The effect of anxiety, depression, and stress on the immune response of Covid-19 patients treated in a special isolation room of dr. Soetomo Hospital Surabaya

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Abstract

Background: SARS-CoV-2 infection causes anxiety, depression, and stress that affect the immune response. **Objective**: This study aims to analyze the effect of anxiety, depression, and stress on the Neutrophil-Lymphocyte Ratio (NLR) and Interleukin 1 beta (IL-1β) of Covid-19 patients. **Method**: Analytical observational research with consecutive sampling technique. The inclusion criteria were new patients with a confirmed COVID-19 diagnosis, treated at Dr. Soetomo Hospital Surabaya, aged 21 – 60 years, does not have a severe mental disorder, can communicate verbally and is able to answer questionnaires. Measurements of anxiety, depression and stress (DASS-21), NLR, IL-1β were performed while the patient was admitted on days 0^{th}, 6^{th}, and 12^{th}. Spearman correlation test analysis to assess the relationship between DASS-21 scores and NLR and IL-1β scores, Mc Nemar's test comparison to assess differences in depression and stress on days 0^{th} and 6^{th}, Wilcoxon's signed rank test for differences in anxiety and IL-1β on days 0^{th} and 6^{th}, and paired t-test to assess differences in NLR on days 0^{th} and 6^{th}. As well as multivariate analysis to assess the effect of anxiety, depression, and stress on NLR and IL-1β. **Results**: From 38 research subjects, there were 29 subjects who dropped out after the 6^{th} day, 28 subjects experienced clinical improvement and underwent self-isolation at home, and 1 subject experienced worsening of symptoms so that it was difficult to conduct interviews. Most of the subjects (95%) had anxiety scores ranging from mild to very severe on day 0^{th} of treatment, but most had normal depression scores (86.8%), and normal stress scores (92.1%). There were differences in anxiety scores (0.016), and IL-1β levels (p=0.001) on the 6^{th} day. There were no differences in depression, stress, and NLR scores during the observation period 0^{th} and 6^{th}. In the multivariate analysis, there was no significant effect of anxiety, depression, and stress on NLR. Anxiety had an effect on IL-1β on the 6^{th} day of observation, with p=0.030; OR(95%CI) = 0.853 (0.738 – 0.985). **Conclusion**: Anxiety affected IL-1β levels in mild-moderate COVID-19 patients on day 6^{th}, but had no effect on NLR on days 0^{th} and 6^{th}. Depression and stress had no effect on NLR or IL-1β of mild-moderate COVID-19 patients on days 0^{th} and 6^{th}.

Keywords: Anxiety; Depression; Stress; Neutrophil-Lymphocyte Ratio; Interleukin 1 Beta; Covid-19; Mental Health.

1. Introduction

Corona Virus Disease (COVID-19) cases spread rapidly from one city to all countries in just 30 days and were declared by WHO as a Public Health Emergency International Concern (Sohrabi et al., 2020; Wu & McGoogan, 2020). The World Health Organization (WHO) classifies COVID-19 into four, namely mild degrees, moderate degrees (Pneumonia), severe degrees (severe pneumonia) and critical degrees. The average COVID-19 infection has an incubation period of 2-14 days with symptoms of fever, cough, shortness of breath,

fatigue and muscle aches, phlegm production, headache, coughing up blood, nausea and vomiting and diarrhea (Guan et al., 2020; Rodriguez-Morales et al., 2020; Sohrabi et al., 2020; Sun et al., 2020).

Most of the patients with confirmed COVID-19 experience anxiety about the highly contagious and fatal disease. In addition, COVID-19 patients must undergo quarantine or treatment in isolation rooms so that it has an impact on their social life. Feelings of guilt about the effects of transmission on family and people around as well as stigma aimed at patients and families are separate stressors for COVID-19 patients which can lead to symptoms of depression (Kong et al., 2020).

Anxiety is an intense, excessive, and persistent feeling of worry accompanied by autonomic symptoms such as palpitations, heavy breathing, sweating, and feeling weak and powerless (Stuart & Sundeen, 2006). Stress is described as an experience that causes feelings of anxiety and frustration, because it pushes us beyond the limits of our ability to cope. Stress can affect various systems in the body (metabolism, cardiovascular, and immune system) that are involved as a short and long term consequence of experiencing stress (McEwen, 2020). While depression is a condition of decreased mood characterized by feelings of sadness, loss of interest, feeling easily tired, hopeless and difficult to concentrate (Torres, 2020).

