Scientific Journal of
Pulmonary & Respiratory Medicine

Research Article

To Study the Efficacy of Repeated Induced Sputum *versus* Bronchial Washings to Diagnose Acid Fast Bacilli -

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Submitted: 23 March 2020; **Approved:** 16 April 2020; **Published:** 09 May 2020

Cite this article: Deshpande T, Godbole GP, Magar P, Kakad S, Pawar U. To Study the Efficacy of Repeated Induced Sputum versus Bronchial Washings to Diagnose Acid Fast Bacilli. Sci J Pulm Respir Med. 2020;2(1): 001-006.

ABSTRACT

Background: Induction and processing of sputum samples in a standardised manner is a key component to provide valuable information for clinical decision making. The methods for induction and processing have been mentioned by the various agencies working in tandem with the WHO. It is said that the induced sputum has more yield than conventional sputum collection.

Diagnostic flexible bronchoscopy has become a fairly commonly prescribed intervention in sputum ZN stain negative cases with suspicion for tuberculosis. Cost of procedure, co-operation of the patient for the procedure, anaesthetic implications of the procedure, possibility of complications and the discomfort caused to the patient during and after the procedure are some of the hurdles that must be addressed to perform flexible broncchoscopy in patients.

This study aims to compare yield of induced sputum with yield of bronchial washings from flexible bronchoscopies in a tertiary care centre.

Methods: Sample Size: 384 patients

Type of Study: Case Control Study

Duration of study: Two and a half years

Detailed Methodology: Out of the 384 cases 192 patients were subjected to induced sputum to diagnose pulmonary tuberculosis and remaining 192 patients were subjected to flexible bronchoscopy once initial sputum AFB was found to be negative. The data was tabulated and statistically analysed. Exact Fisher Test was applied to look for statistically significant difference. Conclusions were drawn from the statistical analysis.

Results: There was no statistically significant difference in yield with regard to acid fast bacilli on ZN stain when induced sputum was compared to flexible bronchoscopic bronchial washings.

Conclusions: Repeated sputum inductions are as effective if not more, than bronchial washings from flexible bronchoscopy in diagnosis of acid fast bacilli. The selection of patients for either of these diagnostic techniques must however be made with the clinical scenario of the patient in mind.

Keywords: Tuberculosis; Acid fast bacilli; ZN stain; Microscopy; Induced sputum; Bronchoscopy

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]. Induced sputum has been used in clinical practice in a number of different ways [2]. A task force was set up by the European Respiratory Society (ERS) and it published its recommendations for standardisation of sputum induction and processing in 2002 [3-8]. Induction and processing of sputum samples in a standardised manner is a key component to provide valuable information for clinical decision making [9-14].

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. Though bronchoscopy has very high yield and can be performed with relative ease, there remain certain hurdles for performing flexible bronchoscopy in especially resource limited settings.

Cost of procedure, co-operation of the patient for the procedure, anaesthetic implications of the procedure, possibility of complications and the discomfort caused to the patient during and after the procedure are some of the hurdles that must be addressed to perform flexible bronchoscopy in patients.

3% hypertonic saline nebulization for 20 minutes has been useful in inducing sputum in patients who otherwise have been unable to expectorate or have collected predominantly salivary samples. It is said that the induced sputum has more yield than conventional sputum collection.

This study aims to compare the yield of induced sputum with the yield of bronchial washings from flexible bronchoscopies in a tertiary care centre.

AIMS & OBJECTIVES

To compare yield of induced sputum versus bronchial washings attained after flexible bronchoscopy for diagnosis of acid fast bacilli.

Inclusion criteria

1. Patients clinically suspected to have pulmonary tuberculosis
2. Patients with no results on sputum gram stain examination with radiological picture of tuberculosis.

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 AND METHODS

Sample Size: 384 patients

Type of Study: Case Control Study

Duration of study: Two and a half years

Detailed methodology

Out of the 384 cases 192 patients were subjected to induced sputum to diagnose pulmonary tuberculosis and remaining 192 patients were directly subjected to flexible bronchoscopy once initial sputum AFB was found to be negative.

The induced sputum group (study group) patients despite induced sputum found to be negative for acid fast bacilli were then

subjected to flexible bronchoscopy for diagnosis.

