Evaluation of Category I of Anti-tuberculosis Therapy in Intensive Phase Pulmonary TB by Conversion of Acid-Fast Bacilli Sputum

Oki Nugraha Putra1*, Amitasari Damayanti2, Nani Wijayanti Dyah Nurrahman1, Tsania Devi1, Wildatul Aluf1

1Study Program of Pharmacy, Faculty of Medicine, Hang Tuah University, Surabaya, Indonesia
2Departement of Clinical Pharmacy, Dr. Ramelan Hospital, Surabaya, Indonesia

ABSTRACT

Acid fast bacilli (AFB) sputum microscopy is used to diagnose tuberculosis (TB) and to evaluate the effectiveness of anti-tuberculosis drugs in TB eradication program. The AFB sputum microscopy should be performed before treatment, two months after intensive phase and four months after advance phase treatment. The aim of this study was to evaluate the sputum conversion rate of AFB in pulmonary TB patients who received category I of anti-tuberculosis (anti-TB) drugs. Evaluation of sputum conversion was performed before initiation of anti TB drugs and at the end of the intensive phase. This cohort prospective study was done from February to May, 2018. Nine pulmonary TB patients fulfilled the criteria during the study. This AFB sputum microscopy was evaluated by using Ziehl Neelsen and read by means of International Union Against Tuberculosis and Lung Disease (IUATLD) scale. Nine sputum smear positive were followed for two months. After two months (end of the intensive phase), the smear conversion rate was 100%. All patients received standard dose of anti-TB drugs in fixed dose combination (FDC). It can be concluded that the sputum conversion of AFB in pulmonary TB patients who received category I of anti-TB was success at the second month of intensive phase.

Keywords: pulmonary tuberculosis; smear conversion rate; anti TB drugs; intensive phase

INTRODUCTION

Tuberculosis is still serious problem of infectious disease all over the world (WHO, 2011). Data from Riskesdas, 2018, showed that the prevalence of pulmonary TB was 0.4%, not different from 2007. The prevalence of pulmonary TB in East Java is 0.2, with the prevalence of cough more than 2 weeks is 5.0 and bloody sputum is 2.4 (Kemenkes RI, 2013). In Indonesia, there is an estimated one TB case among three cases that have not been observed by the TB program (Kemenkes RI, 2015). Cases of pulmonary TB in East Java has increased each year. Of the 38 districts in East Java in 2015, Surabaya has the highest number of pulmonary TB with the number of patients reaching 4,028 in 2014. Based on this number, there were 2,000 new TB cases, and the rest are TB patients who have not recovered since 2010. Based on data from the City Health Office of Surabaya in 2016, it was known that the indicator of success rate in 2015 was 79.21%, whereas standard national indicator for success rate is more than 85%. The result showed that the success rate in Surabaya in 2015 has not met the national target.

Adequate treatment of pulmonary TB is very important to reduce morbidity, mortality, and to prevent the spread of TB (Singla R, et al, 2007). Transmission of TB can be controlled by good findings and curing. Findings and curing are based on the results of examination of the patient’s sputum, therefore examination of sputum with direct microscopic acid-fast bacilli (AFB) becomes fundamental. Similarly, the examination of the level of positivity of BTA at the beginning of the examination is due the level of positivity is also useful for assessing the degree of infectious disease. Sputum smear-positive tuberculosis patients are the most significant source of infection because droplet from the sputum carry TB bacilli (CDC, 2015). The sputum conversion rate or SCR in TB patients is the percentage of smear positive pulmonary that converted to smear negative after two months of the intensive phase. WHO recommends to use sputum conversion rate for monitoring TB control programs, and as a tool for assessment in TB patients with still positive smears (WHO, 2010).

Under Indonesian Health Ministry for national programme for Tuberculosis, sputum conversion rate (SCR) at the end of two months of intensive phase is an important indicator, which is ideally expected to be at least 80% (Kemenkes RI, 2014). When the smear sputum is negative, the patients are no longer a source of infectious (ATS, 2005). Patients who fail sputum conversion, especially at the end of the intensive phase have a poor clinical outcome (Kuaban C et al, 2009; Dembele SM et al, 2007). If the sputum results are still negative at the end of the second month, then the intensive phase is extended for one month.
The objective of this study was to assess the sputum smear conversion rate and to analyze the correlation between the initial smear grading and the failure treatment among new cases of smear-positive pulmonary TB receiving category I anti TB drugs in intensive phase.

