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Post-bronchoscopy sputum: Improving the diagnostic yield in smear negative pulmonary TB

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Summary

Introduction: Patients with suspected active Pulmonary Tuberculosis (PTB) who are Acid-Fast Bacilli (AFB) smear negative or non-productive of sputum may undergo bronchoalveolar lavage. However, post-bronchoscopy sputum (PBS) sampling is not routine. The aim of this study was to establish the potential diagnostic value of PBS sampling.

Methods: A retrospective study of patients attending a London University hospital with microbiologically confirmed PTB between January 2004 and December 2010. Patients who were AFB smear negative or non-productive of sputum were eligible if sputum sampling was performed within 7 days of bronchoscopy.

Results: Over the study period, 236 patients had microbiologically confirmed smear negative PTB of which 57 patients were eligible for the study. 15 patients (26.3%) were infected with HIV. 19 patients (33.3%) converted to AFB sputum smear positivity post-bronchoscopy and 5 patients (8.8%) were exclusively AFB sputum smear positive on PBS microscopy. *Mycobacterium tuberculosis* was cultured from the PBS of 43 patients (75.4%) and of these, 4 (7.0%) were exclusively PBS culture positive.

Abbreviations: AFB, Acid-fast bacilli; BAL, bronchoalveolar lavage; HIV, human immunodeficiency virus; PBS, post-bronchoscopy sputum; PTB, pulmonary tuberculosis; TB, tuberculosis.

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Conclusion: PBS analysis can provide a simple method of rapidly diagnosing pulmonary tuberculosis. In this cohort, *M. tuberculosis* culture yield was increased by 7% through PBS sampling. This study has important infection control implications with nearly one third of patients becoming more infectious after bronchoscopy.

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Introduction

Sputum smear microscopy to detect acid-fast bacilli (AFB) is a rapid, inexpensive, and highly specific tool for identifying persons with active pulmonary tuberculosis (PTB).¹ However, many patients fail to produce sputum; and even amongst those who are productive of sputum, a significant percentage who have active PTB on clinical and radiological grounds are auramine smear negative despite repeated examination. A particular challenge for clinicians concerns the rising incidence of human immunodeficiency virus (HIV) related TB, with an associated increase in smear negative PTB.^{2,3} Smear negative HIV related TB has an increased mortality compared to smear positive disease^{2,4} and this may in part be related to delays in diagnosis and initiation of treatment.⁵

Patients who are not productive of sputum or are consistently sputum AFB smear negative undergo either fiberoptic bronchoscopy for bronchoalveolar lavage (BAL) or sputum induction using nebulized hypertonic saline. The choice of technique is largely dependent on local policy but the two procedures are widely considered equivalent in facilitating sampling of deep-seated bronchial secretions for microbiological and cytological analysis.^{6–8} The sensitivity of BAL microscopy for the detection of AFB is variable in PTB^{7,9} and sensitivities for positive BAL culture of *Mycobacterium tuberculosis* vary from 44 to 95%.^{8,10,11} Thus, a proportion of patients with PTB will remain undiagnosed by BAL alone.

Little is known about the clinical utility of sputum sampling after bronchoscopy (post-bronchoscopy sputum – PBS) and its diagnostic potential in smear negative PTB suspects. Two previous studies have examined the yield of various techniques including PBS for the diagnosis of smear negative PTB^{12,13} but numbers were small ($n = 13–25$) and as a result conclusions regarding its potential value are difficult to establish. One larger study in Ethiopia has also previously investigated PBS sampling in AFB smear negative TB suspects but only included HIV infected individuals¹⁴; post-bronchoscopy sputum culture provided comparable results to BAL culture in this setting.

Current guidance from the American Thoracic Society and the Centers for Disease Control and Prevention¹⁵ as well as the National Institute for Clinical Excellence⁶ suggests that all patients with suspected PTB be isolated until three AFB smear negative sputum samples have been collected. However, it is our clinical experience that a proportion of these patients will convert to AFB sputum smear positivity post-bronchoscopy despite being non-productive or consistently sputa smear negative prior to the procedure. Such patients may be inappropriately deisolated posing an infection risk to health workers and other patients or contacts.

The aim of this study was therefore to determine the additional microbiological yield of PBS sampling and to assess any implications on infection control, in a heterogeneous population within the context of a developed and low incidence setting.

