

Rapid Mycobacterial Tuberculosis Detection in Bronchoalveolar Lavage Samples by Polymerase Chain Reaction in Patients with Upper Lobe Infiltrates and Bronchiectasis

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Abstract

In areas where tuberculosis is endemic, a positive sputum acid-fast bacilli (AFB) smear is frequently regarded as almost diagnostic of pulmonary tuberculosis (PTB). The main problem arises when the AFB smear is negative. The main aim of this study was to determine the clinical utility of rapid mycobacterial tuberculosis (MTB) detection in bronchoalveolar lavage (BAL) samples by polymerase chain reaction (PCR) in 52 patients who underwent diagnostic bronchoscopy for suspected PTB. These patients had either upper lobe infiltrates (n = 31) or bronchiectasis (n = 21).

Mycobacterial culture is usually used as the gold standard of diagnosis. We chose to define active PTB based on positive mycobacterial cultures and/or histological evidence of caseous necrosis and AFB, and/or when there was clinical plus radiological improvement following therapy. We compared AFB smear, respiratory mycobacterial culture, BAL PCR for MTB and clinical active PTB.

Four patients who were smear and culture negative had clinical and radiological clearance following anti-tuberculous therapy showing that using mycobacterial culture as a gold standard may have its limitations. When Kappa (a chance-corrected measure of agreement) was calculated for acid-fast bacilli smear and BAL PCR against our definition of active PTB, it was 0.28 (fair agreement) and 0.73 (substantial agreement), respectively. BAL PCR gave a sensitivity, specificity, positive and negative predictive values of 66.7%, 100%, 100% and 88%, respectively, for the group with upper lobe infiltrates. We also demonstrated that BAL for PCR has a good concordance with the final diagnosis of active tuberculosis.

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Key words: Bronchiectasis, Bronchoalveolar lavage (BAL), Direct amplification test, Pulmonary tuberculosis, Upper-lobe infiltrates

Introduction

Pulmonary tuberculosis (PTB), the incidence of which is rising, poses a major health problem. It is caused by *Mycobacterium tuberculosis*. The isolation of *Mycobacterium tuberculosis* by culture has been the "gold standard" for the diagnosis of tuberculosis, as it gives a specificity that approaches 100% and permits susceptibility testing of the isolates. Unfortunately, the slow growth of *Mycobacterium tuberculosis* of up to 4 to 6 weeks results in delays in diagnosis. For many decades, a positive acid-

fast bacilli (AFB) smear has been the only rapid diagnostic test for tuberculosis but this test is insensitive and also nonspecific. Statistically, this method needs relatively large number of bacteria (5000 to 10,000 bacilli per millilitre of specimen) to be present¹ and does not allow identification at the species level.

In areas where tuberculosis is endemic, empiric anti-tuberculous therapy would have been initiated as soon as the AFB smear returned as positive. The main problem in these areas is when the AFB smear is nega-

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tive. The advent of rapid diagnostic techniques might thus facilitate rapid clinical decision making..

Our main aim was to determine the clinical utility of rapid mycobacterial tuberculosis (MTB) detection in bronchoalveolar lavage (BAL) samples by polymerase chain reaction (PCR) in two groups of patients; those with upper-lobe infiltrates on chest radiograph, and those with bronchiectasis. We chose to study BAL PCR rather than sputum PCR as there has been no data on BAL PCR at the time we embarked on this study. Fibreoptic bronchoscopy and BAL also increase the chance of an early diagnosis.²

Materials and Methods

We recruited 52 patients (38 males, 14 females) who underwent diagnostic bronchoscopy and bronchoalveolar lavage for suspected PTB between February 1994 and January 1995. Their mean age was 57 ± 15.7 years (range 24 to 87 years). We chose to study only patients with upper lobe infiltrates and those with bronchiectasis who had suspected PTB. Radiologically, 31 had upper lobe infiltrates and 21 had bronchiectasis. All the patients with bronchiectasis presented with haemoptysis. Among those with bronchiectasis, 11 were confirmed to have bronchiectasis in a lobar distribution based on high resolution cat scan of the thorax.

Two sputum samples for AFB smear and two samples of laryngeal swabs for mycobacterial cultures were obtained from all the patients. Fibreoptic bronchoscopy was performed in all the patients and bronchoalveolar lavage was done by wedging the bronchoscope in the affected segmental bronchus and instilling 100 ml of warm saline. The returned BAL aliquots were sent for AFB smear, mycobacterial culture and polymerase chain reaction (15 ml). After bronchoscopy, the bronchoscopes were soaked for 20 minutes in Cidex® (Glutaraldehyde 2.2%) solution to minimise the problem of cross-contamination. Transbronchial lung biopsy was performed in selected cases and cultures from extrapulmonary sites

taken where applicable.

