

Improving Diagnosis of Pulmonary Tuberculosis Among HIV/AIDS Patients: Literature Review and Experience in a Teaching Hospital in Indonesia

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ABSTRACT

HIV infection hampers diagnosis of pulmonary tuberculosis (PTB) because many pathogens cause pulmonary infection in HIV people and the load of Mycobacterium tuberculosis is lower in HIV patients.

We conducted a literature review and prospectively examined clinical, radiological, and laboratory diagnosis of PTB in 71 HIV-patients (29 inpatients and 42 outpatients) in a teaching hospital in West Java, Indonesia.

For both in- and outpatients, signs and symptoms were sensitive but not specific for PTB. Chest X-ray (CXR) was sensitive but less specific. Among hospitalized PTB suspects, 28,8% could not expectorate sputum. Compared to culture, ZN had a sensitivity of 11.1% and 66.7% for in- and outpatients, respectively. From the literature, fluorescence microscopy, liquid culture, and nucleic acid assays can improve diagnosis of PTB in HIV, while IFNg-release assays lack sensitivity, especially in advanced HIV.

The current practice of using CXR and microscopy lacks sensitivity for diagnosing PTB in HIV patients. Sputum culture is more sensitive but slow. Fluorescence microscopy might be a quick, relatively sensitive and feasible option in Indonesia. However, because of the frequent absence of sputum, especially in patients with advanced HIV-AIDS patients, there is an urgent need for alternative diagnostic methods using blood or urine.

Key words: *pulmonary tuberculosis, HIV/AIDS patients, chest X-ray.*

INTRODUCTION

At the end of 2007, approximately 33.2 million persons with HIV infection and one-third were co-infected with tuberculosis (TB). TB is the cause of death for half of all persons with AIDS. Since 1990, TB infection rates have increased 4-fold in countries that are heavily affected by HIV.¹ Indonesia has the fastest growth HIV-epidemic in Asia.² The HIV-prevalence in the general population is still low (0.16%), but up to 50% have been reported in certain risk groups. HIV-infection among TB-patients appears low in Indonesia, 2.0% in Bandung and Jakarta and 1.9% in Yogyakarta.^{1,3,4} Among HIV-infected patients, however, TB is very prevalent, in a cohort of HIV patients in Bandung, 36% had a history of TB treatment.⁵

People infected with HIV have 10 times the risk of developing TB compared with normal people, and pulmonary TB (PTB) is still the commonest form.⁶ WHO guidelines recommend screening for TB in all HIV/AIDS patients, however, diagnosis of PTB in HIV/AIDS patients is difficult. First, besides *M. tuberculosis*, there is a range of other microorganisms which can cause lung infections in HIV. Second, sputum microscopy, the cornerstone of diagnosing pulmonary TB, has a lower sensitivity in HIV-infection, as HIV/AIDS patients usually have a lower sputum concentration of *M. tuberculosis*, or may not be able to produce good quality sputum. Finally, chest X-ray (CXR) abnormalities in HIV-associated TB are often atypical and may even be

absent.⁷⁻⁹ Fewer than half of TB cases in HIV infected patients are diagnosed before death¹⁰ and current diagnostic tools perform badly in HIV-positive patients. There is an urgent need to develop rapid, simple, specific and sensitive tuberculosis diagnostic tools for detection of TB in HIV-positive patients.

This paper consists of two parts. We first review the scientific literature about current tools for diagnosis of TB in HIV-positive patients. And then, we report operational research related to diagnosis of HIV-associated PTB from an Indonesian referral hospital.

