



# Usefulness of post-bronchoscopic sputum cytology with respect to bronchoalveolar lavage and brush cytology using fiber-optic bronchoscopy in suspected cases of lung cancer

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## ABSTRACT

**Background:** Bronchogenic cancer is the most common cause of cancer-related mortality in both sexes all over the world, especially across urban population. However, early diagnosis and timely intervention may minimize the mortality rate to a certain extent and increase the 5-year survival rate to 70–80%. Among the diagnostic modalities available, bronchoscopic biopsy, though gold standard, is less feasible in peripheral centers where we depend more on cytological techniques such as bronchoalveolar lavage (BAL) fluid and sputum cytology for quick diagnosis. **Aims and Objectives:** This study was conducted at a tertiary care center for studying usefulness of post-bronchoscopic sputum cytology and also to correlate sputum cytology with brushing and washing cytology taking biopsy as the gold standard in the diagnosis of lung cancer. **Materials and Methods:** A cross-sectional observational study was done in Nil Ratan Sircar Medical College, Kolkata, over a period of 18 months in clinically and radiologically suspected patients of bronchogenic carcinoma. Bronchoscopic samples using a fiber-optic bronchoscope were collected from 50 such patients. In every case, following sequence of events was performed: Pre-biopsy washing (BAL fluid), brushing, biopsy, post-biopsy washing (BAL), and post-bronchoscopy sputum. Cytological examination done and compared with bronchoscopic biopsy specimens of the same. **Results:** All the available information were meticulously documented in tables and charts along with other variables such as age, sex, morphological types, and cytological and histological diagnosis. SPSS software was used to calculate the efficacy and statistical significance, if any, of these different diagnostic tools and its correlation with the final histological diagnosis. **Conclusion:** We found that BAL cytology had higher sensitivity and positive predictive value in early and accurate diagnosis of lung malignancy. However, post-bronchoscopy sputum cytology had no additional benefit with respect to BAL and brush cytology (in addition to bronchial biopsy) in diagnosing the same.

**Key words:** Bronchoalveolar lavage fluid; Bronchoscopic biopsy; Lung cancer; Sputum

## INTRODUCTION

Today, lung cancer is the most common cause of cancer-related mortality in both sexes in the world. In India, it was considered infrequent, but in the recent past, a trend of increase in its incidence has been noticed. Its incidence

is increasing at an alarming rate of 0.5% per year,<sup>1</sup> and now, it has been estimated to be the most frequent among all the new cases of cancers in male in this country, especially across all urban registries. The increasing incidence could be due to increase in smoking habit, change in lifestyles of the people, increased environmental pollution, and at the same time,

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due to availability of different modern diagnostic modalities to detect lung cancer. To treat the disease successfully, it should be diagnosed at the earliest possible stage. Several studies have demonstrated that early detection, localization, and aggressive treatment of lung cancer result in the 5-year survival rate of 70–80%.<sup>2</sup> Recent developments in molecular study of lung cancer along with subsequent targeted therapeutic approaches have given a new ray of hope. Nowadays, bronchoscopy is an invaluable tool for diagnosis of lung cancer and various diagnostic tools have been developed using flexible fiber-optic bronchoscopy (FOB).<sup>3</sup> Among them, histopathological examination of bronchial biopsy specimen remains the confirmatory or the gold standard test in these situations. However, bronchial biopsies cannot be satisfactorily performed in more peripheral sites or in narrow bronchial lumen. Hence, alternative methods including cytological methods for diagnosis are required. Sputum is the collection of mucoid material that contains cells from the buccal cavity, the pharynx, the larynx, the trachea, the bronchial tree and the pulmonary alveoli, as well as inflammatory cells and microorganisms. Sputum cytology is an easy and often the earliest cytological method for the detection of malignant lung tumor. Cytological assessment of specimens obtained through FOB-guided washing and brushing specimens of the respiratory tract offer excellent and accurate information about the lesion as well as the site of the lesion. In this context, cytological assessment of specimens obtained through sputum, bronchoscopic washing, and brushing of the respiratory tract is important, and often, the initial diagnostic technique carried out in a patient with suspected malignant lung lesion.<sup>4</sup> The utilities of cytology are extensive, and sometimes, they help in planning the treatment without the requirement for an open biopsy. This cross-sectional, observational study was conducted at a tertiary care center over the period of 18 months with the aim to study usefulness of sputum cytology and also to correlate sputum cytology with brushing and washing cytology taking biopsy as the gold standard in the diagnosis of lung cancer.

