linked D-dimer. There are three

assays that are available in detecting D-dimer: immunofluorescent assays, enzyme-linked immunosorbent assays (ELISA), and latex agglutination assays.<sup>8</sup>

Absence or presence of D-dimer molecules has variety of uses in different pathologic condition. D-dimer analysis is critical in diagnosis of deep vein thrombosis, pulmonary embolism, aortic dissection and disseminated intravascular coagulation (DIC). Elevated D-dimer concentration was seen in COVID-19 patient and had been shown to be associated with disease severity and mortality.<sup>8</sup> A study that was conducted by soni *et al*, found that elevated D-dimer  $>2.01\mu\text{g/mL}$  was a predictor of mortality in COVID-19 patient ( $P < 0.01$ ; HR, 3.165; 95% (2.013-4.977)).<sup>4</sup>

CRP, NLR and ALC are another inflammatory biomarker that routinely checked in COVID-19 patient. Critical and severe COVID-19 patient tends to have a low lymphocyte count compare with mild and moderate COVID-19 patient. Its also reported in a study of a total 450 subject, subject with severe COVID-19 patient tended to have higher leukocytes count, lower lymphocyte count, but higher NLR. Another study also reported COVID-19 who had fatal and severe COVID-19 had significantly increased in leukocytes, and significantly decreased lymphocyte.<sup>2</sup>

C-reactive protein as inflammatory biomarker found to significantly increase in COVID-19 patient. CRP has been proposed to be associated with disease severity and as early predictor for COVID-19 severity.<sup>2</sup>

A retrospective study conducted by Billian *et al* that study correlation of D-dimer level with other biomarker in COVID-19 and Bacterial pneumoniae found that, in untreated COVID-19 patient, D-dimer was found to be positively correlate with high-sensitive C-reactive protein (hsCRP) ( $r=0.426$ ,  $p<0.01$ ) and Neutrophyl count ( $r=0.464$ ,  $P<0.001$ ), but negatively correlate with lymphocyte count ( $r=-0.464$ ,  $p<0.01$ ).<sup>9</sup> Another study conducted by Guo *et al* found a positive correlation of D-dimer with NLR ( $r=0.5195$ ,  $p<0.001$ ).<sup>10</sup> In line with those study, we also found significantly positive correlation of D-dimer concentration with CRP and NLR ( $r=0.825$ ,  $p < 0.001$ ; and  $r= 0.735$ ,  $p < 0.001$  consecutively), but negative correlated with ALC ( $r=-0.591$ ,  $p<0.001$ ).

The reason responsible for the correlation of D-dimer with other inflammatory biomarker in COVID-19 patient is because, in COVID-19 patient. Under inflammatory condition, there's dysregulation of alveolar homeostatic balance that lead in to an increase in prothrombotic activity. Endothelial injury that caused by pro inflammatory cytokine also cause activation of coagulation cascade in severe sepsis patient. In this study we found significant correlation of D-dimer with other inflammatory biomarker, this suggest that inflammation is one of the factor that causes activation of coagulation in COVID-19 patient.<sup>9,10</sup>

## 5. Conclusion

COVID-19 is an infectious disease cause by SARS-CoV-2 virus. There are many biomarker that had been reported to be associated with COVID-19 disease severity. Elevated D-

dimer concentration was seen in COVID-19 patient and had been shown to be associated with disease severity and mortality. Expression of proinflammatory cytokines under inflammatory condition play a role in activation of coagulation cascade in COVID-19 patient. In this study we found that Elevated concentration of D-dimer serum were correlate with other inflammatory biomarker in COVID-19 patient.

### **Ethical Clearance**

Our study was approved by ethical committee of RSUD Wangaya Hospital.

### **Conflict of Interest**

There is no conflict of interest in this case report.

### **Author Contribution Statement**

All of author contributed equally.

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