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Comparative Analysis of Hematological and Inflammatory Biomarkers in Moderate and Severe COVID-19 Patients

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ABSTRACT

In COVID-19 patients, abnormal blood clotting is common, characterized by elevated D-dimer and fibrinogen levels, reduced platelets, and prolonged clotting times. The second week of infection can trigger a cytokine storm, marked by heightened proinflammatory Interleukin-6 (IL-6) levels, associated with Acute Respiratory Distress Syndrome (ARDS) and organ failure. This study compared hematological biomarkers, D-dimer, and IL-6 in moderate and severe COVID-19 cases. In a cross-sectional study, 81 patients meeting inclusion criteria were examined at a leading private COVID-19 referral hospital in Malang Regency. Data from clinical records and lab results encompassing blood counts, D-dimer, and IL-6 levels were collected. D-dimer was assessed through immunoturbidimetry (STA-Procoag-PPL, Diagnostica Stago S.A.S.), while IL-6 was measured using a chemiluminescent immunoassay (Cobas e411 Elecsys, Roche). Data distribution normality was assessed using Kolmogorov-Smirnov and Shapiro-Wilk tests. Non-normally distributed data were analyzed using the Mann-Whitney U test for numerical data and the Fisher exact test for comorbidity-severity correlation. Moderate COVID-19 cases disproportionately affected females, while severe cases had an even gender distribution. The median age was comparable, but mild cases were typically younger. Hemoglobin, hematocrit, leukocyte, neutrophil, platelet, and procalcitonin levels were normal in both groups, with lowered lymphocyte counts. Severe cases displayed a higher Neutrophil-to-Lymphocyte Ratio (NLR). D-dimer and IL-6 were significantly elevated in extreme cases. This study underscores potential gender and age-related discrepancies in COVID-19 severity, emphasizing the significance of monitoring specific blood parameters for disease progression indicators. Further investigation is vital to unveil underlying mechanisms and clinical implications, aiding the management of COVID-19 patients.

Keywords: Moderate, severe COVID-19, D-dimer, interleukin-6 level

INTRODUCTION

The Coronavirus Disease (COVID-19) pandemic, declared by the WHO in March 2020, has claimed countless lives globally. Originating in Wuhan, China, in December 2019, COVID-19 has afflicted over 752 million individuals, resulting in 6.8 million deaths.[1] Indonesia, as of January 2023, has reported approximately 6.7 million cases and over 160 thousand deaths.[2] By April 18, 2021, there were 140,363,081 confirmed cases worldwide, with 3,004,285 deaths (Case Fatality Rate=CFR 2.1%) across 222 affected and 190 locally transmitting countries. Indonesia had 1,604,348 cases and 43,424 deaths, with a higher CFR of 2.7%. East Java recorded 147,977 cases and 10,689 deaths by April 2021.[3]

COVID-19 exhibits diverse clinical severities, categorized as mild, moderate, severe, and critical Acute Respiratory Distress Syndrome (ARDS). Mild and moderate cases comprise 40% of instances, with severe cases at 20%; mild cases often recover within a week.[3] Influential factors in COVID-19 severity include the immune system, comorbidities, and prompt diagnosis and treatment.

Studies highlight the potential link between D-dimer levels and COVID-19 severity. Elevated D-dimer and Fibrin Degradation Product (FDP) levels and reduced antithrombin (AT) levels are found, with severe cases exhibiting pronounced changes compared to mild cases. Elevated D-dimer levels can signal ARDS-related mortality and persist post-SARS-CoV-2 clearance, offering prognosis insights.[4,5]

Interleukin 6 (IL-6) is a pivotal biomarker for diagnosing, prognosing, and monitoring Cytokine Release Syndrome (CRS) and associated organ damage. It predicts CRS severity and ventilator support necessity, correlating with ICU admission likelihood and recovery prospects.[6]

Prompt treatment is crucial, especially for severe cases, warranting research to predict disease progression and guide therapy. Laboratory tests,

including complete blood count (with a focus on lymphocyte count, NLR, and platelet count), hemostasis (including D-dimer), and inflammatory markers (such as IL-6), aid COVID-19 patient assessment during the pandemic.