METHODS

Study Design
This two months cohort prospective, was conducted at Sidotopo Wetan Primary Health Care Centre, Surabaya. The diagnosis criteria for pulmonary TB in this study were at least two positive sputum smears test results. All new smear positive naive or newly-diagnosed pulmonary TB outpatients were enrolled from February to May, 2018. For each TB patients, sputum smear was done by Ziehl-Neelsen stain to confirm the diagnosis of pulmonary TB and to exclude a drug resistant TB. Acid fast baccilli (AFB) or smear sputum were positive if appears red-colored bacteria, fine rods, sometimes granules, accompanied by other non-smear bacteria and blue-colored leucocytes.

The sampling technique in this study was non random sampling with purposive sampling technique. The researchers determines the sampling by specific characteristics that are in accordance with the objectives of the study, so that it is expected to answer the research problems. Inclusion criteria in this study as follows: newly diagnosed smear positive pulmonary tuberculosis category I or started first-line antituberculosis treatment, aged 17-60 years old. Exclusion criteria were extra pulmonary TB, TB patients with comorbid disease (diabetes mellitus, liver disease, autoimun history), pregnancy, lactation, pneumonia, and patients with HIV. All patients were followed up for 2 months after initiation of TB therapy. The drop out criteria were death during two months of study, inability for collecting sputum, and transfer to the hospital or any other institutions.

The smear grading based on International Union Against Tuberculosis and Lung Disease (IUATLD) scale, as follows: negative (smear contains no AFB in 100 fields), 1+ (10-99 AFB in 100 fields), 2+ (1-9 AFB/field in at least 50 fields), and 3+ (>10 AFB/field in at least 20 fields). Inclusion criteria in this study as follows: newly diagnosed smear positive pulmonary tuberculosis category I or started first-line antituberculosis treatment, aged 17-60 years old. Exclusion criteria were extra pulmonary TB, TB patients with comorbid disease (diabetes mellitus, liver disease, autoimun history), pregnancy, lactation, pneumonia, and patients with HIV. The drop out criteria were death during two months of study, inability for collecting sputum, and transfer to the hospital or any other institutions.

Before study participation, patients were asked for demographic information (sex, age, comorbid diseases), clinical symptoms and signs of pulmonary TB, and smoking habits were obtained from medical record. At least three sputum of each TB patients (spot-morning-spot) are collected over 2 consecutive days, spot sample when patients suspected of TB, one morning sample for tomorrow, and one spot sample on the second day. Sputum smear examination in this study was done at beginning (before initiation of TB treatment) and at the end of 2nd month. One of the smear results shows a positive results, the patient is diagnosed TB.

TB Treatment
All new pulmonary TB patients received FDC in intensive phase based on their weight, to assure the right dose for two months. Each FDC contains four drug regimens (isoniazid, rifampicin, pyrazinamide, and ethambutol). The dosage of TB drugs was modified or adjusted when severe adverse effects of TB drugs occurred. The adherence of taking medicines is monitored by the supervisor of “Pengawas Menelan Obat (PMO)” or Directly Observed Treatment Shortcourse (DOTS), represented by the patient’s family. Everytime the patient comes to the TB unit, the patients will be asked about the amount of medication that has been taken or swallowed.

Data Collection and Analysis
The study mainly involved the primary data from the result of sputum smear of pulmonary TB patients. The secondary data was used to assess the demographic data and history of illness and medical of each patients. The term sputum-positive cases were the pulmonary TB patients who convert to sputum negative at the end of 2nd months or at the end of intensive phase. The term sputum negative cases were the pulmonary TB patients who still sputum negative at the end of 2nd months after TB treatment.

The Saphiro Wilk test was used to assess the normality of the variables. The study results were presented as mean with standard deviation (SD) and as percentage. Chi-square and paired t-test was performed to evaluate differences before and after treatment of two months TB drugs. The data were analyzed by statistical software SPSS 15.0. Statistical significance was accepted when P value <0.05.

RESULTS
Nine sputum positive pulmonary TB patients were initiated on treatment. All patients completed the 2 months of intensive phase. The demographic characteristic is shown in Table 1. One patient had initial high sputum-grade (3+). By Chi-Square analysis, there is no significant relationship between sex and grade of
Table 1. Demographic characteristics of TB Patients

| Sputum smear positive patients (n=9)                  |
|------------------------------------------------------|
| Mean age (years)                                     | 46.1±10.5 |
| Body Weight (Kg)                                     | 48.0±6.5  |
| Sex                                                  |           |
| Male                                                 | 6         |
| Female                                               | 3         |
| Comorbid                                             |           |
| Without comorbid                                     | 8         |
| With comorbid                                        | 1         |
| Initial sputum grade before initiation of TB drugs   |           |
| 0, n (%)                                             | 0 (0%)    |
| 1, n (%)                                             | 4 (44.4%) |
| 2, n (%)                                             | 4 (44.4%) |
| 3, n (%)                                             | 1 (11.2%) |

