Comparative study between bronchoalveolar lavage and induced sputum in the diagnosis of inflammatory lung diseases

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Background Airway sampling is implicated in the workup of inflammatory lung diseases.

Objective The aim of this study was to compare between induced sputum (IS) and bronchoalveolar lavage (BAL) in the diagnosis of inflammatory (including infectious) lung diseases regarding safety, cell composition (total and differential), microbiology workup, and cytology.

Patients and methods This was a prospective comparative study that was carried out between February 2015 and February 2016. We enrolled 30 patients with inflammatory (including infiltrative and infectious) lung diseases whom presented to the Chest Department of Alexandria Main University Hospital. IS was performed in all included patients by inhalation of hypertonic saline (3%), using an ultrasonic nebulizer. In the same cohort, BAL was performed using flexible bronchoscopy within 1 week of IS. Samples from both techniques were sent for cytological (total and differential cell counts), microbiological, and cytopathological workup.

Results The study sample included 26 (86.7%) female and 4 (13.3%) male patients. Their mean age was 43.57±16.30 years. BAL samples were more voluminous than IS samples (52.83±18.69 and 15.33±5.03 ml, respectively; $P<0.001$). Total cell counts were significantly higher in IS than in BAL (292.5 and 105.5 cell/cm^2, respectively; $P<0.001$). No statistically significant differences were noted between both groups regarding the differential cell counts, culture results, or cytology. Complications in the form of hemorrhage occurred in two (6.67%) patients during BAL. No complications were recorded in the IS group.

Conclusion IS is comparable to BAL. Although BAL is more voluminous, the total cell count in IS is higher. No differences were noted between both techniques regarding differential cell count, culture, and cytology results. IS is safe and can replace BAL in the workup of inflammatory, including infiltrative and infectious, lung diseases.

Egypt J Bronchol 2017 11:81–87

Egyptian Journal of Bronchology 2017 11:81–87

Keywords: bronchoalveolar lavage, induced sputum, infiltrative lung diseases, inflammatory lung diseases

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Received 18 September 2016 **Accepted** 11 November 2016

Introduction

For many years, induced sputum (IS) was used for the diagnosis of different respiratory diseases. It is being increasingly used to study the pathophysiology of various lung diseases. It is a safe, noninvasive, repeatable procedure with minimal or no complications [1,2].

Bronchoalveolar lavage (BAL) is a minimally invasive procedure that has been used in diagnosis of various respiratory diseases but has a lot of limitations and cannot be used for follow-up [3,4].

This study aimed to compare IS and BAL with respect to the diagnosis of inflammatory (including infiltrative and infectious) lung diseases regarding safety, cell composition (total and differential), microbiology workup, and cytology.

Patients and methods

This was a prospective, comparative, clinical study carried out between February 2015 and February 2016. We enrolled 30 adult patients of both sexes with inflammatory (including infiltrative and infectious) lung diseases who presented to the Chest Department of Alexandria Main University Hospital, in whom airway sampling was indicated. Any patient with respiratory distress who refused to participate or had any contraindications for BAL was excluded from the study. All included patients were not receiving any medications before enrollment into the study.

Informed written consent was obtained from all patients before enrollment to the study, according to the guidelines of the local institutional ethics committee. Detailed history was taken including the symptoms of the disease, their frequency and duration, smoking history, drug history, and occupational exposure. Complete clinical examination, radiography and computed tomography (CT) of the chest, and routine laboratory investigations were carried out.

 DOI: 10.4103/ejb.ejb_78_16

Bronchoscopy, bronchoalveolar lavage

(1) All patients consumed nothing orally 6 h before bronchoscopy. The procedure was explained to the patient before bronchoscopy. Intravenous access was established.
(2) All patients were given 5–15 mg of midazolam intravenously in an incremental manner, as indicated by the patients' condition, before and/or during the procedure.
(3) Local lidocaine (10%) was sprayed transnasally and transorally before the procedure. Topical anesthesia at the larynx was completed using 2% lidocaine; usually, a total of 4–6 ml is used. Additional 2-ml aliquots of 2% lidocaine were instilled at the carina, at the division of the right lower lobe and the right middle lobe entrance on the right side or the division of the left upper and the left lower lobe bronchi on the left side.
(4) BAL was performed before any other bronchoscopic interventions to decrease the risk of contamination or bleeding. The site at which BAL was performed was determined according to the patient's chest CT. In cases of localized infiltration, BAL was performed from the draining bronchi according to CT findings. For bilateral diffuse disease, BAL was performed from the bronchi draining the most affected parts, if the distribution of the lesions was heterogeneous. If homogeneous distribution existed, BAL was performed from subsegmental bronchi of either the lingula or the right middle lobe.