### Aims and objectives

This study was conducted at a tertiary care center of eastern India for studying usefulness of post-bronchoscopic sputum cytology in diagnosing lung malignancy and also to correlate sputum cytology with brushing and washing cytology taking biopsy as the gold standard by using fiber optic bronchoscopy in the suspected cases of lung cancer.

## MATERIALS AND METHODS

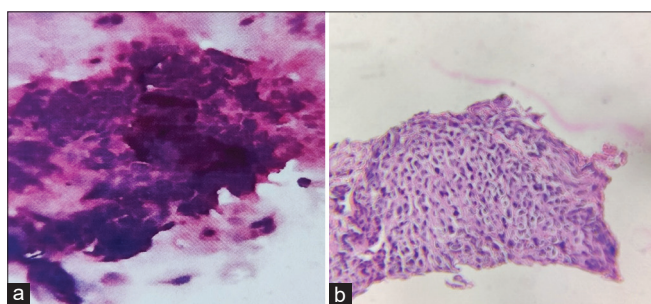
It was a cross-sectional observational study conducted in the Department of Pathology in association with the Department of Respiratory Medicine, Nil Ratan Sircar

Medical College and Hospital (NRSMCH), Kolkata. The study was approved by the Institutional Ethics Committee of NRSMCH. After getting the permission, the samples for cytological and histological examination were collected from the indoor/outdoor patients in whom clinical findings, radiological examination suggested lung malignancy. Chronic cough, hemoptysis, significant weight loss, pallor, and lymphadenopathy were among the most significant clinical findings that were considered. Among the radiological findings, mass with or without consolidation was the most characteristic indicator apart from pleural effusion. Among these, suspicious patients, who were considered for bronchoscopy, endobronchial growth, and narrowing of bronchial lumen (due to compression from outside), were the predominant presentations. Patients with hemorrhagic diathesis, poor general condition, and sputum positive for acid-fast bacilli were excluded from the study. A total of 50 cases were studied in the stipulated time frame of 18 months (January 2014–July 2015) which fulfilled our inclusion and exclusion criteria. Bronchoscopic samples were obtained by Pentax flexible FOB done by the pulmonologists following standard protocol. Bronchial brushings were obtained by the use of a stiff bristle disposable brush (outer diameter of brush is 2 mm and outer diameter of sheath is 1.8 mm). In every case we performed the following sequence of events: Pre-biopsy washing (bronchoalveolar lavage [BAL] fluid), brushing, biopsy, post-biopsy washing (BAL), and post-bronchoscopy sputum. In the present study, sputum samples, taken 30 min after bronchoscopic procedures by deep cough, were collected in a wide-mouthed sterile plastic container and were brought without any fixative. Only one sputum sample of adequate quantity and quality was taken. The delay was avoided as far as possible because samples usually degenerate after 8–10 h of collection. Sputum cytodiagnosis was carried out using the “fresh pick and smear” method, which employed examination of sputum for blood-tinged, reddish, discolored, or solid area with the preparation of thin and even smears from these selected portions. Cytological examination with Leishman-Giemsa stain, hematoxylin and eosin (H and E) stain, and Papanicolaou stain of sputum sample is done in each case. Brushing material smeared directly onto at least four clean glass slides. The two air-dried smears were stained with Leishman-Giemsa stain and two slides are fixed with ethanol-ether mixture for Pap and H and E stain. Bronchial wash fluids (BAL fluids) taken both before brushing and after biopsy were first centrifuged (1500 rpm for 5 min) and then prepared into air-dried and ethanol fixed smears (total four slides as before) and stained with Giemsa, H and E, and Pap stain, respectively. Bronchial biopsy specimens were fixed in 10% formalin, sectioned cut at 3–4  $\mu$  thickness, and stained with H and E.

## RESULTS

Most patients were in their fifth and sixth decade of life with age, the range of 31–80 years. Of 50 study subjects, lung cancer was confirmed in 38 (76%) cases by histopathology of bronchial biopsy. Among patients with lung cancer, 79% were male and 21% were female. Squamous cell carcinoma was found to be the most common lung cancer (47.4%) (Figure 1a and b), followed by adenocarcinoma (23.7%), small-cell carcinoma (15.8%), large cell neuroendocrine (5.2%), and large cell anaplastic carcinoma. All except two cases of bronchial biopsy could be differentiated into a specific type of non-small-cell carcinoma. The overall sensitivity of our post-bronchoscopy sputum sample was 7.9%, specificity 100%, positive predictive value 100%, and negative predictive value 25.53% (Tables 1 and 2).

