The Association of Monocyte Lymphocyte Ratio and IFN-γ/IL-4 Ratio on Status of Sputum Conversion in Tuberculosis Patients with Post Intensive Phase

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Abstract

Background: Conversion status post-intensive phase of treatment in patients with tuberculosis (TB) is an important thing in determining the choice of the next treatment plan. Alternative parameters need to be studied to determine the factors that contribute to this status. Potential parameters thought to have a role are Monocyte Lymphocyte Ratio (MLR) as a hematological marker and Interferon Gamma/Interleukin-4 (IFN-γ/IL-4) as a molecular marker of cytokines and chemokines. **Objective:** analyzed the association between MLR and IFN-γ/IL-4 ratio on status of sputum conversion in TB patients with post-intensive phase. **Method:** The design of this study was cross-sectional with consecutive sampling, which was conducted in the period March to July 2021. The data used in this study included MLR, IFN-γ /IL-4 ratio, and sputum smear conversion. The statistical tests used included chi-square, fisher's exact test, and logistic regression with $p < 0.05$. **Result:** Low MLR (98.2%) and high MLR (55.6%) scores, both had positive sputum conversion (OR = 44.8; CI 4.169 – 481.452; $p = 0.001$). Meanwhile, the positive sputum conversion showed low IFN-γ/IL-4 ratio (97.8%) and high IFN-γ/IL-4 ratio (80%; OR = 11.25; CI 1.169 – 108.280; $p = 0.036$). There was a significant association between low MLR and sputum conversion positive (OR = 27.103; CI 2.289 – 320.883; $p = 0.009$). Meanwhile, IFN-γ/IL-4 ratio and sputum conversion did not have a significant association (OR = 5.248; CI 0.414 – 66,454; $p = 0.201$). **Conclusion:** there is a significant association between MLR, IFN-γ/IL-4 ratio, and the combination of MLR and IFN-γ/IL-4 ratio on sputum conversion in TB patients with post-intensive phase.

Keywords: *IFN-γ, IL-4, intensive phase, MLR, sputum conversion*

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Introduction

Tuberculosis (TB) is still a global health problem. The World Health Organization (WHO) in 2016 estimated that 10.4 million people experienced various manifestations of TB and

mortality reached 1.7 million people[1]. On the other hand, TB is the number one cause of death from a single infectious agent more than HIV/AIDS globally. Indonesia became one of the 5 countries with the largest TB incidence in 2017 along with India, China, the Philippines, and Pakistan[2]. Accuracy of treatment and continuity of therapy is the urgency of the problems faced by Indonesia in the era of directly observed treatment short-course (DOTS). Antituberculosis drugs (ATD) are an integral therapy modality that is available with a combination of at least four drugs for a minimum of 6 months. The pathogenesis of pulmonary TB or treatment of TB can trigger the manifestation of hematological disorders such as red blood cell changes, imbalance of granulocyte cells (neutrophils, basophils, and eosinophils), and platelets, monocytosis, and changes in lymphocyte levels[3].

In supporting efforts to eliminate TB by 2030, research on an effective biomarker as an indicator for rapid monitoring and evaluation of the response to TB therapy that can facilitate success and the development of treatment strategies becomes crucially applied[4, 5]. Acid-fast bacilli (AFB) sputum status after 2 months of TB therapy is currently still used as a marker of response to therapy and recovery. AFB smear examination and mycobacterium tuberculosis (MTB) sputum culture still have limitations. The length of time required to obtain culture results has an impact on diagnostic evaluation and treatment being less efficient. AFB smear examination also has limitations as a treatment evaluation. Most TB patients are unable to cough with sputum production after 2 months of therapy. This is because the clinical cough has improved and difficulties in the expectoration process of sputum. In addition, there are not a few cases of smear-negative sputum, such as cases of TB in children, TB/HIV patients, and individuals with severe immune disorders[4, 6].

