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Correlation of Interferon Gamma (IFN-γ), Platelet to Lymphocyte Ratio (PLR), and Mortality of Covid-19 Patients

Giovanni Antonio Wijanto[1], Agustin Iskandar[2], Catur Suci Sutrisnani[3]

[1]Universitas Brawijaya, Faculty of Medicine,
Clinical Pathology Department

[2]Universitas Brawijaya, Faculty of Medicine,
Clinical Pathology Department

[3] Universitas Brawijaya, Faculty of Medicine,
Clinical Pathology Department

Abstract:- Severe and critical cases of COVID-19 can occur due to a cytokine storm involving many inflammatory mediators including platelets, lymphocytes, cytokines and chemokines. Platelets are one of the factors that affect inflammation indirectly, while lymphocytes will be greatly affected in the state of COVID-19 where both can decrease due to various reasons. One of the cytokines involved in COVID-19 is IFN-γ which can trigger other cytokines in a cytokine storm in COVID-19 and various other conditions related to COVID-19. In COVID-19, there is also an increase in Platelet-To-Lymphocyte Ratio (PLR) and IFN-γ. The increase in PLR and IFN-γ is expected to be one of the means of predicting patient outcomes. To see the correlation between PLR, IFN-γ, and mortality, we conducted a logistic regression test, path analysis, and survival analysis. There were no significant differences in IFN-γ levels, PLR, and length of treatment between survivor and non-survivor patients. In addition, there were no significant differences in all characteristics of subjects with mild-moderate, severe, and very severe severity. It can be concluded that IFN-γ and PLR were not correlated with each other and when combined, IFN-γ and PLR showed no difference in prognosis obtained from survival analysis.

Keywords:- *COVID-19, Interferon Gamma, Platelet-To-Lymphocyte Ratio, Mortality.*

I. INTRODUCTION

The world is experiencing multiple impacts due to the novel coronavirus (SARS-CoV-2 or COVID-19) pandemic. It began with an outbreak of pneumonia of unknown cause in Wuhan city, Hubei province, China and was reported to the World Health Organization (WHO) office in China on 31 December 2019.[1] As of May 2023 there were a total of 766,895,075 cases of COVID-19, causing 6,935,889 deaths (global case fatality rate = 2.1%) while in Indonesia there were 6,806,544 cases with 161,754 deaths (Indonesian case fatality rate = 2.8%).[2] Although the status of COVID-19 has become endemic and remains circulating in the global population for years to come, COVID-19 may cause outbreaks in areas where SARS-CoV2 was previously eliminated.[3]

Cytokine storm is a key factor in the pathogenesis of COVID-19 severity, one of the cytokines that plays a role is IFN-γ. IFN-γ has pleiotropic properties and is thought to be one of the cytokines that has a significant effect in the process of cytokine storms that cause apoptosis together with TNF-α (Karki et al., 2020), which affects multiorgan damage and death due to COVID-19.[4,5] Several studies have shown that IFN-γ increases significantly compared to TNF-α in the occurrence of cytokine storms in COVID-19. Several studies have shown an increase in IFN-γ in COVID-19 patients. The pathomechanism of increased IFN-γ in COVID-19 begins with the entry of SARS-CoV2 into cells through ACE2 and TMPRSS2 receptors, then viral replication occurs followed by the process of pyroptosis and Damage Associated Molecular Pattern (mediated by ATP, nucleic acids, and oligomeric ASC). This is responded by other cells around the infected cells by increasing the production of proinflammatory cytokines and chemokines that attract monocytes, macrophages and T cells. Inflammation is further increased because T cells will produce IFN-γ. Excessive infiltration of proinflammatory cells can lead to cytokine storms, multiorgan failure and death.[6]

PLR seems to reflect inflammation-related changes in platelet and lymphocyte levels and pro-thrombotic state, which is one of the main mechanisms of the lesions caused by SARS-CoV-2. The PLR, which is thought to reflect the inflammatory process, is thought to provide a prognostic element or monitoring of COVID-19 disease activity. PLR was also found to be increased in severe COVID-19 patients compared to mild-moderate COVID-19 patients. The increase in PLR in severe COVID-19 patients can reach approximately 4 times. This is in accordance with several studies on COVID-19 that show a decrease in lymphocytes is more severe than a decrease in platelets so that PLR shows the potential to determine the prognosis of COVID-19 patients, especially in facilities with limited resources.[7,8]

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This study aims to determine whether IFN-γ and PLR can be predictors of mortality in COVID-19 patients.

II. METHODS

A. Design

This study is an analytical descriptive study using a retrospective cohort design. The study used human venous blood samples for measurement of IFN-γ and PLR levels. Both parameters will be analysed with the output of COVID-19 patients who are the subject of the study. The purpose of this study is to determine the correlation between IFN-γ and PLR levels with mortality in COVID-19 patients treated at RSUD Dr Saiful Anwar Malang.

