Correlation of Monocyte Lymphocyte ratio and Interferon gamma Interleukin 4 Ratio on Sputum Positivity in Patients with Pulmonary Tuberculosis

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Abstract

Objective: The study aimed to analyze the correlation between monocyte lymphocyte ratio (MLR) and interferon-gamma interleukin-4 ratio (IFN-γ/IL-4) on sputum positivity of acid-fast bacilli (AFB).

Method: The design of this study used a case-control design which was carried out in the period January – June 2020. Case defined as bacteriologically confirmed tuberculosis, while control defined as clinically confirmed tuberculosis. The technique for collecting participants was consecutive sampling. This study analyzed the MLR, IFN-γ/IL-4 ratio, and tuberculosis status.

Result: The average age of the participants is 45.74 ± 16.54 years with a median value of 46.50 (20 – 77) years. A total of 54.5% of the study subjects had a MLR value of 0.64 and 45.5% of the study subjects had an IFN-γ/IL-4 ratio value of 31.2. Only the association between the MLR value group showed significance on the results of AFB sputum examination with *p*-value = 0.046

Conclusion: the association of MLR on sputum positivity of AFB was significant, while no significant between IFN-γ/IL-4 and sputum positivity of AFB.

Keywords: *monocytes lymphocytes ratio, IFN-γ/Interleukin-4 ratio, acid-fast bacilli, tuberculosis lung*

Introduction

Tuberculosis (TB) is currently a public health problem in the world, even though efforts to control the strategy of directly observed treatment short-course (DOTS) have been implemented in many countries since 1995. Based on data from the Global Tuberculosis Report from the World Health Organization (WHO) in 2015, there were 9.6 million people with TB worldwide. This disease caused about 1.3 million deaths and was the 9th leading cause of death over the last five years in 2012–2016[1].

Indonesia is one of the five countries that account for the largest incidence of TB. Indonesia ranks 2nd in the world for the largest number of TB incidence cases after India[1, 2]. According to WHO data, a major problem hampering control efforts and driving the TB epidemic is the large reservoir of undiagnosed smear negative pulmonary TB disease which comprise ~30 to 50% of the total TB burden. Every year, one in three

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people who fall ill with TB are left undiagnosed or not registered by health systems mainly in TB high burden countries[3]. The existence of a non-sputum biomarker as an indicator for starting therapy and evaluating the success of therapy can facilitate treatment success and be able to develop more effective treatment strategies[4].

In patients with pulmonary TB, lymphocytes play an important role in providing an immune system response that will fight against MTB that enters the body. In a previous study, it was stated that TB patients with low lymphocytes had a higher recurrence rate (15.6%) and more severe clinical manifestations such as respiratory failure in 34.3% of patients. A decrease in the number of lymphocytes or lymphopenia can result in the development of bacteria that increases sharply because the body's ability to fight infection is disrupted. An increase in the number of bacteria can cause permanent damage to the lungs which can be accompanied by more serious complications, including pleural effusion or pneumothorax[5].

The balance of cytokine activity between Th1 and Th2 cells in the adaptive immune system has an important role in protection against MTB infection. Decreased response of TB patients to the anti-tuberculosis drug (ATD) as evidenced by low sputum conversion (86.4% of the sample) in samples with low levels of Th1 cytokines (IFN-γ) and high Th2 cytokines (IL-4)[6]. Increased activation and production of Th2 cell cytokines accompanied by decreased Th1 cell activation are associated with the development of infection and progression of TB[7]. IFN-γ is the main cytokine in natural immunity secreted by Th1 cells, which plays a role in the formation of granulomas and the elimination of MTB bacteria through the activation of macrophages towards the M1 phenotype. While IL-4 is the principal cytokines produced by Th2 cells that act as an anti-inflammatory and contribute to the survival of MTB bacteria in dealing with the immune system[8].

As an important element of immunity against TB, the levels of monocytes, lymphocytes, and IFN-γ and IL-4 cytokines in peripheral blood indicate the level of immunity to infection by MTB. A peripheral blood examination is a routine examination in clinical practice, but the monocyte lymphocyte ratio (MLR) and the IFN-γ/IL-4 ratio as a simple biomarker are rarely used in clinical practice[9].