Knowledge of the hematological manifestations of tuberculosis infection is important as a basis for determining pathogenesis. Previous studies have shown that myeloid cells are host cells for the growth of MTB bacteria. Lymphoid cells are cells that play a major role in components of the immune system against tuberculosis. Therefore, the levels of monocytes and lymphocytes called the Monocyte Lymphocyte Ratio (MLR) can describe the status of the immune system against infection[7]. In peripheral blood components, the MLR ratio reflects the immune clearance (efficiency) mechanism and can assess the severity and progression of TB disease[8]. Changes in normal MLR values can be caused by MTB infection that can alter a subset of hematopoietic stem cells or directly infect bone marrow mesenchymal stem cells[9]. MLR inactive TB infection has a significantly higher value than normal individuals and this is associated with the severity of the lesions in TB[10].

Specific biomarkers of TB pathogens, especially cytokines and chemokines as key molecules that regulate the immunological response, have been extensively studied, especially on their potential role as diagnostic and prognostic biomarkers in tuberculosis. The current biomarkers are not reliable as indicators in predicting the clinical outcome of MTB infection, especially in the treatment aspect[4]. Interferon-gamma (IFN-γ) is one of the cytokines that have a crucial role in the protective response

of cell-mediated immunity against TB so that it can be developed as a non-sputum biomarker. IFN-γ is secreted by activated T cells (Th1) and has clinical implications for the formation of granulomas and the elimination of MTB bacteria through the activation of macrophages towards the M1 phenotype(11). The utilization of IFN- γ in MTB infection is currently used in the diagnosis of infection through Interferon Gamma Release Assays (IGRA)(12). On the other hand, IL-4 is a principal cytokine produced by Th2 cells that acts as a cofactor in the activation of humoral immunity by stimulating proliferation and differentiation in B-cells and T-cells. In addition, IL-4 has an anti-inflammatory mechanism and contributes to the survival of MTB bacteria in the face of the immune system(13).

Based on the background of these problems, the authors want to evaluate the MLR and IFN-γ/IL-4 ratio as an easy biomarker but have never been done in monitoring and evaluating the conversion status of AFB with post-intensive phase ATD.

Method

Participants in this study were bacteriologically confirmed pulmonary TB patients receiving intensive phase of ATD therapy. Participant inclusion criteria included participants diagnosed with tuberculosis who had a bacteriological diagnosis at the time of initial diagnosis, had completed the intensive phase of ATD, and were age over 18 years old. Participant exclusion criteria included TB patients with comorbid malignancies, heart disease, SLE, Sarcoidosis, RA, liver fibrosis, and Alzheimer's and patients receiving immunosuppressant or corticosteroid therapy. Participants had filled out an informed consent form before this research was started.

The study design in this study used a cross-sectional design using a consecutive sampling technique. The number of participants in the study was 66 participants, where the time of data collection was carried out in the period March to July 2021. The data used in this study included MLR, IFN-γ/IL-4 ratio, and sputum smear conversion.

MLR is the absolute number of monocytes compared to the absolute number of lymphocytes, data obtained from the results of a complete blood count at the end of the intensive phase of treatment. The MLR value is displayed based on the median value, the minimum value, and the maximum value after the normality test on the data distribution is performed. The MLR value is also searched for the cut point value based on the ROC cut point value. The IFN-γ/IL-4 ratio is a comparison of the value of the proinflammatory cytokine IFN-γ to the anti-inflammatory cytokine IL-4, the data obtained from the results of blood serum examination at the end of the intensive phase of treatment, examined using the enzyme-linked immunosorbent assay (ELISA) method. IFN-γ/IL-4 ratio based on median value, minimum value, and maximum value after normality test on data distribution. The value of the IFN-γ/IL-4 ratio is also searched for the value of the cut point based on the value of the ROC.

Active TB was patients with pulmonary TB with clinical symptoms, radiological features, and microbiological evidence (TCM sputum/AFB sputum) showing signs of active TB. Clinical symptoms of active TB are typical symptoms of TB infection, namely coughing up phlegm for 2 weeks or more, coughing up blood, fever or

chills for more than 1 month, night sweats without physical activity. Radiographic features of active TB are radiological examinations with X-Ray in the thoracic region with the anterior (chest) facing the film and the rays will be directed from the posterior (back). Sputum results of patients with pulmonary TB: The results of the examination of sputum samples from patients with pulmonary TB to confirm the causative bacteria, using AFB sputum staining or molecular rapid tests. The molecular rapid test for TB is a molecular rapid test using the Xpert MTB/Rif method. Examination of AFB Sputum: Examination of sputum (phlegm) for the diagnosis of pulmonary TB, using Ziehl – Neelsen (ZN) staining. Interpretation of sputum examination results, namely if 2 times positive, or 1 time positive, 1-time negative, is called bacteriologically confirmed TB. If the sputum is negative twice, then a radiological evaluation (thorax photo) is carried out, if the radiological is suggestive of tuberculosis, it is called clinically confirmed TB.

