Conference Paper

The Agreement Level of Tuberculin Skin Test (TST) and T-SPOT.TB Examinations in Detecting Latent Tuberculosis Infection in Illicit Drug Users

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Abstract

World Health Organization (WHO) reported that a third of the world's populations develop latent tuberculosis infection (LTBI). Illicit drug users are at risk of suffering LTBI which potentially developing into active tuberculosis (TB). There is no gold standard diagnostic for LTBI and it is presumed that immune responses play an important role in LTBI. This study aimed o evaluate the ideal diagnostic tool to fill the absence of a gold standard and the effect of illicit drugs on the immune system. This was a cross sectional study conducted in illicit drug clinic, Methadone Maintenance Therapy (MMT) program of Dr. Moewardi Hospital, and MMT program of *Puskesmas* Manahan Surakarta, from February to March 2018. Total subjects were 24 respondents consisting of 5 respondents with TST (+) and 4 with T-SPOT.TB (+). We measured the agreement level of TST and T-SPOT.TB was moderate (k= 0.591, p= 0.003), the relationship of absolute cluster of differentiation 4 (CD4) to TST induration (r= 0.077, p= 0.719), the relationship between absolute CD4 and T-SPOT.TB spot-forming units (SFUs) ESAT-6 (r= -0.238, p= 0.262); CFP-10 (r= -0.117, p= 0.585); and the highest ESAT-6/CFP-10 (r= -0.033, p= 0.879). Tuberculin skin test and T-SPOT.TB have their own strengths and weaknesses, but in this study TST is slightly superior to T-SPOT.TB with moderate agreement level.

Keywords: LTBI, TST, T-SPOT.TB, illicit drug

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1. Introduction

Tuberculosis (TB) is the ninth cause of death in the world [1]. Tuberculosis is an infectious disease and is predicted to cause a high rate of latent TB infection (LTBI). Patients with LTBI have no sign or symptom of active and non-infectious TB but are at risk of becoming active TB [2]. A third of the world's population suffers from LTBI and 2-15% of patients can become active TB. The change of Latent TB infection into active TB is influenced

by several factors, one of which is the main immune status. World health organization (WHO) recommends LTBI examination and treatment in risky populations, such as illegal drug/narcotics, psychotropic substances and other addictive substances (drugs) users [1, 3].

The global burden of drug use is estimated to reach 185 million users. Extraordinary TB event cases have been reported in methadone treatment facilities in the United States [4]. The result of a survey conducted by the National Narcotics Agency (BNN) and the University of Indonesia's Health Research Center (*Puslitkes* UI) showed that 1.9% of the drug prevalence in 2008 increased to 2.2% in 2011 and about 4 million Indonesians aged 10-60 years old are using drugs. The incidence of TB in drug users is 100 times higher than that in general population. Prevalence of LTBI among drug users is 10-59% [6]. Several *in vivo* and *in vitro* studies reported that drugs affect adversely the body's immune system thereby reducing the immune response or resulting in immunosuppression [7].

The gold standard for LTBI diagnosis has not been available yet. Tuberculin skin tests (TST) have been used since more than 100 years ago and there has been no competitor until Interferon-Gamma Release Assays (IGRA) began to be introduced in early 2000 [8]. World Health Organization recommends *in vivo* immunodiagnostic examinations such as TST or in vitro examinations such as IGRA for LTBI diagnosis. Interferon-Gamma Release Assays examination includes, among others, T-SPOT TB (Oxford Immunotec). Action mechanism of TST is to measure delayed-type hypersensitivity (DTH) cellular immunity against tuberculin purified protein derivative (PPD). T-SPOT.BT examination is an enzyme-linked immunospot test (ELISPOT) measuring the number of peripheral blood mononuclear cells producing INF-γ following the stimulation with early secretory antigen target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10) [9].

The objective of the current study was to evaluate the LTBI prevalence using TST and T-SPOT.TB among drug users. It also aimed to evaluate the sensitivity, specificity and suitability of T-SPOT.TB compared with those of TST. In addition, it was also intended to evaluate the relationship between absolute CD4 and TST and T-SPOT.TB among drug users. The results of TST and IGRA (T-SPOT.TB) research are important as an input for doctors and program managers, thus leading to the accuracy of LTBI diagnosis and treatment as well as the reducing TB burden and LTBI in Indonesia.

