ORIGINAL ARTICLE

Evaluation of Haemoglobin, Total Leukocytes, and Neutrophil/Lymphocyte Ratio as A Predictors of C-Reactive Protein Levels in Patients with Pulmonary Tuberculosis from Pontianak, West Kalimantan

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ABSTRACT

Introduction: Pulmonary tuberculosis (TB) caused by Mycobacterium tuberculosis can lead to a decrease in haemoglobin, leukocytosis, neutrophilia and lymphocytopenia, followed by the secretion of pro-inflammatory cytokines such as TNF-α, IL-1, and IL-6 which later on will stimulate hepatocytes to synthesize acute phase proteins such as C-reactive protein (CRP) as a marker of inflammation. **Objective:** The study prospectively involved 48 patients diagnosed with pulmonary TB at Pulmonary Disease Treatment Unit of West Kalimantan Province during April - June 2021. **Materials and Methods:** The examination of haemoglobin, number of leukocytes, neutrophils and lymphocytes was carried out with a haematology analyzer, and the neutrophil/lymphocyte ratio (NLR) was calculated manually, while the determination of CRP level used semiquantitative latex method and the results were converted to mg/L. **Results:** The majority of respondents experienced a decrease in haemoglobin (77.10%), leukocytosis (60.41%) and NLR in the range 1.56–5.80 (62.50%). Kendall's tau-b test revealed significant negative correlations between haemoglobin levels and CRP (r -0.289, p 0.031 < 0.05), positive correlations between the number of leukocytes and CRP (r 0.446, p 0.001 < 0.05), and positive correlations between the number of NLR and CRP (r 0.489, p 0.000 < 0.05). **Conclusions:** The increase in CRP corresponds to an increase in the number of leukocytes and NLR value, but inversely with the haemoglobin level. Thus, the examination of Hb, leukocyte count and NLR is expected to be used as an alternative examination to monitor the course of TB disease, in particular to predict the value of CRP as a marker of inflammation.

Keywords: Haemoglobin, Total Leukocytes, Neutrophil/Lymphocyte Ratio, C-Reactive Protein, Pulmonary Tuberculosis

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INTRODUCTION

Pulmonary tuberculosis (TB) is one of the infectious lung diseases that are the leading cause of death. A single pathogenic infection causes pulmonary tuberculosis, Mycobacterium tuberculosis, characterized by persistent granulomatous inflammation with substantial lung tissue damage (1). Geographically, the most TB sufferers in 2019 were in the Southeast Asia region, 44%. Then eight countries account for two-thirds of the global total, one of which is Indonesia, which is 8.5% (2). The World Health Organization (2020) shows that the number of pulmonary TB globally reaches 10 million, and Indonesia ranks second in the world with the most TB sufferers after India. TB cases in Indonesia in 2019 were recorded as 543,874 cases, a decrease compared to all TB cases detected in 2018, which were 566,623 cases. Based on the report on the Prevention and Control of Infectious Diseases section of the West Kalimantan Provincial Health Office in 2019, there were 8,364 cases recorded with a case notification rate (CNR) number or a pulmonary TB case finding rate of 165 per 100,000 population. CNR is a number that shows the number of new issues found and recorded among 100,000 residents in a particular area. Meanwhile, the percentage of a cure for pulmonary TB patients with smear-positive in West Kalimantan was 75.5%, with

details of 4,633 patients being treated, 3,500 patients were declared cured (3).

Mycobacterium tuberculosis that enters the body causes inflammation as the body's mechanism to defend itself (4). The release of various pro-inflammatory cytokines such as interleukin (IL)-6 on bacterial invasion then induces liver cells to synthesize acute phase proteins such as C-reactive protein (CRP) and fibrinogen which functions as non-septic opsonins in the process of bacterial phagocytosis. CRP will increase sharply during inflammation and the systemic inflammatory process (5). Inflammatory mediators, especially cytokines IL-6, IL-1, and tumor necrosis factor (TNF) play an essential role and affects changes in haemoglobin level (6). An increase in pro-inflammatory cytokines in the blood reduces the availability of bone marrow iron for erythropoiesis, causing anemia (7). Anemia in patients with pulmonary tuberculosis occurs due to the suppression of erythropoiesis by inflammatory mediators. Nutritional deficiencies and malabsorption syndromes can also exacerbate anemia in patients with pulmonary tuberculosis (8). The research results conducted by Yoon, Son, Ph, & Um (2013) proved that the range of CRP levels in patients with bacterial pneumonia was 7.59–28.87 mg/L higher than in patients with pulmonary tuberculosis with a range of 0.74–13.88 mg/L. The same study also showed an increase in the number of leukocytes and the ratio of neutrophils/lymphocytes due to the inflammatory reaction caused by bacterial infection (9,10). High leukocyte counts reflect neutrophil counts as a marker of persistent inflammation or failure to clear bacteria (11). Neutrophils are the foremost immune cells responsible for killing Mycobacterium tuberculosis in the early stages of infection. Neutrophils sense and ingest bacteria as soon as they enter the human body. This process involves bactericidal enzymes and peptides contained in neutrophil granules (12). Meanwhile, specific lymphocytes are localized at the site of infection which indicates the presence of persistent infection causing a decrease in the number of lymphocytes in the peripheral blood (11).

