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The Relationship Between Neutrophil-Lymphocyte Ratio (NLR), C-Reactive Protein (CRP), Interleukin 6 (IL-6), and D-Dimer Levels on the Severity of Covid-19 Patients at RSU Muhammadiyah University of Malang

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ABSTRACT

Background: COVID-19 virus infection has the potential to become severe in individuals who are susceptible or who have comorbid diseases. Natural immunity status in infected sufferers can cause an increase in the number of neutrophil cells and a decrease in the number of lymphocyte cells (increased neutrophil-lymphocyte ratio), serum inter-leukin-6 (IL-6), C-reactive protein (CRP) levels which are associated with severity. covid-19. Hypercoagulability that occurs in COVID-19 infection causes an increase in D-dimer levels, reflecting a response to prothrombotic phenomena and is associated with an increased risk of mortality in COVID-19 patients.

Objective: To determine the relationship between Neutrophil-lymphocyte ratio (NLR), C-reactive protein (CRP), Interleukin-6 (IL-6), and D-dimer levels on the severity of Covid-19 patients at Muhammadiyah Malang University (UMM) Hospital

Method: Using a retrospective design by taking a total sampling of medical record data for COVID-19 patients treated at UMM Hospital in 2021-2022.

Results and Discussion: Spearman correlation on NLR 0.426 (p=0.000), OR=3.67, 95% CI=1.622-8.226, CRP 0.343 (p=0.000) with OR 5.143, 95%CI=1.128-23.448, D- Dimer 0.326 (p=0.000), OR 2.497, 95% CI=1.142-5.458. Meanwhile, the correlation and IL-6 estimation values could not be calculated because the sample size was insufficient. CRP levels in COVID-19 patients are the best predictor of worsening compared to other parameters.

Conclusion: NLR values, CRP levels, and D-Dimer levels are related to the degree of severity in COVID-19 patients.Keywords: Factor, Conversion, RT-PCR COVID-19

Keywords: Covid-19, inter leukin-6, C-Reactive Protein, Neutrophil-Lymphocyte Ratio, D-dimer, degree of severity.

INTRODUCTION

The World Health Organization (WHO) reported on May 4 2023 that Covid-19 infection is an established and ongoing health problem but is no longer a public health emergency of international

concern. (WH0, 2023). This statement means that COVID-19 still needs continued attention to prevent it from becoming a pandemic case again.

The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS CoV-2) virus, which causes COVID-19, infects humans mainly through the respiratory tract and triggers a series of immunological responses. The route for the virus to enter the host cell is determined by the S protein on the virus' surface, which is a glycoprotein that binds to the special receptor acetylcholine esterase-2 (ACE2). After the virus infects the host cell, the antigen-presenting cells (APC) will recognize and present the antigen by involving the major histocompatibility complex (MHC), which will be identified by the virus-specific cytotoxic T lymphocyte (CTLs) lymphocyte cells.(Rauf et al., 2020). The status of natural (innate) immunity in infected sufferers can cause an increase in the number of neutrophil cells, serum inter-leukin-6 (IL-6), and C-reactive protein (CRP) levels, as well as a decrease in the number of lymphocyte cells. An increase in the number of neutrophil cells and a decrease in lymphocyte cells is associated with severity and death. (Rauf et al., 2020). Increased IL-6 levels are associated with increased severity of COVID-19 and worsening of the disease at the cellular level. Increased IL-6 strengthens viral infection by inhibiting Cluster of Differentiation 8 (CD8+) T cell responses, synergizing with IL-17, and increasing the factors Programmed Death 1 (PD-1) and programmed death-ligand 1 (PDL-1). Rapid replication of Severe Acute Respiratory Syndrome COVID-19 (SARS-CoV-2) can cause respiratory infections and increase IL-6 levels. Previous research found that patients with elevated IL-6 levels (>80 pg/mL) were twice as likely to experience respiratory failure as patients with lower IL-6 levels.(Nguyen et al., 2022). COVID-19 patients also experience a state of hypercoagulability, where D-dimer levels reflect a response to prothrombotic phenomena. Elevated D-dimer levels are associated with an increased risk of mortality in COVID-19 patients.(Nemec, Ferenczy and Montgomery, 2022).