BAL fluid cytology [Pre-biopsy (pre-brushing) and post-biopsy washing] showed high specificity of 92.31%, but a very low sensitivity of 32.43% and 35.14%, respectively. Sensitivity and specificity of brushing were found to be 74.36% and 81.82%, respectively. Positive predictive value of pre-biopsy washing, post-biopsy washing, and brushing is 92.31%, 93.55%, and 92.86%, respectively. Both sensitivity and accuracy of combined tests (wash, brush, and sputum taken together) increase significantly (Table 2). The diagnostic efficacy of post-bronchoscopic sputum is statistically insignificant. There was no statistical difference between pre- and post-biopsy wash cytology and if we consider brush and wash cytology together, combined diagnostic efficacy becomes statistically significant (Table 3).



**Figure 1:** (a and b) Photomicrographs of squamous cell carcinoma. (a) Sputum cytology smear shows clusters of polymorphic cells with eosinophilic cytoplasm and hyperchromatic nuclei [PAP stain  $\times 100$ ]. (b) Histology section shows clusters of atypical squamous cells with hyperchromatic nuclei, intercellular bridges, and high mitotic activity [H and E stain  $\times 100$ ]

**Table 1: Diagnostic efficacy of post-bronchoscopy sputum for malignancy**

2*2 contingency table	Disease positive	Disease negative
Test positive	3	0
Test negative	35	12
Total	38	12

## DISCUSSION

Sputum cytology is an example of exfoliative cytology, which is based on spontaneous shedding of cells derived from the lining of an organ into a cavity from where they can be removed by non-invasive means. It is a simple, accurate, reliable, cost-effective, non-invasive procedure for premalignant and malignant diseases. It is sometimes called as poor man's bronchoscopy. Sputum can be collected by one of the following two means – early morning spontaneously produced sputum and induced sputum. In this present study, we studied only post-bronchoscopy-induced sputum. However, it is suggested that the quality of spontaneously produced sputum and aerosol-induced sputum was comparable, with a better cell viability in the former.<sup>5</sup> The pick and smear method was the most reliable method, and it has many advantages over other methods. In our study, sputum samples, produced after bronchoscopy procedure, were brought without any fixative. The delays were avoided as degeneration took place after 8–10 h of collection. However, specimens with high mucous content, like sputum, might be preserved for 12–24 h, if refrigerated.<sup>6</sup> The other methods to induce sputum production are induction with a single dose of INS316 in patients with mild chronic bronchitis, induction with hypertonic saline solution, and injection of neostigmine. Cytological examination of Leishman-Giemsa stain, H and E stain, and Papanicolaou stain of sputum is accepted as a useful diagnostic tool in carcinoma of lung.

In our study, post bronchoscopy positive sputum cytology detected only three out of 38 confirmed cases of lung cancer with sensitivity of only 7.9%, whereas BAL cytology has higher sensitivity of 31.57% and 34.13%. This finding corresponds with the study conducted by Wongsurakiat et al.,<sup>7</sup> where the sensitivity of post-bronchoscopy sputum was 7.7% and BAL cytology was 46.7%. In another study conducted by Yuksekol et al.,<sup>8</sup> sensitivity of post-bronchoscopy sputum was 31.8% for visible endobronchial lesion under FOB. It increases to 42.9% when the lesion is not visible, that is, for peripheral lesion. In this study, bronchoscopic lavage fluid cytology had lower sensitivity than post-bronchoscopic sputum cytology (22.7% vs. 31.8% and 25% vs. 42.9%), whereas in our study, we got the reverse finding. Das et al.,<sup>9</sup> conducted similar study where he found higher sensitivity for BAL cytology (62%) in respect to post-bronchoscopic sputum cytology (14%) in endoscopically non-visible tumors. In another study conducted by Kitamura et al.,<sup>10</sup> the diagnostic rate with the PBS was 26.8% which is far less than overall diagnostic rate of 66.7% and no patient was able to diagnosed only with PBS.