2. Methods

This was a diagnostic test research with cross-sectional design. This research was conducted in the drug users clinic, methadone maintenance therapy (MMT) program of Dr. Moewardi General Hospital Surakarta and MMT *Puskesmas* Manahan Surakarta during February-March 2018. The target population of the study was drug users. The sampling technique employed was consecutive sampling. The minimum sample size was 24 respondents. Inclusion criteria were drug users aged >18 years and willing to participate in the study. Exclusion criteria were drug users with clinical symptoms/ signs of pulmonary and extrapulmonary TB, history of pulmonary and extrapulmonary TB, in anti-tuberculosis drug therapy, human immunodeficiency virus (HIV), acute/ chronic renal failure, and diabetes mellitus. Discontinuous criteria were patients not returning within 48-72 hours for TST assessment, blood lysis/ clotting samples, and resignation.

Drug users who meet the inclusion criteria were given education if available, and then they were asked to sign the informed consent. Data collected during the respondent's first visit included anamnesis, physical examination, and investigations, as well as respondents' characteristics such as age, gender, contact history, and the Bacille Calmette-Guérin (BCG) scar. Characteristics of drugs used were identified using WHO's alcohol, smoking and substance involvement screening test version 3.1 (ASSIST v3.1) to assess addiction, drug use length, and drug type [10]. Supporting examination included cluster of differentiation examination 4 (CD4), absolute T-SPOT.TB examination, and TST injection. The second visit was 48-72 hours later to interpret TST, and GeneXpert MTB/ RIF sputum examination was conducted with NaCl 3% nebulized sputum induction if the results were TST (+) and/ or T-SPOT.TB (+).

2.1. Tuberculin skin test

TST procedure was performed by injecting 0.1 milliliters (ml) of PPD RT23 2 tuberculin unit (TU) (serum statens institute, Copenhagen, Denmark) using Mantoux technique. TST injection was carried out in the forearm volar surface area, far from the vein surface. Injection should be carried out with a single-use tuberculin gauge syringe No. 27 intradermally (just below the skin surface) with a bevel needle facing up. The injection was carried out until a lump of 6-10 millimeters (mm) diameter appeared. The result of injection in the form of induration was evaluated 48-72 hours later, measured by a ruler, and considered to be positive if induration >10 mm [11].

2.2. T-SPOT.TB

T-SPOT.TB examination (Oxford Immunotec) was performed on peripheral blood mononuclear cells separated using a centrifuge process from 6-ml peripheral venous blood. Peripheral blood mononuclear cells were put on plates that have been coated with anti IFN-γ antibodies (2.5 × 105 cells per well). The test consisted of two antigen wells containing Mtb specific antigens, ESAT-6 and CFP-constituting Mtb-specific antigens. Positive control contained PHA, while negative control did not contain mitogen or antigen. The dish was incubated all night at 37 ˚C at 5% atmospheric CO2. Spot forming units (SFUs) were calculated using the ELISPOT reader, and the results were positive if the SFUs on the Mtb ESAT-6 antigen and/or CFP-10 is ≥ 8 [12].

2.3. GeneXpert MTB / RIF

GeneXpert MTB / RIF examination was carried out by sputum or by sputum induction with nebulization of NaCl 3%, and then sputum was collected into a sample tube with incubation for 15 minutes at room temperature. The sample was diluted into the cartridge using a pipette. The cartridge was inserted into the GeneXpert machine and the test was ready to begin. Interpretation of results was measured based on the fluorescence signal and algorithm calculation appearing on the monitor screen [13].

2.4. Other laboratory tests

The measurement of absolute CD4 was carried out using the Becton Dickinson (BD) FASCount System, USA. The BD FASCount system tool employed flow cytometry techniques. Flow cytometry uses light scattering, light excitation and emission principles of a fluorochrome molecule to generate specific multiparameter data from a particle and cell in 0.5 to 40 micrometers in diameter.

2.5. Statistical analysis

Data analysis was performed using SPSS 21 (IBM) for Windows. Basic data was processed descriptively, presented with frequency distribution and percentage. The agreement level of TST and T-SPOT.TB was analyzed by using Test of Agreement (Kappa Cohen). According to Landis and Koch in 1977, the kappa (k) value is considere slight if k ≤ 0.20, fair if 0.20 <k ≤ 0.40, moderate if 0.40 <k ≤ 0.60, substantial if 0.60 <

k ≤ 0.80, and optimal if 0.80 <k 00 1.00 [14]. The sensitivity and the specificity of T-SPOT.TB were presented in the 2x2 tabulation. The sensitivity and the specificity of T-SPOT.TB examination and the cut points were determined using the receiver operating characteristic (ROC) curve. The correlation test used was Pearson product moment (if it meets the requirements of normality) and Spearman's rank (if it does not meet the requirements of normality). The significance value is p <0.05 [15].

