Improving Diagnostic of Pulmonary Tuberculosis in HIV Patients by Bronchoscopy: A Cross Sectional Study

Prayudi Santoso[1], Arto Y. Soeroto[1], Rianita Juniati[1], Yovita Hartantri[1], Rudi Wisaksana[1], Bachti Alisjabana[1], Heda M. Nataprawira[2], Ida Parwati[3]

[1] Department of Internal Medicine, Faculty of Medicine Universitas Padjadjaran - Hasan Sadikin Hospital, Bandung, Indonesia.
[2] Department of Child Health, Faculty of Medicine Universitas Padjadjaran - Hasan Sadikin Hospital, Bandung, Indonesia.
[3] Department of Clinical Pathology, Faculty of Medicine Universitas Padjadjaran - Hasan Sadikin Hospital, Bandung, Indonesia.

Corresponding Author:
Prayudi Santoso, MD., FCCP, FINASIM. Division of Respirology and Critical Care Internal Medicine, Department of Internal Medicine, Faculty of Medicine Universitas Padjadjaran–Hasan Sadikin Hospital. Jl Pasteur No. 38, Bandung, 10430, Indonesia. email: prayudimartha@yahoo.com.

ABSTRAK

Latar belakang: *diagnosis TB paru pada pasien HIV merupakan suatu tantangan karena fitur klinis atau tampilan radiologis yang tidak spesifik. Pasien HIV dengan sel CD4 ≤200 sel/μL yang terinfeksi M. tuberculosis memiliki kapasitas yang lebih rendah dalam menampung M. tuberculosis, membentuk granuloma, nekrosis, atau kavitas. Kondisi ini disebabkan oleh melemahnya inflamasi yang kemudian mengurangi produksi sputum dan dapat menyebabkan hasil negatif palsu. Penelitian ini bertujuan menilai perbedaan tingkat positivitas basil tahan asam (BTA) dan kultur M. tuberculosis dari sputum non-bronkoskopi dibandingkan dengan sputum bronkoskopi (bronkoalveolar) pada pasien tersangka tuberkulosis (TB) paru HIV dengan CD4≤200 sel/μL.* ***Metode:*** *penelitian potong lintang dilakukan pada pasien HIV dewasa yang dirawat di Rumah Sakit Hasan Sadikin dengan CD4 ≤200 sel/μL yang disangka memiliki TB paru dengan uji analitik komparatif berpasangan. Semua pasien diminta memberikan dahak secara spontan atau dengan induksi sputum pada hari pertama. Pada hari berikutnya dilakukan pemeriksaan bronkoskopi dengan bilasan bronkoalveolus. Bahan yang diperoleh dari kedua cara diperiksa secara mikroskopis dengan pewarnaan Ziehl Neelsen (ZN) dan kultur M. tuberculosis dengan media padat Ogawa. Positivitas, sensitivitas dan peningkatan sensitivitas BTA dan kultur M. tuberculosis pada kelompok non-bronkoskopik dan bronkoskopik kemudian dibandingkan.* ***Hasil:*** *terdapat perbedaan tingkat positivitas ZN pada kelompok non-bronkoskopi dibandingkan bronkoskopi yaitu 7/40 (17,50%) vs 20/40 (50,00%) (p<0.001). Perbedaan antara kultur kelompok non-bronkoskopi dengan kelompok bronkoskopi yaitu 16/40 (40,00%) vs 23/40 (57,50%) (p=0,039). Bilasan bronkoalveolus menunjukkan tingkat positivitas pemeriksaan dahak BTA lebih tinggi sebesar 32,5% (dari 17,5% menjadi 50%) dan juga kultur sebesar 17,5% (dari 40,0% menjadi 57,5%).* ***Kesimpulan:*** *bilasan bronkoalveolar dapat meningkatkan tingkat positivitas pemeriksaan sputum BTA dan kultur M. tuberculosis pada pasien tersangka TB paru dengan HIV positif dan CD4≤200 sel/μL.*

