

Procalcitonin (PCT) levels in pulmonary tuberculosis patients with acid fast bacilli positive and negative smear at Haji Adam Malik General Hospital, Medan, Indonesia



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ABSTRACT

Background: Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis* which enters the droplet nuclei into the mouth or nasal passages to reach the pulmonary alveoli. The sputum smear examination for smear-positive and negative smear pulmonary tuberculosis is challenging due to requiring a longer time to get the results. Therefore, a specific marker is needed that is able to detect bacterial infections immediately, such as procalcitonin (PCT). This study aims to determine the levels of PCT in pulmonary tuberculosis patients with acid-fast bacilli positive and negative smear at Haji Adam Malik General Hospital, Medan, Indonesia.

Method: A cross sectional was conducted among 40 pulmonary tuberculosis patients who met the inclusion and exclusion criteria at Haji Adam Malik General Hospital, Medan, Indonesia. The subjects were 20 positive smears and 20 negative smears. PCT examination with miniVIDAS BRAHMS was carried out using the Sandwich principle using the ELFA method (Enzyme-Linked Fluorescent Assay). Sputum examination was

carried out by smear of Zhiel Neelsen smear, which was analyzed by the Mann-Whitney (Non-Parametric) test using SPSS version 17 for Windows.

Results: Most of the respondents were males in both positive and negative smears group (65.0% and 70.0%, respectively) and not significantly different ($P > 0.05$). The age of subjects was slightly older in positive smear (49.75 ± 17.993 years) compare with negative smear group (42.50 ± 14.816 years) but not statistically significant ($P = 0.172$). The PCT levels in pulmonary tuberculosis patients with positive smear (0.1550; 21.65 ng/mL) differ significantly from the PCT levels in pulmonary tuberculosis patients with negative smear (0.05; 3.14 ng/mL) ($p = 0.0001$). The cut off value using ROC found 0.06 ng/mL (AUC: 0.842) with a sensitivity of 80% and a specificity of 80%.

Conclusion: There was a significant difference in the level of procalcitonin in patients with pulmonary tuberculosis who smear-positive and smear-negative.

Keywords: PCT, Pulmonary Tuberculosis, Positive, Negative, Smear

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INTRODUCTION

Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis*.¹ *Mycobacterium tuberculosis* and droplet nuclei enter the mouth or nasal passages, upper respiratory tract and bronchi to reach the lung alveoli.¹ One-third of the world's population has been infected with tuberculosis germs.^{1,2} In Africa, the number of cases is almost two times larger than Southeast Asia, which is 350 per 100,000 inhabitants.² According to the WHO region, the most significant amount of tuberculosis cases occurred in Southeast Asia, namely 33% of all tuberculosis cases in the world. Still, if seen from the total population, there were 182 cases per 100,000 population.² In 2011, Indonesia ranked 4th in the world for the number of tuberculosis cases after India, China and South Africa.³ Indonesia is the first high burden tuberculosis country in Southeast Asia to achieve the Millennium Development Goals (MDG) target for finding tuberculosis cases above

70% and figures 85% recovery in 2006.³ Every year there are 250,000 new cases of tuberculosis and around 140,000 deaths from tuberculosis.³

Diagnosis of tuberculosis can be established based on clinical symptoms (respiratory symptoms in the form of cough > 2 weeks, coughing up blood, shortness of breath, chest pain, and systemic symptoms of fever accompanied by malaise, cold sweat, anorexia and decreased body weight), radiology, bacteriological examination (bacteriological examination what is meant is direct microbiological examination carried out 3 times (at time/morning/at time) with Ziehl-Neelsen staining, culture (culture examination of *Mycobacterium tuberculosis* bacteria by conventional method is employing Egg base media, Lowenstein-Jensen recommended.⁴ Perform intended culture to get a definite diagnosis, and can detect *Mycobacterium tuberculosis* as well as other than tuberculosis (MOTT) and rapid

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tests (by the GeneXpert RT-PCR method), and other investigations (IGRA and Tuberculin Skin Test(TST)).⁵⁻⁷

Based on the results of sputum pulmonary smear sputum examination, the positive smear pulmonary tuberculosis included at least 2 out of 3 sputum specimens showed positive smear results. The results of one sputum specimen showed positive smear and radiological abnormalities showed active tuberculosis, the effects of examination of one sputum specimen showed Positive smear and positive culture.⁸ Besides, the negative smear indicates if the sputum examination 3 times showed negative smear, clinical features and radiological abnormalities showed active tuberculosis.⁸

Differentiation of the causes of pulmonary infections becomes very important to limit irresponsible use of antibiotics. Some laboratory tests can be aware of infections such as leukocyte count, erythrocyte sedimentation rate, C-reactive protein (CRP), tumour necrosis factor, and interleukin 1 (IL-1) and IL-6.⁹ However, various tests are non-specific, so the aetiology of pulmonary infection is complicated to determine in a short time. The diagnosis must wait for the results of the culture blood for several days. Therefore, a specific marker is needed that is able to detect bacterial infections quickly.