3. Results

The respondents consisted of 30 drug users with 6 excluded respondents, so the total subjects of this study were 24 respondents. Six (25%) of respondents developed latent TB infection in this study, 5 (20.8%) respondents were TST (+) and 4 (16.7%) respondents were T-SPOT.TB (+). Three (12.5%) respondents were TST (+) and T-SPOT.TB (+), 2 (8.3%) respondents were TST (+) and T-SPOT.TB (-), 1 (4.2%) respondent was TST (-) and T-SPOT.TB (+), and 18 (75%) respondents were TST (-) and T-SPOT.TB (-). The flow of research results can be seen in Figure 1.

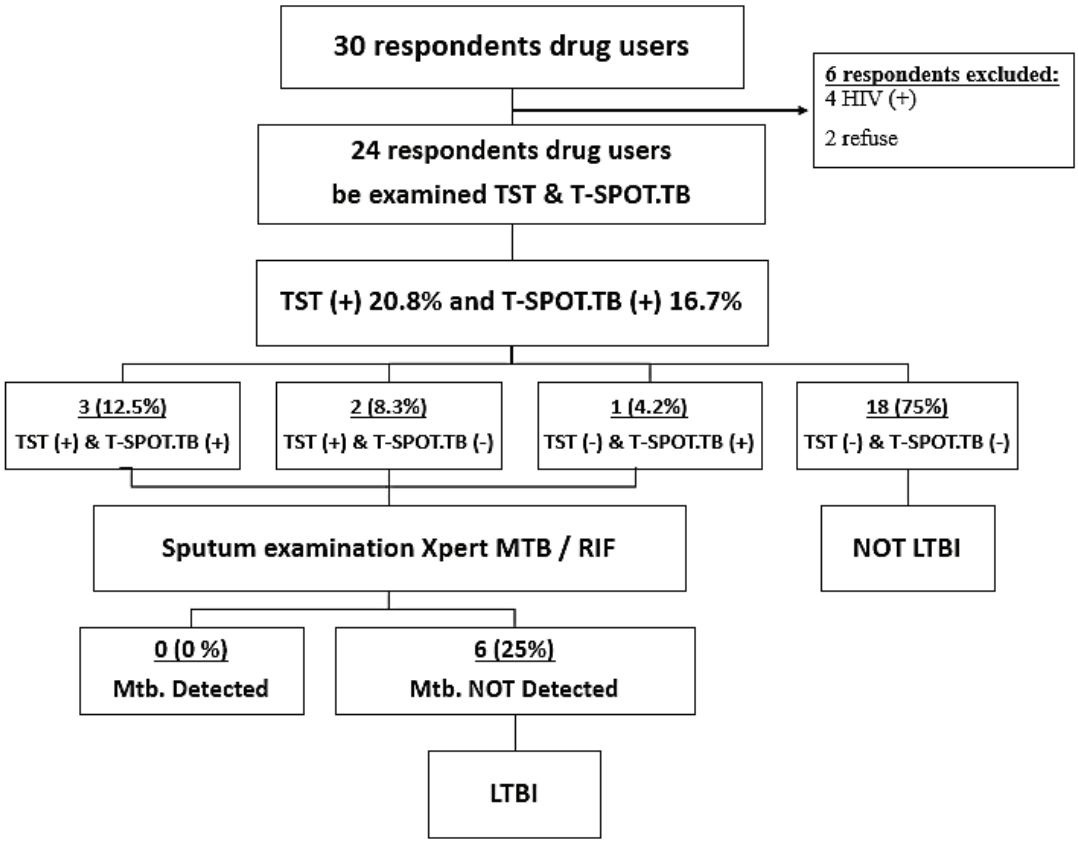

Figure 1: Flow of research and the results. TST = tuberculin skin test; LTBI = latent TB infection.

The average age of respondents was 33.54 years, with 19 years being the youngest and 42 years being the oldest. The highest age distribution in this study range between 18 and 40 years occurring in 21 (87.5%) respondents, and 4 (19.0%) of them belonging to positive LTBI. The sex ratio was 21 (87.5%) male:3 (12.5%) female. Latent TB infection, by sex, occurs in 5 (23.8%) males and 1 (33.3%) female. The history of contact with TB patients can be found in 5 (20.8%) respondents and 1 (20.0%) of them was positive LTBI. BCG scar on right upper arm can be found in 16 (66.7%) respondents and 3 (18.8%) of them are positive LTBI. Mean absolute CD4 is 740.21 cells /uL with standard deviation of 244.81. The lowest absolute cluster of differentiation 4 is 300 cells /uL and highest is 1323 cells /uL, median absolute CD4 is 671.5 cells /uL. Mean of absolute CD4 TST (+) is 705.8 cells / uL and T-SPOT.TB (+) is 671.75 cells / uL. Characteristics of drug abuse respondents are presented in Table 1.