MATERIALS AND METHOD

Study design

This study used an analytical observational method with a retrospective cohort research design to determine the relationship between Neutrophil-lymphocyte ratio (NLR), C-reactive protein (CRP), D-Dimer, interleukin-6 (IL-6) levels and the worsening of Covid-19 patients in RSU Muhammadiyah University of Malang from January 2021 to December 2022.

Degree of COVID-19 severity

Patients who are hospitalized are those in the moderate to critical severity level. Determination of the degree of severity based on clinical symptoms, laboratory and radiographic abnormalities, hemodynamics, and organ function, National Institutes of Health (NIH).

1) Moderate degree: Individuals who have clinical symptoms of pneumonia (fever, cough, shortness of breath, rapid breathing) but no signs of severe pneumonia or radiological evidence of lower respiratory tract disease and who have oxygen saturation (SpO2) ≥ 94% on room air.
2) Severe degree: patients with clinical signs of pneumonia (fever, cough, shortness of breath, rapid breathing), individuals who have (SpO2) ≤ 94% in room air; the ratio of partial pressure of arterial oxygen to fraction of inspired oxygen (PaO2/FiO2) <300 with signs of tachypnea with respiratory rate >30 breaths/minute or pulmonary infiltrates >50%.
3) Critical degree: Individuals experiencing acute respiratory failure, septic shock, and multiple organ dysfunction. Patients with severe COVID-19 disease can become critically ill with the development of acute respiratory distress syndrome (ARDS), which tends to occur about one week after the onset of symptoms. (Cascella et al., 2022)

Parameter measurement

The laboratory parameters examined were the Neutrophyl Lymphocyte Ratio (NLR), which was checked using an automatic hematoanalyzer, C-reactive protein (CRP) levels, D-dimer levels and inter leukin-6 (IL-6) levels, which were checked using an immunoassay.

RESULTS AND DISCUSSION

There were 143 patients undergoing Neutrophil Lymphocyte ratio (NLR) examination, 119 patients for C-reactive protein (CRP), 116 patients for D-Dimer, and five patients for interleukin-6 (IL-6). Most patients were found to be between the ages of 40 and 59 years, male, with moderate severity criteria. The most common comorbid diseases are diabetes mellitus and hypertension.

The age range mentioned above is probably due to productive daily activities with high mobility and social activities (Kalantari, 2020). The most common age after the age range above is old age >60 years. In old age, there is a decrease in the production of naive T and B cells, so the function of innate immune cells is impaired, which can cause cells involved in innate immunity not to be activated efficiently during infection. The development of adaptive immune responses is not well coordinated. These changes reduce the effectiveness of lysing the virus and increase the likelihood of triggering a dysregulated immune response (Kang and Jung, 2020).

The majority of hospitalized COVID-19 patients are male. This aligns with research conducted by (Maryati et al., 2022). In this situation, men may have the habit of being active smokers. Active smoking can damage lung tissue and cells, making it easier for SARS-CoV-2 to attack lung tissue, causing more severe symptoms and increasing the risk of death (Haddad et al., 2021). According to research conducted by Jun Mi, the number of patients infected with COVID-19 in male gender also has a higher percentage compared to female patients. The immune response is more intense and stronger in women than men, whereas pro-inflammatory cytokines and chemokines appear to be higher in men. In the case of cytokine storms, the IL-6 receptor is more expressed in male lung

epithelial cells, indicating that men are more susceptible to cytokine storms. The cell type-specific expression of the Angiotensin Converting Enzyme 2 (ACE 2) receptor on type II alveolar epithelial cells is higher in men than in women, so the respiratory system in men is more susceptible to COVID-19 infection.(Jun Mi et al., 2020).

