



## Comparison of serum neopterin levels in pulmonary tuberculosis patients with positive acid fast bacilli and inactive pulmonary tuberculosis

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### ABSTRACT

**Background:** Tuberculosis (TB) is caused by infection with *Mycobacterium tuberculosis*. Sometimes it is difficult to distinguish between active and inactive pulmonary TB based on chest X-ray images. TB biomarkers are needed to diagnose active TB when TB bacilli are not detected either through microscopic examination, culture or geneXpert MTB/Rif. Neopterin can be used as an additional diagnosis and therapy monitoring as well as assessing activation of macrophage cells. This study aims to examine and comparing serum neopterin levels in new cases of pulmonary TB with positive AFB and inactive pulmonary TB.

**Methods:** This was an analytic study with Cross-Sectional design conducted in Dr. Soetomo General Hospital Surabaya, Indonesia. The subjects of the study were the new cases of pulmonary TB with positive AFB and inactive pulmonary TB who met the inclusion and exclusion criteria. Blood serum was collected and examined to measure neopterin serum levels in patients by ELISA technique.

**Results:** There was a significant difference of mean neopterin serum levels between the new cases of pulmonary TB with positive AFB and inactive pulmonary TB with a p-value < 0.001 (74.92 nmol/l vs. 9.84 nmol/l). There was a significant difference in serum neopterin levels in each AFB grading in the new cases of pulmonary TB with positive AFB with a p-value < 0.001 (60.10 nmol/l in AFB 1+, 80.81 nmol/l in AFB 2+, and 110.70 nmol/l in AFB 3+).

**Conclusion:** Serum neopterin levels in the new cases of pulmonary TB with positive AFB were higher than inactive pulmonary TB.

### 1. Introduction

Tuberculosis (TB) remains a global public health concern. Indonesia is one of 30 countries with a high TB burden in the world. In 2017, Indonesia is in the 3rd rank after India and China with 842,000 TB cases or 319 cases per 100,000 populations [1]. An accurate diagnosis was needed to prevent its transmission. The current TB diagnosis was based on acid fast bacilli (AFB) smear examination. However, this method is less sensitive, because it only gives positive results if there is > 10<sup>4</sup> organisms/ml of sputum. Culture has an important role in diagnosing TB because it has better sensitivity and specificity than AFB smear examination. Although culture is a gold standard for the diagnosis of TB, with sensitivity and specificity of 99% and 100% respectively, it takes a long time of around 8 weeks to get results which causes a significant delay to establish a diagnosis and start therapy. A new diagnostic technique was needed that can diagnose TB more quickly and accurately [2,3]. Diagnosis of active TB was based on *Mycobacterium tuberculosis* detected in sputum using sputum smear examination and culture as conventional methods, and GeneXpert MTB/RIF as a rapid molecular test [4]. Clinicians often face difficult cases to make a diagnosis of whether TB in some patients is an active or not active TB based on chest X-ray examination, while bacteriological examination of both microscopic, culture and genotyping has not been successful in

detecting TB bacilli. The role of TB markers are needed in such cases as well as other clinically and laboratory confirmed [3].

Biomarkers for TB were found in recent years, including neopterin. Neopterin is a biomarker produced by macrophages activated by *Mycobacterium tuberculosis* infection. Neopterin is a catabolic product of guanosine triphosphat (GTP), produced and released by macrophages through stimulation of *Interferon gamma* (IFN- $\gamma$ ). Neopterin is produced in macrophages or monocytes of the body and will subsequently be released into the body's plasma fluid [2,3]. Neopterin is a sensitive marker for cell-mediated immune response. Because of this, the neopterin levels of body fluids show cell-mediated immune response in different infectious diseases which involve T-cells and macrophages. *Mycobacterium tuberculosis* is an intracellular pathogen with the ability to live for a long time in macrophages. *Mycobacterium tuberculosis* that is phagocytosed by macrophages cannot be killed by polymorphonuclear leukocytes. Neopterin is released by the stimulation of intracellular viruses and bacteria, such as TB, and provides information about the status of cell-mediated immunity [5].