Kata kunci: *bronkoskopi, diagnostik mikrobiologi, HIV, sputum, tuberkulosis.*

ABSTRACT

Background: *diagnostic of pulmonary TB in HIV patients is a problem due to non specific clinical features, or radiological appearance. HIV patients with CD4≤200 cells/mL infected with M. tuberculosis have less capacity in containing M. tuberculosis, developing granulomas, casseous necrosis, or cavities. This condition is caused*

by weakend inflammatory which later reduced sputum production and may cause false negative result. This study aimed to assess differences in the positivity level of acid fast bacilli (AFB) and cultures of M. tuberculosis from non-bronchoscopic sputum (spontaneous and induced sputum) compared to bronchoscopic sputum (bronchoalveolar lavage) in HIV positive patients suspected pulmonary tuberculosis with CD4<200 cells/μL. ***Methods:*** *this cross sectional study was conducted in adult HIV patients treated in Hasan Sadikin Hospital with CD4≤200 cells/μL suspected with pulmonary tuberculosis by using paired comparative analytic test. All patients expelled sputum spontaneously or with sputum induction on the first day. On the next day, bronchoalveolar lavage (BAL) was performed. The two samples obtained from two methods were examined by AFB examination with staining Ziehl Neelsen (ZN) and cultured of M. tuberculosis on solid media Ogawa on all patients. Positivity, sensitivity and increased sensitivity of AFB and culture of M. tuberculosis in the non bronchoscopic and bronchoscopic groups were compared.* ***Results:*** *there were differences in the positivity level of AFB with ZN staining between non-bronchoscopic and bronchoscopic groups which were 7/40 (17.5%) vs 20/40 (50.0%) (p<0.001). The differences between the cultures of non-bronchoscopic and bronchoscopic groups were 16/40 (40.0%) vs 23/40 (57.5%) (p=0.039). Bronchoscopic sputum increased the positivity level of the ZN AFB examination by 32.5% (from 17.5% to 50.0%) as well as on culture examination by 17.5% (from 40.0% to 57.5%).* ***Conclusion:*** *Bronchoalveolar lavage can improve the positivity level of smears and cultures in patients suspected of pulmonary TB in HIV patients with CD4<200 cells/μL.*

Keywords: *bronchoscopy, HIV, microbiological diagnostics, sputum, tuberculosis.*

INTRODUCTION

Tuberculosis (TB) is still a major health problem in Indonesia which has the second highest number of cases in the world after India.[1] The problems of TB are increasingly complex due to complications from Human Immunodeficiency Virus (HIV) infection which may increase the risk of acquiring pulmonary tuberculosis. The risk of developing tuberculosis in HIV patients is 20 to 37 times greater than non-HIV patients.[2]

Diagnostic of pulmonary TB in HIV patients is problematic due to non specific clinical features, or radiological appearance. HIV patients with CD4 ≤200 cells/μL infected with *M. tuberculosis* have less capacity in containing *M. tuberculosis*, developing granulomas, casseous necrosis, or cavities.[3,4] This condition is caused by weakend inflammatory which later reduced sputum production and may cause false negative result. This cause significant important problem in the diagnosis of tuberculosis.[3,5] Chest X-ray is unable to distinguish *M. tuberculosis* infection from *Pneumocystis jiroveci*, fungal infection, or other microorganism.[6] Therefore, microbiologic test is necessary to confirm causative agent in suspected TB-HIV patients.[7]

Previous studies and literatures reported that the best method to gain a good quality specimen is by bronchoalveolar lavage (BAL).[3,8-10]

The aim the study is to assess differences in the positivity of acid fast bacilli (AFB) and cultures of *M. tuberculosis* in the specimen acquired from non-bronchoscopic sputum (spontaeous and induced sputum) compared to bronchoscopic sputum (bronchoalveolar lavage) in HIV positive patients suspected of pulmonary tuberculosis with CD4<200 cells/μL.

METHODS

A cross-sectional study with paired comparative diagnostic analysis was conducted in Hasan Sadikin Hospital, a referral hospital in West Java, Bandung, Indonesia. Calculation of sample based on test formula of two proportions of Mc Nemar, with α = 0.05, hypothesis of two ways test, 95% confidence interval and power test 80%. We enrolled all patients older than 14 years old who were admitted to the hospital and clinic with a diagnosis of HIV and pulmonary infection. The study was conducted between November 2011 and October 2013.

