# D-Dimer Analysis in COVID-19 Patients

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## ABSTRACT

The COVID-19 incidence is increasing around the world. Some countries are experiencing worsening conditions, even deaths. One coagulation marker that noticeably increases in COVID-19 patients is D-dimer. This study aimed to analyze D-dimer levels of COVID-19 patients. Retrospective study using medical records of 84 COVID-19 patients, conducted from April to August 2020 at UNHAS Hospital. Patients were grouped based on the severity of the disease as non-severe and severe. D-dimer levels were measured using the Alere Triage® D-dimer with the fluorescent immunoassay method. The statistical test used was Mann-Whitney, D-dimer prognostic levels were calculated with ROC analysis to get the cut-off. Significant if the p < of 0.05. The sample consisted of 74 non-severe and ten severe COVID-19 patients, mostly in the 30-39 age group. D-dimer levels in non-severe (0.31±0.38 µg/L) significantly differ from severe group (3.09±2.56 µg/L) (p<0.001). The Receiver Operating Characteristics (ROC) curve showed D-dimer sensitivity and specificity of 90.0% and 89.2%, respectively at the ≥ 0.80 µg/L cut-off, Negative Predictive Value (NPV) of 98.5%, and Positive Predictive Value (PPV) of 52.9%. D-dimer levels increased in severe COVID-19 patients due to an increased inflammatory response resulting in excessive thrombin. The ROC D-dimer curve indicated a cut-off rate of 0.80 µg/L, providing optimal sensitivity and specificity. D-dimer has a significant difference in non-severe and severe COVID-19 patients and shows good value to determine the severity of COVID-19 disease with a cut-off value ≥ 0.80 µg /L.

**Keywords:** COVID-19, D-dimer, predictor

## INTRODUCTION

COVID-19 cases were first reported in December 2019, starting with a series of acute atypical respiratory illnesses occurring in Wuhan, China, quickly spreading to other regions. This virus was named Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2, 2019-nCoV). The spread of SARS CoV-2 was initially thought to have started through zoonotic transmission linked to the seafood market in Wuhan, China. Human-to-human transmission played a significant role in subsequent outbreaks. The disease caused by this virus is called Coronavirus 2019 (COVID-19) and was declared a pandemic by the World Health Organization (WHO) on March 11, 2020.[1-10]

COVID-19 case figures in October 2020 have been confirmed to occur in more than 38,789,204 million cases worldwide and have resulted in more than 1,095,097 deaths. More than 180 countries have reported confirmed cases of COVID-19 on all continents. COVID-19 cases were first reported in Indonesia on March 2, 2020, and in October 2020, there were 349,160 confirmed cases, resulting in as many as 12,268 deaths. The main symptoms in most positive COVID-19 patients are cough, fever, history of fever, and shortness of breath. Most comorbidities in COVID-19 patients are hypertension, diabetes mellitus, heart disease, and Chronic Obstructive Pulmonary Disease (COPD), while the age group with the highest mortality rate is ≥ 60 years (39.9%), 46-59 years (39.3%), and 31-45 years (14.5%).[3,4,11,12]

COVID-19 infection begins with the interaction of the viral spike protein with human cells through the Angiotensin-Converting Enzyme 2 (ACE2) receptors, which can be found in the human lower respiratory tract and small intestinal enterocytes. After entering the cell, genome encoding facilitates the expression of genes that help the SARS-CoV-2 virus adapt to the host. Recombination, gene exchange, gene insertion, or deletion will cause changes in the genome that lead to future outbreaks.[3,8,13]

COVID-19 has been linked to hemostatic disorders. Significant increases in D-dimer levels were found in those who did not survive and those who suffered from severe disease. D-dimer is a unique marker of fibrin degradation formed by the sequential action of 3 enzymes: thrombin, factor XIIIa, and plasmin. First, thrombin cleaves fibrin-producing monomers of fibrin, which polymerize and serve as a

template for factor XIIIa and plasmin formation. Second, thrombin activates plasma factor XIII bound to polymer fibrin to produce active transglutaminase, factor XIIIa. Factor XIIIa catalyzes the formation of covalent bonds between D-domains in polymerized fibrin. Finally, plasmin degrades cross-linked fibrin to release fibrin degradation products, including D-dimers.[6,14]