The procedure for lavage

(1) The bronchoscope was positioned at a subsegmental bronchus. Good positioning was indicated during the wink test [5] by a bronchoscope that can be fully maintained in position by the bronchoscopist and an airway that does not fully close immediately on gentle suction.
(2) Five aliquots of 20 ml sterile normal saline (a total volume of 100 ml), placed in a water bath at 37°C, were instilled and immediately aspirated into a collection trap. Each participant was observed for 120 min after the procedures.
(3) The retrieved BAL fluid was expelled gently into a labeled sterile container; the volume of BAL fluid was recorded and then sent to the laboratory within 1 h for further assessment.
(4) Any complication during the procedure was noticed, and postprocedural observations were monitored and recorded. A clinical examination was performed before discharge.

Laboratory analysis of bronchoalveolar lavage specimens

The analysis was performed at the Central Laboratory, Alexandria Faculty of Medicine, Egypt. The BAL was examined. White blood cell (WBCs) counts as well as differential WBC counts were performed using the Neubauer hemocytometer (VWR Scientifics, West Chester, Pennsylvania, USA) [6–8].

Total white blood cell counts

A drop (10 μl) of whole, undiluted BAL fluid was applied on each quadrant of the Neubauer hemocytometer, and WBCs were identified on the basis of their morphology and counted. The number of WBCs was calculated per cubic millimeter on the basis of the standard cell counting procedure using the Neubauer hemocytometer.

Furthermore, an aliquot of BAL fluid was mixed with 3% WBC solution (3% acetic acid solution in distilled water with a few drops of Leishman's stain). According to the degree of clarity of the fluid and the presence or absence of reddish tinge indicating hemorrhagic fluid, the dilution of the fluid was adjusted accordingly, the cells were counted using the Neubauer hemocytometer, and the counted cells were calculated after considering the dilution factor. The cells were classified to either mononuclear cells or polymorphonuclear cells according to nuclear segmentation.

Differential white blood cell counts

For the sake of classification of cells in the BAL, a stained, thick film was created. The BAL fluid was centrifuged, the supernatant was discarded, and the deposit was resuspended gently, mixed with equal volume of patient serum (or serum albumin); the mixture was centrifuged once more. Finally, 10 μl from the deposit was spread in a circular manner on the center of a microscopic slide, the preparation was left to dry, stained with Leishman's stain, and examined under the microscope for differential cell count. WBCs were classified as macrophages, neutrophils, lymphocytes, eosinophils, mast cells, or other cells according to standard morphological criteria [7].

Sputum induction

Sputum induction was performed for all patients within the week before bronchoscopy. All patients were pretreated with 200 μg salbutamol administrated by an ultrasonic nebulizer. All patients wore nose clips during the procedure. Patients inhaled sterile 3% hypertonic saline for 30 min. Patients were encouraged to cough throughout the procedure. Five minutes after the start of nebulization and every 2 min thereafter, patients were

asked to rinse their mouth and throat carefully, dry their mouth using tissue paper to minimize salivary contamination, and then try to cough into a sterile container. Nebulization was stopped after 30 min or earlier if a sputum sample of good quality was obtained – that is, at least 1 ml of sputum containing mucous plugs; all the saliva samples were discarded separately [9].

Sputum processing

Dithiothreitol (DTT) was freshly prepared at a dilution of 1 : 10 with distilled water. DTT was added in a ratio of 2 : 1 of the IS sample. The sample was incubated for 30 min at room temperature and mixed mechanically by shaking vigorously for several times. Next, the sample was diluted by PBS to a volume twice the volume of sputum plus DTT. The mixture was left to settle for another 30 min at room temperature. Ten microliter of the homogenized sputum was used to determine the total cell count using a hemocytometer counting chamber. Homogenized sputum was centrifuged at 1000 rpm for 10 min at room temperature [10,11].