Procalcitonin (PCT) is a biomarker that is more specific to bacterial infections and can be detected earlier than other symptoms or signs of infection, such as fever, changes in leukocyte counts, or blood cultures.¹⁰ The PCT hormone consists of 116 amino acids with a molecular weight of ± 13 kDa. This protein is encoded by the CALC-1 gene, which is located on chromosome 11 and is produced by thyroid gland C cells as calcitonin prohormones. Increased levels of PCT are not always associated with systemic infections. Low levels of PCT also do not always mean there is no bacterial infection. The results of these false negatives can be caused by the initial stage of infection, local infection, subacute bacterial endocarditis, or infection by atypical bacteria (primarily intracellular germs).^{10,11}

Mycobacterium tuberculosis is one of the intracellular bacteria so that they cannot be reached by circulating antibodies. The first exposure to *Mycobacterium tuberculosis* will stimulate local cellular inflammation and bacteria make proliferation in phagocytic cells.¹² At the same time, a specific T cell immunity is formed in infected individuals. After immunity is established, granulomatous reactions can occur at the location of persistent bacteria or the next exposure to bacteria.¹²

There are several reasons why PCT levels in pulmonary tuberculosis are lower than pneumonia. The first pattern of cytokines secreted is different from tuberculosis infections and general bacterial infections; for example, interferon-gamma (IFN- γ) is a cytokine that is more important to inhibit mycobacterial growth than general bacteria.¹³ According to in vitro observations, IFN gamma weakens PCT secretion from human adipose tissue. Second, serum PCT concentrations increase slightly in intracellular infections, including those caused by *Mycoplasma*, viruses and *Pneumocystis jiroveci*. Because the cascade of inflammatory cytokines determines PCT synthesis and release during systemic infection, the intensity depends on the number of organisms entering the systemic circulation.¹⁴ The number of organisms in pulmonary tuberculosis is lower than general bacterial pneumonia.¹⁴

Previous studies by Naderi M et al. in 2009 found that PCT levels in patients with pulmonary tuberculosis <0.5 ng / mL were 29/49 people (63.1%).¹⁵ Another study by Baylan O et al. in 2006 also suggest that the PCT levels <0.5 ng/mL among pulmonary tuberculosis patients were 58.7%.¹⁶ Also, a study conducted by Ugajin M et al. also found that the PCT serum in patients with HIV-negative pulmonary tuberculosis was low and was a biomarker useful for distinguishing pulmonary tuberculosis with pneumonia.¹⁴ However when serum PCT is > 0.5 ng / mL, it is a poor prognostic marker in pulmonary tuberculosis patients, and it has a shorter survival than PCT <0.5 ng/mL. The difference results of previous studies indicate that the further evaluation of PCT to determine the diagnosis of pulmonary tuberculosis is necessarily essential. Based on those mentioned above, this study aims to assess the level of PCT among pulmonary tuberculosis patients with acid-fast bacilli positive and negative smear at Haji Adam Malik General Hospital, Medan, Indonesia.

METHOD

This research was conducted with a cross-sectional method at Department of Clinical Pathology, Faculty of Medicine, Universitas Sumatera Utara, Haji Adam Malik General Hospital, Medan, Indonesia in collaboration with the Department of Pulmonology and Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Sumatera Utara/ Haji Adam Malik General Hospital, Medan. This research began in July 2018-May 2019.

The subjects of this study were pulmonary tuberculosis patients who met the inclusion criteria. The inclusion criteria were age ≥ 18 years, pulmonary tuberculosis patients supported by clinical symptoms, radiology and positive or negative smear sputum examination, willing to take part in the study. However, the exclusion criteria were tuberculosis patients with other infectious diseases, leukocytes blood $> 13000 / \mu\text{L}$, with a sample of 40 patients with pulmonary tuberculosis, consisting of 20 smear-positive and 20 smear-negative. Procalcitonin examination with mini VIDAS BRAHMS was carried out using the Sandwich principle using the ELFA method (Enzyme-Linked Fluorescent Assay). Sputum examination was carried out by smear of Zhiel Neelsen smear.

This research was conducted after obtaining approval from the Research Committee on Health in the Faculty of Medicine, University of Sumatra, Medan. The results of the study were statistically analyzed using computerization. Procalcitonin levels in pulmonary tuberculosis patients with positive smear and negative smear were analyzed using the Mann-Whitney test (Non-parametric). All of data were analyzed using SPSS version 17 for Windows.

RESULTS

In Table 1, the characteristics of the research subject in the form of gender and age was presented.