METHODS

This research employed an analytic observational approach with a cross-sectional design. Primary data sources included D-dimer levels, serum/plasma IL-6, procalcitonin, complete blood count (specifically NLR and lymphocyte count) in COVID-19 patients, and secondary data from medical records. Subjects were adults diagnosed with COVID-19 at Wava Husada Kepanjen Hospital, a COVID-19 referral hospital, between April 18 and June 28, 2021. Laboratory tests encompassed complete blood count, D-dimer, serum procalcitonin, and IL-6 within 24 hours. Severity levels were categorized as moderate (SpO2 ≥ 94% on room air) and severe (SpO2 <94%, PaO2/FiO2 <300 mmHg, respiratory rate >30 breaths/minute, or >50% pulmonary infiltrates).

The study involved 81 subjects. A complete blood count was conducted via flow cytometry using a Sysmex XN-2000 Hematology system. D-dimer levels were quantitatively measured with STA®-Procoag-PPL assay, while IL-6 and procalcitonin were measured using the Elecsys® ECLIA method. Data were processed using SPSS version 20. The Kolmogorov-Smirnov and Shapiro-Wilk tests assessed data distribution normality. Mann-Whitney U test analyzed abnormal distribution data (lymphocyte count, D-dimer levels, IL-6) between severe and moderate cases. Fisher exact test determined the comorbidity-severity correlation, and the regression correlation test assessed IL-6, lymphocyte count, and NLR relationship.

Ethical approval was granted by the Ethics Committee Team of Wava Husada Kepanjen Hospital (Ethical Eligibility Certificate No. SDN/2021/04/805, dated April 14, 2021).

RESULTS AND DISCUSSIONS

Characteristics of the research subjects in terms of age distribution and gender of the research subjects can be seen in Tables 1 and 2.

Among moderate COVID-19 cases, more females were present, yet not statistically significant. Severe cases had even gender distribution. Median age was similar, favoring younger individuals for moderate cases. Hemoglobin, hematocrit, leukocyte, neutrophil, platelet, and procalcitonin levels were normal and comparable between groups. Severe cases showed slightly higher NLR values, and both groups displayed low lymphocyte counts with no significant differences. D-dimer and IL-6 levels were elevated in severe cases compared to moderate ones.

Table 1. The characteristics of the research subjects in terms of age distribution and gender

Parameters	Severity of Disease		p-value
	Moderate	Severe	
	Mean (95% CI)	Mean (95% CI)	
Gender (f (%))			0.284
Male	6 (33.3%)	32 (50.8%)	
Female	12 (66.7%)	31 (49.2%)	
Age in year	56.5 (39.25-66)	57 (52.00-65.00)	0.554
Neutrophil count in $x10^3/\mu L$	6.48 (3.63-7.68)	6.68 (4.27-11.3)	0.506
Lymphocyte count in $x10^3/\mu L$	0.98 (0.64-1.63)	1.17 (0.74-1.69)	0.605[a]
NLR	4.775 (2.975-9.04)	5.95 (3.55-10.80)	0.460
D-dimer in µg/L	0.995 (0.47-1.865)	1.58 (0.92-4.67)	0.034[a]*
IL-6 in pg/mL	14.9 (7.1-29.1)	29.6 (15.5-83.0)	0.016[a]*
PCT in ng/mL	0.14 (0.5-0.2)	0.205 (0.08-0.3975)	0.391
Hemoglobin in g/dL	12.85 (12.225-13.8)	13.7 (12.3-14.5)	0.093
Leukocyte count in $x10^3/\mu L$	8.497 (5.48-9.81)	8.26 (5.81-12.19)	0.874
Platelet count in $x10^3/\mu L$	224.5 (153-278.875)	230 (169-353)	0.450
Hematocrit in %	38 (34.575-39.225)	39.9 (36.7-43.2)	0.058

Notes: a = Mann-Whitney U test, * = significantly different, PCT = Procalcitonin

Table 2. Fisher's exact test for comorbidities in COVID-19 based on the severity of the disease