The association between MLR values and the risk of infection by MTB was first reported in 1920 by Florence Sabin, et al. They get MLR values on peripheral blood tests reflecting the extent of extension and progression of TB. In another study, it was reported that an increase in the MLR value was associated with the risk of TB in adults, children, and postpartum women with HIV infection. While the decrease in the ratio of IFN-γ/ IL-4 is said to be related to the incidence of more severe lung infections such as milier TB and necrosis of lung tissue. In mice given MTB infection, increased IL-4 values were correlated with peribronchial and lung interstitial necrosis[10]. In this study, we wanted to analyze the MLR and IFN-γ/ IL-4 ratio on blood examination to sputum positively of pulmonary TB patients as markers of diagnosis of active TB infection.

Method

The participants in this study were TB patients undergoing treatment at the hospital. Participant's inclusion criteria included patients diagnosed with pulmonary TB, aged >18 years, bacteriologically and clinically confirmed pulmonary TB patients. Participants' exclusion criteria included pulmonary TB patients who had received ATD and pulmonary TB patients with comorbid malignancies, heart disease, SLE, sarcoidosis, RA, asthma, liver fibrosis, and Alzheimer's. Written informed consent was obtained from all participants.

The design of this study used a case-control design which was carried out in the period January – June 2020. The number of participants in this study was 66 participants (case group = 33 participants and control group = 33 participants) of which case was a bacteriologically confirmed TB patient and control was a clinically confirmed TB patient. The participant collection technique used consecutive sampling. This study analyzed the MLR, IFN-γ/IL-4 ratio, and tuberculosis status.

MLR is the absolute number of monocytes compared to the absolute number of lymphocytes, the data obtained from the results of routine complete blood tests when diagnosed with pulmonary TB. The MLR value is categorized into 2, namely high and low based on the median value after the normality test on the data distribution is carried out. 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 was obtained from the results of blood serum examination when initially diagnosed with pulmonary TB, examined using the enzyme-linked immunosorbent assay (ELISA) method. The IFN-γ/IL-4 ratio is categorized into 2, namely high and low based on the results of the normality test of the measurement results.

Active TB was pulmonary TB with clinical symptoms, radiological features, and microbiological evidence (rapid test molecular/AFB sputum) show 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 infiltrate, fibrosis, cavity, calcification, tuberculoma and pleural effusion. Bacteriologically confirmed TB was determined by sputum examination using Xpert MTB/Rif Method, or AFB staining. Clinically confirmed TB is one who did not fulfil the criteria for bacteriological confirmation but had symptoms or sign suggestive TB and supported by typical radiographic features.

Descriptive statistical analysis used to describe the characteristics of the subjects based on the MLR ratio and the IFN-γ/IL-4 ratio. ROC curve analysis used to determine the intersection point of the MLR and the IFN-γ/IL-4 ratio. In addition, the AUC (area under the curve) value used to determine the sensitivity and specificity values of each marker. Bivariate analysis used to determine the association between increased MLR and decreased IFN-γ/IL-4 ratio with pulmonary TB sputum examination. The association between the two variables is significant if the p-value <0.05.

Result

Characteristic of Participant

The average age of the participants was 45.74 ± 16.54 years with a median value of 46.50 (20 – 77) years. The participant was dominated by the male gender (69.7%). A total of 22.7% of the study subjects had a positive HIV status and 50% of the study subjects had a positive AFB sputum examination result (the same proportion as a negative AFB sputum examination result). Details of participant characteristics in this study can be seen in table 1.

The median value of absolute monocyte levels was 0.59x x109/L, the median value of absolute lymphocyte levels was 1.04x x109/L, the median value of the MLR was 0.56, the median value of IFN-γ levels was 2.63 pg/mL, the median value of IL-4 levels was 0.09 pg/mL, and the median value of the IFN-γ/IL-4 ratio was 30.43 (table 2).

Association between MLR and IFN-γ/IL-4 Ratio on AFB Sputum Examination

Our study found that monocyte levels, interferon-γ levels, MLR values, and IFN-γ/IL-4 ratios were higher in the group with positive smear sputum examination,

while IL-4 levels were slightly higher in the group with the results of the sputum smear examination were negative. Absolute lymphocyte levels between the two groups did not differ much. However, all the associations of inflammatory markers in this study were not significantly associated with AFB sputum results (Table 2).

Receiver Operating Characteristics Analysis for Determination of Area Under Curve, Cut off Point, and Sensitivity and Specificity of MLR and IFN-γ/IL-4 Ratio

The researchers then performed a Receiver Operating Characteristics (ROC) analysis was performed on various markers of inflammation on the results of AFB sputum examination in TB patients expressed in AUC values. The monocyte level has an AUC of 0.583, the absolute lymphocyte level has an AUC value of 0.488, the IFN-γ level has an AUC value of 0.546, the IL-4 level has an AUC value of 0.468, the MLR has an AUC value of 0.577, and the IFN-γ/IL-4 ratio has an AUC value of 0.543. The interpretation of the AUC values of various inflammatory markers on the results of AFB sputum shows a weak level of accuracy.

