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Correlation of Neutrophil to Lymphocyte Ratio with Interleukin-10 in Diagnosis and Monitoring of Coronavirus Disease-19 Patients

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Abstract

BACKGROUND: The enforcement of diagnosis and monitoring of therapy success in SARS-Cov-2 infection, which causes COVID-19 disease, necessitates laboratory tests that may assess and identify patients prior to developing critical circumstances requiring additional treatment. The Neutrophil to Lymphocyte Ratio (NLR) and Interleukin-10 (IL-10) testing are two laboratory procedures used.

AIM: This study aims to determine the correlation between NLR and Interleukin-10 (IL-10) in diagnosing and monitoring COVID-19 patients.

METHODS: An observational analytic cross-sectional design enrolled about 73 COVID-19 patients who met the inclusion criteria and were willing to participate in the study. The levels of NLR and IL-10 were assessed by Sysmex XS-800i Automated Hematology Analyzer and sandwich ELISA methods. Data were analyzed using SPSS version 17 for Windows.

RESULTS: A median of NLR values was 4.02 (1.24-47.89), following IL-10 concentration was 1.870 (0.110–33.368) pg/mL. There was a significant difference in NLR values between critical and non-critical categories (p = 0.000), geriatric and non-geriatric groups (p = 0.006), as well as in groups with comorbid and without comorbidities (p = 0.006). Meanwhile, a significant difference in IL-10 levels was only found between critical and non-critical categories (p = 0.000). There was a moderately significant positive correlation between NLR and IL-10 in COVID-19 patients (r = 0.411; p = 0.000).

CONCLUSION: There is a significant positive correlation between NLR values and IL-10 levels in COVID-19 patients.

Introduction

Coronavirus disease 2019 (COVID-19), which started in Wuhan, China, has spread worldwide and has been declared an international pandemic by the WHO. Globally, confirmed cases have occurred in 216 countries and currently, Indonesia is a country with local transmission [1].

COVID-19 is caused by a novel enveloped RNA beta-coronavirus currently named Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) [2]. COVID-19 patients can be categorized into mild, moderate, severe, and critical types. Prevention of mild and moderate cases to progress is crucial in preventing mortality. However, the factors that lead to worsening are still not clearly understood [3]. Symptoms of shortness of breath are the main characteristics of severe and critical COVID-19 patients. The median duration from the appearance of symptoms to the onset of shortness of breath is between 5 and 8 days. It is necessary to have a simple laboratory test that is able to predict the progression of early-stage COVID-19 [3].

Neutrophil to Lymphocyte Ratio (NLR) is one of the important laboratory parameters in monitoring COVID-19 patients related to changes in the characteristics of the lymphocyte count [3]. A decrease in the number of lymphocytes is associated with disease progression, so it is estimated that the number of lymphocytes can be used as a potential predictor. NLR has been widely used as a marker of the severity of the bacterial infection and to determine the prognosis of patients with pneumonia, tumors, and other malignancy [4], [5]. An increase in NLR indicates a poor clinical prognosis. SARS-CoV-2 plays a role in T lymphocytes and the destruction of T lymphocytes is an important factor that causes the patient's condition to worsen [3]. In addition, patients with severe clinical symptoms may experience bacterial infections due to low immune system function. Hence, it is estimated that NLR is one of the important factors in determining disease worsening and has significant predictive value [3], [6].