The most common comorbid diseases experienced by COVID-19 patients treated at UMM Hospital are Diabetes Mellitus Type 2 and Hypertension. Ganguli et al. reported that hypertension and diabetes mellitus were the most common comorbid diseases in Covid-19 patients treated in Bangladeshi hospitals (Ganguli et al., 2022). Likewise, Guan et al. reported that patients with confirmed Covid-19 with comorbid hypertension, diabetes mellitus, and coronary heart disease had a severe degree of severity (Guan et al., 2020).

Table 1. Characteristics of Patients Undergoing NLR Examination

Characteristics	Category	Amount	(%)
Age	≥ 60 years	53	25,7
	40-59 years	64	31,1
	≤ 39	26	12,6
Gender	Female	69	48,3
	Male	74	51,7
Severity	Moderate	84	51,5
	Severe	38	23,3
	Critical	21	12,9
Outcome	Live	110	76,9
	Dead	33	23,1
Comorbidity	DM Type 2	39	27,3
	Hypertension	13	9,1
	DM Type 2 dan Hypertension	9	6,3
	No comorbidity	83	57,3

Table 2. Characteristics of Patients Undergoing CRP Examination

Characteristics	Category	Amount	(%)
Age	≥ 60 years	40	33.6
	40-59 years	53	44.5
	≤ 39	26	21.8

Gender	Female	51	42,9
	Male	68	57,1
Severity	Moderate	81	68,2
	Severe	30	25,2
	Critical	8	6,7
Outcome	Live	101	84,9
	Dead	18	15,1
Comorbidity	DM Type 2	26	21,8
	Hypertension	13	10,9
	DM Type 2 dan Hypertension	7	5,9
	No comorbidity	73	61,3

(Secondary data)

Table 3. Characteristics of Patients Undergoing D-Dimer Examination

Characteristic	Category	Amount	(%)
Age	≥ 60 years	44	37.9
	40-59 years	50	43.1
	≤ 39	22	19,0
Gender	Female	54	46,6
	Male	62	53,4
Severity	Moderate	74	63,8
	Severe	28	24,1
	Critical	14	12,1
Outcome	Live	93	80,2
	Dead	23	19,8
Comorbidity	DM Type 2	24	20,7
	Hypertension	8	6,9
	DM Type 2 dan Hypertension	7	6,0
	No comorbidity	77	66,4

(Secondary data)

Table 4. Characteristics of Patients Undergoing IL-6 Examination

Characteristic	Category	Amount	(%)
Age	≥ 60 years	1	20
	40-59 years	1	20
	≤ 39	3	60
Gender	Female	2	40
	Male	3	60
Severity	Moderate	2	40
	Severe	1	20
	Critical	2	40
Outcome	Live		
	Dead	3	60
Comorbidity	DM Type 2	2	40
Penyakit	Hypertension	1	20
Komorbid	DM Type 2 dan Hypertension	1	20
	No comorbidity	3	60

(Secondary data)

On NLR, CRP, and Dimer levels examination, the degree of severity appeared increasingly severe, showing a higher mean value. While only a small number of patients were examined for IL-6 (5 patients), there was an increase in the group with the severe degree category.