Pulmonary tuberculosis with acid-fast bacilli positive smear was 20 people consisting of 13 (65%) male and 7 (35%) female. In comparison, 20 people with pulmonary tuberculosis with acid-fast bacilli negative smear were 20 people, 14 (70%) male and 6 (30%) female (Table 1). For gender, there was no significant difference between pulmonary tuberculosis patients with acid-fast bacilli positive smear and pulmonary tuberculosis with acid-fast bacilli negative smear ($p=1.000$). The average age of pulmonary tuberculosis patients with acid-fast bacilli positive smear was 49.75 ± 17.993 years and the negative smear was 42.50 ± 14.816 years, but there was no significant difference ($p=0.172$) (Table 1). In addition, the PCT levels were significantly higher in positive smear group (0.1550; 21.65 ng/mL) compared with negative smear group (0.05; 3.14 ng/mL) ($p=0.0001$) among pulmonary tuberculosis patients (Table 1).

Based on the Receiver Operating Curve (ROC), the Area Under Curve (AUC) was 0.842 (Figure 1). This illustrates the ability of examiners by measuring serum procalcitonin levels to distinguish between Tuberculosis patients with acid fast bacilli positive smear and negative smear of 84.2% (Figure 1).

From Table 2, the Area Under Curve (AUC) of PCT is 84.2% (95% CI 71.1% -97.4%). This AUC is categorized as good in diagnosing patients with pulmonary tuberculosis. The cut-off PCT levels can help enforce the diagnosis of pulmonary tuberculosis is 0.06 ng/mL. The value of sensitivity and

Table 1 Baseline characteristics of the subjects and the difference PCT levels between both groups

Variable	Acid Fast Bacilli (N=40)		P
	Positive Smear (N=20)	Negative Smear (N=20)	
Gender (n,%)			
Male	13 (65.0)	14 (70.0)	1.000 ^a
Female	7 (35.0)	6 (30.0)	
Age (Mean \pm SD)	49.75 \pm 17.993	42.50 \pm 14.816	0.172 ^b
PCT (ng/mL) (Median,Range)	0.1550; 21.65	0.05; 3.14	0.0001 ^c

^aChi-Square; ^bIndependent T-Test; ^cMann-Whitney Test; SD: standard deviation; PCT: procalcitonin; P=statistically significant if P less than 0.05

Table 2 Cut-off value, Sensitivity, Specificity, PPV, and NPV of PCT level in Pulmonary Tuberculosis Patients with Acid Fast Bacilli Positive and Negative Smear

AUC (CI %)	Cut-off (ng/mL)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
84.2 (71.1-97.4)	0.06	80.0	80.0	80.0	80.0

PPV: positive predictive value; NPV: negative predictive value; CI: confidence interval

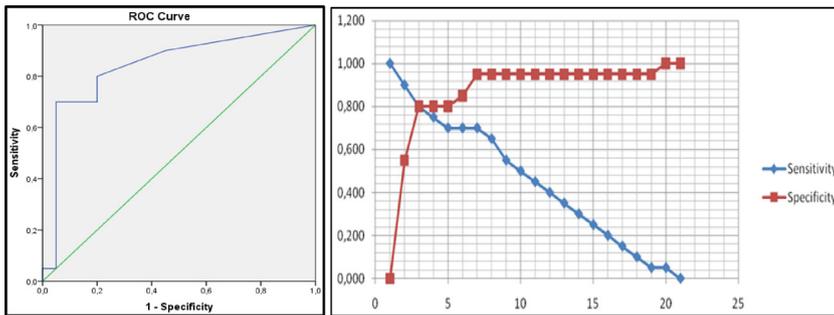


Figure 1 Receiver Operating Curve (ROC) for PCT levels to distinguish between positive and negative smear among pulmonary tuberculosis patients

specificity of Procalcitonin in diagnosing pulmonary tuberculosis is 80.0% sensitivity and 80.0% specificity. In addition, the PPV and NPV value were also 80.0% (Table 2).

DISCUSSION

Determinants of pulmonary tuberculosis disease are population and environmental factors. The population includes gender, age, nutritional status, socio-economic conditions. In this study, it was found that the average age of patients with pulmonary tuberculosis with positive smear was higher compared with negative smear. This is in accordance with the research of Dotulong JFJ et al. in 2015, whereas 75% of Tuberculosis patients are the most productive age group of 15-50 years.¹⁷ Shameem M et al. in 2015 in their study also obtained average age of pulmonary tuberculosis patients was in the productive age.¹⁸ For the gender, in this study, positive AFB tuberculosis in male was higher than that of female, and male negative smear was also higher than female. Ghobadi H et al. in 2014 also found that patients with pulmonary tuberculosis were higher in 23/42 male (55%) compared with 19/42 female (45%).¹⁹

At the productive age, the incidence for the risk of contracting *Mycobacterium tuberculosis* is more significant, because, at that age, more frequent activities outside the work environment and more often communicate with many people.²⁰ The risk of contracting depends on the level of exposure to sputum splashes. Patients with pulmonary tuberculosis with acid-fast bacilli positive smear give a higher risk of transmission from patients with pulmonary tuberculosis with negative smear.