Methods

A retrospective analysis was performed of all patients with microbiologically confirmed PTB on the TB Register at St Mary's Hospital, a central London University hospital, between 1st January 2004 and 31st December 2010. Information was collected regarding patient demographic profiles, HIV infection status, microbiology results and treatment outcomes from the London TB register – a prospectively recorded resource. Missing data was retrieved from the case notes. At this centre, sputum induction is not performed and all patients with suspected PTB who are not productive of sputum or are sputum AFB smear negative undergo fiberoptic bronchoscopy for diagnostic BAL. Bronchoscopy is performed by doctors specializing in respiratory medicine. BAL specimens are obtained by wedging the bronchoscope into the segment of lung most likely to yield diagnostic specimens as defined by radiographic evidence of maximal pulmonary infiltrates. 30 ml aliquots of 0.9% Saline are instilled and fluid is aspirated until the bronchoscopist is satisfied with the lavage return. When diffuse infiltrates are seen on imaging, the right middle lobe is chosen as the default target. Each sputum or BAL specimen is decontaminated using 4% NaOH and then centrifuged at 3000 revolutions per minute for 15 min. After decanting the supernatant, the pellet is washed and re-suspended in phosphate buffered saline. From this, a smear is prepared for auramine staining (with positives being confirmed by Ziehl-Neelson) and 0.5 ml is transferred into an MGIT tube for incubation using the Bactec™ MGIT™ 960 system (BD, New Jersey USA). Sputum and BAL samples are incubated for up to 6 weeks.

Sputum samples were collected from all inpatients undergoing bronchoscopy in the hours after the procedure and then on subsequent mornings whilst still in hospital. Patients who underwent bronchoscopy as outpatients were given sputum pots to take home and were asked to provide samples if they became productive of sputum after the procedure. Patients with microbiologically confirmed PTB were eligible for inclusion into the study if they were sputum AFB smear negative or non-productive of sputum prior to bronchoscopy and had sputum samples sent within one week of the procedure. Patients who were treated empirically on clinical, radiological or biochemical grounds were excluded from the study. Patients who were AFB smear positive prior to bronchoscopy as well as those who did not have a PBS sample sent within the defined

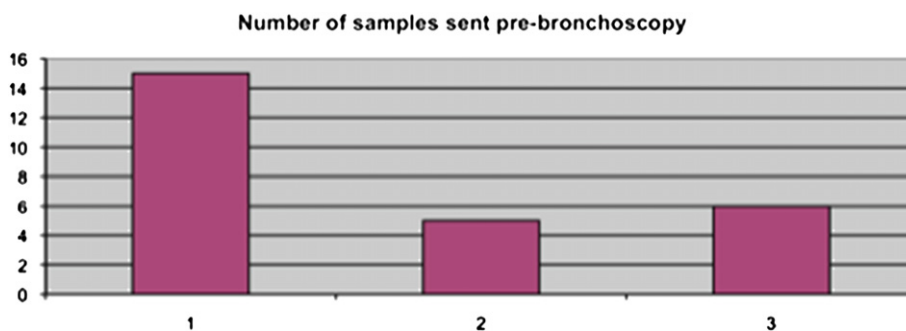


Figure 1 Number of pre-bronchoscopy sputum samples sent per patient.

timeframe were also excluded. In the absence of any published work, 7 days was taken as an arbitrary but pragmatic inclusion time point. Only the first positive PBS sample was recorded even if it was preceded by negative samples. In the absence of a positive PBS sample but in the event of numerous negative samples, only the first sample was included; ie only one PBS sample was included per patient. Patients who were initially AFB smear positive but whose samples subsequently cultured non-tuberculous mycobacteria were excluded.

The study protocol was discussed with and approved by the Chairman of the West London Research and Ethics Committee 2 and the Joint Research Office at St Mary’s Hospital.

Results were analyzed using Microsoft Excel for Mac 2008.

Results

Over the study period, 419 patients had smear negative PTB all of whom underwent bronchoscopy. 183 patients were treated empirically with no positive microbiology but other features consistent with a diagnosis of PTB. The remaining 236 patients had microbiologically proven smear negative PTB and of these a total of 57 patients met the inclusion criteria of the study by virtue of the fact that they had at least one PBS sample sent within 7 days of the procedure. 35 were male (61.4%) and 15 were infected with HIV (26.3%). The HIV status was unknown in 4 patients (7%). The mean age was 41.1 years (range 16–89). 23 patients (40.4%) were of Black African origin, 15 (26.3%) were White Caucasian and 8 (14.0%) were from the Indian

Subcontinent. 53 patients (93.0%) had abnormal radiology which consisted of a chest radiograph or a computerized tomography scan of the thorax which was consistent with possible TB infection.