Cytokine release is an important factor that exacerbates disease progression. Various studies have described abnormal levels of cytokines and chemokines in patients such as IL-1, IL-2, IL-4, IL-6, IL-7, IL-10, IL-12, IL13, IL-17, Macrophage Colony- Stimulating Factor (M-CSF), Granulocyte Colony-Stimulating Factor (G-CSF), Granulocyte-Macrophage Colony Stimulating

Factor (GM-CSF), 10 kD-Interferon Gamma-Induced Protein (IP-10), IFN-γ, Monocyte Chemoattractant Protein- 1 (MCP-1), Macrophage Inflammatory Protein 1-α (MIP 1-α), Hepatocyte Growth Factor (HGF), TNF- α, and Vascular Endothelial Growth Factor (VEGF) [7], [8]. In SARS-CoV-2 infection, there is a decrease in antiviral defenses associated with the innate immune response and an increase in the production of inflammatory cytokines or the so-called cytokine storm. Higher levels of IL-6 and IL-10 and lower levels of CD4+ and CD8+ have also been observed in patients with COVID-19 which correlate with disease severity [7], [8].

Interleukin-10 (IL-10) is a type 2 cytokine that inhibits the production of proinflammatory cytokines (e.g., IFN-γ, TNF-, IL-1β, and IL-6) in various cell types and prevents dendritic cell maturation by blocking IL-12 [9]. IL-10 inhibits the expression of Major Histocompatibility Complex (MHC), which has an important role in cell immunity [9]. Various studies have detected IL-10 in patients with COVID-19 and associated its levels with disease severity and progression [8], [9]. However, previous studies provided inconsistent results when combined with other pro-inflammatory cytokines in the progression of COVID-19. Based on those mentioned above, this study aims to analyze the correlation between NLR and IL-10 levels in COVID-19 patients undergoing treatment at Universitas Udayana Academic Hospital, Bali, Indonesia.

Methods

This study is an analytical observational study with a cross-sectional design to determine the correlation of NLR with IL-10 in diagnosing and monitoring COVID-19 patients. The research was conducted in the isolation room and the Clinical Pathology Laboratory of the Universitas Udayana Academic General Hospital, starting in January 2021 until the number of samples was met. The study sample was 73 confirmed COVID-19 patients hospitalized at Universitas Udayana Academic General Hospital, Bali, Indonesia, who met the inclusion criteria using the consecutive technique.

Neutrophil to Lymphocyte Ratio (NLR) was assessed by calculating the absolute number of neutrophils divided by the absolute number of lymphocytes from a complete blood count. Meanwhile, Interleukin-10 (IL-10) examination was performed on plasma samples examined using the sandwich-ELISA method. After obtaining informed consent from the patient, 3 ml of venous blood was taken, which was accommodated in the EDTA tube for complete blood examination using the Sysmex XS-800i Automated Hematology Analyzer.

The data obtained will then be subjected to data processing and statistical analysis using SPSS version 17 for Windows. All data obtained in this study were analyzed descriptively. The results will be presented in the form of Mean±Standard Deviation (SD) if the data is normally distributed and in the form of the median (minimum-maximum) if the data is not normally distributed. The respondent's characteristic data is displayed in the form of absolute numbers and percentages. The correlation analysis between NLR and IL-10 levels used the Spearman correlation test because the data were not normally distributed with a significance level of =0.05. Meanwhile, the multivariate test was performed using linear regression to assess the relationship between NLR and IL-10 levels after controlling for confounding variables analytically. Statistical significance was evaluated based on the 95% Confidence Interval (CI 95%) and p-value.

Results

In this study, the mean age of the patients was 52.28 ± 14.88 years, with the percentage of geriatric patients (≥ 60 years) of 30.1% and non-geriatric patients (<60 years) of 69.9%. The number of male patients was more than the number of female patients, namely 46 (63%) male patients and 27 (37%) female patients (Table 1).