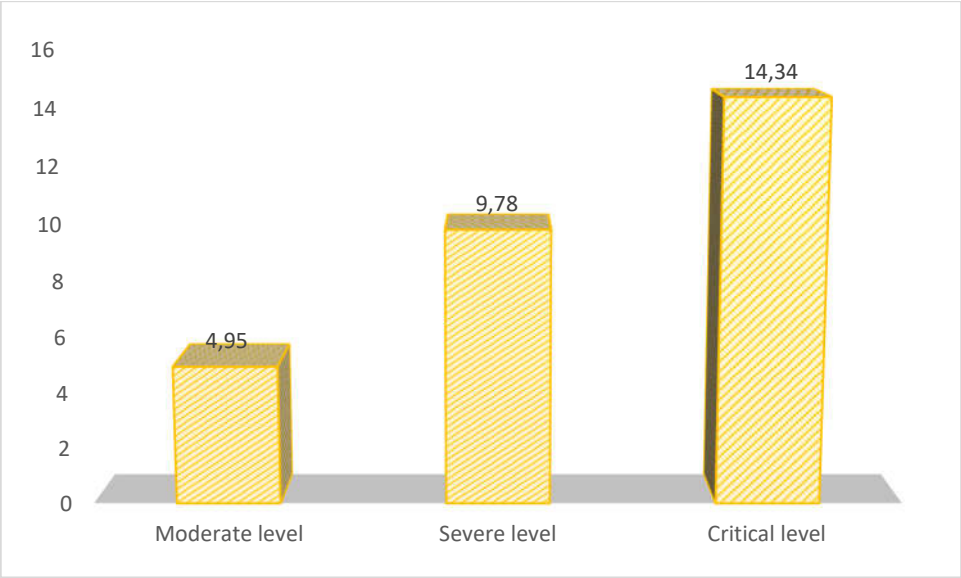

Figure 5.1 Average value of Neutrophil Lymphocyte Ratio

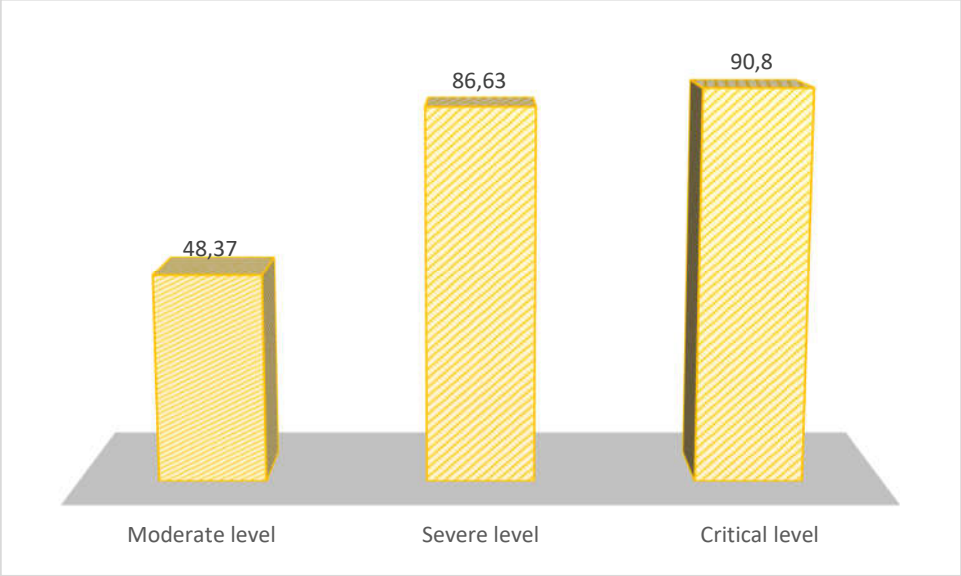

Figure 5.2 Average Value of C-Reactive Protein Levels (mg/L)