B. Subjects

The population subjects in this study were inpatients at RSSA who were diagnosed as confirmed Covid-19 which had been proven by the RT-PCR method. This study has received approval from the RSSA ethics committee (400/011/K.3/302/2021). The inclusion criteria for subjects in this study are:

- Male and female patients with Covid-19 confirmed by RT-PCR method from nasopharyngeal or oropharyngeal swab specimens.
- Age 17 - 60 years
- Onset time ≤14 days from the time the patient has symptoms to the confirmation of Covid-19

The exclusion criteria for subjects in this study are as follows:

1. Women who are pregnant or breastfeeding
2. Having coagulation disorder disease (immune thrombocytopenia purpura, myelodysplastic syndrome, TTP, etc.) before being diagnosed as COVID-19. The data is obtained through the patient's medical record.
3. Have comorbidities that may obscure the results of the study, such as malignancy, liver disease, acute hepatitis, HIV-AIDS.

C. Measurement

IFN-γ levels were examined using the sandwich Enzyme-Linked Immunosorbent Assay (ELISA) method. The tool used for reading is ZENIX-320 Microplate Reader. The reagent used was LEGEND MAXTM Human IFN-γ ELISA KIT reagent. This kit has a minimum detection level of 5.6 pg/mL and can use serum or plasma samples. Determination of IFN-γ levels in serum was determined by the calibration curve method using a spectrophotometer. The examination of platelet and lymphocyte levels requires a venous blood sample collected in an EDTA BD Vacutainer tube. The blood was then examined using a Sysmex XN-1000 haematology analyser that applies the Fluorescence Flow Cytometry (FFC) method.

D. Statistical Analysis

Differences in IFN-γ and PLR levels between survivor and non-survivor groups were tested by t-test on normally distributed data and Mann Whitney test for non-normally distributed data. Analyses to assess the correlation between IFN-γ and PLR were performed by Pearson test on normally distributed data and Spearman test on non-normally distributed data. Analysis to examine the relationship between IFN-γ levels and PLR values to mortality using logistic regression analysis.'

III. RESULTS

A. Demographics

A total of 84 patients were confirmed as COVID-19 from RT-PCR results who met the inclusion criteria during the study period from October 2020 to June 2021. This study aims to determine the performance of IFN-γ and PLR as predictors of mortality in COVID-19 patients. Table 1 shows the characteristics of the study subjects in general.

Characteristic	**n = 84** **f (%) or median (Q1-Q3)**
Gender	
Male	51 (60,7%)
Female	33 (39,3%)
Age (in year, mean±SD)	57,30 ± 12,42
IFN-γ (pg/mL)	14,66 (12,07-19,92)
Platelet (**µ**L)	243000 (185000-317750)
Absolute Lymphocyte	(734,56-1370,52)
PLR	220 (1,46-3.42)
Length of Stay (Days)	10,0 (6-17)
Outcome	
Alive	47 (56,0%)
Death	37 (44,0%)
Comorbid	
Diabetes Mellitus	
Yes	45 (53,6%)
No	39 (46,4%)
Heart Failure	
Yes	16 (19,0%)
No	68 (81,0%)
Obesi**ty**	
Yes	43 (51,2%)
No	41 (48,8%)
Renal Insufficiency	
Yes	23 (27,4%)
No	61 (72,6%)
H**ypertension**	
Yes	52 (61,9%)
No	32 (38,1%)

B. IFN-γ Correlation with PLR as a Predictor of Mortality

From the results of the analysis of differences in IFN-γ and PLR between survivor and nonsurvivor COVID-19 patients, both were found to have a significant relationship. To find out whether there is a correlation between IFN-γ and PLR as a predictor of mortality, a Spearman correlation analysis was conducted. The results of the Spearman correlation between IFN-γ and PLR as a predictor of mortality obtained a sig of 0.602 (sig> 0.05) which indicates that there is no significant relationship. The magnitude of the

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correlation coefficient is 0.058 (<0.2) in the very weak category with a negative direction.

C. Logistic Regression of the Effect of IFN-γ with PLR on Mortality of COVID-19 Patients

Logistic regression mulitvariate analysis was used to predict the relationship between IFN-γ and PLR levels and mortality (effect) using the logistic regression test and the results showed that the ability of the independent variables (IFN-γ and PLR) to predict the dependent variable (mortality of COVID-19 patients) was 56.0% in the model, and the remaining 44.0% was influenced by other factors outside the model.