Of the 57 patients, 26 (45.6%) were sputum AFB smear negative prior to bronchoscopy and 31 patients (54.4%) were non-productive of sputum. At least one and up to 3 pre-bronchoscopy sputum samples were taken per productive patient (Fig. 1). All patients had PBS samples sent within 7 days of the procedure - 39 of 57 samples (68.4%) were sent within the first 48 h (median 2 days) (Fig. 2). PBS samples were smear positive when taken from a range of 1–7 days post-procedure (mean 2.18 days, median 2 days) and overall, the time to *M. tuberculosis* culture ranged from 4 to 28 days (mean 13.6 days, median 13 days).

19 patients (33.3%) were sputum AFB smear positive on PBS sampling (Fig. 3). Of these patients, 7 were sputum smear negative and 12 were non-productive pre-bronchoscopy. 5/57 patients (8.8%) were exclusively sputum AFB smear positive on PBS sampling alone, i.e. these patients converted to smear positivity despite being non-productive of sputum or pre-bronchoscopy sputum AFB smear negative as well as BAL smear negative. All 5 cultured *M. tuberculosis* by 6 weeks from BAL samples. One of these five patients was HIV positive.

49 of 57 patients (86.0%) cultured *M. tuberculosis* within 6 weeks from pre-bronchoscopic sputum samples, BAL samples, or a combination of the two (Fig. 4). In four patients, culture samples were contaminated and so could not be examined but in the remaining 4 patients (7.0%) *M. tuberculosis* was exclusively cultured from PBS samples within 6 weeks with negative pre-bronchoscopy sputum and

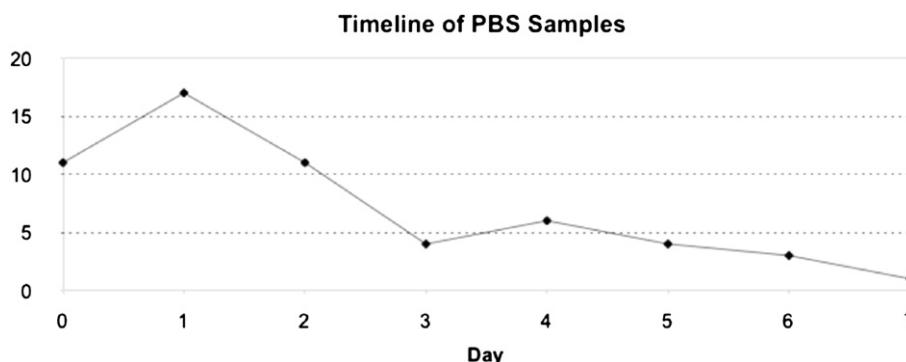


Figure 2 Timeline of PBS samples collected.

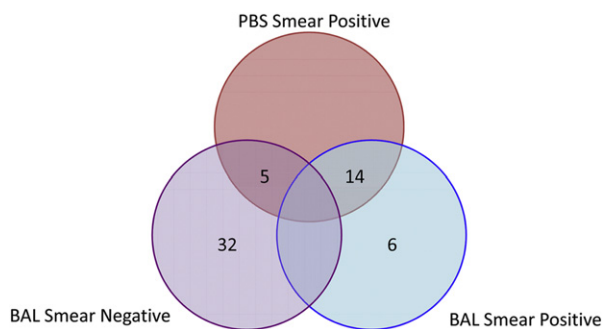


Figure 3 Summary of AFB smear results.

BAL culture samples. Three of these four patients were infected with HIV. A summary of these results can be found in Fig. 5.

Discussion

This study demonstrates that sampling sputum post-bronchoscopy for AFB smear and *M. tuberculosis* culture can provide an additional diagnostic tool which has to date been underutilized in the diagnosis of PTB. 7.0% of patients in our cohort were exclusively PBS culture positive for *M. tuberculosis* and sputum sampling after bronchoscopy provided the only means of establishing the diagnosis using the gold standard. These data show that in this subset of patients with microbiologically confirmed PTB, PBS sampling for *M. tuberculosis* culture had a yield of 75.4% (43 of the 57 patients enrolled into the study were PBS *M. tuberculosis* culture positive). The culture yield from BAL was 73.7%. 5 patients (8.8%) were exclusively AFB smear positive on PBS sampling alone. Of these, 1 patient was HIV infected and in immunosuppressed patients, where the differential diagnosis is wide, a positive AFB smear sample is a finding of key clinical significance. In all 5 patients, the diagnosis was subsequently confirmed with a positive *M. tuberculosis* culture from the BAL sample but an early positive microscopy finding can allow the rapid initiation of treatment when there is diagnostic uncertainty.