Anxiety, stress, and depressive symptoms experienced by COVID-19 patients affect the patient's immunological condition because aggravating the dysregulation of the inflammatory response caused by anxiety and depression can trigger an inflammatory response through various pathways such as an increase in neutrophils and some proinflammatory cytokines, as well as a decrease in lymphocyte proliferation (Channappanavar & Perlman, 2017). Untreated major depressive patients also showed increased NLR compared to healthy individuals and major depressive patients receiving SSRI drugs (Demircan et al., 2016).

Neutrophil Lymphocyte Ratio (NLR) is a prognostic factor that indicates a dysregulation of the inflammatory response that affects the severity prognosis of patients with COVID-19. Patients with age ≥ 50 and NLR ≥ 3.13 will usually have a severe clinical presentation, and are expected to gain immediate intensive care unit access if necessary (Liu et al., 2020). In addition to neutrophils and lymphocytes, the inflammatory response also involves cytokines. Interleukin 1 beta (IL-1β) is one of the mediators of communication between the immune system in the peripheral and the immune system in the central nervous system, whose role in depressed individuals has been confirmed (Dowlati et al., 2010; Howren et al., 2009).

Mitigation of anxiety and depression in patients with confirmed Covid-19 is expected to contribute to improved outcomes by reducing the risk of aggravating the inflammatory response, such as an increase in NLR. This study aims to provide data and input for a more holistic treatment by taking into account the mental well-being of the patient to reduce the risk of an uncontrolled immune response that results in an increased risk of mortality for the patient. The data in this study is the effect of anxiety, depression, and stress on the Neutrophil Lymphocyte Ratio (NLR) and interleukin 1 beta (IL-1β) of COVID-19 patients treated in a special isolation room at Dr Soetomo Hospital Surabaya.

2. Methodology

This study uses an observational analytic study with a prospective cohort design and uses Consecutive sampling conducted in the Special Isolation Room for COVID-19 Dr. Soetomo Hospital Surabaya in May 2021 – July 2021.

The study population was COVID-19 patients and the study sample was mild to moderate COVID-19 patients who met the criteria. Inclusion criteria were 21-60 years old, patients confirmed by SARS Cov-2 RT-PCR examination, clinically mild-moderate criteria (WHO classification), willing to participate in research and able to communicate verbally and in writing in Indonesian. Exclusion criteria were patients with severe cognitive deficits, currently receiving antidepressant and anxiolytic psychopharmaceuticals, history of pregnancy or postpartum, history of autoimmune disease or taking steroids and other immunosuppressants, infected with HIV, currently undergoing chemotherapy or malignancy, and the patient had previously been treated in another isolation room. for 7 days or more.

The research instrument for measuring stress levels was using the Depression Anxiety Stress Scale 21 (DASS 21) questionnaire, measuring the levels of neutrophils and lymphocytes using Flowcytometry, and measuring the levels of Interleukin 1β using the ELISA technique. Measurements were taken when the patient was admitted on days 0^{th}, 6^{th}, and 12^{th}.

The statistical test used in this research is descriptive and inferential statistics. Descriptive statistics were used in presenting the demographic data of the study sample as well as the results of anxiety, depression, stress, NLR, and IL-1β scores. Inferential statistics were Spearman correlation test analysis to assess the relationship between DASS-21 scores with NLR and IL-1β scores, Mc Nemar's comparative test to assess differences in depression and stress on day 0^{th} and 6^{th}, Wilcoxon's signed rank test for differences in anxiety and IL-1β on 0^{th} day and 6^{th} day, as well as paired t-test to assess differences in NLR days 0^{th} and 6^{th}, as well as multivariate analysis (logistical regression) to assess the effect of anxiety, depression, and stress on NLR and IL-1β.

This research has received a certificate of ethical qualification by the Health Research Ethics Committee of Dr. Soetomo Hospital Surabaya, Indonesia, number 0209/KEPK/VI/2021 on 15 June 2021.

3. Results

Samples that met the inclusion criteria in this study were 38 patients. Subjects were followed from day 0^{th} of treatment in the infectious ER to treatment at RIK. Anxiety, Depression, Stress, NLR, and IL-1β data were taken periodically on treatment days 0^{th}, 6^{th}, and 12^{th}. Research subjects were followed until their condition improved (out of the hospital due to recovery, self-isolation, or moving to a non-Covid-19 treatment room), or worsened (falling into a state of decreased consciousness and difficult to interview).