In this study, the cut-off point of the ratio of monocytes and lymphocytes was 0.64 with a sensitivity level of 54.5% and a specificity level of 69.7%. Meanwhile, the cut-off point for the IFN-γ/IL-4 ratio is 31.2 with a sensitivity level of 54.5% and a specificity level of 57.6%. In this study, the highest sensitivity value was in absolute monocyte levels and the highest specificity value was in the MLR. The intersection points of the ratios of inflammatory markers were then used to form a bivariate analysis of the two groups (Figure 1).

Bivariate Analysis of MLR and IFN-γ/IL-4 Ratio based on ROC/AUC Cut-off Point with AFB Sputum Examination

We then divided the MLR and IFN-γ/IL-4 ratio using the intersection point of the ROC/AUC curve analysis into 2 groups. A total of 54.5% of the study subjects had a MLR value of 0.64 and 45.5% of the study subjects had an IFN-γ/IL-4 ratio value of 31.2. Only the association between the MLR value group showed significant results on the results of the AFB sputum examination with p-value = 0.046 (Table 3).
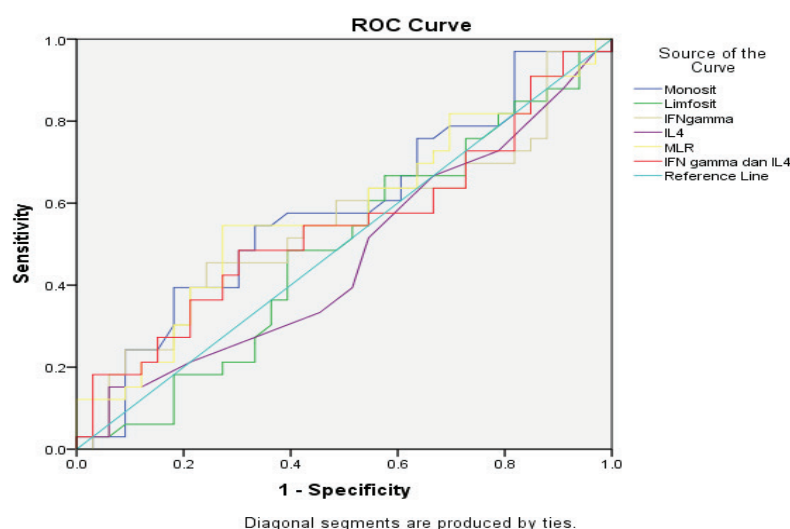
Figure 1. ROC (Receiver Operating Characteristics) analysis between cytokines inflammation on sputum positivity of AFB examination

Table 1. Characteristics of participants

Variables	n (%)
Gender Male Female	46 (69.7) 20 (30.3)
HIV Positive Negative	15 (22.7) 51 (77.3)
Acid-Fast Bacilli Positive Negative	33 (50.0) 33 (50.0)
X-ray Infiltrate Fibrosis Fibroinfiltrate Cavity Consolidation Cavity and fibroinfiltrate Cavity and consolidation Normal	6 (9.1) 1 (1.5) 14 (21.2) 11 (16.7) 29 (43.9) 2 (3.0) 1 (1.5) 2 (3.0)

Table 2. Correlation between MLR and IFN-γ/IL-4 ratio on sputum positivity of AFB

Variable	Mean ± SD	Median (Min-Max)	Sputum AFB		p
			Positive	Negative	
Monocyte	0.78 ± 0.97	0.59 (0.03 – 7.90)	0.65 (0.03 – 1.76)	0.58 (0.05 – 7.90)	0.246
Lymphocyte	1.51 ± 3.16	1.04 (-0.49 – 25.86)	1.04 (0.49 – 25.86)	1.04 (0.35 – 5.61)	0.863
MLR	0.67 ± 0.66	0.56 (-1.60 – 2.86)	0.69 (-1.02 – 2.86)	0.53 (-1.60 – 1.45)	0.284
IFN-γ	6.32 ± 7.97	2.63 (0.15 – 35.71)	3.47 (0.15 – 32.89)	2.48 (0.15 – 35.71)	0.521
IL-4	0.14 ± 0.35	0.09 (0.03 – 2.89)	0.09 (0.03 – 2.89)	0.10 (0.04 – 0.23)	0.657
IFN-γ/IL-4	70.97 ± 100.01	30.43 (0.79 – 518.61)	28.6 (0.79 – 519.40)	28.6 (1.33 – 446.40)	0.551

Note: AFB = acid-fast bacilli.