PBS sampling may not only provide an exclusive means of achieving a rapid diagnosis of pulmonary TB through AFB smear and microscopy and increase the yield of *M. tuberculosis* culture, but also reveal a change in patients' infective status. These data demonstrate that bronchoscopy converted 33.3% of non-infectious patients (non-productive or sputum AFB smear negative) to a higher infective status

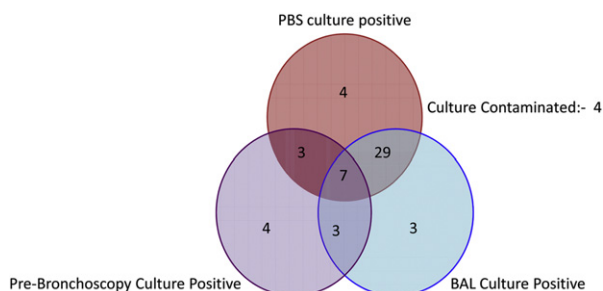


Figure 4 Summary of *M. tuberculosis* culture results.

post-procedure (productive of sputum and AFB smear positive), requiring respiratory isolation. This finding has significant public health and infection control implications. There is currently no guidance as to how to manage such patients, but where the pre-test probability of PTB is high, and if the patient is still coughing, we advocate respiratory isolation post-bronchoscopy until confirmation of at least one further negative acid-fast smear sample. We are unable to comment as to whether subsequent PBS samples could produce even further yield after an initial negative PBS sample and suggest that prospective studies could be designed to specifically answer this question.

The proportion of patients with HIV co-infection in this specific smear negative PTB cohort was 26% which is high when considered against reported rates in London of 17–25% that include the total burden of pulmonary and extrapulmonary TB.¹⁶ This is in keeping with the growing body of evidence that HIV associated PTB is often difficult to confirm reflecting a lack of sputum production and AFB smear negativity. Bronchoscopy and post-bronchoscopy sputum sampling provided the means of culture proven diagnoses in this group and should be considered particularly when HIV infection exists. It is especially worth noting that of the 4 patients in whom the only positive culture was from the PBS sample, 3 were HIV positive. In the setting of HIV the results of our study have further implications beyond merely proving PTB. It has been estimated that 5% of the total number of new TB cases worldwide are due to multi-drug resistant (MDR) strains and in some settings MDR-TB has also been shown to be almost twice as common in HIV infected patients.¹⁷ As the frequency of MDR-TB rises, increased efforts should be made to recover a culture isolate to determine drug susceptibilities and this data suggests that PBS sampling can provide a simple and affordable method by which this could be further optimized.

The mechanism of conversion to AFB smear and *M. tuberculosis* culture positivity post-bronchoscopy is yet to be clearly elucidated. Targeted bronchoalveolar lavage directed by radiological appearances could cause mobilization of deep-seated bacillary-laden secretions, which are then expectorated in the following days. The longest time to a positive PBS sample was seven days post-procedure and so it would appear unlikely that airway irritation and increased cough frequency caused by the bronchoscope itself, as is the case with induced sputum sampling, could solely explain the phenomenon. We made an estimate of sputum bacillary load using days to culture as a surrogate in an attempt to identify subgroups of patients in whom there was a greater chance of conversion to smear positivity post-procedure. This did not differ significantly in patients infected with HIV nor those that were smear negative versus those who were non-productive prior to bronchoscopy.

There are several recognized limitations to our observations. This retrospective study is limited by the constraints of any case note analysis. Stratification for confounding variables would be difficult with a cohort of this size and so any conclusions drawn would need to be handled with care. The HIV status of a small number of patients in this study is unknown and although it is our practice to establish the retroviral status of every patient attending our services, the