Specimens were divided into two groups: the non-bronchoscopic specimens which was spontaneously expectorated and induced sputum sample (non-bronchoscopic group) and the bronchoscopic specimens which was sputum

taken by bronchoscopy (bronchoscopic group).

HIV infection was determined by ELISA (Allere/USA). The inclusion criteria for this study was the HIV patient with CD4 ≤200 cells/μL and presumptive TB based on clinical findings and chest X-ray. Patients with respiratory failure, chronic kidney disease, congestive heart failure, and diabetes mellitus, or on TB treatment were excluded.

Before expectorating out sputum, patients were educated on how to cough a good quality sputum. If the patient were unable to expectorate, sputum was induced with nebulizition using 5–10 mililiter of NaCl 3% as recommended.[11] Bronchoscopy was done by principal investigator using Olympus Bronchoscopic based on standar operational in our department after informed consent was given.

This study is part of the Etiologic Pulmonary Infection in HIV Patients study. In this study, 40 HIV-TB patients specimens were collected from expectorated and induced sputum sample. On the next day, bronchoalveolar lavage (BAL) were perfomed on the same subject. All specimen were subjected to microscopic ZN examination and cultured on solid media Ogawa.

The protocol of study had been approved by the Ethics Committee of Hasan Sadikin Hospital in Bandung, registration number: 264/FKUP-Hasan Sadikin/KEPK/Kep./EC/2010. Written informed consent was obtained from each subjects.

Primary Endpoint

The primary endpoint of our study was to assess the positivity rate of AFB and *Mycobacterium tuberculosis* sputum culture in non-bronchoscopic group compared to bronchoscopic group.

Statistical Analysis

The data was analyzed using SPSS 20.0 (Chicago, USA). The data of positive and negative smear of non-bronchoscopic sputum and sputum from bronchoalveolar lavage was presented in a 2x2 table. The data collected at the beginning was statistically processed by calculating the sensitivity, specificity and accuracy using Mc Nemar test. Significance of test results, was set at p≤0.05.

RESULTS

Characteristics of subjects were presented according to age, gender and CD4 levels. Normality of the data was tested using the Shapiro-Wilk test. Based on the data normality test, the age and gender showed normal distribution (p>0.05), whereas CD4 levels were not normally distributed (p<0.05).

The fourty subjects consisted of 23 men (57.0%) and 17 women (43.0%). The average age was 32.5 years old (20–44 years old). Median CD4 levels was 22 with a range of 0–190 cell/mm^3.

Comparison of AFB with ZN Staining and *M. tuberculosis* Culture Results

The non-bronchoscopic specimen (n=40) revealed positive result with ZN staining in 7 samples (17.5%) and positive culture result in 16 samples (40.0%). The bronchoscopic specimen resulted in smear positive with ZN staining in 20 samples (50.0%) and positive TB culture result in 23 samples (57.5%), p<0.001 (**Table 1**).

The difference between culture results of non-bronchoscopic patients and bronchoscopic patients was 16/40 (40.0%) compared to 23/40 (57.5%). The difference was significant, as shown by value of p=0.039.

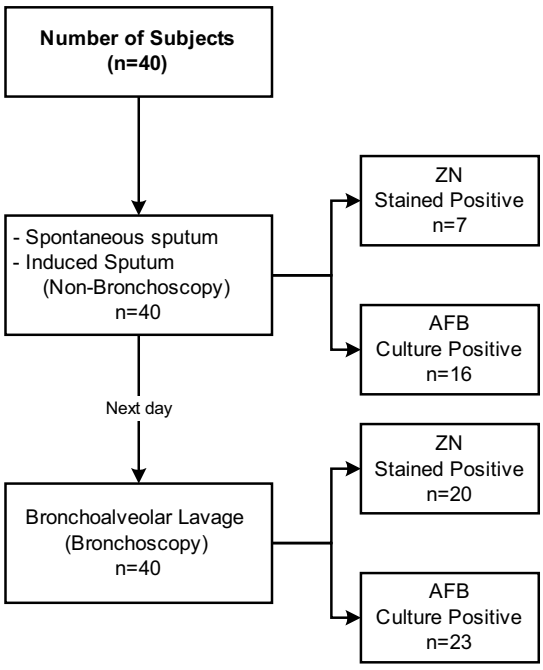

Figure 1. Charts results

Table 1. Results of AFB with ZN staining and culture of TB from non-bronchoscopic patients and the bronchoscopic patients