Descriptive statistical analysis that aims to describe the characteristics of the subject based on the MLR ratio and the IFN-γ/IL-4 ratio. Normality test using Kolmogorov-Smirnov is used to analyze whether the data that has been collected has a normal distribution or not. Bivariate analysis was conducted to determine the comparative association between MLR levels or the ratio of IFN-γ/IL-4 and pulmonary TB sputum results. Because the independent variable has a categorical scale and the dependent variable has a categorical scale, a comparative test was conducted using the Fisher Exact test. Multivariate analysis with logistic regression was conducted to determine whether there was an association between MLR and the ratio of IFN-γ/IL-4 to pulmonary TB sputum results. Statistical analysis was declared significant if $p < 0.05$.

Result

Characteristic of participant

The subjects of this study had a mean age of 41.17 ± 14.67 with 44 (66.6%) of the male. There is diversity in the level of education and type of work of the subjects of this study. 11 (16.7%) subjects had an elementary education level or equivalent. Then for Middle school/high school/diploma or equivalent, 9 subjects (13.6%), 36 subjects (54.5%), and 3 (4.5%) subjects, respectively. The rest of, research subjects have undergraduate education status. Subjects' occupations consisted of 22 subjects (33.3%), employees 7 (10.6%), 17 subjects (25.8%), and 20 subjects (30.3%).

All patients from the beginning had a positive initial status on the Molecular Rapid Test sputum examination. About 63 subjects (95.5%) were given ATD in the first category while the rest were in the second category. In the research subjects, there were 45 subjects (68.2%) who had comorbidities. Types of comorbidities possessed by the subject included HIV in 6 subjects (27.3%), diabetes mellitus in 11 subjects (50.0%), asthma in 1 subject (4.5%), COPD in 1 subject (4.5 %), hypertension in 1 subject (4.5%), and chronic renal failure in 2 subjects (9.1%). The status of the final sputum examination in the subjects showed that as many as 61 subjects (92.4%) had undergone conversion. The nutritional status of the research subjects included no malnutrition in 54 subjects (81.8%), while 12 (18.2%) were malnourished.

On examination of the subjects' blood cells, the median of monocytes and lymphocytes were 0.5 (0.21 – 1.31) and 1.87 (0.53 – 107), respectively. The ratio of these two cells, known as the MLR, has a median of 0.26 (0 – 1.12). In the cytokine examination of the subjects, the medians of interferon-gamma and interleukin 4 were 0.87 (0.07 – 22.45) and 0.12 (0.04 – 0.85). The ratio of the two, namely IFN-γ/IL-4, has a median of 6.85 (0.74 – 299.37). The overall data on the characteristics of the research subjects are presented in Table 1.

Correlation of MLR and IFN-γ/IL-4 on sputum AFB positive in tuberculosis patient with post-intensive phase

MLR participants are divided into 2, namely <0.596 and ≥ 0.596. Based on the analysis, it was found that the majority of participants had MLR <0.596 with a positive sputum conversion of 98.2%, and most of the MLR participants ≥0.596 with a positive sputum conversion of 55.6% (OR = 44.8; $p = 0.001$). Meanwhile, the results of the measurement of IFN-γ/IL-4 ratio were categorized into 2, namely low <20,844 and high ≥20,844. The majority of participants had IFN-γ/IL-4 ratio <20,844 values with sputum conversion positive as much as 97.8% and those with IFN-γ/IL-4 ratio ≥20,844 values with sputum conversion positive as much as 80.0% (OR = 11.25; $p = 0.036$; table 2).