Sensitivity of pre-biopsy washing was found to be 31.57 and that of post-biopsy washing was 34.13% in patients of

**Table 2: Results of different cytological techniques compared to gold standard (bronchial biopsy)**

Cytological technique	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Accuracy
Sputum	7.9	100	100	25.53	30
Bronchoalveolar lavage	34.13	91.67	92.85	34.21	50
Brush	76.31	75	90.62	50	76
Combined	84.57	69	90	58.4	82

**Table 3: Statistical significance of different cytological procedures**

Different procedures	Chi-square test	P	Significance
Sputum	1.008	0.314	Not significant
Difference of pre-wash and post-wash (BAL) cytology	0.0985	0.753654	Not significant
BAL and brush cytology (together)	14.9139	0.000113	Significant

BAL: Bronchoalveolar lavage

lung cancer confirmed by bronchial biopsy. The previous studies by Park et al.,<sup>11</sup> and Karahalli et al.,<sup>12</sup> had found almost comparable result of bronchial washing (BAL) cytology in suspected lung cancer cases.

In the present study, the bronchial brushing could detect malignancy in 25 out of 38 (65.7%) cases and we found a sensitivity of 76.31% and specificity of 75%. This finding of the present study is similar to the result of Mak et al.,<sup>13</sup> Chen et al.,<sup>14</sup> and Bodh et al.<sup>15</sup> Sensitivity of brush cytology (76.31%) is much higher than sputum cytology (7.9%) in our study.

At our center, we got additional yield from bronchial washing but not with post-bronchoscopy sputum. BAL did give additional positive results in subjects with central tumors in whom bronchial brush and biopsies were found negative. Bronchial biopsies cannot be performed in more peripheral sites or in patients with luminal obstruction or at risk of hemorrhage. Hence, alternative methods for diagnosis are sometimes required. The bronchial washing, brushing, and sputum are a safer technique with much lesser risk of hemorrhage or mortality.

In our study, we find that the sensitivity and diagnostic accuracy increase significantly if we combine two methods, brush and wash. Combination of these diagnostic techniques – brushing, pre- and post-washing (BAL) has sensitivity of 84.57%, accuracy 82%, and positive predictive value of 90%. Our finding has been supported by the studies done by Mak et al.,<sup>13</sup> Jones et al.,<sup>16</sup> and Bodh et al.,<sup>15</sup> but studies done by Trevisani et al.,<sup>17</sup> and Karahalli et al.,<sup>12</sup> disagreed with our results. Addition of sputum to these techniques does not increase overall diagnostic accuracy.

### Limitations of our study

Post-bronchoscopy sputum samples have low sensitivity. Early morning fresh spontaneous samples might give different result as pointed by other studies. Multiple simultaneous samples were not taken in our study which could pick up higher positive cases as shown in different studies. There are different factors contributing to the final yield such as location of the tumor (central versus peripheral) and tumor type. On bronchoscopic examination, the gross morphology of majority of these cases of adenocarcinoma was compression type lesion, that is, extrinsic compression of the bronchus by the lesion, and thus, there may be a possibility of getting less representative material by cytology techniques and bronchial biopsy in such tumors. Furthermore, in mucinous type of adenocarcinoma, bronchial biopsy specimen may contain pools of mucin, very few neoplastic cells with a relative lack of atypia that makes the diagnosis of adenocarcinoma more difficult as observed by Butnor.<sup>18</sup> Majority of the previous studies that have used other techniques such as rebronchoscopy, surgery, computed tomography-guided fine-needle aspiration cytology, tumor markers, and autopsy, to prove the cases of lung cancer have shown that bronchial biopsy does not provide diagnostic yield in all cases of lung cancer. Chances of missing the diagnosis by bronchial biopsy are more in peripheral lung tumors.

### CONCLUSION

Although it is difficult to derive a definite conclusion from such a small sample of subjects in a single-centered study and analysis of a larger cohort from multiple institutions including patients from different geographical regions would reflect the true pattern, we found post-bronchoscopy sputum cytology had no additional benefit with respect to BAL and brush cytology (in addition to bronchial biopsy) for an early and accurate diagnosis of lung malignancy.

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### Authors Contribution:

**AB-** Concept and design of the study, collected the data, and made the initial manuscript; **MP-** Interpreted the results, reviewed the literature, and corrected the manuscript; **SKB-** Statistical analysis and interpretation; and **RMM-** Concept, coordination, final manuscript preparation, and revision.

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