Table 3. correlation between MLR and IFN-γ/IL-4 ratio on sputum AFB based on categorization

Variable	Sputum AFB		p
	Positive	Negative	
MLR ≥0.64 <0.64	18 (54.5) 15 (45.5)	10 (30.3) 23 (69.7)	0.046*
IFN-γ/IL-4 ≤31.2 >31.2	15 (45.5) 18 (54.5)	19 (57.6) 14 (42.4)	0.325

Note: AFB = acid-fast bacilli; *significant < 0.05.

Discussion

In this study we found that MLR was associated with AFB sputum examination. Monocytes are an essential component of the innate immune system that plays a role in connecting with the adaptive immune system. The MLR in peripheral circulation shows the capacity of an individual to carry out an effective immune response so that it is correlated with the growth inhibition of *Mycobacterium sp* in vitro[11]. Monocytes that will turn into macrophages at the site of infection after 24-28 hours are markers of chronic inflammation. Inflammatory stimuli and increased numbers of chemoattractant molecules lead to an increase in the number of monocytes in the peripheral circulation (monocytosis) and at the site of infection, especially in the specialized form of alveolar macrophages in TB infection. Monocytes are the site of MTB proliferation, on the other hand, lymphocytes are components that provide resistance (clearance) so that their number decreases in the peripheral circulation (resistance index). This is supported by previous studies which found lymphopenia (22.1%), neutrophilia (14.2%), monocytosis (23.5%), increased MLR, and increased neutrophil/lymphocyte ratio in

TB patients[12].

Increase MLR value in active TB are associated with MTB infection that interferes with a subset of hematopoietic stem cells or directly infects bone marrow mesenchymal stem cells. Infection of MTB attacks the myeloid and lymphoid pathways to varying degrees, causing variations in MLR. The MLR value has quantitative variations with different phases of treatment, so it is used to evaluate treatment effectiveness[11, 13].

The median value in the study of IFN-γ levels was 2.63 pg/mL, the median value of IL-4 levels was 0.09 pg/mL, and the median value of the IFN-γ/IL-4 ratio was 30.43. In a previous study, the mean IFN-γ/IL-4 ratio was 9.3 ± 4.6 pg/mL in patients newly diagnosed with pulmonary TB, and higher in the latent TB population 9.8 ± 3.8 pg/mL. and the healthy population was 10.2 ± 3.4 pg/mL[14]. The normal value of IFN-γ is <2 pg/mL, so this study shows an increase in this marker but not as high as other previous studies. Another study by Hussain in Pakistan showed a higher mean increase in IFN-γ levels up to 48.69 ± 28.78 pg/mL in the case group

and 12.99 ± 5.7 pg/mL in the control group with a significant difference between the two. In addition, there is a negative correlation between interferon-gamma levels and the duration of ATD[15].

Cells that play a role in cell-mediated immunity are lymphocytes, especially T helper (Th) lymphocytes which are divided into Th1 (releasing the pro-inflammatory cytokine IFN-γ) and Th2 (secreting the anti-inflammatory cytokine IL-4). IL-4 plays a role in preventing macrophage activation induced by IFN-γ so that the effects of the two cytokines are opposite. The increase in IFN-γ levels but not significant is explained by the condition of newly diagnosed active pulmonary TB infection so that they have not received therapy or the therapy given has not reached 1 month. This condition indicates the patient's immune response is good so that IFN-γ increases, but is not strong enough to fight the TB infection. IFN-γ levels generally will increase significantly after receiving ATD. Another influencing factor is the nutritional status of the patient (especially protein intake) which plays a role in the regeneration of damaged tissue. Good nutritional status can increase the clearance process of MTB which is characterized by an increase in cytokines IFN-γ, TNF-α, and Inducible Nitric Oxide Synthase (iNOS). Another factor not assessed in this study was genetic polymorphism. A low IFN-γ/IL-4 ratio was also found in previous studies but did not provide a significant association as in this study. Elevated IL-4 is associated with latent TB infection, reactivation, extrapulmonary TB and more severe infection, therefore, further study should provide data on the patient's clinical condition[16].