So far, the diagnosis of tuberculosis has been confirmed by acid-fast staining, chest radiography, culture and polymerase chain reaction (PCR) based examination; while the examination to monitor the course of the disease, especially because its relationship with inflammation has not been established. This study aims to measure haemoglobin levels, leukocyte counts and neutrophil/lymphocyte ratios as predictors of CRP as a marker of inflammation. Furthermore, whether these parameters have the potential as a supporting examination to monitor the course of tuberculosis.

MATERIALS AND METHODS

Samples

The sampling technique in this study uses the total population. The sample were patients diagnosed with pulmonary TB at the Pulmonary Disease Treatment Unit of West Kalimantan Province during the study period, namely April - June 2021. Patients diagnosed with pulmonary TB were then given an explanation and asked to fill out an informed concern sheet. During that period as many as 48 people were diagnosed and willing to become respondents.

Criteria of Participant Recruitment

Inclusion criteria were patients who were positively diagnosed with pulmonary TB based on positive results from one or more examinations, namely microscopic examination of acid-fast staining, culture, thorax imaging, and Xpert MTB; 20–60 years old and willing to be a respondent. Exclusion criteria were having comorbidities or other infectious diseases.

Ziehl-Neelsen (ZN) Staining Procedure

The smears were arranged in serial order on the staining bridge, with smear side up and flooded with filtered 0.1% carbol fuchsin. The smear was steamed and allowed to stain for 5 minutes, rinsed with water, and drained. They were decolorized with 3% HCl alcohol, rinsed with water, and exhausted. They were then counterstained with 0.1% methylene blue solution for 1 minute and rinsed with water. The smear was allowed to air dry and examined microscopically using the oil immersion (100x) objective (13).

Xpert MTB/RIF assay procedure

Prepare the Xpert-MTB/RIF cartridge. Give identity to the right or left side of the cartridge by using a marker or barcode sticker. Open the top cover of the cartridge. Remove the processed phlegm using the pipette provided. Fill the pipette beyond the 2 ml mark on the pipette. Slowly insert the pipette into the sample chamber contained in the cartridge, then remove the sample slowly. Avoid air bubbles. Close the cartridge cover tightly, immediately process the sample using the GeneXpert machine (14).

Haemoglobin level, total leukocytes number and neutrophil/lymphocyte ratio (NLR) count

The examination method used by researchers is flow cytometry. Sysmex XN-450 is an automated haematology analyzer for analyzing particle or cell suspensions with sizes from 0.2–150 µm. Sysmex XN-450 for measuring haemoglobin level, total leukocytes number, and neutrophil/lymphocyte ratio (NLR).

Examination of CRP in this study using the latex agglutination method. CRP latex test is a qualitative and semi-quantitative test. The principle of CRP examination using the latex agglutination method is that the latex

particles are coated with an anti-human CRP antibody and agglutinated when mixed with the patient's serum containing CRP. The last agglutination titer from the latex method was then multiplied by 6 to obtain the result in mg/L units (15).

Statistical analysis

The data's distribution and normality were determined by the Shapiro-Wilk One-Sample test, and correlation test were using Independent Sample Kendall's Tau-b. The confidence level used is 95%, so the precision or inaccuracy limit is (α)=5%=0.05.

RESULT

The research data obtained in this study were haemoglobin levels, total leukocytes number, NLR values, and CRP's from whole blood patients diagnosed with pulmonary TB from April to June 2021.

Most of the respondents were male (39 people, 81.25%) and came from the age group of 56–60 years (10, 20.8%), while the age group with the least number of respondents was 20–30 years (1, 2.08%) (Table I).

CRP values varied from 6 mg/L to 96 mg/L with the highest frequency at that time was 12 mg/L (18, 37.5%) and the lowest was 6 mg/L (2, 4.2%). The majority of respondents had low Hb values, 7 women (14.5%) and 30 men (62.5%), and none of the respondents had high Hb values. In measuring the number of leukocytes, most of the respondents (29, 60.41%) had high leukocytes values (above 10,000 cells/uL), compared to respondents who had normal leukocytes values, which were 19 (39.59%). The NLR values obtained varied from 1.67 to 13.55, where 30 (62.5%) respondents were in the range 1.56–5.80 and the rest (18, 37.5%) were in the range > 5.80 (Table II).