		Non-bronchoscopic patients n (%)	Bronchoscopic patients n (%)	p
Ziehl Neelsen	+	7 (17.5)	20 (50.0)	<0.001
	-	33 (82.5)	20 (50.0)	
TB culture	+	16 (40.0)	23 (57.5)	0.039
	-	24 (60.0)	17 (42.5)	

* Mc Nemar test

There was a 22.5% increase of positivity in the non-bronchoscopic culture group between ZN and culture. The bronchoscopy group also underwent the same examination as in non-bronchoscopy group which included, ZN examination and *M. tuberculosis* culture. There was an increase of 7.5% in the positivity of the group examined by *M. tuberculosis* culture in bronchoscopy group. (**Table 2**)

Table 2. Increase of positive results between non-bronchoscopy and bronchoscopy

	Test Positive with TB, %	Increase Test Positive with TB, %
Non-Bronchoscopy ZN	17.5	17.5
Non-Bronchoscopy Culture	40.0	22.5
Bronchoscopy ZN	50.0	10.0
Bronchoscopy Culture	57.5	7.5

Additional test positive with TB was 32.5% and sensitivity was 73.9%. The increased sensitivity has 30% in bronchoscopic ZN. Proportion of truly positive test was 85% as shown by the PPV. (**Table 3**)

DISCUSSION

As we know the highest cause of death of HIV patients in TB endemic countries is TB. Therefore, early detection of TB in HIV patients is very important so that the patients are not late for treatment and reduce the disease transmission. The detection of AFB in sputum will provide certainty of microbiologic diagnosis so that it will reduce the number of patients given therapy based on clinical symptoms, or *ex juvantibus* therapy by looking at therapeutic response. When the treatment is given only based on clinical diagnosis, there will be two possible consequences: overdiagnosis, or underdiagnosis.[6]

Diagnostic confirmation of pulmonary TB in HIV patients becomes problematic when clinical symptoms is suspicious, but smear result was negative. This may occur in 24-61% of patients with active pulmonary TB.[2]

Microbiologic examination is the most important test for detecting the etiologic microorganism. The problem is the need of good quality sample which is often very difficult to obtain in HIV-TB patients. That is why we conducted the study to confirm the value of

Table 3. The contribution of non-bronchoscopic and bronchoscopic groups for the diagnosis of tuberculosis

	No Bronchoscopy with ZN	Bronchoscopy with ZN
Test Positive with TB	7 (17.50%)	20 (50.00%)
Additional Test Positive with TB	7 (17.50%)	13 (32.50%)
Sensitivity (95% CI)	43.75 (19.75 – 70.12)	73.91 (51.59 – 89.77)
Incremental Sensitivity (95% CI)	43.75 (19.75 – 70.12)	30.16 (15.58 – 44.42)
Specificity (95% CI)	100 (85.75 – 100)	82.35 (56.57 – 96.20)
PPV (95% CI)	100 (59.04 – 100.00)	85.0 (62.11 – 96.79)
NPV (95% CI)	72.73 (54.48 – 86.70)	70.0 (45.72 – 88.11)

Definition of abbreviations: ZN=Ziehl Neelsen; NPV=negative predictive value; PPV=positive predictive value

BAL.[8] In this study we found that in HIV-TB patients with CD4 cells less than 200 cells/uL, the positivity of AFB with ZN staining and culture was higher in bronchoscopic group than non-bronchoscopic group. To our knowledge, this study is the first study using bronchoscopy for diagnosing pulmonary tuberculosis in HIV patients in Indonesia. Positivity on sputum culture in non-bronchoscopic group and also in bronchoscopic group were higher than ZN smear examination. Culture of *M. tuberculosis* is clearly more sensitive than examination of sputum smear ZN because it requires fewer bacilli that is at least 100/ml compared to the acid fast bacilli that requires a minimum of 10.000 bacilli/ml to produce positive smear examination. Positivity of smear examination of sputum ZN correlated with concentrations of AFB in sputum.[6]