From the results of the multivariate test with logistic regression, it is known that IFN-γ levels do not significantly affect the mortality of COVID-19 patients (p=0.423; OR 1.004 (0.994 - 1.014)). OR>1 means that the higher IFN-γ levels increase the chance of possible death of COVID-19 patients. PLR also had no significant effect on the mortality of COVID-19 patients (p=0.059; OR 1.780 (0.979 - 3.238)). OR> 1 means that the higher the PLR level increases the chance of possible death of COVID-19 patients.

D. Combination of IFN-γ and PLR levels as predictors of COVID-19 patient mortality

The AUC between IFN-γ and PLR can also be combined. The results of the AUC of IFN-γ and PLR levels in determining the mortality of COVID-19 patients were 49.0% (95% CI 36.0%-62.0%) with a value of p=0.875. The AUC value obtained was low and statistically insignificant. The AUC results of IFN-γ and PLR can be seen in the following figure.

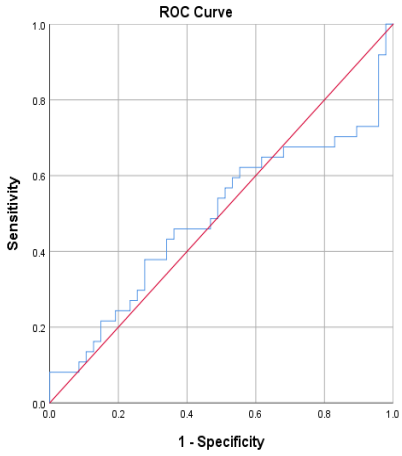

Fig 1. ROC Curves of IFN-γ and PLR Levels of COVID-19 Patients

IV. DISCUSSION

In this study, IFN-γ and PLR did not have a significant relationship. This was shown by the results of the Spearman correlation which showed no significant relationship between IFN-γ and PLR on mortality. We also conducted a path analysis between the variables IFN-γ and PLR, and mortality as the outcome, where the path analysis showed that IFN-γ and PLR were not interrelated, and each variable, namely IFN-γ and PLR, did not affect mortality.

On logistic regression analysis, we found that IFN-γ and PLR could predict 56.0% of mortality in this study model. In addition, we also found that IFN-γ had an odds ratio of 1.004 (p=0.423), and PLR had a higher odds ratio of 1.780 (p=0.059). In this study, there was no significant value of either IFN-γ or PLR in predicting mortality. In line with this finding, the association size of exposure was found to be 1.004 and 1.780 which are also low enough to influence mortality. This is because IFN-γ and PLR are influenced by many factors, where in several studies it was found that low or high IFN-γ could affect COVID-19 mortality so that its use in predicting mortality is still a controversy. In many studies PLR is said to have increased in COVID-19 but PLR is determined by platelet and lymphocyte values that have changed in COVID-19 so that the use of PLR in assessing the risk of COVID-19 mortality is difficult to do.

The absence of correlation between IFN-γ and PLR may occur due to many factors. At the time of this study, there were no studies that examined the correlation between IFN-γ and PLR. But biologically, IFN-γ is a cytokine produced mainly by cells of the innate immune system, including lymphocytes such as NK cells, innate lymphoid cells (ILCs), and cells of the adaptive immune system, such as T helper 1 (TH1) cells and CD8+ and CD4+ cytotoxic T lymphocytes. So there is a possibility that there is a correlation between decreased IFN-γ production and lymphopenia, where based on existing studies lymphopenia often occurs in severe or critical COVID-19 patients accompanied by abnormalities in CD4 + & CD8 + cells. The state of lymphopenia in normal platelet conditions will cause an increase in PLR values, but in conditions accompanied by thrombocytopenia such as in most severe or critical COVID-19, PLR values can be normal or even decreased depending on the degree of thrombocytopenia and lymphopenia that occurs. This may be one of the factors affecting the correlation between IFN-γ and PLR.[7-9]

There are several limitations in this study. Measurement of IFN-γ and PLR levels was only carried out once at the beginning of the patient's arrival at the hospital without knowing and considering the time when symptoms first appeared. In addition, the increase in IFN-γ and PLR levels is not only influenced by COVID-19, comorbid factors such as renal insufficiency and obesity also contribute to the increase in these two variables so that IFN-γ and PLR cannot accurately describe their role in COVID-19. This study also did not measure platelet and lymphocyte function, both of which are involved in the pathophysiology of COVID-19.

V. CONCLUSION

Research on IFN-γ and PLR with other research designs to have better results can be considered. Comorbid factors in

COVID-19 patients need to be considered so as not to cause bias in IFN-γ and PLR values, especially in comorbidities that increase IFN-γ and PLR levels. The use of other markers that look at the function of platelets and lymphocytes can be considered because existing studies show an association between COVID-19 and platelet and lymphocyte function.

Compliance with Ethical Standards

Patients consent: obtained

Conflict of interest. The authors declare no conflict of interest relevant to this manuscript.

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