LITERATURE REVIEW

We limited our review to current available methods of diagnosing TB. Excellent reviews have been published on diagnostic methods which are still under development.¹¹

We searched PubMed combining “Tuberculosis”, “diagnostics” as free text and MeSH terms, combined with other terms including “HIV/AIDS”, “review”, “sputum microscopy”, “induction”, “instruction”, “sputum specimen”, “sputum processing, centrifugation, bleach, sedimentation”, “liquid culture, BACTEC, MGIT”, “interferon γ , antigen, serodiagnostic”, “PCR”, “nucleic acid test, molecular diagnostic”, “negative smear”, “fluorescence microscopy, Auramin, Auramin-Rhodamin, Ziehl Neelsen”. Articles referenced in retrieved papers were also located.

Chest X-ray

Chest x-rays are part of routine diagnosis and screening of tuberculosis in many countries including Indonesia.¹² However, CXR suffer from low inter-observer reliability,¹² are subjected to the quality and availability of equipment,¹³ and have a reduced usefulness in HIV-positive patients who often produce atypical CXR.¹⁴ Opinion regarding CXR as a screening tool is divided. In Botswana, only one TB case out of 563 CXR screened HIV patients was found.¹⁵ In South Africa,¹⁶⁻¹⁸ Kenya,^{13, 19} Tanzania and Burundi,²⁰ CXR was of value both for case detection and cost-efficacy. Concern remains regarding to the low specificity of CXR, at worst resulting in further unnecessary testing in 49% of those screened.¹⁷ When combined with symptoms, the value of CXR for diagnosis increases. Among HIV-positive South African miners, the addition of CXR to symptoms of TB increased sensitivity from 75.0% to 90.1%.¹⁷ In Kenya, an extra 6% of HIV patients with a cough may receive correct diagnosis when CXR is used.⁹ (Table 1) Improving quality control and using a standardised scoring system may increase CXR efficacy.¹³

Symptom Screening

Symptom screening does not require expensive equipment or health personnel specialisation, but sensitivity and specificity of symptoms are reduced in heavily immune suppressed HIV-patients. Screening by cough alone in HIV-positive patients has low sensitivity^{17, 21-25} (Table 1), with up to 86% of TB being missed.¹⁷ When other symptoms are included, sensitivity will increase. In Cambodia, sensitivity rose from 55% to 100%²⁴ when fever and weight loss were included and from 56% to 77% in Ethiopia upon inclusion of night sweats and fever.²² Nevertheless screening for several symptoms compromises specificity; South African^{17, 21} Ethiopian²² and Cambodian²⁴ research reports a loss of specificity between 4% and 39% compared with screening for cough alone.

Tuberculin Skin Test (TST)

Tuberculin skin testing (TST), which is used in many countries to diagnose latent and active TB, suffers from reduced sensitivity in HIV-patients, especially those with severe immunosuppression.²⁶ TST may be of value for detecting TB, but no distinction can be made between active and latent TB, and numerous studies have shown that HIV-positive patients are significantly less likely to be TST positive compared with HIV-negative patients.²⁷⁻²⁹ (Table 1) Even when the cut-off for positivity is lowered TST has a poor performance in HIV-positive patients compared with HIV-negative patients.²⁶⁻²⁸ Consequently, TST results in HIV-positive patients should be interpreted with caution.

Interferon gamma release assays measure T-cell response to TB antigens in the blood and are commercially available for diagnosis of latent TB infection. However, their potential in populations with high rates of HIV is uncertain. In Tanzania the sensitivity of QFT-IT (QuantiFERON TB-Gold In-Tube) was higher in HIV-negative than in HIV-positive patients (81% vs 65%). Furthermore, high rates of indeterminate results due to low CD4 count in HIV-positive patients have been observed.³⁰ (Table 1)

Sputum Diagnosis

Sputum examination remains as the cornerstone of diagnosing TB. Aspects related to sputum collection, processing, and microscopy are reviewed below, white culture and nucleic acid testing in the next section.