A multicentre retrospective study during the first two months of the epidemic in China showed 260 out of 560 patients (46.4%) confirmed with COVID-19 had elevated D-dimers ($\geq 0.5$ µg/L). Increased D-dimer was more pronounced in severe cases than in mild cases (59.6% vs. 43.2%).[2]

One of the main problems in handling COVID-19 is the high number of patients admitted to health centers or hospitals during the pandemic. This fact has resulted in an imbalance between available resources and the number of incoming patients, especially patients who need intensive care support, so risk stratification steps are required to increase initial management according to the severity of the COVID-19 patient. Therefore an effective marker is necessary to determine the seriousness of COVID-19 patients.[15]

To the researchers' knowledge, there has never been a study that reported differences in D-dimer levels on the severity of COVID-19 patients accompanied by determining the levels of predictors and D-dimer cut-off's in Makassar. Therefore, based on this background, D-dimer was analyzed to see whether it can be used as a marker of severity of COVID-19 patients at the Hasanuddin University Hospital (RSUH) Makassar, a marker referral hospital for COVID-19 in South Sulawesi Province since April 2020.

## METHODS

This retrospective cross-sectional study used secondary data from the medical records of patients diagnosed with COVID-19 undergoing treatment at RSUH, conducted during the April-August 2020 period. The D-dimer examination using a fluorescence immunoassay method for the quantitative determination of cross-linked fibrin degradation products containing D-dimer in EDTA anticoagulated whole blood with the reference value ($< 0.5$ µg/L). The results of D-dimer examination were further classified into the criteria for non-severe COVID-19 if the symptoms that occur were symptoms of non-pneumonia or mild pneumonia, and severe COVID-19 if the visible symptoms include respiratory problems (shortness of breath), respiratory rate $\geq 30$ times per minute, blood oxygen saturation $\leq 93$, PaO2/FiO2 ratio $<300$, and/or there was a pulmonary infiltrate $> 50\%$ within 24-48 hours.

The samples were then analyzed. Statistically, the D-dimer data in the two groups were not normally distributed, so the Mann-Whitney test was performed to see the difference in D-dimer levels. A Receiver Operating Characteristics (ROC) analysis was performed to obtain a cut-off value. The test results were significant if the p-value $< 0.05$.

Approval of ethical eligibility was obtained from the Health Research Ethics Commission (KEPK) of Hasanuddin University Medical Faculty/Hasanuddin University Hospital/Dr. Wahidin Sudirohusodo Makassar with Number 524/UN4.6.4.5.31/Pp36/2020.

## RESULTS AND DISCUSSION

Of the 392 COVID-19 patients at RSUH, only 84 subjects had D-dimer data, so the total samples that met the inclusion criteria were 84, all aged 6-71 years old. In general, the average D-dimer level was $0.64\pm1.29$ µg/L (Table 1).

D-dimer levels in severe COVID-19 were higher than non-severe COVID-19. In addition, the D-dimer SD range in Severe COVID-19 was more comprehensive ($3.09\pm2.5$ µg/L) than non-severe

**Table 1.** Sample characteristics

| Parameter | n (84) | (%) | Minimum | Maximum | (Mean) | ± SD |
|---|---|---|---|---|---|---|
| **Gender** | | | | | | |
| Male | 40 | 47.6 | | | | |
| Female | 44 | 52.4 | | | | |
| **Age (years)** | | | 6 | 71 | 40.0 | 15.0 |
| < 30 | 21 | 25.0 | | | | |
| 30-39 | 29 | 34.5 | | | | |
| 40-49 | 10 | 11.9 | | | | |
| 50-59 | 10 | 11.9 | | | | |
| ≥ 60 | 14 | 16.7 | | | | |
| **Severity** | | | | | | |
| Non-severe | 74 | 88.1 | | | | |
| Severe | 10 | 11.9 | | | | |
| D-dimer content (µg/L) | | | 0.10 | 9.90 | 0.64 | 1.29 |