The patient characteristics in table 1 show that most of the subjects (52.6%) were male, and the highest ethnic group was Javanese (73.3%). Most of the subjects (84.2%) were married, the most occupations were private employees (28.9%), domiciled the most in Surabaya (55.3%), had an education level dominated by high school (34.2%), the most comorbidities (13 .2%) were obese, and most (76.3%) were in the Bargaining phase (Kubler Ross) with their illness. Normality test using Saphiro-Wilk on age characteristics, and Body Mass Index (BMI) is normally distributed.

Table 1. Distribution of Research Subject Characteristics

Demographic Characteristics	n	% Mean ± Standard deviation
Age	38	42,13 ± 11,660
Gender		
Male	**20**	**52,6**
Female	18	47,4
BMI		26,42 ± 4,906

Underweight	1	2,6
Normal	**17**	**44,7**
Overweight	12	31,6
Obese	8	21,1
Ethnic group		
Java	**28**	**73,3**
Madura	8	21,1
Batak	1	2,6
Minang	1	2,6
Profession		
Civil servant	8	21,1
Private Employee	**11**	**28,9**
Entrepreneur	8	21,1
Others	1	2,6
Not Working	10	26,3
Education		
No school	3	7,9
Primary school	7	18,4
Junior High School	4	10,5
High School	**13**	**34,2**
Diploma	4	10,5
Bachelor	7	18,4
Marital status		
Married	**32**	**84,2**
Unmarried	5	13,2
Divorced	1	2,6
Domicile		
Outside Surabaya	**17**	**44,7**
Surabaya	21	55,3
Comorbid		
DM	3	7,9
Hypertension	2	5,3
Obese	**5**	**13,2**
DM, Hypertension	3	7,9
None	25	65,8
Kubler Ross phase		
Denial	4	10,5
Bargaining	29	76,3
Acceptance	5	13,2

Normality test (Saphiro-Wilk), normally distributed if $p > 0.05$

3.1. Overview of anxiety, depression, and stress on day 0^{th}, 6^{th}, and 12^{th}

The description of anxiety, depression, and stress on day 0^{th}, 6^{th}, and 12^{th} in Table 2 shows that most of the subjects on day 0^{th} had a normal depression score (86.8%), had an anxiety score at a moderate level (39.5%), and a normal stress level score (92.1%). On day 6^{th}, most of the subjects had a normal depression score (97.4%), had an anxiety score that varied from mild to very severe (60.5%), and a normal stress level score (92.1%). Meanwhile, on day 12^{th}, all subjects had normal depression scores (100%), most had normal anxiety scores (68.4%), and all had normal stress scores (100%).

Table 2. Distribution of depression, anxiety and stress on day 0^{th}, 6^{th}, and 12^{th}

	0^{th} day n (%)	6^{th} day n (%)	12^{th} day n (%)
Depression			
Normal	33 (86,8%)	37 (97,4%)	9 (100%)
Mild	3 (7,9%)	1 (2,6%)	

Moderate	2 (5,3%)		
Anxiety			
Normal	5 (13,2%)	15 (39,5%)	4 (68,4%)
Mild	8 (21,1%)	3 (7,9%)	2 (10,5%)
Moderate	15 (39,5%)	11 (28,9%)	2 (13,2%)
Severe	3 (7,9%)	5 (13,2%)	1 (7,9%)
Very severe	7 (18,4%)	4 (10,5%)	
Stress			
Normal	35 (92,1%)	37 (97,4%)	9 (100%)
Mild	1 (2,6%)	1 (2,6%)	
Moderate	2 (5,3%)		

The Wilcoxon test showed that there was a difference in the level of anxiety (p=0.016) on day 0th and day 6th. McNemar's comparison test showed that there was no significant difference in the level of depression (p=0,219) and stress level (p=0,500) on the 0th and 6th treatment days, obtained 9 subjects (Table 3, 4, and 5).

