Comparative Cytological Techniques in the Diagnosis of Non Neoplastic and Neoplastic Bronchopulmonary Lesions

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Abstract
Objective: Diagnostic cytology is one of the suitable modality for both non-neoplastic and neoplastic conditions of the lung. The aim is to use different cytological methods in the diagnosis of lesions of the respiratory tract. It has been generally acclaimed as one of the most successful application in the evaluation of patient with suspected lung malignancy.

Materials and Method: It was a prospective study of 127 cases. The present study was conducted in the department of pathology at a tertiary care hospital. It is a cross-sectional study, carried out on all patients who were admitted with lung diseases during a period of two years. Both male and female patients of all age group with the specified respiratory tract complaints like cough, breathlessness, hemoptysis, fever and chest pain were subjected to bronchoscopic examination.

Results: There were 81 males and 46 females. Male : female ratio was 1.76 : 1. The mean age was 50±16.14 years. The lowest age was 14 years and the oldest age was 85 years. It was included in both the non-neoplastic and neoplastic study groups. Sensitivity of BB was 91.8%; while that of BAL was only 59.2%. specificity of BB was 87.5% and that of BAL was 75%. Accuracy of BB was 90.7% while that of BAL was 63.1%.

Conclusions: Bronchial brushing is a much superior technique in the diagnosis of bronchopulmonary lesions. It demonstrates far better sensitivity, specificity and accuracy. Combination of various cytohistological techniques complements each other and enhances the diagnostic efficacy of various non-neoplastic and neoplastic lung diseases.

Keywords: cytology, lung, techniques.

Introduction
Cytology of the pulmonary lesions provides valuable diagnostic information. These cytological procedures constitute the most useful and least expensive investigative tools available for the detection of pulmonary diseases. Respiratory tract cytology is well established throughout the world as a vital diagnostic procedure in the evaluation of any patient with suspected pulmonary lesions especially lung cancer. The aim of this study is to assess the efficacy and diagnostic importance of BAL/bronchial washings, brushings when compared to biopsy in the investigations of patients who are suspected of having any bronchopulmonary lesion both clinically and radiologically.
Materials and Method
This study was carried out prospectively in the department of Pathology at a tertiary care hospital. It was a hospital based cross-sectional study. The patients had one or more of the following features; growing peripheral lesion on chest X-ray, positive sputum cytology, and clinical symptom refractory to medication or visible endobronchial mass.
Detailed clinical history, physical examination, hemogram, chest X-ray and bronchoscopy was performed on all the cases. All the subjects were informed about the study and a written consent was obtained from all.
Paediatric patients, patients with bleeding disorders or recent myocardial infarction were excluded from the study.
Bronchoscopy was performed through the transnasal approach, using an Olympus BF- 2TR fibreoptic bronchoscope.
BAL was obtained by introducing a bronchoscope in the lower respiratory tract and specimens obtained by means suction apparatus after infusing 60ml or more saline. A minimal volume of 5 ml of a pooled BAL sample was needed for BAL cellular analysis.
Bronchial washings was obtained by repetitive instillation of 3-5 ml of a sterile balanced salt solution through the bronchoscope and reaspiration of fluid. Brushings (BB) are obtained by the use of a small circular stiff-bristle brush.
Materials obtained by broncho-alveolar lavage and brushing were fixed by various fixatives. The slides were stained with May-Grünwald-Giemsa (MGG), Papanicolaou (Pap), and hematoxylin and eosin (H and E) stains. The overall cytodiagnostic efficacy as well as that of various cytological samples were analyzed. Endobronchial biopsy was performed using a flexible long biopsy forceps and tissue bits were fixed in 10% formalin and processed for histopathological examination.

Results
The present study group comprised of 127 patients. The age of patients ranged from 14 yrs to 85 yrs. There were 81 males and 46 females. Male: female ratio was 1.76:1. There were 73 smokers and 08 non-smokers.
The most common presenting complaint was cough followed by breathlessness. The laterality of lung lesion, most commonly was in right lung and right upper lobe was most commonly involved.
Neoplastic lesions was diagnosed in 49 patients and non-neoplastic lesions were seen in 78 patients. Biopsy was done in 65 cases (TABLE I).

| Table no. I Diagnostic accuracy of different modalities in different lung lesions
<table>
<thead>
<tr>
<th>Type of carcinoma</th>
<th>BAL</th>
<th>Brushing</th>
<th>Biopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus</td>
<td>3</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>atypia</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>candida</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>dysplasia</td>
<td>19</td>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td>candida</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>eosinophilia</td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>granuloma</td>
<td>1</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>Hydatid cyst</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Alveolar proteinosis</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>inflammation</td>
<td>80</td>
<td>25</td>
<td>4</td>
</tr>
<tr>
<td>mucor</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>6</td>
<td>15</td>
<td>19</td>
</tr>
<tr>
<td>Adenosquamous</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
</tbody>
</table>

Bronchial washing showed 29 true positive (TP), 12 true negative (TN), 4 false positive (FP) and 20 false negative (FN) cases whereas bronchial brushing showed 45 TP, 14TN, 2 FP and 4 FN cases as confirmed on biopsy (TABLE II).
Table No. II Diagnostic evaluation of various lesion confirmed on Bronchial washing and bronchial brushing