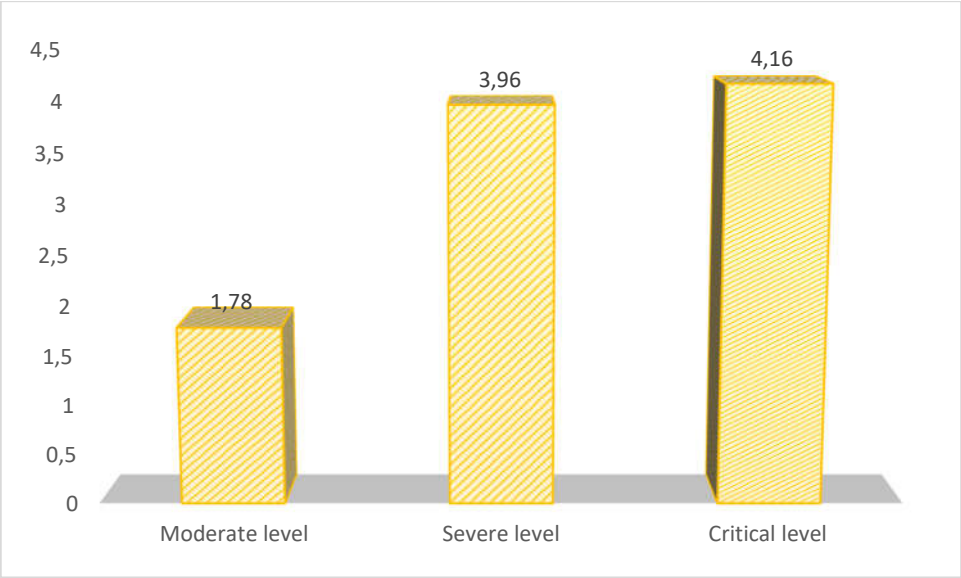

Figure 5.3 Average Value of D-Dimer Levels (mg/L)

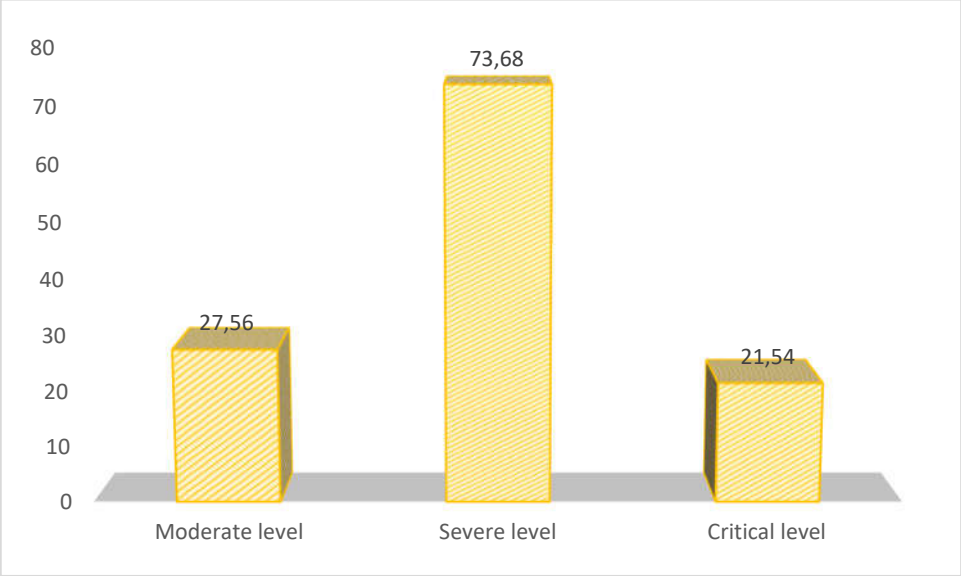

Figure 5.4 Average Value of Inter Leukin-6 Levels (pg/L)

Bivariate analysis shows the relationship between NLR and the severity of COVID-19 patients at RSU UMM using the Spearman correlation value, showing a significant correlation between NLR and severity (correlation coefficient of 0.426 (p-value=0.000), indicating a positive relationship between the level of NLR with the severity level of the patient. This means that the higher the NLR value, the more severe the severity experienced by COVID-19 patients. The data is then converted into an ordinal scale by dividing the NLR into average (<3.13) and increased groups, while the degree of severity into severity groups moderate and a combined group of severe and critical, the Odds Ratio value was obtained, 95% CI=1.622-8.226. The severe degree of disease is caused by excessive

cytokine production, resulting in a state of lymphocyte exhaustion, which has the potential to open the entrance to opportunistic infections. COVID-19 virus -19 can also infect T lymphocyte cells without involving ACE, further contributing to lymphocytopenia and T cell exhaustion. In severe patients, there is a correlation between the advanced stages of lymphocytopenia, and around 83% of COVID-19 patients show lymphocytopenia when hospitalized. (Sapir et al., 2022) (Rauf et al., 2020). NLR is a predictive factor for predicting the early stages of patients infected with COVID-19 who are likely to experience critical illness. Patients aged ≥ 50 and having an NLR ≥ 3.13 are expected to have a critical illness, so they should have rapid access to an intensive care unit if necessary. (Liu et al., 2020)