The PCR assay was performed as follows: DNA was extracted by heat inactivation at 80°C for 20 minutes, followed by digestion and decontamination by N-Acetyl L-Cysteine-2% NaOH solution before lysis by ultrasonication at 20mHz for 15 minutes at 65°C. The insertion sequence 6110 (IS6110) was then amplified with primers and the product visualized by gel electrophoresis under ultraviolet light. This protocol was modified from that described by Nolte et al.³

All patients were followed up for one year. Patients who were diagnosed to have PTB were given anti-tuberculous treatment and had repeat chest radiographs done on the 2nd and 6th month of therapy. Although most authors use mycobacterial culture as the gold standard of diagnosis,^{4,5} we chose to define clinical active PTB on a broader basis according to a positive mycobacterial cultures, histological evidence of caseous necrosis and AFB, or clinical plus radiological improvement following therapy.

Results

Of the 52 patients (38 males, 14 females), 9 had active PTB based on the criteria described. All of them had upper lobe infiltrates. None of the patients with bronchiectasis had active PTB. The diagnosis of PTB was based mainly on positive mycobacterial culture from the respiratory tract (n = 5) (Table I). Among the patients who were smear and culture negative, PTB was based on transbronchial lung biopsy (n = 1), histology from a fistula-in-ano and upper lobe infiltrates (n = 1) and clinical and radiological clearance following anti-tuberculous therapy (n = 2).

Table II shows the discordance between the mycobacterial cultures, BAL PCR and the final diagnosis. In the group with upper lobe infiltrates, there were 3 false negatives and in the group with bronchiectasis, there were 3 false positives. The concordance between the mycobacterial cultures, histology BAL PCR and the final diagnosis is shown in Table II. There was a concordance

TABLE I: GENERAL SUMMARY OF RESULTS

Total no of patients = 52	Sputum AFB smear positive (n = 2)	BAL PCR positive (n = 9)	Tuberculous culture or histology positive (n = 7)	Final diagnosis of pulmonary tuberculosis (n = 9)
Upper lobe infiltrates (n = 31)	2	6	5 (culture from respiratory specimens) 1 (Fistula-in-ano culture) 1 (transbronchial lung biopsy showed caseous necrosis)	9 (All had radiological clearing after treatment) NB: 2 were smear, culture and PCR negative
Bronchiectasis (n = 21)	0	3	0	0

AFB: acid-fast bacilli; BAL PCR: bronchoalveolar lavage for polymerase chain reaction; PCR: polymerase chain reaction

TABLE II: CONCORDANCE AND DISCORDANCE BETWEEN MYCOBACTERIAL CULTURES, HISTOLOGY, BAL PCR AND CLINICAL DIAGNOSIS OF ACTIVE PULMONARY TUBERCULOSIS

		Acid-fast smear	Resp c/s	PCR	No. of subjects	Clinical diagnosis of active TB	Remarks
Concordant	Upper lobe infiltrates	+	+	+	2	+	True positive
		—	+	+	2	+	True positive
		—	—	+	1	+	True positive
		—	—	—	22	—	True negative
	Bronchiectasis	—	—	—	18	—	True negative
Discordant	Upper lobe infiltrates	—	+	—	1	+	False negative
		—	—	—	2	+	False negative
	Bronchiectasis	—	—	+	3	—	False positive

Active TB: final diagnosis of active pulmonary tuberculosis based on our definition; BAL PCR: bronchoalveolar lavage for polymerase chain reaction; Histology: from transbronchial lung biopsy; PCR: polymerase chain reaction; Resp c/s: mycobacterial culture from respiratory specimens

of 87.1% and 85.7% in the group with upper lobe infiltrates and bronchiectasis, respectively.

Among these 9 patients with active PTB, only 2 were AFB smear positive (Table III). This gave a sensitivity for the AFB smear test of only 22.2% whereas the sensitivity for mycobacterial culture was 55.6%. The specificity, positive and negative predictive values of AFB smear were 100%, 100% and 75.9%, respectively.

The usefulness of BAL PCR in diagnosing active PTB in both the groups is shown in Table IV and V, respectively. In the group with upper lobe infiltrates, BAL PCR gave a sensitivity, specificity, positive and negative predictive values of 66.7%, 100%, 100% and 88%, respectively, according to our definition of active tuberculosis. The sensitivity of BAL PCR was 85.7% if the gold standard for diagnosis of tuberculosis was based on mycobacterial culture.

Kappa⁶ (a chance-corrected measure of agreement) was calculated as an index of the agreement between the diagnostic test and the final gold standard diagnosis. When it was calculated for AFB smear for the group with upper lobe infiltrates, Kappa was 0.28 (fair agreement). On the other hand, it was 0.73 (substantial agreement) for BAL PCR in the same group of patients.