Infection occurs when a person breathes a droplet nucleus containing a tubercle bacillus that reaches the pulmonary alveoli. These tubercular bacteria are phagocytosis by alveolar macrophages; most of these bacilli are destroyed. Small amounts can multiply intracellularly. In this study, there was

a significant difference in the level of Procalcitonin between groups of pulmonary tuberculosis patients with Positive and Negative smear. In accordance with the research of Naderi M et al., the previous study found that the procalcitonin levels in patients with pulmonary tuberculosis <0.5 ng/mL were 63.1%.¹⁵ A similar finding was also found in the study of Baylan O et al. where the procalcitonin levels were <0.5 ng / mL of 58.7%.¹⁶

Ugajin M et al. found that the procalcitonin serum in patients with HIV-negative pulmonary tuberculosis was low and was a biomarker useful for distinguishing pulmonary tuberculosis with pneumonia.¹⁴ However when serum procalcitonin is > 0.5 ng/mL, it is a poor prognostic marker in pulmonary tuberculosis patients and has a shorter survival than procalcitonin <0.5ng / mL.¹⁴

Procalcitonin (PCT) is a biomarker that is more specific to bacterial infections and can be detected earlier than other symptoms or signs of infection, such as fever, changes in leukocyte counts, or blood cultures. The body's response to a bacterial infection will trigger the release of various proinflammatory cytokines (IL1- β and TNF- α) by lipopolysaccharide (LPS).²¹ The cytokine then increases PCT production. Unlike bacteria, the virus will trigger IFN- γ secretion which will provide negative feedback on PCT production.²¹ Increased levels of PCT are not always associated with systemic infections. Low levels of PCT also do not always mean there is no bacterial infection. The results of these false negatives can be caused by the initial stage of infection, local infection, subacute bacterial endocarditis, or infection by atypical bacteria (primarily intracellular germs).²²

There are several reasons why the level of procalcitonin in pulmonary tuberculosis is lower. The first pattern of cytokines secreted differs between tuberculosis infections and general bacterial infections; for example, interferon-gamma (IFN- γ) is a cytokine that is more important to inhibit the growth of mycobacteria than typical bacteria.²³ According to in vitro observations, IFN gamma weakens procalcitonin secretion from human adipose tissue. Second, the serum procalcitonin concentration increases slightly in intracellular infections, including those caused by *Mycoplasma*.²⁴ Because the synthesis and release of procalcitonin is determined by the cascade of inflammatory cytokines during systemic infection, the intensity depends on the number of organisms entering the systemic circulation. So, the number of organisms in pulmonary tuberculosis is lower than general bacterial pneumonia.

According to the BRAHMS criteria, procalcitonin levels <0.05 ng/mL is a typical value for

healthy individuals with no systemic inflammatory reaction; <0.5 ng/mL low systemic inflammatory reaction, local inflammation and local infection, and procalcitonin levels $\geq 0, 5$, and <2.0 ng/mL occurred sepsis, but increased levels of procalcitonin can be caused by a series of conditions.²⁵ Patients must be monitored and procalcitonin should be reassessed within 6-24 hours, ≥ 2.0 and <10 high risks for developing severe sepsis and has a high risk of organ dysfunction. This procalcitonin level ≥ 10 is a feature of sepsis or septic shock caused by bacteria and can cause organ dysfunction.²⁵ Thus the results of this study which found overall procalcitonin levels <0.5 ng / mL, means pulmonary tuberculosis is a local inflammation or local infection.

CONCLUSION

There were significant differences between Procalcitonin levels in pulmonary tuberculosis patients with acid-fast bacilli positive smear and Procalcitonin levels in patients with acid-fast bacilli pulmonary tuberculosis with negative smear. From this research, by using the cut-off value of 0.06 ng/mL, the sensitivity, specificity, PPV, and NPV were high. In some instances that are difficult to ascertain the definitive diagnosis of pulmonary tuberculosis, procalcitonin can be used to help definitive diagnosis.

CONFLICT OF INTEREST

There is no competing interest regarding with manuscript.

ETHICS CONSIDERATION

Ethics approval has been obtained from the Ethics Committee, Faculty of Medicine, Universitas Sumatera Utara, Haji Adam Malik General Hospital, Medan, Indonesia prior to the study being conducted.

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AUTHOR CONTRIBUTION

All of the authors are equally contributed to the study from the conceptual framework, data gathering, data analysis, until interpreting the results on publication.

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