The Spearman correlation in the CRP examination group was 0.343, indicating a moderate positive relationship between CRP levels and severity. The p-value is 0.000<0.05, indicating this relationship is statistically significant. This means a significant relationship exists between CRP values and the severity of symptoms in COVID-19 patients. The higher the CRP level in the body, the more severe symptoms the patient tends to experience. Meanwhile, the Odds Ratio value is 5.143, 95%CI=1.128-23.448. The SARS-CoV-2 virus enters respiratory epithelial cells via the Angiotensin-converting enzyme 2 (ACE2) receptor and can trigger an immune response by producing inflammatory cytokines accompanied by a weak interferon (IFN) response. The proinflammatory immune response of pathogenic T helper 1 cells and Cluster of Differentiation 14+ (CD14+) membrane-bound immune receptors mediate monocytes. This is followed by the infiltration of macrophages and neutrophils into the lung tissue, resulting in a cytokine storm. Specifically, SARS-CoV-2 can rapidly activate pathogenic T helper 1 cells to secrete proinflammatory cytokines, such as granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-6 (IL-6). GM-CSF further activates CD14 + CD16 + inflammatory monocytes to produce large amounts of IL-6, tumor necrosis factor-α (TNF-α), and other cytokines (Hu, Huang and Yin, 2021). Production of IL-6 by monocytes, dendritic cells and macrophages in patients with severe COVID-19 causes systematic production of cytokines and C Reactive Protein (CRP). CRP production will continue in response to inflammation produced by a cytokine storm that causes an increase in CRP levels (Daneshkhah et al., 2020). In patients with Covid-19 infection, CRP levels correlate with disease severity and are a good predictor of poor outcomes. (Luo et al., 2020)

The Spearman correlation in the D-Dimer examination group was 0.326, indicating a sufficient positive relationship between D-Dimer levels and severity. The p-value is 0.000<0.05, which suggests that this relationship is statistically significant. This means a significant relationship exists between the D-Dimer value and the severity of symptoms in COVID-19 patients. The higher the level of D-Dimer in the body, the more severe symptoms the patient tends to experience. The Odds Ratio value was 2.497, 95% CI=1.142-5.458. The inflammatory response to COVID-19 infection can then cause damage that contributes to thromboinflammation, namely a coagulatory response to systemic inflammation through thrombin formation. Upon infection, monocytes, endothelial cells, and

platelets release proinflammatory cytokines and pro-coagulant microparticles, causing increased leukocyte adhesion and decreased vasculo-protective molecules. The above situation causes NETosis, namely the activation and release of neutrophil extracellular traps, which recruit more inflammatory leukocyte cells and cytokines. In the end, there can be a loss of homeostasis and microvascular damage, known as disseminated intravascular coagulation (DIC) (Sapir et al., 2022). Inflammatory cytokines and virus-specific Toll-like receptors (TLRs) induce monocyte tissue factor (TF) expression, activating the coagulation cascade. Activation of platelets by TLR signalling results in increased platelet reactivity and platelet aggregation. Activation of neutrophils results in the release of neutrophil extracellular traps (NETosis), which leads to activation of coagulation and adhesion to platelets, red blood cells and platelet adhesion molecules. In parallel, activation of coagulation via TF also results in thrombin generation and fibrin formation, which allows cross-linking between platelets and other cellular constituents and results in thrombus formation, leading to activation of coagulation. Thrombi that have formed through various mechanisms will later be degraded by the plasmin enzyme, producing D-dimers (de Bont, Boelens and Pruijn, 2019). D-dimer levels increase as the condition of Covid-19 patients worsens (Hannah M, 2022).