Discussion

Most studies on PCR for MTB use mycobacterial culture as the gold standard for the diagnosis of active

tuberculosis.^{4,5} However, we felt that this would miss out a proportion of people who might have active tuberculosis but are smear and culture negative. In a large study in Hong Kong investigating patients with active PTB (clinically and radiologically) who were sputum AFB smear negative, 35% were culture positive for MTB and the remaining 65% had smear and culture negative tuberculosis.⁷ Clinical tuberculosis even in the absence of positive smears and cultures should be treated.² Therefore we designed our study such that we could follow-up the patients over a one-year period so as to pick up such patients who would otherwise be mislabelled as having no active tuberculosis. In our study, there were 4 of such patients showing that using mycobacterial culture as a gold standard has its limitations.

TABLE IV: THE USEFULNESS OF BAL PCR IN DIAGNOSING ACTIVE PULMONARY TUBERCULOSIS IN PATIENTS WITH UPPER LOBE INFILTRATES (n = 31)

	Active PTB	No active PTB
BAL PCR positive	6	0
BAL PCR negative	3	22

BAL PCR: bronchoalveolar lavage for polymerase chain reaction; PTB: pulmonary tuberculosis
sensitivity = 66.7%; specificity = 100%;
positive predictive value = 100%; negative predictive value = 88%

TABLE V: THE USEFULNESS OF BAL PCR IN DIAGNOSING ACTIVE PULMONARY TUBERCULOSIS IN PATIENTS WITH BRONCHIECTASIS (n = 21)

	Active PTB	No active PTB
BAL PCR positive	0	3
BAL PCR negative	0	18

BAL PCR: bronchoalveolar lavage for polymerase chain reaction; PTB: pulmonary tuberculosis
specificity = 85.7%; negative predictive value = 100%

TABLE III: THE USEFULNESS OF ACID-FAST BACILLI SMEAR IN DIAGNOSING ACTIVE PULMONARY TUBERCULOSIS

	Active PTB	No active PTB
AFB smear positive	2	0
AFB smear negative	7	22

AFB: acid-fast bacilli; PTB: pulmonary tuberculosis
sensitivity = 22.2%; specificity = 100%;
positive predictive value = 100%; negative predictive value = 75.9%

Our series showed that 9 of the 31 patients (29%) with upper lobe infiltrates had PTB. None of our patients with bronchiectasis had active PTB, suggesting that active PTB is rather uncommon even in Asian patients with bronchiectasis. Chan et al⁸ reported that 9 of 91 patients (9.9%) with bronchiectasis had active *Mycobacterium tuberculosis* infection. There were 3 false-positives (5.8%) altogether, all of which came from the bronchiectasis group and these patients are still being followed-up. Kaul et al⁹ found that residual amplifiable human or MTB DNA could remain in sterilized bronchoscopes and be a source of false positive PCR in 2 of 55 samples (3.6%). Other possible causes of false positive results include dead mycobacterial DNA and cross-contamination in the laboratory.

Of the 9 patients with active PTB, only 2 were AFB smear positive, giving a sensitivity of 22% against our diagnosis of active tuberculosis and 40% if compared against respiratory specimen mycobacterial culture. In contrast, 6 were BAL PCR positive, giving a much higher sensitivity of 100% using respiratory mycobacterial culture as the gold standard but only 66.7% according to our definition of active tuberculosis. Kappa analysis also showed that BAL PCR had a much higher agreement beyond chance than AFB smear with our gold standard of diagnosis. Most studies use sputum samples for PCR for MTB. The sensitivity of sputum samples PCR for MTB ranges from 60% (smear negative) to 95% (smear positive) compared with culture.⁴ Only a few authors have used BAL as part of their specimens to analyse the PCR for MTB.^{10,11} The sensitivity of BAL samples PCR for MTB was 88% in one study.¹⁰ The clinical value of a rapid diagnostic test for tuberculosis depends largely on its positive and negative predictive values rather than its sensitivity and specificity. In our study, BAL for PCR had positive and negative predictive values of 100% and 88%, respectively, in patients with upper lobe infiltrates.

The role of rapid diagnostic tests for tuberculosis in the West has been defined by the American Thoracic Society¹² but its role in Asia where tuberculosis is endemic has yet to be defined. Although we have limited data, our study shows that BAL PCR is a useful adjunct for the rapid diagnosis of tuberculosis in Asian patients with

upper lobe infiltrates, thereby enhancing tuberculosis control. It would be especially useful in patients with upper lobe infiltrates who are unable to expectorate sputum.

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