The quality and number of collected specimens affect diagnostic results. A systemic review in 2007 concluded that the average sensitivity of the first sputum slide (53,5%) increased following the addition of second slide (64.9%), but not further with a third slide³¹

These findings apply to HIV-positive and negative patients and have prompted the WHO to propose a reduction of specimen numbers for examination in settings with well established laboratory networks and fully functional EQA programmes.³²(Table 2)

In many cases, HIV-seropositive patients, especially those with advanced immunosuppression (“AIDS”) and low CD4 cell-counts are unable to produce sputum spontaneously, hampering diagnosis of PTB. Sputum instruction leads to a significant increase in smear positive case detection in HIV-negative patients, but so far this has not been examined in HIV-patients. In Indonesia and Pakistan, the smear positive case detection rate was increased by 15.1% and 5%, respectively, when sputum submission guidelines were provided.^{4, 33} Increase the volume of sample is also effective, using > 5 ml sputum sample increased SM sensitivity from 72.5 to 92%.³⁴ These methods have not been explored in HIV-positive populations. For both HIV positive and negative patients, improving sample quality by induction through nebulized saline has been proven to be safe and cost-effective with a high diagnostic yield and high agreement with results from bronchoscopy.^{7, 35-37} (Table 2)

Sputum microscopy (SM) is the primary tool for TB diagnosis. However, a lower sensitivity in HIV-patients is often seen due to their lower sputum bacillary load. Sputum microscopy may also be false-negative if the sputum concentration of mycobacteria is below 10,000 organisms/mL.^{22, 26, 38} Alternative sample processing

methods may increase SM sensitivity. A systemic review showed that centrifugation or overnight sedimentation with chemical processing increases sputum smear sensitivity. For HIV-positive patients, the data remains insufficient, the studies which have been conducted in HIV-positive populations only reported one increase in sensitivity (11%).³⁹ Further investigation is required to verify the use of sputum processing in HIV-positive patients (Table 2).

Fluorescent microscopy (FM) is an alternative way to Ziehl-Neelsen (ZN) staining for the detection of AFB. The procedure is thought to be faster, more-cost effective, and more sensitive than ZN.⁴⁰⁻⁴³ A systemic review showed that the average sensitivity to be 10% higher than ZN.⁴⁰ For HIV-positive patients, FM is also more sensitive than ZN. (Table 2) In a study from Kenya, FM was twice as sensitive as ZN for HIV-positive cases use culture as a gold standard⁴³ and an Indian study that reported 26% more TB cases detected when FM was used compared to ZN in a population including 15% HIV-positive patients.⁴²

Sputum Culture and Molecular Testing

Sputum culture using liquid or solid media is regarded as the gold standard of TB diagnostic tools. It is more sensitive than sputum smear, allowing detection of TB in sputum smear negative cases such as HIV-positive patients.²⁶ In a setting in South Africa, 49% of sputum smear negative HIV patients were sputum culture positive. However, studies have found detection rates of TB in HIV-positive patients to vary widely (19%-

Table 1. Chest x-ray, symptoms, TST

Aspect	Conclusion	Comment
Chest x-ray	Sensitivity 27% - 97% Specificity 51% - 67%	Low specificity increases over-diagnosis
	Combination of symptoms and CXR increases sensitivity	
	Screening with ZN followed by CXR is more cost effective than initial CXR screening	CXR screening must be followed by examination
Symptoms	Inter-rater reliability ranges between moderate to good; Kappa 0.50 – 0.84	
	Screening by cough alone sensitivity 14% - 82%, specificity 33% - 96%	HIV patients are often asymptomatic
TST	Screening for more than one symptom increases sensitivity but reduces specificity	
	HIV-positive patients less likely to be TST positive than HIV-negative patients	Interpret TST for HIV patients with caution
IFN-γ Release Assays	Sensitivity 65% in HIV+, lower in patients with advanced immunosuppression (low CD4-count)	Expensive

