



TO STUDY THE EFFICACY OF POST BRONCHOSCOPY SPUTUM IN ADDING TO THE YIELD OF BRONCHIAL WASHINGS IN DIAGNOSIS OF ACID FAST BACILLI

General Medicine

Dr. Tejas Deshpande*	Junior Resident, Department of Respiratory Medicine, Navale Hospital Campus, Narhe, Pune-411041. *Corresponding Author
Dr. Gauri Paresh Godbole	Professor & Head, Department of Respiratory Medicine, Navale Hospital Campus, Narhe, Pune-411041.
Dr. Pankaj Magar	Associate Professor, Department of Respiratory Medicine, Navale Hospital Campus, Narhe, Pune-411041.
Dr. Anandkumar Luniya	Assistant Professor, Department of Respiratory Medicine, Navale Hospital Campus, Narhe, Pune-411041.
Dr. Hardik Patel	Clinical Associate, Jaslok Hospital, Mumbai

ABSTRACT

Introduction: Sensitivities for positive BAL culture of Mycobacterium tuberculosis vary from 44 to 95%. 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 and its diagnostic potential in smear negative PTB suspects. This study aims to study the efficacy of post bronchoscopy sputum in adding to the yield of bronchial washings in diagnosis of AFB.

Aim: To study the efficacy of post bronchoscopy sputum in adding to the yield of bronchial washings in diagnosis of acid fast bacilli.

Methodology: Retrospective study

Results: The combination of bronchial washings and post bronchoscopy sputum examination is superior to bronchial washing examination alone in the detection of acid fast bacilli.

Conclusion: The study proves the efficacy of post bronchoscopy sputum examination in adding to the yield of bronchial washings examination in diagnosis of acid fast bacilli.

KEYWORDS

Sputum, Bronchial washings, Tuberculosis, Diagnosis

INTRODUCTION:

Sputum analysis has been used as a diagnostic technique for centuries, and reports on sputum in different diseases, containing important aspects of sample processing, were published more than a century ago [1]. 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].

In the era dominated by invasive procedures and aggressive treatment options, diagnostic flexible bronchoscopy has become the standard in patients with sputum ZN stain negative with suspicion for tuberculosis. 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 i.e. 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 and as a result conclusions regarding its potential value are difficult to establish.

This study aims to study the efficacy of post bronchoscopy sputum in adding to the yield of bronchial washings in diagnosis of acid fast bacilli in a tertiary care centre.

Aims & Objectives:

To study the efficacy of post bronchoscopy sputum in adding to the

yield of bronchial washings in diagnosis of acid fast bacilli in a tertiary care centre.

Inclusion Criteria:

1. Patients diagnosed to have pulmonary tuberculosis on bronchial washings and post bronchoscopy sputum examinations and having routine sputum AFB negative.
2. Patients with no results on sputum gram stain examination.

Exclusion Criteria:

1. Patients not willing to participate in the study
2. Patients with co-infection with other organisms as demonstrated by sputum gram stain report.

Materials & Methods:

Sample Size: 192 patients

Type of Study: Retrospective study

Duration of study: Two and a half years

Funding: None

Detailed Methodology: Retrospective data of the past 2 and half years was collected. In this duration, a total of 192 flexible bronchoscopies were performed for diagnosing sputum smear negative pulmonary tuberculosis.

Data was collected for each of these cases.

Positivity for AFB on bronchial washings alone was compared with combined positivity of bronchial washings + post bronchoscopy sputum.

The data was tabulated and statistical analysis was done.

Exact Fisher Test was applied to look for statistically significant difference.

Conclusions were drawn from the statistical analysis.

Method of Flexible Bronchoscopy:

- Flexible bronchoscopy done after acquiring fitness from anaesthetist.
- Stand by ventilator, ICU bed and anaesthesia back up maintained for the entire length of the procedure.
- Patient maintained nil by mouth for 6 hours prior to procedure.
- Xylocaine sensitivity test done previous evening.
- Vitals and other physical examination findings recorded prior to procedure and fitness for procedure reassessed.
- Premedication with 2% Xylocaine nebulization for 20 minutes administered.
- 10% Xylocaine spray administered over the posterior pharyngeal wall of the patient.
- Bronchoscopy performed with patient in supine position and bronchoscopist standing at head end of the patient facing the foot end of the bed.
- Midazolam used for sedation of the patient during the procedure.
- Local instillation of 2% Xylocaine jelly at anterior nares of chosen nostril done prior to insertion of bronchoscope.
- Spray as you go technique used with 2% Xylocaine for administering local anaesthesia during the procedure.
- Normal lung/ less affected lung visualized first followed by the lung with more extensive disease.
- Washings taken with 50 to 100ml Normal Saline instillation divided into aliquots of 10ml each.
- Site for washings decided on the CT scan of the patient.
- Post procedure patient maintained in propped up position and kept in ICU for 2 hour post procedure for observation.
- After said observation patient shifted to the ward and given sips of water 4hours after procedure followed subsequently by incremental intake of semisolids and then solid foods.