Previous studies reported different results. Sigal et al. stated that there was no significant correlation between neopterin levels with response of TB therapy [6], while El-Shimy et al. reported that there was a significant correlation between increased serum neopterin levels and severity of TB disease based on radiological test. Serum neopterin levels

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were decreased along with anti-TB therapy [7]. This study was aimed to examine and comparing serum neopterin levels in new cases of pulmonary TB with positive AFB and inactive pulmonary TB.

## 2. Methods

This was an analytic study with Cross-Sectional design. The populations of this study were all new cases of pulmonary TB with positive AFB and inactive pulmonary TB in Dr. Soetomo Hospital Surabaya. Inclusion criteria in this study were patients with age  $\geq 21$  years old. Exclusion criteria were patients with pneumonia, HIV, cancer, asthma, COPD, Hepatitis C, autoimmune disease and diabetes mellitus.

Sputum smear examination was done using Ziehl–Neelsen staining according to the WHO recommendation. AFBs identified were graded according to the International Union against Tuberculosis and Lung Disease (IUATLD) and the WHO smear grading scale. Findings were scored as follows: 1–9 AFB/100 fields (AFB 1+); 1–9 AFB/10 fields (AFB 2+); and 1–9 AFB/ field (AFB 3+).

Blood serum was collected and examined to measure neopterin serum levels in patients by ELISA technique in the clinical pathology laboratory, Dr. Soetomo Hospital Surabaya. SPSS 21.0 is used for statistical analysis. Statistical results were considered significant if the  $p$ -value was  $< 0.05$ .

Written informed consent was obtained from all participants and the study protocol was approved by ethics committee in health research in Dr. Soetomo Hospital with ethical clearance number 118/Panke.KKE/II/2015 and has been approved on February 16th, 2015.

Serum neopterin levels are the result of the neopterin examination of blood serum by ELISA. The normal value of serum neopterin is (nmol/l): Male with age 19 to 75 years is  $5.3 \pm 2.7$  nmol/l, and age more 75 years is  $9.7 \pm 5.0$  nmol/l, while female with age 19–75 years is 8.7 nmol/l and age more than 75 years is 19.0 nmol/l.

New cases of pulmonary TB are patients who have never been treated for TB or have taken anti-TB drugs for less than 1 month. New cases of pulmonary TB are also stated of two times AFB smear results are positive or one time AFB smear results are positive followed by TB appearance found in chest X-ray. Inactive pulmonary TB is patients who have been cured and completed TB treatment with negative smear results and supported by a fibrotic or calcified appearance in chest X-ray.

## 3. Results

There were 50 patients in this study with 25 samples of each group. Mean age of new cases of pulmonary TB was 39.5 years old and 42.6 years old in inactive pulmonary TB. Profiles of 2 pulmonary TB groups were presented in Table 1.

There was a significant difference of neopterin serum level between new cases of pulmonary TB with positive AFB and inactive pulmonary TB with independent  $t$ -test of  $p$ -value  $< 0.001$  as presented in Table 2.

Pearson correlation between age and serum neopterin level in new cases of pulmonary TB with positive AFB and also in inactive

**Table 1**  
Profiles of pulmonary TB groups.

Variables	New cases of pulmonary TB	Inactive pulmonary TB	$p$ -value
Sex			
Men	15 (60%)	19 (76%)	0.225
Women	10 (40%)	6 (24%)	
Age	39.5 $\pm$ 11.1 (18–64)*	42.6 $\pm$ 10.8 (23 - 65)*	0.318
18–25 y.o	3 (12%)	1 (4%)	
26–35 y.o	6 (24%)	5 (20%)	
36–45 y.o	10 (40%)	10 (40%)	
46–55 y.o	4 (16%)	6 (24%)	
56–65 y.o	2 (8%)	3 (12%)	

\* mean  $\pm$  SD (range).