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 sputum induction:

- Sputum induction is conducted by inhalation of nebulised sterile saline solution (isotonic or hypertonic) followed by coughing and expectoration of airway secretions.
- Since saline inhalation may cause bronchoconstriction, careful safety measures should be taken, including the measurement of lung function before induction, pre-treatment with inhaled salbutamol and monitoring of lung function during the process.
- Spirometry (Forced Expiratory Volume in 1 s (FEV1)) is preferred over the measurement of Peak Expiratory Flow (PEF) determination and the use of a single dose of 200 mg salbutamol is recommended for pre-treatment.
- FEV1 should be measured before (baseline) and 10 min after salbutamol inhalation. It is important to note that baseline FEV1 does not have predictive value for the occurrence and severity of bronchoconstriction caused by induction.
- Resuscitation equipment should be available in the place where the sputum induction is undertaken and a physician should be available to supervise the procedure, which can be carried out by an experienced technician.
- Induction is carried out using a sterile, freshly prepared saline solution.
- The use of 4.5% sodium chloride solution is recommended for general use. The use of hypertonic saline results in more sample than the use of isotonic saline; however, importantly there is no difference in cellular composition between samples induced by isotonic or hypertonic solutions [15].
- For nebulisation, an ultrasonic nebuliser is recommended.
- In general, 15-20 min is enough to provide an adequate amount of sample, during which the subject is asked to cough and expectorate at 5 min intervals.
- In each period, lung function is measured to detect potential bronchoconstriction and if FEV1 decreases by more than 20% compared with post-salbutamol baseline, induction is stopped.

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 [16]:

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 labeling 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-Nielsen stain (carbol fucshin, 3% acid alcohol, methylene blue)
- Staining sink
- Staining rack
- Slide drying rack
- Forceps

- Timer
- Vortex mixer
- Distilled water
- Wash bottle

Smear preparation *[16]:*

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 [16]:

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 [16]:

1. Using a bright field microscope, Ziehl-Nielsen 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 [16]:

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

The above table 1 indicates that out of the 192 patients in the study group 187 were found to be positive for AFB. The remaining 5 patients who were found to be negative for AFB were subjected to flexible bronchoscopy and diagnosed as positive for AFB on their bronchial washing ZN stains.

The table indicates that the mean number of inductions of sputum required to get a positive result was 2. This meant a slightly more cumbersome process for the patient, but at the same time a less invasive one.

STATISTICAL ANALYSIS

Fisher exact test applied to the data collected.

The Fisher Exact Test resulted in a p value = 0.0609 which is more than 0.05. This indicates a statistically insignificant difference.

This indicates that the induced sputum is an equally effective modality in diagnosis of acid fast bacilli when compared to bronchial washings acquired from flexible bronchoscopy. The sputum might

Table 1: Master table.

Group	Number of patients	Mean number of times procedure done	Patients found to be positive for AFB	Patients found to be negative for AFB
Bronchial washing	192	1	192	0
Induced Sputum	192	2	187	5

Table 2: Fisher exact test table.

	Positive for AFB	Negative for AFB	Marginal Row Totals
Bronchial Washings	192	0	192
Induced Sputum	187	5	192
Marginal Column Totals	379	5	384 (Grand Total)

have to be induced multiple times to attain a good quality sample and in our study the mean number of inductions required for acquiring a good quality sample was 2.

DISCUSSION

Induced sputum has been used in clinical practice in a number of different ways [2, 9-11, 17].

The differential cell count of induced 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 [9-11]. 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 [13-14]. The measurement has a good reproducibility and its use has been 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 induced sputum eosinophil counts as an outcome measure [13]. 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 [18]. Similarly, in patients with COPD, the method can be used to determine steroid responsiveness based on sputum eosinophil differential count [19]. 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, 20-24].

In our study it was found that sputum induction was as effective if not more, than the bronchial washings acquired during flexible bronchoscopy for detection of acid fast bacilli.

Multiple inductions were required as compared to single sitting flexible bronchoscopy, thus making the process of sputum induction slightly more time consuming. Moreover, we can infer that the selection of patients for induced sputum and flexible bronchoscopy should depend upon the clinical scenario. Patient with haemoptysis or poor general condition with rapidly deteriorating prognosis where time is of the essence or in patients who have altered sensorium or those who are uncooperative for sputum expectoration, flexible bronchoscopy may remain the investigation of choice. In relatively hemodynamically stable patients with anxiety of invasive procedures or in cases where patient is able to expectorate decent amount of sputum or in those patients who cannot afford flexible bronchoscopy, repeated sputum inductions may be useful in clinching the diagnosis.

CONCLUSION

Repeated sputum inductions are as effective if not more, than bronchial washings from flexible bronchoscopy in diagnosis of acid fast bacilli. The selection of patients for either of these diagnostic techniques must however be made with the clinical scenario of the patient in mind.

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