Differential cell counting of induced sputum

Ten milliliter of the sputum was spread on glass slides, air dried, and fixed using heat. These slides were stained using Leishman's stain. The slides were coded and counted blindly for differential cell counts. Two samples of IS and BAL were sent for cytological and microbiological analyses.

Statistical analysis

Data were fed to the computer, and were analyzed using IBM SPSS software package (IBM SPSS Statistics for Windows, version 20.0; IBM Corp., Armonk, New York, USA). Qualitative data were described using numbers and percentages. Quantitative data were described using ranges (minimum and maximum), means, SDs, and medians. Comparison between different groups regarding categorical variables was tested using the χ^2-test. When more than 20% of the cells had expected count less than 5, correction for χ^2 was conducted using Fisher's exact test or Monte Carlo's correction. McNemar–Bowker's and marginal homogeneity test was applied for ordinal data. The distributions of quantitative variables were tested for normality using the Kolmogorov–Smirnov test, the Shapiro–Wilk test, and the D'Agostino test. If it revealed normal data distribution, parametric tests were applied. If data were abnormally distributed, nonparametric tests were used. For normally distributed data, comparisons between the two studied groups were performed using independent *t*-test; paired *t*-test was used to analyze two-paired data. For abnormally distributed data, comparisons were carried out using the Mann–Whitney *U*-test. To compare between the different periods, Wilcoxon's signed-rank test was applied. Correlations between two quantitative variables were assessed using Spearman's correlation coefficient regarding normality of the data. Significance of the obtained results was judged at the 5% level.

Results

Among 30 patients, four (13.3%) patients were male and 26 (86.7%) patients were female. The study was performed on adult patients whose ages ranged between 20 and 70 years with a mean of 43.57± 16.30 years. Regarding smoking history, two (6.7%) patients were active smokers, whereas 28 (93.3%) patients were nonsmokers (Table 1).

Regarding patients' clinical data, cough was the most common presenting symptom followed by expectoration, crepitations, and dyspnea (Table 1). Regarding chest radiograph and CT findings, most of the patients (56.7%) has diffuse bilateral disease (Table 1).

Two cases were excluded from the BAL group, as hemorrhage during the procedure affected the sample and the whole procedure was aborted. This is why the number of patients in the BAL group was

Table 1 Distribution of the studied cases according to personal data, clinical data and imaging (*n*=30)

	N (%)
Age (years)	43.57±16.30
Sex	
Male	4 (13.3)
Female	26 (86.7)
Smoking status	
Smoker	2 (6.7)
Nonsmoker	28 (93.3)
Clinical data	
Cough	30 (100.0)
Expectoration	22 (73.3)
Fever	8 (26.7)
Weight loss	6 (20.0)
Dyspnea	19 (63.3)
Hemoptysis	0 (0)
Wheezes	0 (0)
Crepitations	20 (66.7)
Imaging (chest radiograph and CTs)	
Localized unilateral	9 (30.0)
Diffuse unilateral	2 (6.7)
Localized bilateral	2 (6.7)
Diffuse bilateral	17 (56.7)

Qualitative data were described using *n* (%). Normal quantitative data are expressed as mean±SD. CT, computed tomography.

less than the IS group. For microbiological analysis of the yield, five patients were proven to have active pulmonary tuberculosis (TB) by both techniques after mycobacterial culture. Positive culture for bacterial etiology was found in samples from seven patients (Table 2).

BAL samples were more voluminous than IS samples (52.83±18.69 and 15.33±5.03 ml, respectively; $P<0.001$). Total cell counts were significantly higher in IS than BAL (292.5 and 105.5 cell/cm^2, respectively; $P<0.001$). No statistically significant differences were noted between both groups regarding the differential cell counts, culture results, or cytology. Complications in the form of hemorrhage took place in two (6.67%) patients during BAL. No complications were recorded in the IS group (Table 2).

Table 3 shows the final diagnosis of included patients according to the cellular profile of the yield either by IS or BAL and according to culture in infectious diseases. BAL and IS were found to be lymphocytic (lymphocytes ≥25%) in seven patients. Yields from both techniques showed neutrophilic predominance (neutrophilic count ≥50%) [8] in 20 patients of IS and only in 18 patients in the BAL group as two patients were excluded because of complications. BAL and IS were found to be hypocellular [near normal BAL profile in interstitial lung diseases (ILDs)] in three patients. Four patients were diagnosed with hypersensitivity pneumonitis, but this was mainly based on radiological patterns in the CTs. Sarcoidosis was diagnosed in one patient by transbronchial lymph node biopsy.