Method of AFB staining [14]:

Materials required:

- Tuberculocidal disinfectant
- Waste receptacles (including splash proof receptacle for liquids)
- Discard bucket with biohazard bag insert, containing appropriate disinfectant
- Paper towel soaked in appropriate disinfectant
- Microscope slides, frosted at one end, new and clean
- Pencil for labelling slides
- Study labels
- Hot plate or slide warmer
- Bunsen burner (or spirit lamp)
- Sterile, transfer pipettes with graduations marking volume (individually wrapped)
- Sterile loop or disposable applicator stick
- Ziehl-Neelsen stain (carbol fuchsin, 3% acid alcohol, methylene blue)
- Staining sink
- Staining rack
- Slide drying rack
- Forceps
- Timer
- Vortex mixer
- Distilled water
- Wash bottle

Smear Preparation [14]:

The slides must remain in the biological safety cabinet until they have dried.

1. Label the frosted end of the slide in pencil with the laboratory accession number, screening ID number and/or subject ID number, visit number, sputum specimen number (#1 or #2, unless specimen is from V2 or V3), and date.
2. Working in a biological safety cabinet, vortex the decontaminated sediment (see Section 7: Processing Sputum for Smear Microscopy and Qualitative Culture) to mix thoroughly.
3. Use a transfer pipette to place ~100 µl (2 drops) of well-mixed resuspended pellet from the digested-decontaminated specimen onto the slide, spreading over an area approximately 1 x 2 cm. Air-dry the smear.
4. Place the slides on a hot plate or slide warmer at a temperature between 65°C to 75°C for at least 2 hours (longer time is preferable), to heat-fix the samples. Do not expose slides to UV light.
5. Work systematically through the samples with slides on one side and the discard bucket in close proximity (often best at back of cabinet). Remember to open only one specimen tube at a time.

Dispose of the transfer pipette into the biohazard discard bucket.

Staining Technique [14]:

1. Place slides on staining rack so they are at least 1 cm apart, and flood with carbol fuchsin.
2. Heat the slide to steaming with the flame from a Bunsen burner. An electric heating block may also be used. Apply only enough additional heat to keep the slide steaming for 5 minutes. Do not let the stain boil or dry. Add additional stain if necessary.
3. Wash off the stain with distilled water.
4. Flood slides with 3% acid-alcohol.
5. Let stand for 2-3 min (more acid-alcohol should be used if the smear is heavily stained).
6. Wash off the acid-alcohol with distilled water and tilt the slides to drain.
7. Flood the slides with methylene blue and let stand for 1-2 minute.
8. Wash off the methylene blue with distilled water.
9. Tilt the slides to drain.
10. Allow slides to air dry in the slide rack. Do not blot.

Examination of smear [14]:

1. Using a bright field microscope, Ziehl-Neelsen smears are examined with the 100X oil objective (10X eye piece for a total of 1000X magnification). Take care not to touch the slide with the tip of the dropper when dispensing oil. Always wipe oil from the oil immersion lens after each AFB-positive smear is read.
2. AFB will have similar morphology as fluorescence-stained bacilli. They are variable in shape, from very short rods to long filaments. Often they are bent, contain heavily stained beads, and may be aggregated side by side and end to end to form cords, especially when grown in liquid culture (MGIT). The AFB appear bright red against the background material counterstained blue.

Grading of smear [14]:

Bacilli to field ratio	Grade
None per 100 Oil Immersion Fields(OIF)	Negative
1-9 per 100 OIF	Scanty
10-99 per 100 OIFs	1+
1-10 per OIF (examine 50 OIFs)	2+
>10 per OIF (examine 20 OIFs)	3+

Observations:

Table 1: Master data table

Group	Total Patients	Positive	Negative
Bronchial Washings	192	186	6
Bronchial Washings + Post Bronchoscopy Sputum	192	192	0

The above table indicates that there were 6 patients who were bronchial washing smear negative for acid fast bacilli, but were eventually diagnosed with the supplementation of post bronchoscopy sputum examination for acid fast bacilli.

Statistical Analysis:

Table 2: Exact Fisher Test Applied to the data

	Positive for AFB	Negative for AFB	Marginal Row Totals
Bronchial Washings	186	6	192
Bronchial Washings + Post Bronchoscopy Sputum	192	0	192
Marginal Column Totals	378	6	384 (Grand Total)

P value after application of Fisher Exact Test was found to be = 0.03.