Table 2. Sputum collection, processing and microscopy

Aspect	Conclusion	Comment
Number of Specimens	Sensitivity of 2 or 3 sputum specimens almost similar (SR including 3 studies in HIV+)	Proposed by WHO
Sample collection	Instruction for better sputum quality may increase diagnostic yield (RCT)	Not examined in HIV+
	Collecting large volume sputum samples (> 5cc) may increase sensitivity Sputum induction with nebulized saline is safe and effective for HIV+ adults and children without sputum; better than gastric washing, high agreement with bronchoscopy.	Not examined in HIV+
Sputum processing	Centrifugation / overnight sedimentation of sputum samples with chemical processing increases sensitivity (SR incl 2 studies in HIV+);	Feasible in field condition?
Fluorescence microscopy	Overall sensitivity on average 10% higher compared to conventional microscopy (SR including 2 studies in HIV+) cost-effective; shortens diagnostic process in HIV+	Insufficient evidence in HIV

SR = systemic review; RCT = randomized clinical trial;

96%).^{44, 45, 16} Increased sensitivity, reduced cost and lower bacillary load makes culture likely diagnostic tool of choice for HIV-positive patients in low resource settings.²⁶

A major draw back of solid media is the time of diagnosis; it can be up to six weeks or longer for HIV-positive patients. There are other culture systems exist which speed-up growth and increase sensitivity, however these are technical and expensive²⁶ (Table 3). A relatively new, inexpensive liquid culture technique, called microscopic-observation drug susceptibility assay (MODS) is a potential solution to this problem. MODS uses inverted light microscope and broth culture to rapidly detect growth. Results are promising; In Peru the sensitivity of MODS was 97.8% while 89% and 84% for automated mycobacterial culture and LJ culture, respectively, the average time to diagnose was seven days.^{46, 54} Further evaluation in HIV-positive populations is required.²⁶

Nucleic Acid Amplification (NAA) molecular diagnostics also shorten diagnostic time.⁴⁷ There are two tests currently commercially available, both displaying high sensitivity and specificity in AFB positive samples compared to culture. Reported sensitivity is 79.4 - 91.9% for Amplicor and 90.9 - 95.2% for E MTD, and specificity is 99.6 - 99.8% for Amplicor and 98.8 - 100% for E MTD.^{48, 49} However, test performance is lower in AFB negative samples. Subsequently, only E-MTD is

available for testing in sputum smear negative specimens, with a reasonable performance in HIV-positive patients (sensitivity 89.0%, specificity 97.7%).⁴⁷ Several reports indicate that in HIV patients, NAA tests may also be helpful for diagnosing TB when used on blood and urine.⁵⁰⁻⁵² Despite its possible advantages, NAA diagnostics remain expensive, technically demanding and prone to contamination, especially in high-volume settings.

BANDUNG CASE STUDY

Setting, Design and Methods

This study was done in Hasan Sadikin Hospital (RSHS), a large referral hospital located in Bandung, Indonesia. Between June and December 2008 we recruited all HIV/AIDS patients with suspected PTB in HIV polyclinic, pulmonology ward, or inpatients suspected of having PTB and tested positive for HIV during their hospital stay. Suspected TB was defined by the presence of cough or CXR abnormalities.

To assess the clinical diagnosis of PTB, clinical information was collected through standardized questionnaire, physical examination and from the patient charts. We recorded information about symptoms from patient interview. To assess the diagnostic validity of CXR, CXR's were examined independently by two consultant radiologists using a standardized form. A third

Table 3. Culture, molecular testing and IFN γ release assays

Aspect	Conclusion	Comment
Culture	detection of mycobacteremia in HIV/AIDS pts varies widely between 19%-96% Commercial media (ex BACTEC and MGIT) reduce culture time from 2 to 4 weeks. Microscopic Observation Drug Susceptibility testing (MODS) reduces culture time from 7 to 14 days and is more cost-effective than commercial liquid culture.	Results taken between 6-8 weeks Commercial media are expensive needs inverted microscope;
Nucleic Acid Assay (NAA)	NAA enables rapid detection of <i>M. tuberculosis</i> from clinical samples. More sensitive than microscopy, not as sensitive as culture, also in HIV+ Loop-Mediated Isothermal Amplification (LAMP) showed sensitivity of 97.7% in smear- and culture-positive specimens, 48.8% in smear negative, culture positive specimen with specificity of 99% ; may enable point-of-care testing.	Commercial tests are expensive; Feasible in field settings?