TABLE 1: Characteristics of drug abuse respondents.

Variable	Total n (%)	TST (+) n (%)	T-Spot (+) n (%)
Total	24 (100)	5 (20.8)	4 (16.7)
Sex			
Man	21 (87.5)	5 (23.8)	3 (14.3)
Woman	3 (12.5)	0 (0.0)	1 (33.3)
Age			
18-40 years	21 (87.5)	4 (19.0)	3 (14.3)
41-60 years	3 (12.5)	1 (33.3)	1 (33.3)
>60 years	0 (0.0)	0 (0.0)	0 (0.0)
Contact History			
No	19 (79.2)	5 (26.3)	3 (15.8)
Yes	5 (20.8)	0 (0.0)	1 (20.0)
BCG scar			
No	8 (33.3)	2 (25.0)	1 (12.5)
Yes	16 (66.7)	3 (18.8)	3 (18.8)
Absolute CD4			
Mean ± SD	740.21±244.81	705.80±202.24	671.75±246.23
Median	671.50	753.00	665.50
Length of drugs			
Mean ± SD	17.29+6.13	19.20+4.15	18.00±2.83
Median	17.00	18.00	17.00

Drug abuse respondents consisted of 11 respondents in methadone maintenance therapy and 13 respondents in non-methadone maintenance therapy (active users). Drug users were assessed using WHO's ASSIST v.3.1 in the last 3 months with 3 levels of severity: mild, moderate, and severe. The average drug duration is 17.29 years with standard deviation of 6.13. The lowest drug duration is 2 years and the highest one

is 28 years, the median drug duration is 17 years. The average length of the drug is 19.2 years for TST (+) and 18 years for T-SPOT.TB (+). Drugs addiction occurs not only with one type of substance, but it can be with some substances. Drugs are divided into alcohol, cannabis, cocaine, amphetamines, and opioids (methadone). Number and percentage of drug types, WHO's ASSIST can be seen in Table 2.

Table 2: Characteristics of Drugs Type.

		WHO's ASSIST		
	Total n (%)	Mild n (%)	Moderate n (%)	Severe n (%)
Total	24 (100)			
Alcohol	21 (87.5)	1 (4.8)	7 (33.3)	13 (61.9)
TST(+)		0 (0)	3 (14.3)	2 (9.5)
T-SPOT.TB(+)		0 (0)	3 (14.3)	0 (0)
Cannabis	14 (58.3)	4 (28.6)	9 (64.3)	1 (7.1)
TST(+)		1 (7.1)	3 (21.4)	0 (0)
T-SPOT.TB(+)		0 (0)	2 (14.3)	0 (0)
Amphetamine	22 (91.6)	1 (4.5)	10 (45.5)	11 (50)
TST(+)		0 (0)	3 (13.6)	2 (9.1)
T-SPOT.TB(+)		0 (0)	3 (13.6)	1 (4.5)
Methadone	11 (45.8)	0 (0)	8 (72.7)	3 (27.2)
TST(+)		0 (0)	2 (18.2)	2 (18.2)
T-SPOT.TB(+)		0 (0)	1 (9.1)	3 (27.3)

3.1. The level of agreement between the T-SPOT.TB and the TST examinations in detecting LTBI on drug users

The kappa agreement level of T-SPOT.TB and TST is k= 0.591 with p= 0.003 (statistically significant). The agreement level of k= 0.591 is moderate ($0.40 < k \leq 0.60$). The compatibility of TST to T-SPOT- TB in detecting LTBI among drug users is presented in Table 3.

Table 3: Compatibility of T-SPOT.TB to TST in detecting LTBI in drug users.

T-SPOT.TB	**TST**		**Total**	**k**	**p**
	Positive	**Negative**			
Positive	3	1	4	0,591	0,003
Negative	2	18	20		
Total	5	19	24		
Note: k= kappa; p = <0.05 means that the test is significant.					

3.2. The sensitivity and the specificity of T-SPOT.TB to TSTexaminations in detecting LTBI in drug users.

This study used TST as an alternative to gold standard. The sensitivity and the specificity of T-SPOT.TB are presented in 2x2 tabulation. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR), and negative likelihood ratio (NLR) values are 60.00%, 94.74%, 75.00%, 90 00%, 11.40 and 0.42, respectively as shown in Table 4.

Table 4: The result of sensitivity, specificity, NDP, NDN, RKP, RKN, and accuracy tests on T-SPOT.TB and TST examinations.