Bivariate analysis and Odds Ratio calculations could not be carried out on inter-leukin-6 levels because the number of samples was too small. IL-6 is a proinflammatory cytokine. The initial phase of pneumonia due to SARS-Cov-2 is characterized by viral replication, which causes direct tissue damage by the virus, which is then followed by the final phase when infected host cells trigger an immune response with the recruitment of T lymphocyte cells, monocytes and the recruitment of neutrophils which release cytokines such as tumour necrosis factor-α (TNF α), granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-1 (IL-1), interleukin-6 (IL-6), IL-1β, IL -8, IL-12 and interferon (IFN)-γ. In severe COVID-19, a cytokine storm can occur due to excessive immune system activation, characterized by high cytokine levels, especially IL-6 and TNF-α in the circulation, causing local and systemic inflammatory responses. Furthermore, there is an increase in vascular permeability, causing pulmonary oedema, which is caused by 1) endothelium as a direct result of damage by viruses and perivascular inflammation, which causes microvascular deposition and microthrombi; 2) dysregulation of the renin-angiotensin-aldosterone system (RAAS) caused by increased viral binding to the ACE2 receptor; c) activation of the kallikrein-bradykinin pathway which increases vascular permeability; d) increased contraction of epithelial cells causes cell swelling and disruption of intercellular connections. SARS-Cov-2 also binds to Toll-like receptors (TLR), thereby inducing the release of pro-IL-1β, broken down into mature and active IL-1β, which mediates lung inflation and even fibrosis. (Cascella M et al., 2022)

CONCLUSION

This study was able to identify that gender, case severity, and comorbid history of COVID-19 patients were factors related to the time of negative conversion RT-PCR of COVID-19. We hope

that these factors can provide early clues to identify patients with longer negative RT-PCR COVID-19 conversion times and can provide more optimal treatment strategies and isolation protocols.\

REFERENCES

Ai, T., Yang, Z., Hou, H., Zhan, C., Chen, C., Lv, W., Tao, Q., Sun, Z., Xia, L. (2020). *Correlation of chest CT and RT-PCR testing in coronavirus disease 2019 (COVID-19) in China: a report of 1014 cases. Radiology*. Wuhan: RSNA Journal. https://doi.org/10.1148/radiol.2020200642

Annechien, B., Jan, H. M., Marijke, F. (2005). *Sex hormones and the immune response in humans.* Netherland : Human Reproduction Update 2005;11(4):411–23 http://dx.doi.org/10.1093/humupd/dmi008

Bennasrallah C., Zemni, I., Dhouib, W., Sriha, H., Mezhoud N et al. (2021). *Factors associated with a prolonged negative conversion of viral RNA in patients with COVID-19*. Tunisia: International Journal of Infectious Diseases 105 (2021) 463–469 https://doi.org/10.1016/j.ijid.2021.02.089

Chen, W.J., Yang, J.Y., Lin, J.H., Fann, C.S., Osyetrov, V., King, C.C., Chen, Y.M., Chan, H.L., Kuo, H.W., Liao, F., et al. (2006). *Nasopharyngeal shedding of severe acute respiratory syndrome-associated coronavirus is associated with genetic polymorphisms*. Taipei: *Clinical Infectious Diseases*, Volume 42, Issue 11, 1 June 2006, Pages 1561–1569 https://doi.org/10.1086/503843

Cortegiani, A., Ingoglia, G., Ippolito, M., Giarratano, A., Einav, S. (2020). *A systematic review on the efficacy and safety of chloroquine for the treatment of COVID-19*. Italy: J. Crit. Care https://doi.org/10.1016/j.jcrc.2020.03.005

Hu, X., Xing Y., Jing, J., Wei, N., Jiwei, L., Dan, Z., Xin, S., Ruqin, G., Fachun, J. (2020). *Factors Associated with Negative Conversion of Viral RNA in Patients Hospitalized with COVID-19*. China: Science of The Total Environment 728 (August): 138812. https://doi.org/10.1016/j.scitotenv.2020.138812

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., Zhang, L., Fan, G., Xu, J., Gu, X., et al., (2020). *Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China*. Wuhan: The Lancet Vol. 395 https://doi.org/10.1016/S0140-6736(20)30183-5.