Table 1: Baseline characteristic of respondents

Variable	Mean ± SD	Frequency n (%)	Median (minimum-maximum)
Age (Years)	52.28 ± 14.88		
Age groups (years)			
Geriatric (≥60)		22 (30.1)	
Non-geriatric (<60)		51 (69.9)	
Gender			
Male		46 (63.0)	
Female		27 (37.0)	
Hemoglobin (g/dL)			13.30 (8.20–17.30)
Leukocytes (10^3/μL)			7.83 (3.25–28.23)
Neutrophils (10^3/μL)			5.14 (1.73–25.92)
Eosinophils (10^3/μL)			0.03 (0.00–1.00)
Basophils (10^3/μL)			0.01 (0.00–0.06)
Lymphocytes (10^3/μL)			1.13 (0.22–4.78)
Monocytes (10^3/μL)			0.55 (0.08–1.48)
Platelets (10^3/μL)			221 (34–649)
Clinical condition category			
Critical		36 (49.3)	
Non-Critical		37 (50.7)	
Comorbidities			
Yes		46 (63.0)	
No		27 (37.0)	

The results of complete blood examination on the research subjects showed that the median hemoglobin level was 13.3 (8.2–17.3) g/dL, followed by the leukocyte count of 7.83 (3.25–28.23) × 10^3/L, absolute neutrophil count of 5.14 (1.73–25.92) × 10^3/μL, absolute eosinophil count of 0.03 (0.00–1.00) × 10^3/μL, absolute basophils of 0.01 (0.00–0.06) × 10^3/μL, absolute lymphocytes of 1.13 (0.22–4.78) × 10^3/μL, monocytes absolute of 0.55 (0.08–1.48) × 10^3/μL, and the platelet count in the study subjects was 221 (34–649) × 10^3/μL (Table 1).

The median NLR value for all research subjects was 4.02 (1.24–47.89), while in the critical category group was 13.54 (1.99–47.89), followed by the median NLR value in the geriatric group (≥60 years) of 10.84 (1.30–47.89)

years and the comorbid group of 6.32 (1.30–47.89) (Table 2). The Mann-Whitney test showed significant differences in NLR values between critical and non-critical categories (p = 0.000), geriatric and non-geriatric groups (p=0.006), as well as in groups with comorbid and without comorbidities (p = 0.006) (Table 2).

Table 2: Mann-Whitney test to the NLR values in COVID-19 patients

NLR values	Median (minimum-maximum)	p value
Overall subjects	4.02 (1.24–47.89)	
Clinical category		
Critical	13.54 (1.99–47.89)	0.000*
Non-critical	1.94 (1.24–5.20)	
Age groups (years)		
Geriatri (≥60)	10.84 (1.30–47.89)	0.006*
Non geriatri (<60)	2.80 (1.24–32.00)	
Comorbidities		
Yes	6.32 (1.30–47.89)	0.000*
No	1.99 (1.24–23.63)	

*Statistically significant if p < 0.05.

The median value of IL-10 examination in all study subjects was 1.870 (0.110–33.368) pg/mL. Meanwhile, the median value of IL-10 in critical research subjects was 3.080 (0.660–33.368) pg/mL, followed by the geriatric group (≥60 years) of 1.925 (0.220–26.240) pg/mL, and the group with comorbidities of 2.145 (0.220–33.368) pg/mL (Table 3).

Table 3: Mann-Whitney test to the IL-10 levels in COVID-19 patients

IL-10 (pg/mL)	Median (minimum-maximum)	p value
Overall subjects	1.870 (0.110–33.368)	
Clinical category		
Critical	3.080 (0.660–33.368)	0.000*
Non-critical	1.320 (0.110–7.700)	
Age groups (years)		
Geriatri (≥60)	1.925 (0.220–26.240)	0.467
Non geriatri (<60)	1.760 (0.110–33.368)	
Comorbidities		
Yes	2.145 (0.220–33.368)	0.070
No	1.430 (0.110–7.700)	

*Statistically significant if p value < 0.05.

The Mann-Whitney test results showed a significant difference in IL-10 levels between research subjects with critical and non-critical categories (p = 0.000). However, the results of this study showed that there was no significant difference in IL-10 levels in the geriatric and non-geriatric groups (p = 0.467) or the comorbid and no comorbid groups (p = 0.070) (Table 3). In contrast, the results of the Spearman correlation showed a moderately significant positive correlation between NLR and IL-10 (r=0.411; p=0.000) (Table 4).