The results of the analysis of the combination of MLR and IFN-γ/IL-4 ratio for sputum conversion showed that 44 participants had MLR <0.596 and IFN-γ/IL-4 ratio <20.844 and had positive sputum conversion. In addition, 17 participants who had MLR 0.596 and IFN-γ/IL-4 ratio 20,844 also had positive sputum conversion ($p = 0.003$). Multivariate logistic regression analysis showed that subjects with MLR <0.596 had the possibility of sputum conversion after intensive therapy 27.103 times compared to MLR subjects 0.596, with the probability range in the population ranging from 2,289 to 320,883 times. Meanwhile, subjects with an IFN-γ/IL-4 ratio <20,844 had a probability of sputum conversion after intensive therapy 5,248 times compared to subjects with an IFN-γ/IL-4 ratio of 20,844, with a probability range in the population ranging from 0.414 to 66,454.

The final result showed that MLR was the only predictor that was independently associated with sputum AFB conversion status in patients with active pulmonary TB after intensive phase therapy. The results of this study also showed that MLR <0.596 had no interaction with the IFN-γ/IL-4 ratio <20.844 in predicting AFB sputum conversion status so that the combined OR value of the two predictive factors could not be determined.

Table 1. Characteristic of participant

Characteristic	n = 66
Age (years)	41.17 ± 14.67
Sex	
Male	44 (66.7)
Female	22 (33.3)
Education	
Elementary school	11 (16.7)
Junior high school	9 (13.6)
Senior High School	36 (54.5)
Diploma	3 (4.5)
Bachelor	7 (10.6)
Profession	
Entrepreneur	22 (33.3)
Employee	7 (10.6)
Does not work	17 (25.8)
Other	20 (30.3)
GeneXpert	
Positive	66 (100.0)
Negative	0 (0.0)
ATD category	
1	63 (95.5)
2	3 (4.5)
Comorbid	
Yes	45 (68.2)
No	21 (31.8)
Comorbid type	
HIV	6 (27.3)
Diabetes mellitus	11 (50.0)
Asthma	1 (4.5)
COPD	1 (4.5)
Hypertension	1 (4.5)
Chronic kidney disease	2 (9.1)
AFB	
Positive	5 (7.6)
Negative	61 (92.4)
Status konversi	
Yes	61 (92.4)
No	5 (7.6)
Nutritional Status	
Normal	54 (81.8)
Malnutrition	12 (18.2)

***Cont...* Table 1. Characteristic of participant**

Monocyte	0.5 (0.21 – 1.31)
Lymphocyte	1.87 (0.53 – 107.00)
IFN-γ	0.87 (0.07 – 22.45)
IL-4	0.12 (0.04 – 0.85)
MLR Low High	 57 (86.4) 9 (13.6)
IFN-γ/IL-4 Low High	 47 (71.2) 19 (28.8)

Note: HIV = human immunodeficiency virus; COPD = chronic obstructive pulmonary disease; AFB = acid fast bacilli; IFN-γ = interferon gamma; IL-4 = interleukin 4; MLR = monocyte lymphocyte ratio.

Table 2. Corelation of MLR and IFN-γ/IL-4 ratio on sputum conversion in tuberculosis patient with post intensive phase

Variable	Sputum Conversion		95% CI	OR	p
	Yes	No			
MLR <0.596 ≥0.596	 56 (98.2) 5 (55.6)	 1 (1.8) 4 (44.4)	4.169 – 481.452	44.8	0.001*
IFN-γ/IL-4 ratio <20.844 ≥20.844	 45 (97.8) 16 (80.0)	 1 (2.2) 4 (20.0)	1.169 – 108.280	11.25	0.036*

Note: MLR = monocyte lymphocyte ratio; IFN-γ = interferon gamma; IL-4 = interleukin 4; OR = odd ratio; *significant <0.05

Discussion

Various studies have shown that MLR is reported to be significantly increased in TB patients. This is because each component of the MLR can contribute to the severity of TB. Monocytes in TB patients will experience increased production in response to an existing infection[14]. On the other hand, lymphocytes in TB patients may decrease because peripheral lymphocytes will be recruited to the site of infection[15]. The study of Wang et al showed that there was a significant difference between the

MLR of patients with active TB and the MLR of patients who had completed TB treatment[7]. Other studies have also shown that MLR can be used as an indicator of the effectiveness of the use of ATD[16].