Table 2. Sputum smear before and after TB treatment

| No. of Patients | Sputum smear before the treatment | Sputum smear after the treatment | P Value |
|-----------------|----------------------------------|----------------------------------|---------|
| 1               | 1+                               | -                                |         |
| 2               | 1+                               | -                                |         |
| 3               | 2+                               | -                                | <0.001  |
| 4               | 2+                               | -                                |         |
| 5               | 1+                               | -                                |         |
| 6               | 2+                               | -                                |         |
| 7               | 2+                               | -                                |         |
| 8               | 1+                               | -                                |         |
| 9               | 3+                               | -                                |         |

smear-positive ($p = 0.06$) at the beginning of the study. It means, sex had no correlation with grade of smear-positive. The smear examination before and after of TB treatment is shown in Table 2.

For calculating the sputum conversion rate, the number of smear positive who had smear negative at the end of intensive phase is divided by the number of smear-positive patients before TB treatment and the ratio is multiplied by 100. In this study of 9 smear positive pulmonary tuberculosis case, 100% patients at the end of 2nd month were smear negative. Number of FDC daily for each patients based on body weight was shown in Table 3. Seven patients (77.7%) had minor side effects or adverse effects. All adverse effects observed during this study was shown in Table 4. No patients lead to final termination of TB standard therapy due to adverse effects. Major adverse effects such as hepatoxicity, jaundice, ototoxicity, and psychiatric changes were not observed during this study. No patients had history of smoking (ever smokers or ex-smokers).

Table 3. Daily dose of Fixed-Dose Combination (FDC) based on body weight

| No. of Patients | Body Weight (Kg) | No of FDC tablets daily |
|-----------------|------------------|-------------------------|
| 1               | 42               | 3                       |
| 2               | 53               | 3                       |
| 3               | 42               | 3                       |
| 4               | 38               | 3                       |
| 5               | 54               | 3                       |
| 6               | 60               | 4                       |
| 7               | 46               | 3                       |
| 8               | 47               | 3                       |
| 9               | 50               | 3                       |
Sputum conversion, from smear positive to negative, is one of the most important outcomes in pulmonary TB, besides clinical outcome. Because it illustrates the infection control in TB patients. In this study, TB was diagnosed based on symptoms, clinical signs and confirmed by sputum smear examination. No patient was confirmed by sputum culture and chest x-ray. Several studies shown that nonconversion at the end of the intensive phase is one of the strongest predictors for TB therapy failure, although it is not the best indicator because of its weak predictive value (Kuaban et al, 2009). Based on Table 1, subjects suffering from tuberculosis tended to be more male than female, 6 men dan 3 women. This is similar to the data of Riskesdas, 2013, showed that the prevalence of pulmonary TB incidence greater in men. The incidence of pulmonary TB tend to be more in male, because it associated with different social interaction between men and women, smoking, and alcohol causes a decrease of immune system, so that when the body exposed to TB bacilli, it can cause symptoms and if tested become positive pulmonary TB (Kemenkes RI, 2013). Our study is also similar to the study by Shamnuganathan et al, who showed that the majority of TB patients were male. It could be indicated that sex is a risk factor for TB (Shamnuganathan et al, 2015). Sex steroid hormone in woman, estradiol, could activate macrophage, and natural killer cells from female mice could produce more interferon-gamma (IFN-γ) than that of cells from male mice (Callippe et al., 2008).

In this study, 9 sputum positive pulmonary tuberculosis (category I) patients become smear negative at the end of 2 months of intensive phase or 100% sputum conversion rate. Based on age, our study was not similar with several studies for smear sputum conversion. A study by Yellapa et al, showed that the mean age associated with sputum non-conversion at the end of 6 months of treatment was 45.2 ± 15.3 years (Yellapa et al., 2016). It similar with a study conducted in South India reporting that age more than 45 years is associated with poor sputum conversion rate of 60% (Rekha et al., 2007). Study by Babalik et al showed that smear conversion decreased at age more than 40 years due to decreased immunity. The important immune response in pulmonary tuberculosis is macrophages and T cells. Macrophages are useful for phagocytes of Mycobacterium tuberculosis and introduce antigens resulting from phagocytosis to T cells. T cells will secrete IFN-γ. IFN-γ will stimulate macrophage to be more effective to phagocyte Mycobacterium tuberculosis (Babalik et al., 2012).