Conclusion

The limit value of the MLR associated with smear-positive was 0.64. There is a significant association between the value of the MLR on the results of AFB sputum examination with a sensitivity of 54.5% and a specificity of 69.7%. There was no significant association between the value of the IFN-γ/IL-4 ratio on the results of the sputum smear examination.

References

1. Chakaya J, Khan M, Ntoumi F, Aklillu E, Fatima R, Mwaba P, et al. Global Tuberculosis Report 2020 - Reflections on the Global TB burden, treatment and prevention efforts. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases. 2021.

2. Kusmiati T, Mertaniasih NM, Eko Putranto JN, Suprapti B, Soedarsono, Luthfah N, et al. Correlation of inflammatory cytokines on corrected QT interval in rifampicin-resistant tuberculosis patients. Annals of Medicine and Surgery. 2021;70:102862.

3. Gupta RK, Lucas SB, Fielding KL, Lawn SD. Prevalence of tuberculosis in post-mortem studies of HIV-infected adults and children in resource-limited settings: a systematic review and meta-analysis. AIDS (London, England). 2015;29(15):1987-2002.

4. La Manna MP, Orlando V, Dieli F, Di Carlo P, Cascio A, Cuzzi G, et al. Quantitative and qualitative profiles of circulating monocytes may help identifying tuberculosis infection and disease stages. PloS one. 2017;12(2):e0171358.

5. Prezzemolo T, Guggino G, La Manna MP, Di Liberto D, Dieli F, Caccamo N. Functional

Signatures of Human CD4 and CD8 T Cell Responses to Mycobacterium tuberculosis. Frontiers in immunology. 2014;5:180.

6. Wang C, Chen ZL, Pan ZF, Wei LL, Xu DD, Jiang TT, et al. NOD2 polymorphisms and pulmonary tuberculosis susceptibility: a systematic review and meta-analysis. International journal of biological sciences. 2013;10(1):103-8.

7. Sabbagh DK, Beasley R, Marks GB. The Immunological Mysteries of Tuberculosis. The journal of allergy and clinical immunology In practice. 2019;7(2):649-50.

8. Kak G, Raza M, Tiwari BK. Interferon-gamma (IFN-γ): Exploring its implications in infectious diseases. Biomolecular concepts. 2018;9(1):64-79.

9. Paul WE. History of interleukin-4. Cytokine. 2015;75(1):3-7.

10. van Crevel R, Karyadi E, Preyers F, Leenders M, Kullberg BJ, Nelwan RH, et al. Increased production of interleukin 4 by CD4+ and CD8+ T cells from patients with tuberculosis is related to the presence of pulmonary cavities. The Journal of infectious diseases. 2000;181(3):1194-7.

11. Wang J, Yin Y, Wang X, Pei H, Kuai S, Gu L, et al. Ratio of monocytes to lymphocytes in peripheral blood in patients diagnosed with active tuberculosis. The Brazilian journal of infectious diseases : an official publication of the Brazilian Society of Infectious Diseases. 2015;19(2):125-31.

12. Choudhary RK, Wall KM, Njuguna I, Pavlinac PB, LaCourse SM, Otieno V, et al. Monocyte-to-Lymphocyte Ratio Is Associated With Tuberculosis Disease and Declines With Anti-TB Treatment in HIV-Infected Children. Journal of acquired immune deficiency syndromes (1999). 2019;80(2):174-81.

13. Chaidir L, Ruesen C, Dutilh BE, Ganiem AR, Andryani A, Apriani L, et al. Use of whole-genome sequencing to predict Mycobacterium tuberculosis drug resistance in Indonesia. Journal of global antimicrobial resistance. 2019;16:170-7.

14. Köksal D, Unsal E, Poyraz B, Kaya A, Savaş H, Sipit T, et al. The value of serum interferon-gamma level in the differential diagnosis of active and inactive pulmonary tuberculosis. Tuberkuloz ve toraks. 2006;54(1):17-21.

15. Hussain S, Afzal N, Javaid K, Ullah MI, Ahmad T, Saleem Uz Z. Level of interferon gamma in the blood of tuberculosis patients. Iranian journal of immunology : IJI. 2010;7(4):240-6.

16. Hui Y, Xie JJ, Li L, Xiang HX, Mu HJ, Yin Y, et al. Association between suppressors of cytokine signaling mRNA expression and Th1/Th2 balance in children with asthma. Zhongguo dang dai er ke za zhi = Chinese journal of contemporary pediatrics. 2012;14(10):755-8.