This being less than 0.05 indicates a statistically significant difference.

The statistical analysis thus proves that the combination of bronchial washings and post bronchoscopy sputum examination is superior to bronchial washing examination alone in the detection of acid fast bacilli.

DISCUSSION:

Sputum examination has been used in clinical practice in a number of different ways [15, 16–18, 21].

The differential cell count of sputum is a widely used marker for phenotyping airway inflammation. Publication of several lines of

evidence has demonstrated that sputum eosinophil differential cell counting provides an important means of phenotyping airway inflammation and facilitates personalised treatment choices [16–18]. In the current guidelines for asthma, sputum eosinophils are placed as an evidence-based tool for assessing airway inflammation and, therefore, predicting and assessing corticosteroid response [19–20]. The measurement has a good reproducibility and its use has been shown to improve asthma control. The recent guidelines for clinical end-points in asthma trials, created by the American Thoracic Society and the ERS, have also incorporated the use of sputum eosinophil counts as an outcome measure [19]. The updated guideline recommendations outline a role for inclusion of assessment of sputum eosinophils, in addition to standard measures of asthma control, to guide adjustment of controller therapy in adults with moderate-to-severe asthma. In occupational asthma it can also be used as a diagnostic tool [22]. Similarly, in patients with COPD, the method can be used to determine steroid responsiveness based on sputum eosinophil differential count [23]. As a diagnostic tool, the method is used for diagnosing different pulmonary diseases including lung cancer, interstitial lung diseases, tuberculosis and opportunistic infections in immunocompromised hosts [1, 24–28].

The role of sputum examination in detection of AFB on ZN stain is undebatable and it forms the cornerstone for many National Tuberculosis Control Programs. However, in cases where patients are unable to expectorate and have significant clinical features suggestive of tuberculosis, flexible bronchoscopy comes into the picture. After localizing the lesion with required imaging modalities, the pulmonologist may perform a flexible bronchoscopy to acquire the required sample for examination. The sample may be in the form of bronchial washings, lavages, trans-bronchial node aspirations, trans-bronchial biopsies, and so on, depending upon the presentation of the patient. Utility of multiple invasive techniques during flexible bronchoscopy has been successfully proven to increase the yield of diagnosis of tuberculosis. This study restricts the intervention during bronchoscopy to bronchial washings. The post bronchoscopy sputum is an investigation advised by quite a few pulmonologists, however the efficacy of the same in adding to the diagnosis has not clearly proven. Not many studies are available that show this increased yield of this investigation in addition to the routine bronchial washing examination. 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 hence allowing a greater proportion to have full drug sensitivity testing and therefore appropriate management of potential drug resistant strains. Further studies are now required to establish the duration of smear positivity post bronchoscopy in-patients 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.

In our study, it was proven that the combination of bronchial washing ZN staining and post bronchoscopy sputum ZN staining was statistically superior to bronchial washing ZN staining alone in detection of acid fast bacilli.

CONCLUSION:

The study proves the efficacy of post bronchoscopy sputum examination in adding to the yield of bronchial washings examination in diagnosis of acid fast bacilli. Also, it suggests that the patient post bronchoscopy may be isolated to prevent transmission of the infection to other patients in view of the post bronchoscopy sputum positivity. Serial sputum examinations may be done to confirm return of patient to sputum smear negative status before de-isolation.

REFERENCES:

- Craig CF. The branched form of the bacillus tuberculosis in sputum. *J Exp Med* 1898; 3: 363–370.
- Colebunders R, Bastian I. A review of the diagnosis and treatment of smear-negative pulmonary tuberculosis. *Int J Tuberc Lung Dis* 2000;4:97e107.
- Getahun H, Harrington M, O'Brien R, Nunn P. Diagnosis of smear-negative pulmonary tuberculosis in people with HIV infection or AIDS in resource-constrained settings: informing urgent policy changes. *Lancet* 2007;369:2042e9.
- Mukadi YD, Maher D, Harries A. Tuberculosis case fatality rates in high HIV prevalence populations in sub-Saharan Africa. *AIDS* 2001;15:143e52.
- Whitehorn J, Ayles H, Godfrey-Faussett P. Extra-pulmonary and smear-negative forms of tuberculosis are associated with treatment delay and hospitalisation. *Int J Tuberc Lung Dis* 2010 Jun;14(6):741e4.
- National Institute for Health and Clinical Excellence. Clinical diagnosis and management of tuberculosis, and measures for its prevention and control, www.nice.org.uk; March 2006.
- Anderson C, Inhaber N, Menzies D. Comparison of sputum induction with fibre-optic bronchoscopy in the diagnosis of tuberculosis. *Am J Respir Crit Care Med* 1995;52:1570e4.
- Khan MS, Dar O, Sismanidis C, Shah K, Godfrey-Faussett P. Improvement of tuberculosis case detection and reduction of discrepancies between men and women by simple sputum submission instructions: a pragmatic randomised controlled trial. *Lancet* 2007 Jun 9;369(9577):1955e60.
- Yajko DM, Nassos PS, Sanders CA, Madej JJ, Hadley WK. High predictive value of the acid-fast smear for Mycobacterium tuberculosis despite the high prevalence of Mycobacterium avium complex in respiratory specimens. *Clin Infect Dis* 1994;19(2):334e6.
- Wallace JM, Deutsch AL, Harrell JH, Moser KM. Bronchoscopy and transbronchial biopsy in evaluation of patients with suspected active tuberculosis. *Am J Med* 1981;70: 1189e94.
- Chawla R, Pant K, Jaggi OP, Chandrashekar S, Thukral SS. Fibre-optic bronchoscopy in smear negative pulmonary tuberculosis. *Eur Respir J* 1988;1:804e6.
- Kennedy DJ, Lewis WP, Barnes PF. Yield of bronchoscopy for the diagnosis of tuberculosis in patients with human immunodeficiency virus infection. *Chest* 1992;102:1040e4.
- de Gracia J, Curull V, Vidal R, et al. Diagnostic value of bronchoalveolar lavage in suspected pulmonary tuberculosis. *Chest* 1988;93:329e32.
- Global Laboratory Initiative, First Edition April 2014, Stop TB Partnership in collaboration with WHO.
- Bickerman HA, Sproul EE, Barach AL. An aerosol method of producing bronchial secretions in human subjects: a clinical technique for the detection of lung cancer. *Dis Chest* 1958;33:347–362.
- Jatakanon A, Lim S, Barnes PJ. Changes in sputum eosinophils predict loss of asthma control. *Am J Respir Crit Care Med* 2000; 161: 64–72.
- Pavord ID, Brightling CE, Woltmann G, et al. Noneosinophilic corticosteroid unresponsive asthma. *Lancet* 1999; 353: 2213–2214.
- Green RH, Brightling CE, McKenna S, et al. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. *Lancet* 2002; 360: 1715–1721.
- Reddel HK, Taylor DR, Bateman ED, et al. An official American Thoracic Society/European Respiratory Society statement: asthma control and exacerbations: standardizing endpoints for clinical asthma trials and clinical practice. *Am J Respir Crit Care Med* 2009; 180: 59–99.
- Loughheed MD, Lemiere C, Ducharme FM, et al. Canadian Thoracic Society 2012 guideline update: Diagnosis and management of asthma in preschoolers, children and adults. *Can Respir J* 2012; 19: 127–164.
- Bakakos P, Schleich F, Alchanatis M, et al. Induced sputum in asthma: from bench to bedside. *Curr Med Chem* 2011; 18: 1415–1422.
- Maestrelli P, Calcagni PG, Saetta M, et al. Sputum eosinophilia after asthmatic responses induced by isocyanates in sensitized subjects. *Clin Exp Allergy* 1994; 24: 29–34.
- Eltboli O, Brightling CE. Eosinophils as diagnostic tools in chronic lung disease. *Expert Rev Respir Med* 2013; 7: 33–42.
- Aloneo Fernandez A, Garcera R, F, Mayorales Alises S, et al. Utilidad de la citología de esputo inducido en el estudio de masas centrales en ancianos. [Usefulness of induced sputum cytology in the study of central masses in elderly patients.]. *Rev Clin Esp* 2001; 201: 444–447.
- D'Urso V, Doneddu V, Marchesi I, et al. Sputum analysis: non-invasive early lung cancer detection. *J Cell Physiol* 2013; 228: 945–951.
- Olivieri D, D'Ippolito R, Chetta A. Induced sputum: diagnostic value in interstitial lung disease. *Curr Opin Pulm Med* 2000; 6: 411–414.
- Bothamley GH, Ditiu L, Migliori GB, et al. Active case finding of tuberculosis in Europe: a Tuberculosis Network European Trials Group (TBNET) survey. *Eur Respir J* 2008; 32: 1023–1030.
- LaRocque RC, Katz JT, Perruzzi P, et al. The utility of sputum induction for diagnosis of Pneumocystitis pneumonia in immunocompromised patients without human immunodeficiency virus. *Clin Infect Dis* 2003; 37: 1380–1383.