		TST		
		Positive	**Negative**	**Total**
T-SPOT.TB	Positive	3	1	4
	Negative	2	18	20
	Total	**5**	**19**	**24**
Sensitivity	60,0%			
Specificity	94,74%			
PPV	75,00%			
NPV	90,00%			
PLR	11,40			
NLR	0,42			

3.3. ROC (receiver operator curve) of T-SPOT. TB

The receiver operating characteristic (ROC) curve is a way to determine the cut off point in a diagnostic test. The ROC curve is a graph illustrating the bargaining between sensitivity (ordinate Y) and specificity (ordinate X). The higher the sensitivity value, the lower is the specificity value and vice versa. ROC curve of T-SPOT.TB sensitivity and specificity can be seen in Figure 2.

From the ROC curve, it can be seen that AUC of TSPOT.TB examination is 79.6% (95% CI 53.5% -100%) and p= 0.033 (statistically significant). The best diagnostic value of T-SPOT.TB examination based on the ROC curve is obtained at the intersection of 5 with a sensitivity value of 66.7%, and specificity of 100%. The cut-off point of the ROC analysis results on the T-SPOT.TB examination is presented in Table 5.

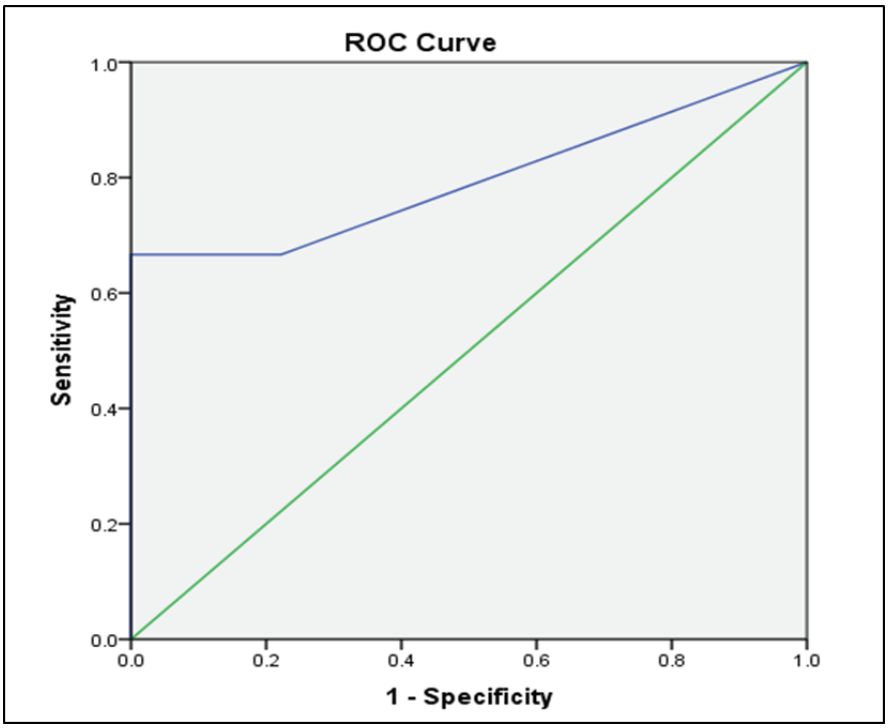

Figure 2: ROC curve of T-SPOT.TB with sensitivity and specificity.

TABLE 5: Results of ROC analysis on T-SPOT.TB examination.

T-SPOT.TB	Cut Off						
	0.5	1.5	5	11.5	22.5	115	201
Sensitivity	0.667	0.667	0.667	0.500	0.333	0.167	0.000
Specificity	0.778	0.944	1.000	1.000	1.000	1.000	1.000

3.4. The relationship of absolute CD4 to TST and T-SPOT. TB examinations.

The mean TST induration is 5.7 mm and the standard deviation is 5.00, with induration of at least 0 mm and a maximum of 15 mm. The mean SFUs ESAT-6 is 10.75, and the standard deviation is 40.88, SFUs are at least 0 and maximally 200. The mean SFUs CFP-10 is 11.08, and the standard deviation is 33.88, SFUs are at least 0 and maximally 150. The average SFUs in the highest value of ESAT-6 / CFP-10 is 14.25 and the standard deviation is 43.08, SFUs are at least 0 and maximally 200.