	Severity		p
	Moderate	Severe	
Hypertension			
None	13	53	0.305
Present	5	10	
Heart failure			
None	18	61	1.00
Present	0	2	
Diabetes mellitus			
None	14	44	0.57
Present	4	19	
CVA/stroke			
None	16	62	0.123
Present	2	1	
Asthma			
None	18	62	1.00
Present	0	1	
Tuberculosis			
None	17	63	0.222
Present	1	0	
Coronary heart disease			
None	18	61	1.00
Present	0	2	
Anemia			
None	18	62	1.00
Present	0	1	

Though not significantly, the number of severe cases exceeded those with moderate disease. Comorbidities were more common in severe cases, mainly diabetes mellitus (30.16% in severe, 28.39% overall), hypertension (15.87% in severe, 18.52% overall), and cerebro-cardiovascular disease (7.94% moderate, 8.64% severe).

The IL-6 levels and lymphocyte count correlation analysis in COVID-19 patients revealed a weak negative correlation (-0.105, p=0.491). Conversely, IL-6 levels and NLR ratio analysis indicated a moderate positive correlation (0.505, p < 0.001).

This cross-sectional observational study was conducted at a prominent private hospital in Malang. Data were sourced from patient records and laboratories. It aimed to evaluate routine lab parameters, focusing on IL-6 and D-dimer, in moderate and severe COVID-19 patients. These accessible tests could serve as early indicators for patient deterioration, enabling timely interventions for improved outcomes.

Adequate screening tests are crucial for identifying high-risk COVID-19 patients prone to severe illness and death, necessitating vigilant monitoring and swift hospitalization. Biomarkers like CRP, ferritin, fibrinogen, D-dimer, and IL-6 are linked to COVID-19 progression.[7-9] Among these, IL-6 stands out for predicting respiratory failure more accurately. IL-6 significance lies in its role in immune dysregulation and ARDS in COVID-19 cases.[6,7,10] No prior research has assessed IL-6, other inflammation markers, and thrombosis markers at the type B referral hospital for initial COVID-19 assessment, nor their potential in predicting disease progression.

Subjects exhibited distinct characteristics: moderate COVID-19 cases aged 39-66 and severe cases aged 52-65. Older individuals are more susceptible, attributed to age-related pulmonary changes and increased comorbidities.[11] Physiological decline and weakened defenses contribute, alongside pre-existing conditions like cardiovascular disease, diabetes mellitus, and obesity, elevating COVID-19 mortality risk, especially in the advanced age.[12]

Elderly individuals and those in long-term care facilities with underlying health issues face a notable risk of severe COVID-19 outcomes and mortality.[7,13] In a pivotal study by Huang *et al.* in Wuhan, 27 out of 41 cases revealed adults were more susceptible due to visiting a seafood market, marking the initial evidence of human-to-human COVID-19 transmission.[14] However, Australia's Department of Health epidemiological report highlighted individuals aged 60 to 89 as most vulnerable to SARS-CoV-2 infection, especially during activities like long-distance travel.[15]

Regarding gender distribution, overall, no significant differences were found between males and females. However, concerning disease severity, more moderate cases occurred in females, while males exhibited more severe cases. This trend wasn't statistically significant. Walter *et al.* and Mukherjee *et al.* reported varying gender distribution results from studies in Wuhan and the US. This study found more severe cases among males, necessitating increased hospitalization and intensive care. Male susceptibility and severity might stem from factors like smoking and higher receptor expression (ACE2 and TMPRSS2), aiding COVID-19 infection.[16,17]

COVID-19 often leads to reduced lymphocyte counts, notably in severe cases. Although lymphocytes express low levels of ACE2, the SARS-CoV-2 receptor, the exact cause of this lymphopenia remains unclear.[18] This study observed lower average lymphocyte counts (980/µL in moderate, 1170/µL in severe) that fell within the normal range (male: 1.46-3.73 x 10^3/µL, female: 1.46-3.73 x 10^3/µL).[19] Other research by Jurado *et al.* found similar findings (average 1.16 x 10^3/µL),

and a study by Pan *et al.* reported a more pronounced lymphocyte drop (0.75 x 10^9/L) in ICU COVID-19 patients compared to non-ICU patients (1.20 x 10^9/L).[20,21]