Discussion

ILDs are a group of pulmonary disorders with different pathogenesis, pathology, management, and prognosis, but similar in presentation and diffuse in nature. Some reports suggest that the number of cases have increased, but no studies have documented recent trends in ILD subgroups [12].

For evaluation of patients, the first step is clinical assessment followed by investigations such as

Table 2 Comparison between the two groups according to different parameters

	IS (*n*=30)	BAL (*n*=28)	*P*
Culture			
Sterile	18 (60.0)	16 (57.1)	1.000
Bacteria	7 (23.3)	7 (25.0)	
TB	5 (16.7)	5 (17.9)	
Volume (ml)	18.0 (7.0–20.0)	52.50 (0.0–80.0)	<0.001*
Total cell count (c/cmm)	292.5 (10.0–3304.0)	105.5 (8.0–2808.0)	<0.001*
Neutrophils (%)	69.50 (0.0–97.0)	57.0 (0.0–93.0)	0.084
Lymphocytes (%)	11.0 (0.0–93.0)	12.0 (0.0–83.0)	0.808
RBCs (%)	0.0 (0.0–83.0)	6.50 (0.0–85.0)	0.120
Eosinophils (%)	0.0 (0.0–6.0)	0.0 (0.0–3.0)	0.593
Alveolar macrophages (%)	49.50 (46.0–145.0)	51.0 (47.0–146.0)	0.078

Qualitative data were described using *n* (%) and was compared using marginal homogeneity test. Abnormally distributed data are expressed as median (minimum–maximum) and compared using Wilcoxon's signed-rank test. BAL, bronchoalveolar lavage; IS, induced sputum; RBCs, red blood cell counts; TB, tuberculosis. *Statistically significant at $P\leq0.05$.

Table 3 Diagnosis according to cellular basis and microbiology

	IS [*n* (%)] (*n*=30)	BAL [*n* (%)] (*n*=28)[a]
On cellular bases		
Lymphocytic predominance	7 (23.3)	7 (25)
Neutrophilic predominance	20 (66.7)	18 (64.3)
Hypocellular sample	3 (10)	3 (10.7)
According to culture		
TB	5 (16.6)	5 (17.8)
Bacteria	7 (23.3)	7 (25)
Pseudomonas aeruginosa	3	3
Staphylococcus aureus	2	2
Staphylococcus pneumoniae	2	2
Sterile culture	18 (60)	16 (57.1)

BAL, bronchoalveolar lavage; IS, induced sputum; TB, tuberculosis. [a]Two cases were excluded from BAL because of complications.

pulmonary function tests and thoracic imaging (radiographs and High Resolution Computed Tomographies (HRCTs)); subsequently, direct invasive methods such as BAL and lung biopsies – either transbronchial biopsy or open surgical lung biopsy – are usually needed to confirm the diagnosis. BAL is recommended as the standard procedure in the European Respiratory Society and American Thoracic Society statements for the diagnostic workup of patients with ILD [13].

BAL is very useful in the workup of ILD, especially in subtypes that are uncommon (e.g. occupational, eosinophilic lung diseases, and Langerhans cell histiocytosis). In addition, it can help in the diagnosis of more common disorders such as sarcoidosis, hypersensitivity pneumonitis, and idiopathic pulmonary fibrosis when lung biopsy is not accessible or contraindicated [14].

It is well known that BAL is a minimally invasive technique, with infrequent complications, but it is not easy to get patient acceptance to the procedure. Moreover, BAL is not recommended for screening, or to evaluate exposures and follow-up, and is contraindicated in some patients [3,4].

Therefore, it was logical to search for an alternative procedure that is safe, noninvasive, and reproducible procedure for sampling inflammatory conditions in the airways. This is why IS may add to this field as it can be carried out easily with less or no complications in comparison with BAL [15,16].

IS is a very useful sampling method for both research and clinical use, and can be used for diagnosis, monitoring treatment response, and follow-up. IS has been compared with BAL in many studies, especially in asthma and chronic obstructive pulmonary disease [17–19].