The successfull conversion rate in this study might be because all patients had no history of smoking and miliary TB. Study by Boer et al, showed that tobacco smoking was found to prolonged or even delay culture conversion during TB treatment in pulmonary tuberculosis patients. The study was also showed that TB patients who current smokers had higher risk of non conversion after TB therapy for two months compared to ex-smokers or never smokers (Boer et al., 2013). Smoking causes several effects on the respiratory system, including affects immunological responses, decreased clearance of inhaled bacteria or viruses. Smoking also inhibits the immunity of anti-TB T-helper type 1 (Th-1) by inhibiting the activation of innate immune and recruitment of lung-T-cell (Shaler CR et al., 2013). Smoke cause disrupted ciliary function in the respiratory tract, so it can increase the risk of TB (Caetano Mota et al., 2012). Another study by Bouti et al, showed the different results. The relationship between factors like age, sex, weight, smoking and delays of conversion of positive sputum smears after two months of treatment had no statistically significant association (Bouti et al., 2013).

In our study, the administration of anti TB drugs or FDC doses was divided according to the weight, in which patients with 30-37 Kg received 2 FDC, 38-54 Kg received 3 FDC, 55-70 Kg received 4 FDC and more than 70 Kg received 5 FDC as shown in Table 3 (Kemenkes RI, 2011). This is in accordance with the dosage given to our patients. There were no overdose or underdose. All TB patients are classified into the right dose. Study by Al-Shaer et al, showed that pulmonary TB patients who received FDC, the sputum smear conversion was significantly greater compared to separate tablet of TB drugs (FDC 36.6 ± 19.5 days vs. separate tablet 56.1 ± 28.8 days) with P value 0.008. It was also showed that FDC was significantly greater among TB patients with pretreatment of bacillary load of TB 3+ (Al-Shaer et al., 2018). The usual or standard for TB therapy regimen is a 2 months regimen, including isoniazid, rifampicin, pyrazinamide, and ethambutol, and then followed by a 4 months regimen of isoniazid and rifampicin. All drugs regimen are available as separate tablets or capsules. Another regimen of TB drugs is fix dose combination or FDC. It means, in one tablet, includes four drug TB drugs. The FDC is easy to arrange dose calculation, prevent errors of prescribing, increase adherence and decrease of pill dumping (Blomberg B et al., 2003).
Some factors have been identified that may prolong of smear conversion. These include high sputum smear acid fast bacilli (AFB) grade, cavitatory lesion, untreated hyperglycaemia or diabetes mellitus, older age, treatment with less than four anti TB drugs (Long et al., 2003). Another result from this study indicate that the side effects of anti-TB drugs is still serious problems during TB treatment in intensive phase. A total of 11 cases of side effects from 9 patients were observed during this study. The highest percentage was arthralgia (37%). Arthralgia is strongly suspected to be caused by pyrazinamide. One of the minor side effects of pyrazinamide is arthralgia. In non-gouty TB patients who received pyrazinamide, hyperuricemia commonly leads to arthralgia. The mechanism is associated with the main metabolit of pyrazinamide, pyrazinoic acid, which inhibits the elimination of uric acid due to inhibition of renal tubular secretion. It suggested that the administration of pyrazinamide be discontinued or dose adjusted. Sometimes, the hyperuricemia is typically asymptomatic, and administration of aspirin or nonsteroidal anti-inflammatory drugs help to reduce pain (Gholami K et al., 2006). However, our patients were not measured for uric acid levels to be associated with arthralgia. The second most side effects of anti-TB drugs in this study was flu-like syndrome. Flu-like syndrome is rare and usually occurs in TB patients who use intermitten TB regimen that include rifampicin. A study by Gulbay et al, showed that the incidence of flu-like syndrome in hospitalized TB patients was observed in 3 cases, 2 of them the administration of rifampicin was discontinued. The result also showed that the interval between the initiation of rifampicin therapy and flu-like syndrome was 11.6 days (7-21 days) (Gulbay et al., 2006).

One patient suffered Morbus Hansen (Leprosy). Leprosy is a chronic infection caused by Mycobacteria leprae, which attack the peripheral nerves, skin and other organs. In patient who are experiencing TB treatment and also leprosy, there is an interaction between rifampicin and dapson. Rifampicin increases dapson excretion, in the process of the dapson metabolic pathway, it involves the hydroxylation of one amino group by cytochrome 3A4 to form hydroxyamine dapson. Rifampicin also increases levels of hydroxyamine (metabolite of dapsones) in the blood (Baxter, 2009). In this case, dapson doses may need to be increased, but this can increase the potential exposure to toxic hydroxyamine metabolites which can cause methemoglobinemia.

Additionally, the limitation of our study is the small sample size. The results may not represent the entire populations or generalized because of a limited number of samples. Besides that, the confounding variables, such as nutrition and body weight in the second month was not evaluated.

CONCLUSION

In conclusion, our study showed a succesfully smear conversion at the end of 2nd month of anti-TB drugs used. More prospective studies about sputum conversion and also larger sample are needed to confirm our results.

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