Neutrophil Lymphocyte Ratio and Acid-Fast Bacilli in Tuberculosis

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Abstract

Background: Tuberculosis (TB) is one of the top 10 causes of death in the world and the most common cause of death with single infectious agent. The neutrophil-lymphocyte ratio is a cheap and fast marker of inflammation that has been widely studied in TB. The aimed of this study was to investigate Neutrophil-lymphocyte ratio (NLR) in TB with positive Acid-Fast Bacilli (AFB) and negative AFB. **Methods**: This was a retrospective study that included TB patients data from the medical records in two hospitals in Palu, Indonesia. A total of 150 TB patients were involved in this study consisting with positive and negative AFB TB suspect. Neutrophil, lymphocyte and NLR data were compared with the Mann-Whitney test. **Results**: The study shows a significant difference of neutrophils and lymphocytes percentage between positive AFB TB patients (80 subjects, 53.3%) and negative AFB TB patients (70 subjects, 46.7%) with ($p < 0.001$) as well as NLR ($p < 0.001$). The percentage of Neutrophils was significantly higher in positive AFB TB compared to negative AFB tuberculosis (81.18 ± 8.52 vs. 55.02 ± 9.80), lymphocyte percentage were found to be significantly lower in positive AFB TB compared with negative AFB TB (12.72 ± 7.51 vs 28.69 ± 12.01). Additionally, NLR were significantly higher in positive AFB TB compared with negative AFB TB (10.20 ± 9.53 vs 2.47 ± 1.56). **Conclusion**: There is a significant increase in the number of neutrophils, a decrease in lymphocytes and an increase in NLR among positive AFB TB compared with negative AFB TB.

Keywords: *Tuberculosis, Acid-Fast Bacilli, Neutrophil, Lymphocyte, Neutrophil Lymphocyte Ratio*

Introduction

Tuberculosis (TB) is one of the top 10 causes of death in the world and the most common cause of death with single infectious agent more than human immunodeficiency virus (HIV). Millions of people suffer from TB every year. In 2017, TB was estimated to cause 1.3 million deaths in HIV-negative patients and 300,000 deaths in HIV-positive patients [(1)]. The activation progress of TB is a combination of bacterial virulence factors and host immune system factors. Recently advanced knowledge of immunology in TB provides an overview of abnormalities in hematology [(2)].

One of the criteria for TB is by examining acid-fast bacilli (AFB). High quality and sufficiency of sputum sampling is very important to obtain good result. Therefore, patients need to understand good sputum sampling. Good sputum is sputum containing material from the lung when productive coughing and not from the nasopharynx only. One of methods to determine quality of sputum sample is to assess sputum leukocyte

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levels [3], but this is time consuming and costly. Despite its lack of sensitivity compare to sputum culture, AFB is still the mainstay of diagnosis of tuberculosis with positive AFB among the pulmonary tuberculosis suspect patients in many region [4]. The use of AFB examination alone without confirmation by culture, the quality is needed to fully assured in its use [5].

Various factors can influence the results of AFB examination, including the quality of the sample and the competency of the examiner so that the AFB examination has its own constraints in determining the diagnosis of TB patients. The number of bacteria in sputum may be related to bacterial virulence, higher virulence bacteria have better endurance and replicate in macrophages or neutrophils faster than low virulence bacteria [6, 7]. Cellular immune responses especially those produced by T lymphocytes play an important role in controlling the replication of *Mycobacterium tuberculosis* (MTB) as well as neutrophils play a role in naive immunity to MTB. Lymphopenia, neutrophilia and monocytosis are the most common hematological abnormalities in TB [8, 9].

The neutrophil-lymphocyte ratio (NLR) is an inflammatory marker that can be used to estimate various clinical conditions including cancer and coronary heart disease and also shows differences based on the type of infection. In addition, NRL is also useful to predict and detect inflammatory conditions and infections. NLR of miliary TB patients at the time of hospital admission can be used as a marker of mortality and the risk of acute respiratory distress syndrome (ARDS) [10-12]. Other studies have also shown that NLR is found to be increased in patients newly diagnosed with TB and NLR can be used as an inflammatory marker that can help management of TB patients and to determine the severity of TB disease [2, 9]. AFB and NLR obtain from leukocyte differential count are widely use and more affordable in Indonesia specially in rural areas. The objective of this study was to see differences of NLR in TB with positive AFB and negative AFB.

Method

This research is a retrospective study utilizing data from medical record of TB patients in two hospitals in Palu City, Indonesia (Undata Hospital in 2016 and Anutapura Hospital in 2017). TB patients were included and examined whether they had carried out AFB examinations, then divided into 2 groups based on examination AFB, positive AFB and negative AFB. Hematological examination data of the two groups was taken to determine the levels of neutrophils and lymphocytes. The patients were excluded from the study if AFB and hematology examination were unavailable. Generally these patients have undergone TB therapy. 88 samples were taken from the Undata Hospital and 62 samples were taken from Anutapura Hospital, the total number of samples was 150 TB patients.

Data distribution was tested by the Kolmogorov-Smirnov Test. Data with normal distribution has a value of $p > 0.05$. The Kolmogorov Smirnov test show that the distribution of neutrophils, lymphocytes and NLR data is not normal. The statistical test used to compare the two groups was the Mann-Whitney test because of abnormal data distribution.

Result

The total number of samples was 150 TB patients consisting of 80 smear of positive AFB TB and 70 negative AFB TB. The age of positive AFB TB patients was 43.20 ± 13.16 years with a range of 21-72 years, the age of patients with negative AFB TB was 41.32 ± 14.07 with a range of 17 - 63 years. Male in the positive AFB TB group was 60% and 40% female, in the negative AFB TB group male was 56% and female 34% (table 1).