Table 3. Differences in depression scores on the DASS 21 questionnaire on Treatment Day 0th and 6th

Depression 0th day	Depression 6th day		Total	P score
	Normal	Mild		
Normal	32 (97%)	1 (3%)	33 (100%)	
Mild-moderate	5 (100%)	0 (0%)	5 (100%)	0,219
Total	37 (97,4%)	1 (2,6%)	38 (100%)	

*McNemar comparison test, significant if p<0.05

Table 4. Differences in anxiety scores on the DASS 21 questionnaire on Treatment Day 0th and 6th

Anxiety 0th day	Anxiety 6th day					Total	P score
	Normal	Mild	Moderate	Severe	Very severe		
Normal	3 (60%)	0 (0%)	1 (20%)	0 (0%)	1 (20%)	5 (100%)	
Mild	6 (75%)	2 (25%)	0 (0%)	0 (0%)	0 (0%)	8 (100%)	
Moderate	5 (33,3%)	1 (6,7%)	7 (46,7%)	2 (13,3%)	0 (0%)	15 (100%)	0,072
Severe	0 (0%)	0 (0%)	1 (33,3%)	2 (66,7%)	0 (0%)	3 (100%)	
Very Severe	1 (14,3%)	0 (0%)	2 (28,6%)	1 (14,3%)	3 (42,9%)	7 (100%)	
Total	15 (39,5%)	3 (7,9%)	11 (28,9%)	5 (13,2%)	4 (10,5%)	38 (100%)	

*McNemar comparison test, significant if p<0.05

Table 5. The difference in the stress level score of the DASS 21 questionnaire on Treatment Day 0 and 6

Stress 0th day	Stress 6th day		Total	P score
	Normal	Mild		
Normal	35 (100%)	0 (0%)	35 (100%)	
MildModerate	2 (66,7%)	1 (33,3%)	3 (100%)	0,500
Total	37 (97,4%)	1 (2,6%)	38 (100%)	

*McNemar comparison test, significant if p<0.05

3.2. Overview of NLR and IL-*1β* at days 0th, 6th, and 12th

The results of NLR and IL-1β examinations showed that the NLR data were normally distributed. The highest mean NLR was found on day 0th, and the lowest was on day 12th. Meanwhile, data on IL-1β levels were not normally distributed. The highest median IL-1β was found on day 6th. The range of IL-1β is very wide, indicating that the variability of IL-1β levels in each subject is quite high. The widest range of IL-1β was found on the 6th day of hospitalization (Table 6).

Table 6. The results of NLR and IL-1β examinations on day 0th, 6th, and 12th

	N	Mean ± Standard deviation	Median (min – max)
NLR			
0th day	38	5,40 ± 3,18	
6th day	38	5,36 ± 3,06	
12th day	9	5,22 ± 2,17	
IL-1β			
0th day	38		521,3 (42,4 – 4.064)
6th day	38		383,6 (5,80 – 4.795)
12th day	9		315,4 (27,7 – 1.597)

The analysis of differences in NLR and IL-1β levels using paired t test showed that there was no significant difference in NLR on day 0th and day 6th (p=0.944). The Wilcoxon test performed showed a significant difference in IL-1β on day 0th and day 6th (p=0.001). There was a decrease in IL-1β levels on the 6th day of observation. The difference test was not carried out on day 12th because there were too few subjects (Table 7).

Table 7. Differences in NLR and IL-1β levels on day 0th and 6th

	Hospitalization Day			
	0th	6th		
	N(%) 38(100,0)	N(%) 38(100,0)	Difference	P
NLR (cell/mm³) Mean ± SD	5,40 ± 3,18	5,36 ± 3,06	-0,042±3,625	0,944 *
IL-1β (pg/mL) Median (min-max)	521,3 (42,4 – 4.064)	383,6 (5,80 – 4.795)	-194,35 (-3541,2 – 4276,9)	0,001**

* Paired t test, significant if p < 0.05
** Wilcoxon test, significant if p < 0.05

3.3. Effect of anxiety, depression and stress on NLR

Spearman correlation test to determine the relationship between anxiety, depression and stress on NLR on days 0th and 6th showed no significant relationship either on 0th day or 6th day (p>0.05) (Table 8). The results of the NLR examination were divided into 2 categories, namely <3.53 and ≥3.53 (Table 9 and 10).

Table 8. Relationship between depression, anxiety and stress with NLR

Relationship	n	NLR	
		r_s	P score
0th day			
Anxiety	38	-0,150	0,368*
Depression	38	0,040	0,813*
Stress	38	-0,131	0,434*
6th day			
Anxiety	38	0,301	0,066*
Depression	38	0,232	0,160*
Stress	38	-0,202	0,223*

* Spearman correlation test, significant if p <0.05

Table 9. Effect of depression, anxiety and stress on NLR on day 0th

Variable	B	P score	OR (95% CI)	R^2
Stress	-0,104	0,104	0,901 (0,795 – 1,021)	0,100
Constant	1,202	0,015		