The relationship of CD4 cell counts to TST and T-SPOT.TB examinations was tested bivariately in all 24 respondents. The data distribution was tested using the Shapiro-Wilk test for small sample size ($\leq$ 50). The data distribution was found to be abnormal with TST p=0.002 and T-SPOT. TB p=0.000 so that the non parametric Spearman's rank test was used for further analysis. Correlation test between absolute CD4 and TST (mm) examination showed r=0.077, and p=0.719. Correlation test on absolute CD4 and SFUs

T-SPOT.TB was divided into ESAT-6, CFP-10, and the highest value between ESAT-6 / CFP-10. ESAT-6 correlation test found r=-0.238, p=0.262, CFP-10 r =-0.117, p=0.585, and the highest value between ESAT-6 / CFP-10 r=-0.033, p=0.879. The result of Spearman's test on absolute CD4 with TST and ESAT-6, CFP-10, and the highest value ESAT-6 / CFP-10 is presented in Table 6.

Table 6: The result of Spearman test on absolute CD4 and TST and T-SPOT. TB.

Examination		Mean±SD TST (mm)	Mean±SD T-SPOT.TB (SFUs)	Mean±SD Absolute CD4 (cell / mL)	Spearman's test
TST		5.75±5.00	-	740.21±244.81	*r*= 0.077, *p*= 0.719, n= 24
T-SPOT.TB	ESAT-6	-	10.75±40.87	740.21±244.81	*r*= -0.238, *p*= 0.262, n= 24
	CFP-10	-	11.08±33.87	740.21±244.81	*r*= -0.117, *p*= 0.585, n= 24
	The highest value is ESAT-6 / CFP-10	-	14.25±43.08	740.21±244.81	*r*= -0.033, *p*= 0.879, n= 24

Note: p = <0.05: significant; r: spearman's rho; n: ESAT-6 sample size: early secretory antigenic target-6; CFP: culture filtrate protein-10; SFUs: spot-forming units.

4. Discussion

This study shows that 25% of respondents were LTBI (+) (TST 20.8% and T-SPOT.TB 16.7%) and 75% of respondents were LTBI (-). This result is not much different from Brassard et al.'s [16] study in 1999 that found 22% TST and Duarte et al.'s [17] study in Portugal in 2001-2003 that found 50% TST and in 2005-2007 that found 20.6% TST. Grimes et al.'s study (2007) found 28% TST and 34% T-SPOT.TB [8]. Higher prevalence was obtained in Yung-Feng Yen, et al.'s study in Taipei in 2011 that found 85.0% TST and 58.7% T-SPOT. TB[18].

Factors likely affecting LTBI (+) in 25% of subjects in this study were age, contact history, BCG scar, absolute CD4 count, duration of the drugs used, and type of drugs used. Males developed more LTBI (23.8%) than females. The majority (87.5%) of respondents were in early adulthood (18-40 years old), with LTBI patients of 19.0%. World drug report in 2015 reported that there were more drug abusers in men than in women and in productive age [19]. Health Effort Building Division of Republic of Indonesia's Ministry of Health in 2010 reported more male drug users than male ones, with the highest

prevalence in those aged between 15 and 44 years [5]. Considering the above data, the high number of male and productive age drug users is directly proportional to the incidence of LTBI.

There were 20.0% of LTBI (+) respondents with a history of contact with TB (at the same home) were; this figure was lower than those without history of contact; i.e., 26.3%. The high number of LTBI (+) respondents without a history of contact may be due to ignorance about TB disease among drug users so that they can be infected during jointly used narcotics equipment such as pipes, and while gathering in tight spaces or those with poor ventilation [20]. The higher LTBI (+) prevalence with TB contact is found in Yung-Feng Yen, et al.'s study (46.7%) [18]. Centers for Disease Control and Prevention (CDC) in 2005 reported the incidence of LTBI with TB contact history of 20-30 % [21]. Considering the data above, the history of contact corresponds to the CDC report. Immunity plays an important role in resisting Mtb infection, so not everyone with TB contact will be infected.

History of BCG immunization is found in 66.7% respondents with 18.8% TST (+) and 18.8% T-SPOT.TB (+). The history of BCG immunization does not affect the positive results of TST and T-SPOT.TB examination, as confirmed by Yung-Feng Yen, et al's study reporting that positive TST was not associated with BCG scar [18]. The history of BCG vaccine, according to the CDC, is not a contraindication to TST; the effect of BCG vaccine decreases as time goes by, and if within 5 years after the BCG vaccine, a positive TST result is obtained, it is most likely due to Mtb [22]. The impact of BCG vaccine on TST specificity, according to WHO, depends on the vaccine strain used, the age at which the vaccine is given, and the dose given [3]. This study found that the BCG vaccine was given at infant time, so that the TST (+) results are actually positive rather than false positive.