Positivity of ZN smear examination by bronchoscopy was higher than sputum cultures for *M. tuberculosis*. This study shows that differences in both smear positivity with ZN staining in bronchoscopic group was higher than non-bronchoscopic group (50% v.s. 17.5%). Bronchoscopic increased the positivity of the ZN AFB examination from 17.5% to 50% as well as on culture examination from 40% to 57.5%. The improvement is quite high. It is reasonable to put in consideration to perfom bronchoscopy in HIV positive patients suspected of pulmonary TB when sputum smear ZN was negative. The difference in positivity is influenced by the quality and quantity of the sample. Bronchoalveolar lavage was obtained from bronchoscopy by way of flushing in alveolar resulting in an adequate amount and lavage derived directly from alveolar.[12,13] The use of bronchoscopy increased the number of microbiologically confirmed cases from 18 to 26, a relative increase of 44% and this is in accordance increased sensitivity by 30% on the examination of AFB ZN with bronchoscopy.[9]

ZN smear examination was more profitable than culture, based on time for detection. Sputum culture with solid media requires time to grow of 3–8 weeks.[14] Liquid media detects approximately 10% more TB cases than solid media, and requires a shorter incubation period for mycobacterial growth.[15] If non-bronchoscopic give negative result, but clinical symptom supported pulmonary tuberculosis, we suggest that bronchoscopy should be done immediately. This was because the positivity of bronchoscopy with AFB ZN was higher than the culture which took longer time. Bronchoscopic examination might shorten the diagnostic time of pulmonary TB in HIV patients so that clinicians could immediately provide TB treatment.

At the time of this study there were no Xpert MTB/RIF checks for sensitive TB diagnostics, since Xpert MTB/RIF examination was prioritized for patients with TB MDR (Multi Drug Resistant) presumptive based on history of treatment, relaps, or treatment after loss to follow up.

Examination of bronchoscopy is an invasive procedure that can only be performed at a large health facility, or possibly at an advanced referral health facility, requiring trained and cost-effective experts. In Indonesia, bronchoscopy facilities are usually available at the advanced referral facilities that are usually present in the provincial capitals. However, a patient referral network has been established for primary and advanced health facilities in Indonesia. The HIV patients suspected of tuberculosis, but not sputum, or phlegm negative and living in non-advanced facilities can be referred to referral hospital for perfoming bronchoscopy. In this study the bronchoscopy relatively safe without any significant complications.

The limitation of this study was that the patients with ordinary expectorants with sputum induction were not separated with spontaneous expectoran. Liquid culture can be done to shorten the actual time and it can grow faster than solid media that is approximately 2 weeks.[15] For further research it is necessary to examine the sample using NAAT (Nucleic acid amplification test) which can detect targeted regions of the *M. tuberculosis* Genome by amplifying specific regions of mycobacterial DNA using Xpert MTB/RIF method which is more sensitive than the ZN AFB examination and can simultaneously detect rifampicin resistance.

There is a difference in the positivity of smear results when different diagnostic and screening measures are performed. Bronchoscopic method will increase positivity compared to non-

bronchoscopy. Culture of *M. tuberculosis* will also increase positivity compared to ZN staining.

The study recommends that if HIV patients as suspected of having pulmonary TB, but microscopic examination by spontaneous cough or sputum induction are negative, BAL should be examined especially in facilities and by experts who can perform bronchoscopy. Further studies are needed by using Xpert MTB/RIF examination and theoretically this examination will add positivity compared to ZN AFB examination. Currently Xpert MTB/RIF are available in almost all referral hospitals in Indonesia, so incorporation of sampling with BAL and diagnostics with Xpert MTB/RIF can be performed.

CONCLUSION

The bonchoalveolar lavage with bronchoscopy can increase the positivity of smear and shorten the diagnostic time of patients suspected of pulmonary TB in HIV patients with CD4 cell counts below 200 cells/uL.