**Table 2**  
Neopterin serum levels in pulmonary TB cases.

Cases	Neopterin serum levels (nmol/l)	
	mean $\pm$ SD (range)	$p$ -value
New case of pulmonary TB with positive AFB	74.92 $\pm$ 17.08 (42.70–118.20)	$< 0.001$
Inactive pulmonary TB	9.84 $\pm$ 2.38 (6.30–15.20)	

**Table 3**  
Correlation between age and sex with patient's serum neopterin level.

Serum neopterin level	P-value		
		New cases of pulmonary TB with positive AFB	Inactive pulmonary TB
Pearson Correlation	Age	0.390	0.460
Chi-Square Test (a)	Sex	–	0.289

Serum neopterin levels categories based on normal values.

- below normal ( $< 2.6$  in men,  $< 8.7$  in women).

- normal (2.6–8 in men, 8.7 in women).

- above normal ( $> 8$  in men,  $> 8.7$  in women).

pulmonary TB showed that there was no significant difference between age and serum neopterin level with  $p$  value  $> 0.05$ . Chi-square test showed that there was no significant correlation between sex and serum neopterin levels in inactive pulmonary TB with  $p$ -value  $> 0.05$ . Whereas in new cases of pulmonary TB with positive AFB cannot be tested, because all patients in this group have serum neopterin levels above normal. This result was showed in Table 3.

One way Anova showed that there was a significant difference between serum neopterin level in new cases of pulmonary TB with positive AFB with  $p$ -value  $< 0.001$ . Results in Table 4 showed that the higher sputum smear, the serum neopterin level in new cases of pulmonary TB with positive AFB will be higher as presented in Table 4.

## 4. Discussion

There were 50 patients in this study which was counted of 25 new cases of pulmonary TB and 25 inactive pulmonary TB. In this study, men was found to be higher by 60% (15 of 25 cases) in new cases of pulmonary TB and by 76% (19 of 25 cases) in inactive pulmonary TB. WHO global TB report 2018 reported that the ratio was higher in men than women [1]. In Indonesia, TB is significantly more common among men than among women [8]. In Dr. Soetomo Hospital, TB patients were higher in men than women with 36/61 (59%) and 25/61 (41%) [9]. In *Mycobacterium tuberculosis* infection, which is an intracellular facultative bacterium, cellular immunity plays an important role in the immune defence mechanism. In the cellular immune response, T lymphocytes are activated from Th1-subtype cells and produce *interferon- $\gamma$*  and TNF which activate cells of macrophages and dendritic cells. As a

**Table 4**  
Comparison of serum neopterin levels in new cases of pulmonary TB with positive AFB.

AFB smear	Serum neopterin level (nmol/l)	
	Mean $\pm$ SD (range)	$p$ -value
1+	60.10 $\pm$ 10.40 (42.70–75.90)	$< 0.001$
2+	80.81 $\pm$ 7.67 (70.10–94.60)	
3+	110.70 $\pm$ 10.61 (103.20–118.20)	

result of activation of interferon- $\gamma$ , the macrophages will stimulate the *GTP-cyclohydrolase-1* enzyme. *GTP-cyclohydrolase-1* enzyme which then synthesizes guanosine triphosphatase (GTP) to 7.8 *Dihydroneopterin triphosphatase* and finally becomes a neopterin [10]. GTP in the macrophage itself is needed for an increase in bacterial killing [11]. Many studies reported that the production of neopterin in pulmonary TB is correlated with the level of disease activation and can also be used as a monitoring therapy. Research conducted by Moses on neopterin levels in 38 TB patients in Nigeria showed that there was an increase in neopterin of 36.5 nmol/ml compared to a control of 11.4 nm [11]. Previous study obtained neopterin levels of 69.54 nmol/l in the case of active TB and 10.71 nmol/l in inactive TB. Whereas IL-2 levels were 164.53 pg/ml in the case of active TB and 95.43 pg/ml in inactive TB [12]. Serum neopterin levels can be used as a helper laboratory findings for the diagnosis of patients with TB [5].