The IFN-γ/IL-4 ratio describes the balance between the response of Th1 cells and Th2 cells to MTB infection[17]. Th1 cell responses through the production of IFN-γ describe the activation of immune cell responses in the mechanism of phagocytosis and MTB destruction, while Th2 cell responses through the production of IL-4 indirectly describe the activation and inhibition of macrophage phagocytosis[18]. The ratio between Th1:Th2 can describe the antimicrobial response, an increase in Th1 response accompanied by a decrease in Th2 response is associated with antimicrobial activity and good outcome, and conversely, a low Th1 response with a high Th2 response is thought to be associated with active TB conditions and poor outcome[19]. Indirectly, the equilibrium of Th1:Th2 is measured by the ratio between IFN-γ/IL-4 which is an integral cytokine, and prototype of Th1 and Th2, respectively[20].

The study of Feng et al found that IFN- levels were significantly correlated with conversion status, the higher the IFN-γ levels, the greater the likelihood of conversion of AFB sputum in the first 2 months of therapy[21]. Another study found that high IL-4 indicates a poor prognosis[22]. Th1 response in patients with active TB was significantly increased compared to non-TB patients before intensive therapy and high Th1 levels were predictive of a good outcome on radiological findings. However, in these studies, measurement of IFN-γ or IL-4 was performed before the intensive phase. The difference in findings between this study and those studies could be due to the difference in the timing of the measurement of the IFN-γ/IL-4 ratio, in this study the measurement was carried out at the end of the intensive phase. A high IFN-γ/IL-4 ratio at the beginning of the intensive phase indicates high macrophage activation and active TB status. High macrophage activation correlates with high TB bacteria elimination ability, so a high IFN-γ/IL-4 ratio at the beginning of the intensive phase is associated with successful conversion at the end of the intensive phase. In contrast, by the end of the intensive phase, the patient had been on treatment. Successful treatment will reduce the number of TB germs so that the patient will experience sputum conversion from smear-positive to smear-negative. The decrease in the number of TB germs will also lead to reduced macrophage activation, as indicated by a low IFN-γ/IL-4 ratio[23].

Previous studies have emphasized the potential of applying hematological parameters as diagnostic markers to predict the course of TB disease, the presence or absence of active TB infection, and the response to ATD. However, research that discusses the correlation between these hematological parameters and the outcome of ATD therapy is still limited[24]. The phenomenon of increasing IFN-γ/IL-4 along with treatment success was not found in the TB patient population with HIV-positive status. This is because in this population there is an alteration of the immune system response[4]. Atopic conditions also cause alteration of the immune system, where there is a significant decrease in Th1 levels in the blood even though the patient has active TB. In this study, patients with a history of atopy were not excluded, thus possibly causing

a nonsignificant association between the IFN-γ/IL-4 ratio and conversion status in multivariate analysis[23].

Conclusion

The characteristics of pulmonary TB patients after intensive phase therapy were having a mean age of 41.17 ± 14.67 years (26 to 56 years); in men 66.6%; with a conversion status of 92.4%. There is an association between MLR and sputum smear conversion status of pulmonary TB patients after intensive phase therapy. If the MLR value <0.596, the possibility of sputum conversion after intensive phase therapy is 44.8 times compared to the MLR value of ≥ 0.596. There is an association between the ratio of IFN-γ/IL-4 with sputum smear conversion status of patients with pulmonary TB after intensive phase therapy. If the value of IFN-γ/IL-4 <20,844 then the possibility of sputum conversion after intensive phase therapy is 11.25 times compared to the value of IFN-γ/IL-4 ≥ 20,844. There is an association between the combination of MLR and the ratio of IFN-γ/IL-4 with sputum AFB conversion status after intensive phase therapy.

Ethical Approval: We have conducted an ethical approval based on the Declaration of Helsinki with the registration of research at the Health Research Ethics Committee in Sanglah General Hospital, Denpasar, Indonesia.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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Table and Legend