ACKNOWLEDGMENTS

The research was financially supported by grant National AIDS Research Commission (KPAN, Komisi Penelitian AIDS Nasional), and HCPI (HIV Cooperation Program for Indonesia). The author would like to thank dr. Melindah who prepared the patients and as bronchoscopy assistant. Dr. Dewi KT, SpPK and dr. Adhi K. Sugianli, SpPK who helped laboratory for our patients.

The authors do not have any conflict of interest to disclosure.

REFERENCES

1. World Health Organization. Global tuberculosis report 2016 (global tuberculosis control). Switzerland: World Health Organization Press; 2016.
2. Getahun H, Harrington M, O'Brien R, Nunn P. Diagnosis of smear-negative pulmonary tuberculosis in people with HIV infection or AIDS in resourceconstrained settings: informing urgent policy changes. Lancet. 2007;369:2042–9.
3. Karakousis PC, Chaisson RE. Mycobacterial infections and HIV infection. In: Fishman A, Elias J, Fishman J, Grippi M, Senior R, Pack A, eds. Fishman's pulmonary disesase and disorders. 4ed. New York: Mc Graw Hill; 2008. p.2487-97.
4. Nachega JB, Maartens G. Clinical aspects of tuberculosis in HIV infected adults. In: Schaaf H, Zumla A, ed. Tuberculosis a comprehensive clinical reference. Europe: Saunders; 2009. p. 524-31.
5. Gupta P, Baloch Z. Pulmonary cytopathology. In: Fishman A, Elias J, Fishman J Grippi MA, Senior RM, Pack AI, ed. Fishman's pulmonary disesase and disorders. 4ed. New York: Mc Graw Hill Medical; 2008. p511-610.
6. TB CARE I. International Standards for Tuberculosis Care, Edition 3. TB CARE I, The Hague, 2014.
7. Fangman JJW, Sax PE. Human immunodeficiency virus and pulmonary infections. In: Fishman AP, Elias JA, Fishman JA, Grippi MA, Senior RM, Pack AI, eds. Fishman's pulmonary diseases and disorders. 4ed. New York: Mc Graw Hill Medical; 2008. p.2241–64.
8. Aderaye G, G/Egziabher H, Aseffa A, Worku A, Lindquist L. Comparison of acid-fast stain and culture for Mycobacterium tuberculosis in pre- and post-bronchoscopy sputum and bronchoalveolar lavage in HIV-infected patients with atypical chest X-ray in Ethiopia. Ann Thorac Med. 2007;2(4):154-7.
9. Worodria W, Davis J, Cattamanchi A, Andama A, Boon S, Yoo S. Bronchosocopy is useful for diagnosing smear-negative tuberculosis in HIV-infected patients. Eur Respir J. 2010;446-56.
10. Adewole OO, Onakpoya UU, Ogunrombi AB, et al. Flexible fiberoptic bronchoscopy in respiratory care: Diagnostic yield, complications, and challenges in a Nigerian Tertiary Center. Niger J Clin Pract. 2017;20(1):77-81.
11. Garcia SB, Perin C, Silveira MMd, Vergani G, Menna-Barreto SS, Dalcin PdTR. Bacteriological analysis of induced sputum for the diagnosis of pulmonary tuberculosis in the clinical practice of a general tertiary hospital. J Bras Pneumol. 2009;35(11):1092-9.
12. Baughman RP, Lower EE. Diagnosis of pneumonia in immunocompromised patient. In: Agusti C, Torres A, Eds. Pulmonary infection in the immunocompromised patient: Strategi for management. UK: John Wiley and Sons. Ltd; 2009. p.65-70.
13. Vachini A, Seijo L, Unger M, Sterman D. Bronchoscopy, transthoracic needle aspiration, and related procedures. In: Fishman AP, Elias JA, Fishman JA, Grippi MA, Senior RM, Pack AI, eds. Fishman's pulmonary diseases and disorders. 4th edition. New York: Mc Graw Hill Medical; 2008. p. 629-48.
14. Al-Zamel FA. Detection and diagnosis of Mycobacterium tuberculosis. Expert Rev Anti Infect Ther. 2009;7(9):1099-108.
15. Dinnes J, Deeks J, Kunst H, et al. A systematic review of rapid diagnostic tests for the detection of tuberculosis infection. Health Technol Assess. 2007;11(3):1-155.