SD = Standard Deviation

COVID-19 (0.31±0.38 µg/L), and there was a statistically significant difference (p < 0.001) (Figure 1).
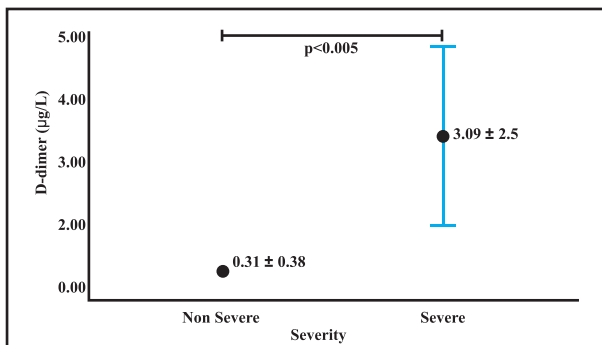


**Figure 1.** Comparison of D-dimers according to the severity

Based on the ROC curve (Figure 2), it can be seen that the Area Under Curve (AUC) for the D-dimer level = 0.981 (p < 0.001). This finding suggests that D-dimers could be used as a marker for COVID-19 severity.
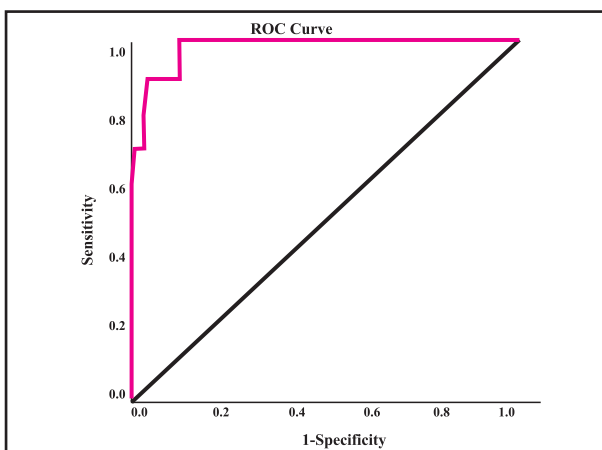


**Figure 2.** D-dimer ROC curve

Based on the coordinate levels of the D-dimer ROC curve, a cut-off of 0.80 µg/L was obtained, which provided optimal sensitivity and specificity (Table 2).

**Table 2.** Sample distribution based on D-dimer cut-off value

| D-dimer cut-off (µg/L) | Severity | | Total |
|---|---|---|---|
| | Weight | Non-severe | |
| ≥0.80 | 9 | 8 | 17 |
| <0.80 | 1 | 66 | 67 |
| Total | 10 | 74 | 84 |

Prognostic value of D-dimer sensitivity= 9/10x100%=90.0%; specificity=66/74x100% =89.2%; PPV=9/17x100%=52.9%; NPV= 66/67x100%=98.5%; accuracy value=((9+66)/84)x100%=89.3%; positive likelihood ratio (LR +) 0.9/1-0.892=8.3; negative likelihood ratio (LR-)= 1-0.90/0.82=0.121

Total samples obtained were 84, with a higher number of females (44 people) than males (40 people). In contrast to Ng *et al.'s* research, which collected data on severity and mortality from COVID-19 from European countries, the United States, South America, China, and South Korea, revealed that more cases of COVID-19 were found in males in all age groups. The tendency of more cases in males than females were due to the influence of biological characteristics, estrogen hormone in females triggers a more robust immune response in overcoming infections, including viral infections, compared to males. The tendency of males to be more active outside can also affect the number of cases of COVID-19 in males. The difference in the finding of the number of COVID-19 cases based on gender in this study, apart from being due to the different sample inclusion process, can also be caused by severe COVID-19 patients experiencing worsening, which theoretically are mostly male, that were referred to the main referral hospital for COVID-19. However, overall in this study population, there were more confirmed cases of COVID-19 in males than in females. Most of them were referred to the main referral hospital for COVID-19, which was in the same area as the sampling location. Overall in this study population, there were more confirmed cases of COVID-19 in males than in females.[1]