In this study, the IL-6 levels of 81 COVID-19 patients were measured, revealing elevation. A significant difference was noted between moderate and severe cases (14.9 pg/mL versus 29.6 pg/mL). Qin *et al*. research in Wuhan found a mean IL-6 level of 21.0 pg/mL among 452 COVID-19 patients.[22] Similarly, Wan *et al*. study at Chongqing Three Gorges Central Hospital showed raised IL-6 levels in mild (13.41 pg/mL) and severe (37.77 pg/mL) COVID-19 cases.[23]

Elevated serum IL-6 levels correlate with poor outcomes and prognosis. Studies suggest IL-6 above 80 pg/mL can identify high-risk respiratory failure patients. It's proposed as a reliable predictor for disease progression and mortality.[24] Sabaka *et al*. research highlighted IL-6 levels exceeding 24 pg/mL as a predictive marker for hypoxemia requiring hospitalization. IL-6 could be an early identifier for severe COVID-19, assisting hospitalization decisions.[7] However, further research is essential to ascertain IL-6 COVID-19 severity screening instrument efficacy.

A meta-analysis of nine studies established a strong correlation between increased IL-6 levels and severe disease. Severe COVID-19 patients displayed an average IL-6 level of 58 pg/mL, while mild cases had 17 pg/mL.[25] Herold *et al*. found IL-6 levels above 80 pg/mL could predict respiratory failure and mechanical ventilation needs, alongside higher neutrophil counts and lower lymphocyte and eosinophil counts, identified as severe disease markers.[6-9] Sabaka *et al.* revealed in their ROC analysis that IL-6 was a more robust hypoxemia marker than CRP, procalcitonin, fibrinogen, ALT, AST, total bilirubin, lymphocyte, neutrophil, and eosinophil counts.[7]

Although this study found no significant correlation between IL-6 levels and lymphocyte numbers, it indicated an inverse relationship between IL-6 and lymphocyte counts in COVID-19 patients. Yang *et al.* research demonstrated dynamic changes in these inflammatory markers reflecting clinical severity in COVID-19 patients.[26] Sari *et al*. study also noted no significant IL-6 levels and lymphocyte counts correlation but found a link to disease severity. Elevated IL-6 levels were associated with higher severity and lower lymphocyte counts, suggesting a potential cytokine storm.[27]

This study noted a mean NLR of 4.775 in moderate cases and 5.950 in severe cases, though not statistically significant. The neutrophil count increased while the lymphocyte count decreased. Liu *et al*. longitudinal COVID-19 analysis over 16 days post-admission found the severe group's neutrophil count continually rose, while lymphocytes showed a two-step pattern, falling consistently during the first week and gradually increasing during the second.[28,29]

These findings aligned with prior research, linking the intensity of lymphopenia, proinflammatory cytokine storms, and disease severity. Virus effects, IL-6, inflammatory tissue exosomes, and elevated blood lactic acid could contribute to lymphocyte deficiency.[29] Notably, neutrophilia, common in COVID-19, mirrored lymphopenia. The NLR trended higher in severe cases in most studies, reflecting elevated neutrophil and reduced lymphocyte counts compared to non-severe cases.[28]

A correlation test between IL-6 levels and NLR revealed a strong correlation. Interleukin-6 increase corresponded to NLR increase, and vice versa. Jurado *et al*. study showed hospitalized patients had IL-6 levels ranging from 15.3 to 94.6 and NLR values from 4.64 to 5.12, consistent with these results. Qin *et al*. research at Tongji Hospital presented similar outcomes. Among 452 patients, non-severe cases had NLR values from 1.8 to 4.9 and IL-6 levels from 3.9 to 41.1. Severe cases had NLR values from 3.3 to 10.0 and IL-6 levels from 9.5 to 54.5. Conclusively, NLR and IL-6 levels escalate with growing patient severity.[22]

Abnormal coagulation function, particularly elevated D-dimer levels, plays a role in COVID-19 development.[30,31] D-dimer levels exceeding 1 µg/mL have been linked to hospitalization. However, the complete correlation between D-dimer and COVID-19 severity remains unclear.[32,33] Yu *et al.* retrospective study and a meta-analysis found significantly elevated D-dimer levels in severe cases, with D-dimer levels above 0.5 µg/mL being associated with severe COVID-19.[32] Lippi and Favaloro indicated lower D-dimer levels in mild/moderate versus severe cases, especially ICU-admitted patients. Yao *et al*. similarly found disease severity correlated with D-dimer levels.[32,34]