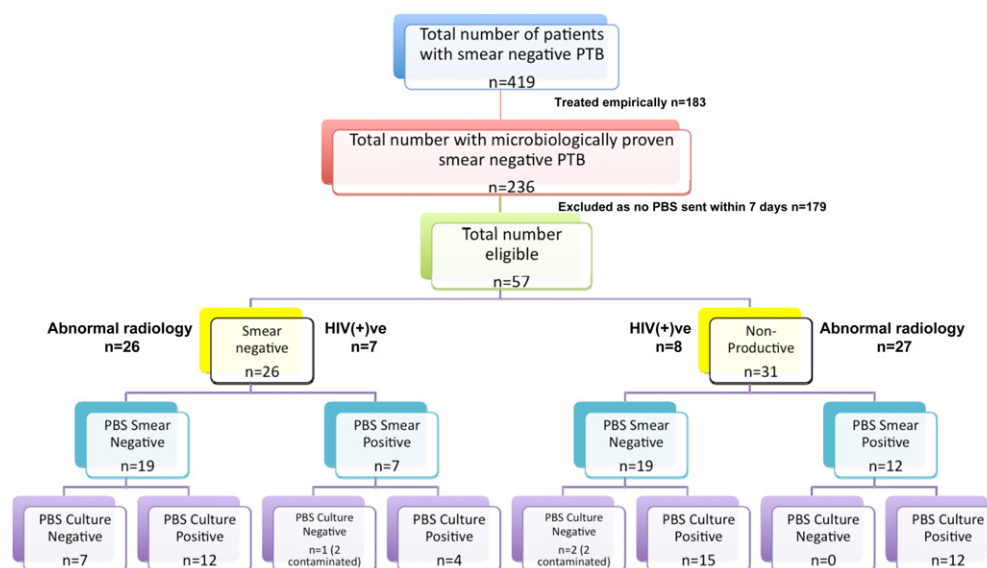


Figure 5 Summary of PBS results.

uptake of an offer of a test was variable. As a result, we may have underestimated the burden of HIV infection in this group of patients but to our knowledge, no patient was subsequently diagnosed and our data is in keeping with prior prevalence reports of co-infection.¹⁶ PTB is commonly managed in the outpatient setting and a further confounding factor may be that individuals most likely to have undergone PBS sampling were those who were isolated as inpatients and who may have more severe or complex disease. Some of the patients attending our respiratory unit proceeded to bronchoscopy before three negative sputa were obtained. When tuberculous disease is suspected, we routinely advocate obtaining three negative sputum samples prior to invasive testing if the patient is clinically stable and in the outpatient setting. However given the diverse nature of the patient mix in our unit where TB is only one of a number of differential diagnoses, where HIV co-infection is common and where acutely unwell inpatients are a significant clinical issue, it is at times inappropriate to delay proceeding to the most sensitive diagnostic test and hence delay treatment initiation. We recognize that this practice may limit the generalized applicability of the yield of PBS when 3 samples are taken. In addition the results of the study can only be generalized to centers that utilize bronchoscopy as a means of increasing the microbiological yield in smear negative PTB. We acknowledge that bronchoscopy is not a risk free procedure requiring expertise and technical equipment that may not be readily available in all settings. Many centers perform sputum induction in preference and in this context the results of this study may not be applicable. Notwithstanding this, our findings demonstrate the additional challenges that are presented in smear negative PTB particularly when HIV co-infection exists and illustrate the additional diagnostic potential that bronchoscopy may yield post-procedure in a simple and clinically applicable manner.

In conclusion, sampling sputum post-bronchoscopy can provide a previously underutilized method of making a rapid diagnosis of PTB and reduce the number of patients

who are treated on an empiric basis, particularly in the context of sputum smear negative or non-productive disease. Importantly it can increase culture yield by up to 7% hence allowing a greater proportion to have full drug sensitivity testing and therefore appropriate management of potential drug resistant strains. In our study this added culture sensitivity was in fact found largely in HIV positive individuals, a group already recognized to have significantly higher drug resistant rates. PBS sampling is also a potentially key infection control issue that should be considered following bronchoscopy. Further studies are now required to establish the duration of smear positivity post-bronchoscopy inpatients who were previously considered non-infectious but in the light of this data, we consider it best practice to only de-isolate such patients when their infective status can be ascertained with at least one post-bronchoscopy sputum sample.

Conflict of interest

We declare that we have no conflict of interest.

Acknowledgments

OMK designed the study, advised regarding data collection and edited the manuscript.

PMG helped design the study, coordinated data collection, analyzed the data and wrote the manuscript

MM and JD collected the data, analyzed the data and wrote the manuscript

AS analyzed the data and edited the manuscript

CR and YS collected and analyzed the data

PM, DC, and MW were involved in data collection and edited the manuscript

AJ provided microbiology records for all the patients and critically read the manuscript

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