Most cases of COVID-19 were found in the 30-39 year old age group who are classified as the productive age group. This is following the research of Guan *et al.* that the productive age has relatively high mobility and activity, so it is suspected to be the cause of the spread of COVID-19.[16]

This study also revealed that the number of non-severe COVID-19 patients (n=74) was higher than those with severe COVID-19 (n=10). This result is in line with the research of Wu and Mc Googan, which stated that 81% of patients with confirmed COVID-19 were in the mild COVID-19 category, the other 19% were severe and critical COVID-19 patients.[17]

Infection of SARS CoV-2 in severe COVID-19 patients will activate the coagulation pathway. They began when SARS CoV-2 infects a blood vessel and disturbs integrity, causing the death of blood vessel cells. Endothelial cells undergo activation and dysfunction, resulting in exposure to the thrombogenic basement membrane and activating the coagulation cascade. Endothelial cell activation is also activated by IL-1β and Tumor Necrosis Factor (TNF) by expressing p-selectin, Von Willebrand

factor, and fibrinogen, which will bind to platelets. Endothelial cells then release cytokines, which will summon other platelets, and more platelets will bind. Platelets also release the Vascular Endothelial Growth Factor (VEGF), which stimulates endothelial cells to increase tissue factor expression, the coagulation cascade's primary activator. The body responds to the coagulation event by releasing fibrin bonds and thrombus. This process has led to an increase in the fibrin degradation product D-dimer in severe COVID-19 patients.[16,18,19]

The increase in D-dimers has been reported as a significant laboratory finding in COVID-19 patients undergoing hospitalization. This study found that the average D-dimer level in patients with severe COVID-19 was higher (3.09 μg/L) than in non-severe COVID-19 (0.31μg/L). The statistical test showed that this difference was very significant (p < 0.001). SARS CoV-2 infection is often accompanied by a high pro-inflammatory response but is not accompanied by a sufficiently good anti-inflammatory response capability, resulting in excessive thrombin formation.[16,18]

A ROC curve analysis was performed to determine the possibility of D-dimers as a marker of severity in COVID-19 patients, showing that D-dimer could be used as a predictor of severity with an AUC=0.981 (p < 0.001). This result is in line with research by Yao *et al.*, which revealed that D-dimers correlate with the severity of COVID-19.[2]

This study shows that the D-dimer cut-off of 0.80 μg/L gives a sensitivity value of 90.0% and a specificity of 89.2%, so it can be said that the D-dimer cut-off is 0.80 μg /L. It can be used to screen COVID-19 patients before they enter into severity. Positive Likelihood Ratio (LR +) = 8.3 and Negative Likelihood Ratio (LR-) = 0.121 (excellent), which means that a D-dimer cut-off of 0.80 μg/L will provide a greater probability of predicting patients into non-severe and severe COVID-19 degrees. This is in line with the findings of the D-dimer study in COVID-19 patients, including; an increase in D-dimer (> 1.5 μg/L) was detected in 36% of patients in a descriptive study of 99 cases of COVID-19 in Wuhan, China.[20,21]

The limitation in this study is the use of secondary data so that the information obtained is limited, in addition to the uneven distribution of the sample in each sample group.

## CONCLUSIONS AND SUGGESTIONS

D-dimers level in severe COVID-19 patients was higher than those in non-severe COVID-19 patients,

and a cut-off ≥ 0.80 μg/L can determine the direction of more severe COVID-19 disease (sensitivity 90%, specificity 89.2%). As a suggestion, further research should use large primary samples by including and comparing other laboratory data that support the severity of COVID-19 patients.

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