Petrilli *et al.* noted abnormal D-dimer levels on admission led to poorer outcomes. Kidney injury affected 43%, thrombosis 20%, and critical illness 45% of patients, with D-dimer independent correlation to outcomes. Normal D-dimer levels are related to better recovery. Inflammation caused by the virus possibly triggers a D-dimer increase, linking inflammation, coagulation pathways, and COVID-19 coagulation events.[34] ICU-treated COVID-19 patients showed elevated D-dimer levels, highlighting venous thromboembolism risks, particularly in severe cases.[32,35]

Beyond thrombosis, high D-dimer levels could indicate severe viral infection, potentially

progressing to sepsis and coagulation dysfunction, common in severe cases. Elevated D-dimer levels might signal an inflammatory response, where cytokines disrupt the coagulation-fibrinolysis balance in the alveoli, activating fibrinolysis and raising D-dimer levels. D-dimer levels above 1 μg/mL pose a poor prognosis risk in COVID-19, and abnormal levels correlate with 28-day mortality.[32]

This study indicated a higher prevalence of comorbidities in the severe COVID-19 category, though not statistically significant. Diabetes mellitus, followed by hypertension and cerebro-cardiovascular diseases, were the most frequent comorbidities. Older males and individuals with cardiovascular disease, diabetes, hypertension, and Chronic Obstructive Pulmonary Disease (COPD) were at elevated risk for severe outcomes and ICU admissions.[36] Various factors, particularly in older adults, contribute to COVID-19 severity, with illness severity classified based on hospitalization need, ICU admission, and mortality, enabling tailored assessments. Diabetes, COPD, hypertension, and malignancy have been linked to poorer prognoses in prior studies.[36,37]

Articles from January to April 2020 demonstrated that comorbidities like hypertension or diabetes heightened the likelihood of severe COVID-19. Older patients, especially those ≥ 65 with comorbidities, faced higher ICU admissions, worse prognoses, and elevated COVID-19 mortality rates. A meta-analysis involving 1.786 patients identified hypertension (15.8%), cardiovascular and cerebrovascular diseases (11.7%), and diabetes (9.4%) as prominent comorbidities.[11,38] A COVID-19-Associated Hospitalization Surveillance Network (COVID-NET) analysis of 1.478 hospitalizations from March 01 to 30, 2020, found 12% of adults had underlying medical conditions. Hypertension (49.7%), obesity (48.3%), chronic pulmonary disease (34.6%), diabetes mellitus (28.3%), and cardiovascular disease (27.8%) were common.[11,39]

Honardoosta *et al.* study revealed a significant link between disease severity and cerebrovascular disease presence (OR 4.85, $p < 0.01$, I2: 2.2%).[36,40] Neurological involvement in COVID-19 is substantial, with SARS-CoV-2 affecting the nervous system via hematogenous or retrograde nervous routes, causing diverse neurological symptoms. CNS-involved patients had lower lymphocyte counts, possibly due to COVID-19-induced immunosuppression. Severe infection-related acute cerebrovascular disease leads to rapid deterioration and high mortality.[36]

Comorbidity patients must take precautions against SARS-CoV-2 infection, given their adverse prognosis. Precautions encompass hand hygiene, distancing, mask usage, and minimizing outings. A global public health campaign is crucial to mitigate comorbidity-driven COVID-19 mortality.

In conclusion, this study highlighted the potential significance of specific biomarkers, such as D-dimer and IL-6, in predicting the severity of COVID-19. While no significant gender-based differences were observed, comorbidities were more common in subjects with severe cases. Understanding the relationships between IL-6 levels, NLR, and lymphocyte count can provide valuable insights into the underlying inflammatory processes associated with COVID-19 severity. However, further research was needed to elucidate the mechanisms involved and improve the early identification and management of severe cases. Evaluating laboratory biomarkers such as D-dimer, IL-6, NLR, and other hematologic parameters concerning disease severity helps identify potential biomarkers to aid in early detection and prognosis assessment of severe COVID-19 cases. These markers could be incorporated into clinical decision-making algorithms and contribute to improved patient management.

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