Ling, Yun, Shui-Bao Xu, Yi-Xiao Lin, Di Tian, Zhao-Qin Zhu, Fa-Hui Dai, Fan Wu, et al. (2020). *Persistence and Clearance of Viral RNA in 2019 Novel Coronavirus Disease Rehabilitation Patients*. China: Chinese Medical Journal https://doi.org/10.1097/CM9.0000000000000774

Liu, K., Chen, Y., Lin, R., Han K., (2020). *Clinical features of COVID-19 in elderly patients: A comparison with young and middle-aged patients*. China: Journal of Infection https://doi.org/10.1016/j.jinf.2020.03.005

Liu, Y., Yan, L., Wan, L., Xiang, T., Le, A., Liu, J., et al. (2020). *Viral dynamics in mild and severe cases of COVID-19*. China: Lancet Infectious Disease doi: http://dx.doi.org/10.1016/S1473-3099(20)30232-2

Mo, P.Z., Deng, L.P., Liu, X.P., Gao, S.C., Liang, K., Luo, M.Q., Chen, T.L., Song, S.H., Ma, Z.Y., Chen, X.P., et al. (2020). *Risk factors for delayed negative conversion of SARSCoV-2 in patients with COVID-19 pneumonia: a retrospective cohort study*. China: Epidemiology and Infection 148, e293, 1–7. https://doi.org/ 10.1017/S0950268820002940

Our World in Data, (2020). *Coronavirus Disease (COVID-19) – Statistics and Research*. Oxford Martin School, The University of Oxford, Global Change Data Lab Available from. https://ourworldindata.org/coronavirus/

PDPI, PERKI, PAPDI, PERDATIN, IDAI. (2020). *Pedoman Tatalaksana COVID-19 Ed. 3*., Jakarta

World Health Organization. (2020). *Criteria for releasing COVID-19 patients from isolation.* https://www.who.int/publications/i/item/criteria-for-releasing-COVID-19-patients-from-isolation

Wuhan Municipal Health Commission (WMHC), (2020). *Report of clustering pneumonia of unknown etiology in Wuhan City*. Available from. http://wjw.wuhan.gov.cn/front/web/showDetail/2019123108989

Xing, Y.H., Ni, W., Wu, Q., Li, W.J., Li, G.J., Wang, W.D., Tong, J.N., Song, X.F., Wong, G.W.K., Xing, Q.S., (2020). *Prolonged Viral Shedding in Feces of Pediatric Patients with Coronavirus Disease 2019*. China: J. Microbiology Immunology 2020 https://doi.org/10.1016/j. jmii.2020.03.021

Xu, Y., Li, X., Zhu, B., Liang, H., Fang, C., Gong, Y., Guo, Q., Sun, X., Zhao, D., Shen, J., et al., 2020. *Characteristics of pediatric SARS-CoV-2 infection and potential evidence for persistent fecal viral shedding*. China: Nat. Med. 26, 502–505. https://doi.org/10.1038/s41591-020-0817-4

Yang, Y., Hu, X., Xiang, L., Fu, P., Fang, W., Li, W., Zhang, L., Sun. F. (2021). *Clinical characteristics of hospitalized mild/ moderate COVID-19 patients with a prolonged negative conversion time of SARS-CoV-2 nucleic acid detection*. China: BMC Infectious Diseases (2021) 21:141 https://doi.org/10.1186/s12879-021-05851-z