The Mann-Whitney test showed a significant differences of neutrophils and lymphocytes percentage between positive AFB TB patients and negative AFB TB ($p < 0.001$), as well as NLR ($p < 0.001$). Neutrophils percentage was significantly higher in positive AFB TB compared with negative AFB TB (81.18 ± 8.52 vs. 55.02 ± 9.80), lymphocytes percentage were significantly lower in positive AFB TB compared with negative AFB TB (12.72 ± 7.51 vs 28.69 ± 12.01). NLR were significantly higher in positive AFB TB compared with negative AFB TB (10.20 ± 9.53 vs 2.47 ± 1.56; table 2).

Tabel 1. Sample characteristics

Characteristics	Positive AFB (n = 80)	Negative AFB (n = 70)
Age, (year)	43.20 ± 13.16	41.32 ± 14.07
Gender (Male/Female)	48/32	39/31
Neutrophil (%)	81.18 ± 8.52	55.02 ± 9.80
Lymphocyte (%)	12.72 ±7.51	28.69 ± 12.01
NLR	10.20 ± 9.53	2.47 ± 1.56

AFB = Acid-Fast Bacilli; NLR = Neutrophil Lymphocyte Ratio

Table 2. Comparison of Neutrophils, Lymphocytes and NLR between positive AFB TB and negative AFB TB

Characteristic	Positive AFB (n = 80)	Negative AFB (n = 70)	p
Neutrophil (%)	81.18 ± 8.52	55.02 ± 9.80	0.000**
Lymphocyte (%)	12.72 ±7.51	28.69 ± 12.01	0.000**
NLR	10.20 ± 9.53	2.47 ± 1.56	0.000**

*Significant < 0.05; **Significant < 0.001

Discussion

Tuberculosis is an infectious disease which the progression and the outcome depend on the immune reactivity of the host (13). The immune system helps control the proliferation of MTB. Reactivation of latent TB occurs in individuals who experience a decrease in the immune system [14, 15].

Inflammation plays an important role in the pulmonary TB pathogenesis as in protection and pathology. Yaranal et al showed that hematologic abnormalities were often found in severe TB cases. Hemoglobin levels, platelet counts, lymphocytes and neutrophils counts change in the chronic inflammatory process and return to normal with effective therapy. These parameters can be used as indicators to assess treatment [2, 13, 16, 17]. In the early stages of the disease proinflammatory cytokines and chemokines released by various cells, induce migration of immune cells to infected areas, form granulomas and initiate host response protection. The cellular population increases in response, involving alveolar macrophages, dendritic cells, neutrophils, NK cells, epithelial cells and other cells [13].

Jadoon et al. showed that decreased lymphocyte levels and increased neutrophil levels were associated with the severity of TB disease [18]. The immune response often has a certain pattern, which were an increase in the number of neutrophils and a decrease in the number of lymphocyte cells. When infection persists, neutrophil production increases and the possibility is not apoptosis. Neutrophil apoptosis in sepsis is beneficial, whereas lymphocyte apoptosis provides a loss [19]. Polymorphonuclear cells (PMN) are cells that play a role in phagocytosis and are the first line of defense against microbes. The highest PMN in the blood is neutrophils. In TB infection, PMN will be attracted to infected areas along with interleukin 8. In patients with active pulmonary TB, PMN becomes the dominant cell found in the lungs [20].

The role of neutrophils in TB is still debatable. Several studies demonstrated the involvement of neutrophils in granuloma formation, initiation of T cell responses, amplification of antimicrobial activity of macrophages, secretion of antimicrobial peptides and killing of tuberculosis. This implies that neutrophils have many different roles in each phase of TB, mediating protection in the onset phase of infection and pathology induction in the later phase of TB. There are some mechanisms of neutrophil induction of pathology in TB, neutrophils were suggested to play the role of trojan horse by hiding MTB from active macrophages. With increasing neutrophils and precursors, neutrophils are likely to suppress T cell responses [21]. Neutrophils help early CD4 activation of T cells by working with dendritic cells in mice infected with virulent MTB. Neutrophils are predominant cells infected with MTB in TB patients and these bacteria replicate intracellular [7, 22]. Research by Xu et al. showed that the percentage of regulatory T cells increased in patients with negative AFB compared with patients with positive AFB TB. Regulatory T cells are thought to play a role in preventing inflammation which causes damage to host tissues in TB [23, 24].

Calculations of NLR are easily obtained from the results of routine laboratory examinations, and can be done in many hospitals. NLR is also easier to obtain and cheaper than other inflammatory markers such as C-reactive protein and procalcitonin [2, 25]. The role of NLR as an inflammatory marker in TB has been widely investigated [26-30]. This study showed that higher neutrophils percentage and lower lymphocyte percentage were found in positive AFB TB compared with negative AFB TB. Both of these contribute to the increased value of NLR in positive AFB TB Tuberculosis with positive AFB is considered to have more bacterial load in sputum and could be more virulent than negative AFB TB. The NLR in this study can illustrate the level of inflammation of TB infection but there are findings that shows high numbers of neutrophils play a role in the replication of TB bacilli.

The limitations of the study include the absence of the duration or type of TB patients treatment, the severity of clinical symptoms and radiological features. Further research is needed to analysis NLR in TB patients based on therapy, relapse, HIV co-infection, nutritional status, molecular test and TB drug resistance. Research with more patients is needed to determine the cut-off value of NLR in positive AFB and negative AFB TB.

Conclusion

This study indicates that in positive AFB TB has an increase in neutrophils percentage, a decrease in lymphocytes percentage and an increase in NLR compared with negative AFB TB.

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

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