Mean CD4 in the total respondents is 740.21 cells / uL while TST (+) is 705.80 cells / uL and T-SPOT.TB is 671.75 cells / uL. The drug users with LTBI (+) has a lower number of CD4 cells and it can be caused by drugs with immunosuppressive effect [23]. The population of drug users has a higher risk of becoming active TB. These data are appropriate for TB pathogenesis, where a decrease in the number of CD4 cells can attenuate the structure of the LTBI granuloma so that *M. tuberculosis* can replicate and go out of granulomas [22]. LTBI treatment can reduce the incidence of LTBI and prevent it from developing into active TB.

Severe drug addiction is at high risk of experiencing health, social, financial, legal, and relationship problems as a result of current patterns of substance use and tends to depend [10]. The average total length of drug use is 17.29 years while the mean TST (+) and T-SPOT.TB (+) is 19.2 years and 18 years. Solomon, et al.'s study in New York in 1996 found that the longer the duration of drug use, the greater is the risk of developing LTBI

[24]. Considering the data aforementioned, the duration of drug use tends to increase the incidence of LTBI.

The respondents in this study were 11 people in methadone maintenance therapy and 13 non-methadone active users with the same LTBI incidence rate, 27.3%. The substances used by the respondents of this study varied and on their way as drug users, they use not merely one substance. The result of WHO ASSIST's severe alcohol consumption is 54.2%, alcohol can change the innate immune response, so that the interaction between alcohol and Mtb will affect the number of cytokines, and then suppress TNF-α production [6]. The result of WHO ASSIST's largest cannabis is moderate (64.3%), TST (+) 21.4% and T-SPOT. TB (+) 14.3% is not much different from Grimes, et al.'s study. TST (+) and T-SPOT.TB (+) values are 24%, respectively [8]. Cannabinoids are active in cannabis and have anti-inflammatory and immunosuppressive effects [6]. Amphetamines are dominated by WHO ASSIST, weighing 50%, while TST (+) and T-SPOT.TB (+) values of 13.6% respectively are found in the moderate WHO ASSIST group. Yung-Feng Yen, et al.'s study found TST (+) and T-SPOT.TB (+) values of 3.3% and 1.8%, respectively on amphetamines [18]. The use of amphetamines can result in immunosuppression. Twenty five respondents with methadone maintenance therapy were dominated by the moderate WHO ASSIST group, 72.7%, while the highest number of WHO ASSIST TST (+) and T-SPOT.TB (+) is 18.2% and 27.1%. Yung-Feng Yen et al.'s study on opioids users found TST (+) of 42.6% and T-SPOT.TB (+) of 43% [18]. Opioids have been evident *in vivo* and *in vitro* to cause immunosuppression, making them more susceptible to infection [23].

5. The Agreement Level of TST and T-SPOT. TB Examinations in Detecting LTBI for Drug Users

The agreement level of T-SPOT.TB and TST examinations as a diagnostic tool for LTBI is assessed using Kappa's level of agreement. Kappa's agreement level of diagnostic value for T-SPOT.TB and TST examinations is k=0.591 with p=0.003 (statistically significant). *k* value of 0.591 shows a moderate level of agreement (0.40 <k ≤0.60) better than that in previous studies. Yung-Feng Yen, et al.'s study [18] found k=0.26 belonging to fair agreement (0.20 <k ≤ 0.40), p= 0.001 while Grimes, et al [8] found k= 0.2856 belonging to fair agreement (0.20 <k ≤ 0, 40) with p = 0.0022.

Moderate agreement level may result from the absence of a true diagnostic gold standard. TST limitations can result in false positives (infected with Mycobacterium other than tuberculosis and previous BCG vaccination) and can be subjective (in relation to biased technique and observer) [26]. Moderate agreement, T-SPOT.TB has not been

able to replace TST in detecting LTBI. WHO's LTBI Guidelines of 2018 states that both TST and IGRA examinations can be used to detect LTBI. The availability and the affordability of these two diagnostic tools will determine what doctors and program managers will choose. Neither TST nor IGRA can be used to diagnose active TB disease or to conduct diagnostic examinations on adults suspected as developing active TB [3].

5.1. Sensitivity and specificity of T-SPOT. TB and TST examination in detecting LTBI in drug users.

There has been no gold standard diagnostic tool for detecting LTBI. This inhibits the determination of TST and T-SPOT.TB examination sensitivity and specificity. Therefore, according to Rutjes AWS, et al., an alternative panel of experts or consensus can be used [27]. The World Health Organization, CDC, and Indonesian Pulmonologist Association (PDPI) state that both TST and IGRA (T-SPOT.TB) examinations can be used for LTBI diagnosis [1, 3, 22]. Researchers use TST as an alternative to the gold standard vacuum. Grimes et al.'s study also used TST as a gold standard to assess the sensitivity and specificity of T-SPOT.TB [8].