Our study found that there was a significant difference in neopterin serum level between new cases of pulmonary TB with positive AFB and inactive pulmonary TB with independent *t*-test of *p*-value < 0.001. Following these findings, Mohamed et al. and Yuksekol et al. showed that the neopterin quantification in various body fluids, especially in the urine and serum, will be a rapid diagnostic method for TB in the future. They suggested that the measurements of neopterin levels in pulmonary TB patients might reflect disease activity earlier than cultures [13,14]. Berktaş et al. showed that the mean serum neopterin levels were higher in patients with active TB compared with healthy individuals. It represents a sensitive marker for activated cell-mediated immunity. It is produced by human macrophages specifically on stimulation with interferon- $\gamma$ . The key pathogenesis mechanism in pulmonary TB is the activation of T cells and macrophages [15]. Turgut et al. also showed in their study that the serum levels of neopterin and interleukin-2 in patients with active TB were significantly higher compared with patients with inactive TB and controls. High levels of neopterin, of which the main source is activated macrophages, demonstrate the importance of the cellular immune response in TB [12]. Our results study also matched with those of Guler et al. who showed that the serum neopterin levels were significantly higher in active TB [16]. Ozdemir et al. also found that the serum levels of neopterin in patients with TB were significantly higher than those in controls [17].

In this study, the mean serum neopterin levels in new case of pulmonary TB with positive AFB was 74.92 nmol/l, while the mean serum neopterin levels in inactive pulmonary TB were 9.84 nmol/l. The results of the independent *t*-test showed that there were significant differences in serum neopterin levels between new cases of pulmonary TB with positive AFB and inactive pulmonary TB with a *p*-value of < 0.001 (Table 2). New case of pulmonary TB patients with positive AFB has higher levels of serum neopterin than inactive pulmonary TB patients. Age was not correlated with the levels of serum neopterin in new cases of pulmonary TB with positive AFB and inactive pulmonary TB with Pearson correlation 0.390 and 0.460 (Table 3). Chi-square test showed that there was no significant correlation between sex and serum neopterin level with *p* 0.289 (Table 3).

AFB smear examination is used to diagnose pulmonary TB disease and evaluation of treatment. Our research showed that the more positive the AFB smear, the higher serum neopterin level in new case of pulmonary TB patients with positive AFB as presented in Table 4. Neopterin production in pulmonary TB is correlated with the level of disease activation [10]. The explanation was that the limited lesions were small, and hence the bacterial load and the antigenic challenge was likely to be low; thus, the activation of the immune system was not enough to produce higher levels of serum neopterin. Results of the study reported that there was a significant correlation between increased serum neopterin levels in pulmonary TB with the radiological extent of the disease (severity of TB disease) [7]. There was no previous study investigate AFB smear with serum neopterin levels. Our study found that serum neopterin levels were higher in AFB 3+. This result

showed that the more amount of *Mycobacterium tuberculosis* in the patient, the higher activation of macrophage cells to fight pathogens and an increase in neopterin production.

## 5. Conclusion

There was an increase in serum neopterin levels in new cases of pulmonary TB with positive AFB. Serum neopterin levels in new cases of pulmonary TB patients with positive AFB are significantly higher than in inactive pulmonary TB. Serum neopterin levels are significantly correlated with AFB smear grading in positive smear. The higher grade of AFB smear, the level of serum neopterin will be higher. Serum neopterin levels may be used for distinguishing the active pulmonary TB than inactive pulmonary TB, in addition to others diagnostic test.

## Ethical statement

The authors attest that this clinical investigation was determined to the Institutional Review Board / Ethics Committee review, and the corresponding protocol/approval number is 118/Panke.KKE/II/2015 and has been approved on February 16th, 2015. We also certify that we have not plagiarized the contents in this submission and have done a Plagiarism Check. Written informed consent was obtained from all participants.

## Declaration of Competing Interest

The authors declared that there was no conflict of interest.

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