Table 10. Effect of depression, anxiety and stress on NLR on day 6th

Variable	B	P score	OR (95% CI)	R^2
Constant	0,773	0,027	2,167	0,000

The results of the regression test at step 3 to determine the effect of anxiety, depression and stress on day 0th NLR showed that only stress variables could be included in the equation, but the results were not significant (p = 0.104; OR (95% CI) = 0.901 (0.795) -1.021)). This means that there is no effect of depression, anxiety and stress on NLR (≥ 3.53 and < 3.53) on day 0th. This result is strengthened by the value of R^2 which shows the contribution of stress to NLR is very small, only 10% (Table 9). The results of the logistic regression test in step 4 show that there are no independent variables that can be included in the equation. This means that there is no effect of depression, anxiety and stress on NLR (≥ 3.53 and < 3.53) on day 6th. This result is reinforced by the R^2 value of 0%. (Table 10).

3.4. Effect of anxiety, depression, and stress on IL-*1β*

The results of the Spearman correlation test to determine the relationship between anxiety, depression and stress on IL-1β on day 0th showed that there was no significant relationship with the direction of the positive relationship and the strength of the weak relationship (0.351) and there was no significant relationship between anxiety, depression, and stress with IL-1β on day 6th (p > 0.05) (Table 11). IL-1β levels were divided into 2 categories, namely <247.6 and ≥247.6 (tables 11, and 12).

Table 11. Relationship of anxiety, depression, and stress with IL-1β

Relationship	n	IL-1β	
		r_s	P score
0th day			
Anxiety	38	-0,022	0,898*
Depression	38	0,069	0,682*
Stress	38	0,351	0,031*
6th day			
Anxiety	38	-0,267	0,105*
Depression	38	0,007	0,964*
Stress	38	0,247	0,134*

* Spearman correlation test, significant if p <0.05

The results of the regression test at step 4 to determine the effect of anxiety, depression and stress on IL-1β day 0th showed that there were no independent variables that could be included in the equation. This shows that there is no effect of depression, anxiety and stress on IL-1β (≥247.6 and < 247.6) on day 0th. This result is strengthened by the R^2 value of 0% (Table 12). Meanwhile, on day 6th, the results of the logistic regression test at step 3 showed that only anxiety variables could be included in the equation. This shows that there is an effect of anxiety on IL-1β (≥247.6 and < 247.6) on day 6th, namely the lower the anxiety score, the lower the level of IL-1β. However, the contribution of anxiety to IL-1β was only 20.1% (R^2 value), the rest (79.9%) was caused by other factors (Table 13).

Table 12. Effect of anxiety, depression, and stress on IL-1β on day 0th

Variable	B	P score	OR (95% CI)	R^2
Constant	2,890	< 0,001	18,0	0,000

Table 13. Effect of anxiety, depression, and stress on IL-1β on day 6th

Variable	B	P score	OR (95% CI)	R^2
Anxiety	-0,159	0,030	0,853 (0,738 – 0,985)	0,201
Constant	2,977	0,003	18,0	

3.5. Relationship of changes in anxiety, depression, stress scores with changes in NLR and IL-*1β*

The results of the analysis of the difference between the 0th and 6th treatment days were carried out to describe the trend or movement of the dependent variable during the stress response process. On day 12th, it could not be analyzed because of the small number of samples (9 research subjects). The results of the Spearman correlation test showed that there was no significant relationship between the difference in scores for depression, anxiety and stress with the difference between NLR and IL-1β on day 0th and day 6th ($p > 0.05$). Thus, changes in depression, anxiety and stress conditions were not associated with changes in NLR values or changes in IL-1β levels during the observation period (Table 14).

Table 14. The relationship of the difference in scores of depression, anxiety and stress with the difference in NLR, and the difference in IL-1β

Relationship	n	Correlation (r_s)	P score
Difference in Depression Score with the difference in NLR 6th day - 0th day	38	0,168	0,313
Difference in Depression Score with the difference in IL-1β 6th day - 0th day	38	-0,072	0,670
Difference in Anxiety Score with the difference in NLR 6th day - 0th day	38	-0,113	0,500
Difference in Anxiety Score with the difference in IL-1β 6th day - 0th day	38	0,005	0,977
Difference in Stress Score with the difference in NLR 6th day - 0th day	38	-0,094	0,575
Difference in Stress Score with the difference in IL-1β 6th day - 0th day	38	-0,009	0,956