Table 4: Correlation of NLR values with IL-10 levels in COVID-19 patients using Spearman's correlation

Variable	IL-10 levels (pg/mL)	
	r	p value
NLR	0.411	0.000*

*Statistically significant if p < 0.05.

Discussion

Most of the research respondents had a mean age of 52.28 ± 14.88 years for 73 research subjects. The number of research subjects in this study is almost the same as the previous studies with COVID-19 patients [3], [10], [11]. Han *et al*., conducted a larger number of research subjects involving 102 COVID-19 patients classified into moderate, severe, and critical clinical symptom groups and healthy control groups [12]. The age range of the study subjects was also similar to previous studies with median ages of 48 and 51 years [7], [10].

The results of this study indicate that most of the research respondents are male. COVID-19 patients are more commonly found in adults over 18 years of age with a predominance in males and mortality is more commonly found in those aged over 65 years and adults with comorbidities such as cardiovascular, respiratory, endocrine, diabetes mellitus, and immunocompromised diseases, which are more prone to serious complications to COVID-19 patients [12], [13].

The description of a complete blood count in COVID-19 patients is in the form of lymphopenia with or without leukopenia. Absolute lymphocyte count <1.0 × 103/μL is associated with severe disease symptoms. The number of eosinophils also decreased. Platelet counts are usually normal or slightly decreased [13], [14].

Several laboratory studies have shown that the SARS-CoV-2 virus is cytopathic, which causes damage to the lungs. The amplification of the virus causes the activation of the host immune response to eliminate the virus, which consists of a natural immune response that recognizes pathogen-associated molecular patterns (PAMPs) and an adaptive immune response that plays a role in the synthesis and release of cytokines, where the occurrence of cytokine storms plays an important role in the pathogenesis of cases. severe in COVID-19 [12].

The study of Han H *et al*. stated an increase in the cytokines TNF-α, IFN-γ, IL-2, IL-4, IL-6, and IL-10 and CRP in COVID-19 patients compared to healthy controls, where IL-10 increased 37% and IL-6 increased twofold compared to healthy controls [12]. It was found that IL-10 levels increased in disease progression and it is estimated that IL-10 can be used as a predictor for high-risk patients who will experience clinical deterioration [12]. A previous study by Huang C *et al*., also stated that when compared to non-ICU patients, ICU patients had higher plasma levels of IL-2, IL-7, IL-10, GSCF, IP10, MCP1, MIP1A, and TNF-α [7].

Interleukin-10 (IL-10) has an immune regulatory property in protecting the lungs against damage and protection against these infectious processes. Interleukin-10 (IL-10) is a negative regulator of the initiation of adaptive T cell responses [9]. It is assumed that this activity fights hyper inflammation and inhibits the antiviral defense in COVID-19. The absence of IL-10 in SARS-CoV-2 infection contributes to early deterioration because IL-10 protects the lung from early immune-mediated damage and plays an

important role in viral clearance. In general, various innate immune cells release IL-10 in early infection, whereas T cells, especially Tregs and NK cells, appear to predominate at an advanced stage. This population of cells is functionally exhausted and/or decreased in the peripheral blood of severe COVID-19 patients [14].

Conclusion

The results of this study indicate that there is a moderately significant positive correlation between NLR values and IL-10 levels in COVID-19 patients. However, the authors realize that further research needs to be carried out with a prospective cohort study design with continuous data collection so that accurate data on IL-10 levels can be obtained as a predictor according to the course of the disease and clinical severity.

Ethics Consideration

Ethics approval has been obtained from the Ethics Committee, Faculty of Medicine, Universitas Udayana, Sanglah General Hospital, Bali, Indonesia, with number 2372/UN14.2.2.VII.14/LT/2021.

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Author Contribution

All authors equally contribute to the study from the conceptual framework, data acquisition, data analysis, until reporting the study results through a manuscript for publication.

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