radiologist was consulted if results between examiners differed. Results were reported as one of four categories: normal, abnormal but no chest infection, suggestive for TB (cavitary, military, upper zone infiltrate), and suggestive for chest infection but not specific for TB. Radiologists were blinded to microbiologically diagnostic results.

Sputum examination included Ziehl Neelsen (ZN) and culture. Outpatients received instruction for sputum submission by a technician and by a trained nurse for inpatients. If unable to expectorate sputum, inpatients were nebulised with a 0.9% NaCl solution to induce coughing. Direct smears were stained according to ZN these results technique and were reported according to the quantitative scale recommended by the WHO and IUATLD. All available sputum samples were cultured on Ogawa slants in pairs and stored at 37°C for 8 weeks or until found positive. Sputum culture was employed as the gold standard in this study. Sensitivity, specificity and positive and negative predictive values were calculated for clinical information, compared to sputum culture and CXR as gold standard.

Results

We recruited 29 in- and 42 outpatients with suspected PTB. Patient characteristics and symptoms are displayed in table 4 and 5. Patients were predominantly young and male and many had been treated for TB before. Outpatients had higher CD4 cell counts than inpatients, and more were already taking ART and PCP prophylaxis. Outpatients often presented with a productive cough rather than with hemoptysis, fever, or night sweats.

Symptom screening, CXR, and ZN had a different diagnosis value in inpatients compared to outpatients. For inpatients, cough alone had high sensitivity but very low specificity for PTB. Combination of cough with fever and weight loss increased sensitivity and specificity. The absence of cough, fever and weight loss guaranteed an absence of PTB. For outpatients, cough alone had a low sensitivity and specificity. Different from inpatients, the combination of cough with fever and weight loss resulted in lower sensitivity but higher specificity. The combination of CXR and symptoms had a sensitivity of 25% (75% of PTB cases would be missed). The positive predictive values (PPV) overall were low for all clinical diagnostic tests. For inpatients the highest PPV was seen for symptom combination (60%), while for out-patients CXR and symptom combination had the highest PPV (66.7%).

Table 4. Description of diagnostic process: Patient demographics and treatment history

Characteristic	Inpatients n= 29	Outpatients n= 42	p
Male sex	75.8%	73.8%	
Age, median (range)	29 (25-51)	29 (22-56)	0.43
CD4 count, cells/mm ³ , median (range)	9 (1-186)	181.5 (2-556)	0.00
On ART	2/19	14/30	0.01
On PCP prophylaxis	2/20	17/40	0.02
AB treatment during assessment	5/19	4/37	0.14
Ever treated for TB	9/20	13/42	0.28

ART = antiretroviral treatment; PCP = *Pneumocystis jirovecii* pneumonia; AB = antibiotic

Table 5. Patient symptoms and signs

Symptom/sign	Inpatients n = 29	Outpatients n = 42	p
Cough	18/20	38/42	1.00
Cough ? 3 weeks	9/17	21/38	1.00
Productive cough	12/18	35/38	0.04
Hemoptysis	1/20	0/42	0.60
Fever	15/20	23/42	0.21
Night sweats	15/20	11/42	0.001
Chest pain	10/20	4/41	0.002
Weight loss	20/20	15/32	0.001
Mean BMI, m ² /kg (SD)	15.6 (3.8)	18.9 (3.9)	0.005
Oral thrush	16/20	11/42	0.000

BMI = Body Mass Index; SD=standard deviation ;
n BMI = 18 inpatients and 36 outpatients

For sputum examination, not all of the study participants (82% of inpatients and 67% of outpatients) supplied sputum samples for examination due to loss of follow-up or an inability to expectorate sputum. Prevalence of culture-confirmed TB was 37.5% and 32.1% for in- and outpatients, respectively. Sensitivity of sputum smear microscopy was considerably higher for inpatients compared to outpatients (66.7% compared to 11.1%, $p=0.05$). Specificity (100% for both group), PPV (100% for both group), and NPV (83.3% for inpatients and 70.4% for outpatients) of sputum microscopy were high.