*Spearman correlation test, significant if $p<0.05$

4. Discussion

The mean age of the respondents was 42 years (standard deviation ±11.6). This is because older age is associated with a higher NLR ratio (Li et al., 2015). Some respondents have comorbid Diabetes Mellitus (DM) (7.9%), Hypertension (5.3%), Obesity (31.2%), and multiple comorbidities, namely DM and Hypertension (7.9%). The systematic review and meta-analysis conducted by Zandifar et al (2020) on hospitalized SARS-CoV-2 patients obtained the following data: Hypertension (16.37%), cardiovascular disease (12.11%), smoking history (7 ,63%) and diabetes (7,87%) (Zandifar et al., 2020). There are similarities between this study and other studies in terms of the types of co-morbidities associated with COVID-19. The difference in the number of populations studied, makes it impossible to compare the prevalence. In general, the subjects in these studies have comorbid physical illnesses that can worsen their clinical condition by weakening the immune response.

Most of the respondents (95%) experienced anxiety ranging from mild to very severe at the beginning of day 0th treatment. 13% of respondents experienced mild-moderate depression, and 7.9% of respondents experienced mild-moderate stress at the beginning of treatment. A study on coping strategies in people who experience loneliness situations, says that adults get the highest scores on the ability of reflection and

acceptance. Most likely as a result of their level of maturity, experience, and wisdom, being able to accept their fate in a calmer way (Rokach, 2001).

This study found no difference in conditions of anxiety, depression and stress on day 0^{th} and day 6^{th}. Along with the length of treatment, there was a tendency to decrease the percentage of subjects who experienced anxiety, depression, or stress. On the 12^{th} day of observation, it was found that all subjects did not show symptoms of depression or stress, and as many as 68% of the subjects showed normal anxiety scores. This finding is in line with several other studies in Wuhan. Two clinical studies, one conducted in the isolation ward of a general hospital care ward (n = 106) and the other in a temporary quarantine facility (n = 714) in Wuhan, China, reported that COVID-19 patients experienced symptoms of depression, severe symptoms of anxiety, suicidal ideation, and clinically significant PTSD symptoms were 9.4%, 15.1%, 24.5%, and 96.2%, respectively. These findings indicate that COVID-19 patients have a greater risk of developing this mental disorder (Xie et al., 2020).

Anxiety, depression, and stress in this study were measured using the DASS-21 questionnaire which is a self-report questionnaire with Likert-style answer choices. Several things can cause bias in the results of this kind of assessment, including:

1. Subjects did not answer honestly, especially sensitive questions. Subjects may answer according to socially applicable norms (social desirability bias).

2. Tendency to answer yes or no, answer in extreme ratings on all questions given (response bias).

3. Differences in question interpretation.

4. The presence of the researcher when completing the questionnaire.

5. Questionnaire is less flexible (Demetriou et al., 2015).

To avoid differences in the interpretation of questions, during the data collection process, patients were given an explanation before filling out the questionnaire and were given the opportunity to ask questions if it was unclear during filling out the questionnaire. However, the presence of the researcher during filling out the questionnaire (as mentioned in point 4) can affect the concentration and answers of the subjects when filling out the questionnaire. The questionnaires used during data collection on day 0^{th}, 6^{th}, and 12^{th} were the same (DASS-21). This can lead to bias, where research subjects tend to answer the same thing the previous time. The DASS-21 questionnaire serves to describe symptoms or emotional states during the past week and is not used for clinical diagnosis. The combination of an assessment based on a questionnaire and a direct patient examination can provide a more complete picture of the patient's psychological condition.

The ethnocentrism factor can also influence the way the subject answers the questionnaire questions. Ethnocentrism is a way of viewing and interpreting things from their own cultural perspective. In cross-cultural psychology studies, problems related to response sets are often discussed. Response set is a cultural tendency to respond in certain ways to a test or response scale, which is more a reflection of cultural tendencies and not its true meaning. There are cultures that encourage the use of extreme responses on the scale, while others prefer responses around the "middle" (Herdiyanto et al., 2016). Most of the subjects came from Javanese ethnicity. Society in Javanese culture has its own sociopsychological construct in interpretation, including interpreting

and answering questions. There is an obedient and shy culture that is embraced by the Javanese, so often the answers to the questions asked are cultural tendencies, and do not describe the actual conditions (Adab et al., 2012).

Individual resilience also plays a role in adaptation to stressors. Factors that promote resilience, such as psychosocial support from family and friends, can help individuals remain socially connected. This psychosocial support also reduces the negative effects of stressful conditions that affect the immune response (Shields et al., 2020). Social support also plays an important function so that individuals do not feel alone during treatment in isolation rooms, thereby reducing the risk of anxiety and depression (Ingravallo, 2020).