Table I: Characteristics of respondents

Characteristics	Count	Percentage (%)
Gender:		
Male	39	81,25
Female	9	18,75
Age:		
20–25	8	16,70
26–30	1	2,08
31–35	4	8,32
36–40	7	14,60
41–45	6	12,50
46–50	6	12,50
51–55	6	12,50
56–60	10	20,80

Kendall's Tau-b Independent Sample statistical test was used to determine the correlation between two variables and the results were as follows: there was a negative correlation between Hb levels and CRP levels (p 0.031 < 0.05) with a correlation coefficient (r) of -0.289 (weak); there is a significant positive correlation between the number of leukocytes and CRP levels (p 0.001 < 0.05) with a correlation coefficient (r) of 0.446 (moderate); and there is a significant positive correlation between NLR and CRP levels (p 0.000 < 0.05) with a correlation coefficient (r) of 0.489 (moderate).

Table II: Distribution of CRP, haemoglobin levels, number of total leukocytes, and NLR

C-reactive protein	Frequencies	%
6 mg/L	2	4.2
12 mg/L	18	37.5
24 mg/L	12	25.0
48 mg/L	5	10.4
96 mg/L	11	22.9
Total	48	100%

Sex	Reference value of haemoglobin	Frequencies	%	Min.	Max.	Median
Women	<12.0	7	14.5	9.3	11.5	10.6
	12.0–16.0	3	6.25	12.5	14.3	12.9
	>16.0	0	0	0	0	0
Men	<14.0	30	62.5	6.9	12.9	12.1
	14.0–18.0	8	16.6	13.1	16.2	14.2
	>18.0	0	0	0	0	0

Total leukocytes (cells/uL)	Frequencies	%	Min.	Max.	Median
4,000–10,000	19	39.59	6,900	9,920	8,110
> 10,000	29	60.41	10,220	17,410	12,400

NLR	Frequencies	%	Min.	Max.	Median
< 1.56	0	0	0	0	0
1.56–5.80	30	62.5	1.67	5.15	3.94
> 5.80	18	37.5	6.08	13.55	6.75

DISCUSSION

The results of research showed that the lower the haemoglobin levels, the higher the CRP levels; the higher the number of leukocytes, the higher the CRP levels; and the higher the NLR value, the higher the CRP levels in pulmonary tuberculosis patients. Anemia in pulmonary TB is associated with nutritional deficiencies, malabsorption syndromes, impaired or impaired use of iron by the body, bone marrow suppression (16), and chronic inflammation (15). Considering in this case the decrease in Hb levels is accompanied by an increase in CRP levels, the occurrence of anemia is more related to inflammation than nutritional causes (15).
In 29 (60.41%) samples, there was an increase in the number of leukocytes accompanied by an increase in CRP levels, which states that in the case of pulmonary tuberculosis, the entry of the bacterium Mycobacterium tuberculosis causes the number of leukocytes to increase as a response to the body's immune system. An increase in leukocytes is accompanied by inflammatory cytokines, such as TNF-α, IL-1, and IL-6. Furthermore, IL-6 stimulates hepatocytes to produce acute-phase proteins such as CRP (17). As a marker of inflammation and infection, CRP will increase drastically up to 1,000-fold when there is injury, infection, and inflammation (18,19). Likewise, when the stimulation ends, the levels will drop rapidly in 18–20 hours (18).

The majority of the increase in CRP levels was in line with the increase in NLR value, but some did not show an increase in NLR despite experiencing an increase in CRP. The increase in CRP may precede the increase in leukocytes number in newly exposed patients. The body responds to the entry of TB bacteria by releasing phagocytes, namely monocytes and neutrophils, as an initial response (20). These phagocytes secrete pro-inflammatory cytokines such as TNF-α, IL-1, and IL-6, stimulating hepatocytes to synthesize CRP. After that, leukocytes proliferate or multiply to increase their number in the blood. An increase in the number of neutrophils and a decrease in the number of lymphocytes indicates that the current infection is in the acute phase (21). Patients with neutrophilia may have recently been infected with Mycobacterium tuberculosis, thereby triggering an innate immune response through an increase in neutrophils. The role of lymphocytes in TB infection is more involved in the chronic inflammatory process, killing bacteria intracellularly and being antigen-presenting cells (APCs) that capture and process antigens (22). In patients with pulmonary tuberculosis, there is depression of Th1 characterized by low levels of IFN-γ. Lymphocytes play an important role in the specific immune system. The process of recognizing and eliminating pathogens is regulated by T lymphocytes, either directly or indirectly, by recruiting B lymphocytes to form specific antibodies. The T-cell response is elicited by a signal generated by mature dendritic cells induced by neutrophils.

The limitation of this study is the absence of clinical findings and thorax imaging of tuberculosis patients related to the patient privacy policy by the institution where this research was conducted.

CONCLUSION

This study showed that there was a significant negative correlation between Hb levels and CRP levels, and a significant positive correlation between leukocytes number and CRP and between NLR and CRP. Therefore, Hb levels, leukocytes number and NLR values can be used as an accompaniment or help clarify the increase in CRP levels in pulmonary TB patients.

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