DISCUSSION

We reviewed the literature regarding diagnosis of PTB in HIV patients. We also conducted a case study in HIV patients comparing the performance of common TB diagnostics in a large teaching hospital in Bandung. We found that many patients had a history of TB-treatment (45% and 31% for in- and outpatients respectively). These numbers are lower than what was found in a study in South-Africa where 52% of patients prior to ART had a history of TB⁵³, and comparable with numbers found in other South-East Asian countries like Cambodia (29% after ART)⁵⁴ and Thailand (37% among inpatients).⁵⁵

We found that the current diagnostic process for PTB performed poorly in HIV patients and was different between in- and outpatients. For inpatients a combination of symptoms seemed the most effective diagnostic tool, while for outpatients the use of CXR showed the best results. However, it must be stressed that these diagnostic methods still had a low accuracy and there is an urgent need for better diagnostic tools.

Microbiological confirmation of TB was often lacking. The high prevalence of smear negative cases found in this study is also in accord with current understanding, the mainly to inability to expectorate

sputum and poor quality of sputum because of low bacillary load. However, the sensitivity of ZN for inpatients (67%) was higher than seen in other studies and is likely due to the severe disease, and to our instruction and sputum induction in these patients, especially as only 67% of inpatients had a productive cough. Sputum instruction appears to be useful in HIV negative population, but it has not been evaluated in HIV/AIDS patients. Sputum induction has been proven useful for HIV patients in literature.^{7, 36} Both methods should be evaluated in a clinical trial with large enough size before making any definitive conclusions about their use in HIV/AIDS patients.

Our literature reviewed only included currently available methods in Indonesia. An excellent review has addressed newer methods.¹¹ Our case study was cross-sectional and small with sputum only available for 52 patients due to loss to follow-up or an inability of patients to produce good quality of sputum. Our study population was selected from a hospital where patients present with advanced disease: thus, it is unknown how these diagnostic tests would perform in HIV patients with less severe disease. However, we believe we have a clear picture of the difficulties of diagnosing PTB in HIV patients which are probably representative for many setting in Indonesia.

CONCLUSION

Diagnosis of PTB in HIV patients is problematic. The role of symptoms, CXR, and microscopy needs further evaluation. Based on our findings and a review of the literature, we make the following recommendations for improving PTB diagnosis in HIV patients in Indonesia. First, a high alertness and systematic screening for PTB among HIV patients is indicated. Active case finding among HIV patients to prevent transmission and death from PTB is crucial. In addition, the use of routine isoniazid preventive therapy should be considered for this population.⁵⁶ Second, to improve the detection of PTB for both in- and outpatients, CXR should be performed, also in the absence of clear symptoms. For inpatients, microbiological tests should be performed and sputum samples should be collected using instruction and induction methods. Culture should be employed for both in- and outpatients as it is useful in confirming cases especially those which are smear negative patients. Regarding the difficulties that arise from diagnosing PTB from sputum, alternative ways of diagnosing PTB should also be considered. Microscopic techniques such as fluorescence microscopy and NAA to detect *M. tuberculosis* in peripheral blood and urine

for example, seem promising in HIV patients.⁵⁰⁻⁵² Finally, as immunosuppression hampers diagnosis of TB, it should not be forgotten that all new ARV patients should be monitored for the unmasking of TB disease.⁵⁷

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