Overview of the immune response of COVID-19 patients using NLR and IL-1β biomarkers. This study divided 2 categories of NLR values, namely <3.53 and ≥3.53, based on the cut-off value used by WHO to predict the possibility of worsening inflammation in COVID-19 patients. The average NLR in this study obtained the highest value on day 0^{th} and the lowest average on day 12^{th}. The mean value of NLR on day 0^{th} and day 6^{th} was obtained above the cut off value. A high NLR value is often associated with a moderately severe inflammatory process, and a poor prognosis for COVID-19 patients (Fuad et al., 2021; Russell et al., 2019). Clinical condition Most of the subjects until the end of the observation met the criteria for mild-moderate pneumonia according to the WHO classification. High NLR values may be related to comorbidities that the subject has, such as metabolic syndrome, obesity, hypertension, diabetes, and cardiovascular disease. Comorbid metabolic disease in COVID-19 patients is often associated with dysregulation of the immune response, leading to an increase in NLR (De Heredia et al., 2012). However, there was no significant difference in NLR values during the first week of observation when the patient was treated. This is because the subject also did not experience a worsening of clinical symptoms during the observation period. NLR is often used as a predictor of possible worsening of symptoms in COVID-19 patients (Zeng et al., 2021). NLR in peripheral blood is associated with systemic inflammatory states and disease activity. NLR also shows prognostic value in autoimmune and infectious diseases (Russell et al., 2019).

The data that was successfully taken from observations during the study obtained levels of IL-1β with a very wide range. The decrease in median IL-1β on day 6^{th} may indicate a decrease in the activity of the proinflammatory cytokine IL-1β. The decrease in IL-1β can be influenced by several things, including pharmacological interventions received by Covid-19 patients, such as the administration of antiviral drugs or immunomodulatory supplements during treatment. Immunomodulators can reduce levels of proinflammatory cytokines in some studies (Rahardjo et al., 2014; Ye et al., 2020).

In this study, there was no statistically significant relationship between changes in anxiety, depression, and stress with changes in NLR values on the 0^{th} and 6^{th} day measurements. This can be related to the determination of the cut off value of the NLR. The cut off value used as a reference in this study is 3.53. Other studies use different NLR cutoff values to predict whether a clinical condition will improve or worsen. Such as the results of a study in Wuhan which stated that Covid-19 patients aged 50 and NLR ≥3.13 will show severe clinical signs, and are expected to gain access to the intensive care unit immediately if necessary (Liu et al., 2020).

Comorbidities also affect the patient's NLR scores, especially the metabolic syndrome. Some research subjects have comorbid metabolic syndrome such as obesity, diabetes mellitus, and cardiovascular disease. A retrospective study of the effect of obesity on NLR proved that obesity affects neutrophil and lymphocyte levels (Furuncuoğlu et al., 2016). Likewise, another study conducted in Turkey found an increased NLR value in subjects with prediabetes and diabetes, especially those with comorbid obesity (Mertoglu & Gunay, 2017).

Cardiovascular diseases such as hypertension also contribute to the increase in NLR (Chen et al., 2019).

The results of a retrospective study of hematogram images as a biomarker, showed a relationship between inflammation and anxiety disorders. It is also postulated that hematological parameters such as NLR can be used as useful markers in the follow-up of patients with psychiatric disorders such as anxiety, depression or acute stress (Uzun & Akıncı, 2021). However, the data from this study are not consistent with the results of previous studies by Uzun and Akinci. Anxiety and depression did not affect the NLR value on the 0^{th} and 6^{th} day observations. The contribution of stress to NLR is also very small, only around 10%. This means that there are many other factors that have more influence on the neutrophil-lymphocyte ratio in these Covid-19 patients besides anxiety, depression and stress.

This study obtained data on the existence of a weak relationship between stress and IL-1β at the time the patient was initially treated, but there was no relationship between anxiety, nor depression with IL-1β levels. There was no effect of depression and stress on the 6^{th} day of observation. The increase in the percentage of patients who had normal anxiety scores on the 6^{th} day of treatment together with IL-1β levels which tended to decrease, showed a significant correlation between the two, although the effect was only 20%. Improvement in clinical condition on day 6^{th} may be related to this. The levels of proinflammatory cytokines such as IL-1β are affected by the severity of the disease in Covid-19 patients (McElvaney et al., 2020).