Several studies have illustrated the usefulness of sputum cell counts to investigate the pathogenesis and pathophysiology as well as to confirm the diagnosis and follow-up of the treatment of asthma. Many studies have found that increased eosinophils in IS increase the efficacy of steroids as a therapy of asthma. Others have found that increased eosinophilic count above the upper limit of 3% of nonsquamous cells in the absence of diagnostic criteria of asthma is diagnostic [20–22]. These results were comparable with BAL results [23].

The role of IS in the diagnosis of occupational asthma has been studied [24]. IS has been used to evaluate individuals suspected to have occupational exposures. Eosinophilic count has been measured in IS of asthmatic isocyanates-sensitized patients [25], and the frequency of bronchial dysplasia has been investigated in sputum of past exposed miners [26]. The results show that IS eosinophils confirm exposures.

However, the role of IS in the evaluation of patients suffering from ILDs in comparison with BAL was first studied in 1999 [27]. Following this study, many studies compared IS with BAL at a cellular level in ILDs, especially sarcoidosis, hypersensitivity pneumonitis, pneumoconiosis, and more recently idiopathic pulmonary fibrosis. Results of both IS and BAL were comparable [4,15,28,29].

In our study, we compared the cellular composition of IS with BAL in inflammatory (including infectious) lung diseases. We obtained satisfactory IS samples with differential cell counts in all patients, and the procedure was well tolerated with no complications.

However, we obtained satisfactory BAL samples from 28 of the 30 patients included in our study. Two patients were excluded because of complications during the bronchoscopy in the form of hemorrhage that was efficiently controlled in both cases. Both IS and BAL samples were divided into three specimens and were sent for total and differential cell counts as well as microbiological and cytological analysis.

It is well known that IS is more viscid than BAL, and that is why saline dilution is used to process IS rather than BAL. Total cell counts showed significant increase in IS compared with BAL samples, and this was reported in many studies earlier [4,15,28,29].

Some studies showed significant increase in neutrophil counts in IS compared with BAL. They ascribed their finding to the fact that IS originates from the upper and mid airways that are well known for neutrophil-rich secretions [30] compared with BAL. As a wash of the distal airways, it is rich in alveolar macrophages [31]. We found some increase (but not significant) in neutrophils in IS compared with BAL, and this may be due to different disease subtypes that were included in our study. Regarding alveolar macrophages, lymphocytes, and eosinophils, we found no significant difference.TB is still a major respiratory disease with many complications and a high

economic burden, and therefore its diagnosis is vital. Sputum collection with identification of acid-fast bacilli by culture remains the standard for diagnosis. However, some patients may have dry cough, scanty secretions, or smear-negative TB. For those patients, BAL, gastric wash, and lung biopsies were used to confirm the diagnosis despite being invasive techniques with many complications. IS was used for diagnosis with an equal role and comparable results with BAL with less complications and high repeatability [32–34]. The above-mentioned studies lend support to our results where TB was diagnosed equally in five patients by both IS and BAL.

Regarding the role of IS and BAL in diagnosis of infectious lung diseases, we found cultures from both techniques were equally positive in seven patients. This finding was in agreement with previous studies [35,36].

Our study has several points of strengths. First, we had several types of inflammatory and infectious lung diseases with multiple phases of severity. Second, we studied cellular (total and differential) analysis, cultures, and cytology of both IS and BAL in the same patient, which enhance the approach to the final specific diagnosis in most cases and enforce the credibility of the results.

Limitations to our study are numerous. It is a single rather than multicenter study. Technical problems and lack of experienced hands and specific laboratories for processing specimens, especially IS, were hardly overcome. Absence of malignant cases and differences in sex in both groups may be added to the limitations, but both may be ascribed to randomization.

Conclusion

IS is comparable with BAL with regard to diagnosis of inflammatory (including infiltrative and infectious) lung diseases. As it is safe, easy to perform, repeatable, with minimal or no complications, it can replace BAL in the management of patients with inflammatory, infiltrative, and infectious lung diseases.

However, bronchoscopy and BAL will be superior if biopsies are indicated. Moreover, the feasibility to follow-up patients with inflammatory or infiltrative lung diseases by IS is more superior to BAL.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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