This study finds that sensitivity and specificity of T-SPOT.TB were 60.00% and 94.74%, respectively, better than the Grimes, et al.'s study that found the sensitivity of 50% and specificity of 79% [8]. The positive predictive value of T-SPOT.TB was 75.00% meaning that if the T-SPOT.TB (+) examination is possible, the patient will develop 75% of LTBI. The negative predictive value of T-SPOT.TB is 90.00%, meaning that if the T-SPOT.TB (-) is checked, the probability of non-LTBI patients is 90.00%. The results of PLR and NLR for T-SPOT.TB examination are 11.40 and 0.42; it can be concluded that T-SPOT.TB has good diagnostic accuracy in detecting LTBI in drug addiction.

The results of the diagnostic test analysis based on the ROC curve can be obtained at T-SPOT.TB examination with a cut point of 5, sensitivity of 66.7%, specificity of 100%, AUC of 79.6% (95% CI 53.5% -100%) and p= 0.033. AUC value of 79.6% shows medium strength (AUC > 70-80%) meaning that T-SPOT.TB can detect LTBI in 79 out of 100 people. The cut-off point recommended by the European Center for Disease Prevention and Control (ECDC) in 2011 for T-SPOT.TB is ≥ 8 [9]. Based on the data above, the cut-off point of ≥ 8 is still a positive standard value of T-SPOT.TB.

5.2. The relationship of absolute CD4 to TST and T-SPOT. TB examinations

The study evaluating the correlation between absolute CD4 and TST and T-SPOT.TB examinations on drug users as far as the author's knowledge has not been available. The author compares Leidl L, et al.'s study (2006) in Kampala linking the immune diagnostics of TB to the number of CD4 cells in HIV as a reference for the results of the current study [28]. The result of the Spearman test's TST (mm) in this study shows r= 0.077, p= 0.719. Leidl L, et al.'s study found TST diameter in mm (r= 0.41 and p= <0.0001) [28]. The correlation of TST induration (in millimeter or mm) between this study and Leidl L, et al.'s is positive, meaning that the lower the absolute CD4, the smaller the TST induration examination and vice versa. This result is appropriate, in which HIV immunocompromised patients have positive induration score of >5 mm and drug users are of >10 mm [1, 3, 11].

The results of the Spearman's test are as follows: SFUs ESAT-6 (r= -0.238, p= 0.262), CFP-10 (r= -0.117, p= 0.585), and the highest value ESAT-6 / CFP-10 (r= -0.033, p= 0.879). Leidl Let al.'s study shows SFUs ESAT-6 (r= 0.03, p= 0.77), CFP-10 (r= 0.13, p= 0.21), and the highest value ESAT-6 / CFP-10 (r= 0.01, p= 0.31) [28]. The result of the current study has correlation direction that is different from Leidl L, et al.'s study, where T-SPOT.TB was not affected by absolute CD4 count. Considering the results, this study found no statistically significant relationship of absolute CD4 count to TST and T-SPOT.TB tests for detecting LTBI in drug dependent users.

5.3. Comprehensive analysis

This study found LTBI in 25% of respondents (TST [+] 20.8% and T-SPOT.TB [+] 16.7%). Agreement level and T-SPOT.TB level are moderate (k= 0.591) and significant (p= 0.003). The sensitivity of T-SPOT.TB is 60% and its specificity is high (94.74%), so that positive result indicates LTBI development. The relationship between absolute CD4 and TST examination is not statistically significant with positive correlation direction, whereas that between absolute CD4 and T-SPOT.TB is not statistically significant with negative correlation direction.

5.4. Research limitations

The limitation of this study is that there is no gold standard for diagnosing LTBI. The author determines the sensitivity and specificity of T-SPOT.TB with TST being a bridge to fill the gold standard void. Diagnostic test results cannot completely describe the accuracy of the T-SPOT.TB examination.

6. Conclusion

Tuberculin skin test and T-SPOT.TB have their own strengths and weaknesses but in this study, TST is slightly superior to T-SPOT.TB with moderate agreement level. There was no significant relationship between absolute CD4 and TST and T-SPOT.TB examinations in detecting LTBI in drug users.

7. Recommendation

There is no gold standard for diagnosing LTBI, so an agreement is needed or a new diagnostic tool should be invented as a gold standard. The availability and affordability of these two diagnostic tools can be considered as diagnostic standards. Government support is needed for some programs to find and to treat LTBI in high-risk populations (drugs users and others) to reduce TB burden and LTBI as a part of a strategy to achieve SDGs.

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