This study also obtained data that there was no statistically significant relationship between changes in anxiety, depression, and stress with changes in IL-1β levels on the 0^{th} and 6^{th} day measurements. This may be related to the determination of the cut off levels of IL-1β used. Determining normal and abnormal cytokine levels is very challenging for diagnostic evaluation. Cytokine levels vary widely among individuals. Its release and subsequent effects may differ based on the activating signal, specific target cells, and physiological factors including psychological well-being, fitness level, and intake. Cytokines can also vary in physiological settings at different sites and environments, and thus studies measuring cytokines under normal and abnormal conditions can only compare results with other studies of the same biologic fluid (e.g., serum, amniotic fluid, and pleural fluid) (Monastero & Pentyala, 2017). The cut-off value of IL-1β in this study was based on a study related to the severity of pneumonia due to Respiratory Synctitial Virus (RSV), which was 247.6 pg/ml. IL-1β concentrations higher than that, are thought to increase the risk of clinical worsening of pneumonia due to RSV (Díaz et al., 2015).

The research data showed that most of the subjects were overweight and obese (Table 1). Obesity is often associated with inflammatory conditions. Adipose tissue plays an important role in this context as they are a major source of cytokines, chemokines, and metabolically active mediators called adipokines. Adipokines, including adiponectin and leptin, are responsible for regulating the inflammatory immune response. Overweight and obese individuals exhibit higher levels of tumor necrosis factor-alpha (TNF-α), interleukin1 beta (IL-1β) and IL-6, all of which are produced by macrophages derived from adipose tissue. All of these pro-inflammatory cytokines regulate adipocyte proliferation and apoptosis, promote lipolysis, inhibit lipid synthesis and lower blood lipids through autocrine and paracrine mechanisms (Wang & He, 2018). Obesity induces phenotypic changes in macrophage polarization and increases proinflammatory cytokines (TNF-α, IL-1β, MCP-1). IL-1β gene expression was significantly increased in visceral adipose tissue of obese patients (Rakotoarivelo et al., 2018).

Another comorbidity found in this study was Diabetes Mellitus (type 2). The inflammatory response may have a dual role in type 2 diabetes mellitus (T2DM). Inflammation may have a causative relationship leading

to insulin resistance, but it can also be intensified by hyperglycemic states, leading to complications of T2DM. Polymorphisms in genes encoding inflammatory cytokines are thought to be associated with increased levels of proinflammatory markers associated with chronic pathological conditions in T2DM (Cruz et al., 2013).

Hypertensive patients are reported to have higher plasma concentrations of proinflammatory cytokines. Offspring of hypertensive parents tend to have higher serum CRP levels than offspring of non hypertensive parents. CRP is thought to induce monocytes to secrete proinflammatory cytokines such as IL-1β, IL-6, and TNF-α (Lieb et al., 2008). Stimuli that cause hypertension, including salt, hyperactivity of the Renin-Angiotensin-Aldosterone System, oxidative stress, and inflammation cause an initial increase in blood pressure, which results in a modified protein. These altered proteins are no longer recognized as self proteins but are recognized as 'neoantigens'. Then the T lymphocytes are activated. The T-cell signaling cascade promotes the entry of macrophages (and other inflammatory cells) into the blood vessels and kidneys, resulting in the release of proinflammatory cytokines. This confirms higher plasma IL-1β levels in hypertensive patients compared to normotensive patients (Dinh et al., 2014).

Research Limitations

1. The scope of the characteristics of the study subjects was limited to patients with mild-moderate criteria, so they could not observe NLR and IL-1β levels in patients with more severe degrees.

2. The number of subjects who drop out after the 6th day due to recovery, self-isolation at home because their clinical symptoms are improving, or clinical deterioration occurs so that it is difficult to conduct interviews, causing data analysis up to the 12th day cannot be done.

3. The presence of uncontrolled confounding factors such as comorbidities, chronic stressors, the bereavement phase (Kubler Ross) that is being passed, administration of antivirals, immunomodulators, personality, psychosocial support, and sociocultural factors, can cause bias.

5. Conclusion

Anxiety, depression, and stress did not affect the NLR values of mild-moderate COVID-19 patients on treatment days 0th and 6th. Anxiety only slightly affected IL-1β levels of mild-moderate COVID-19 patients on day 6th of treatment, which was 20%. Meanwhile, depression and stress did not affect IL-1β levels in mild-moderate COVID-19 patients on day 0th and 6th of treatment.

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