<table>
<thead>
<tr>
<th>Sample</th>
<th>Test result</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TP</td>
<td>TN</td>
<td>FP</td>
<td>FN</td>
<td>Total</td>
</tr>
<tr>
<td>Bronchial washing</td>
<td>29</td>
<td>12</td>
<td>4</td>
<td>20</td>
<td>65</td>
</tr>
<tr>
<td>Bronchial Brushing</td>
<td>45</td>
<td>14</td>
<td>2</td>
<td>4</td>
<td>65</td>
</tr>
</tbody>
</table>

Bronchial brushing showed good sensitivity (91.8%) and specificity (87.5%) compared to bronchial washing which had sensitivity of 59.2% and specificity of 75%. Similarly, the positive predictive value (PPV), negative predictive value (NPV), false negative index (FNI) and false positive index (FPI) of BB were better in brush samples than washings. The accuracy of BB was 90.7 while that of washing was 63.1. morphologic preservation was better in brushing specimens compared to washings. (TABLE III)

Table no. III Comparison of Indices of Bronchial washing and brush cytology.

<table>
<thead>
<tr>
<th>Indices</th>
<th>Washing</th>
<th>Brushing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>59.2 (0.45 – 0.70)</td>
<td>91.8 (0.83 – 0.97)</td>
</tr>
<tr>
<td>Specificity</td>
<td>75 (0.63 – 0.85)</td>
<td>87.5 (0.77 – 0.94)</td>
</tr>
<tr>
<td>PPV</td>
<td>87.8 (0.77 – 0.94)</td>
<td>95.7 (0.87 – 0.99)</td>
</tr>
<tr>
<td>NPV</td>
<td>37.5 (0.25 – 0.50)</td>
<td>77.8 (0.66 – 0.87)</td>
</tr>
<tr>
<td>Accuracy</td>
<td>63.1 (0.50 – 0.74)</td>
<td>90.7 (0.81 – 0.96)</td>
</tr>
</tbody>
</table>

Bracketed figure indicate 95% confidence interval.

The sample size was taken as 65 as only 65 patients underwent all the three interventions BAL/BW, BB AND biopsy. Hence, McNemar’s chi-square sample test was applied.

The cancer detection by BAL/BW gave mcnemar’s chi-square = 11.56 and p-value = 0.0007. (Table IV)

The cancer detection by BB gave mcnemars chi-square=1.29 and p-value = 0.4531. (TABLE V)

Table No. IV Comparison of diagnostic accuracy of Bronchial washing and Biopsy finding.

<table>
<thead>
<tr>
<th>Biopsy Finding</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Bronchial washing</td>
<td>29</td>
</tr>
<tr>
<td>Negative</td>
<td>20</td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
</tr>
<tr>
<td>McNemar’s Chi2</td>
<td>11.56</td>
</tr>
<tr>
<td>p-value</td>
<td>0.0007, HS</td>
</tr>
</tbody>
</table>

Table V Comparison of Diagnostic Accuracy of Bronchial Brushing and Biopsy Findings

<table>
<thead>
<tr>
<th>Biopsy Finding</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Bronchial brushing</td>
<td>45</td>
</tr>
<tr>
<td>Negative</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
</tr>
<tr>
<td>McNemar’s Chi2</td>
<td>1.29</td>
</tr>
<tr>
<td>p-value</td>
<td>0.4531, NS</td>
</tr>
</tbody>
</table>
Discussion
Due to new therapeutic options in thoracic oncology, pathological diagnosis of different bronchopulmonary lesions has become more challenging.

With the advent of flexible fiber-optic bronchoscope, respiratory cytology has taken a new turn as samples like bronchoalveolar lavage, bronchial washings and bronchial brushings could be collected from the respiratory tract which yield significant amount of cytological material.

In view of compliance and to some extent cost-effectiveness in investigations, bronchial brushings, washings and lavage has been preferred by many clinicians as well as pathologists.
The present study was comprised of 127 patients. The age of the patients ranged from 14-85 yrs. Most of the patients were in the age group of 61-70 yrs (22.83%) with mean age of 50.15 ±16.14. There were 81 males and 46 females and the M:F ratio was 1.76:1. In a similar study by Choudhary m et al(1) the age ranged from 18 – 88 yrs and M : F ratio was 3.3:1.

Non- neoplastic lesions
In this study there were a total of 78 non-neoplastic lesions in which there are 45 males and 33 females and the M : F is 1.36:1. Most of the patients were in the age group of 41-50 yrs with mean age of 45.30±16.54 yrs. In a similar study by Tuladhar et al patients ranged in the age group from 19 to 74 yrs with a mean of 50.80±7.7 yrs and M : F ratio was 3.7:1. Similarly, Shiner et al found the male : female ratio to be 1:1 in non-neoplastic patients in their study.(2,3)

In our study we came across three cases of aspergillosis. Out of three there were two cases diagnosed on bal/wash specimens in which septate hyphae with acute angles were seen and they were later confirmed on biopsy. There was one case detected with squamous cell carcinoma on biopsy. The bb specimens only showed inflammation. There were two cases of candida diagnosed on bal/bw and bb only showed inflammation. There was a case of mucor which was diagnosed on biopsy consisting of non-septate, obtuse angle hyphae. The bal/bw and bb showed only inflammatory infiltrate.

In our study, 30 cases (38.46%) were of acid fast bacillus positive tuberculosis. There were no case identified by bronchial brushings and all showed inflammatory exudates. However, 2 out of 30 cases of tuberculosis were identified by washing and rest only showed inflammation. In a study by Wallace et al on proven cases of tuberculosis he found bronchoscopic specimens mostly have a non-specific chronic inflammatory reaction4,1. In Altaf bach et al.5,1 study bronchial wash smear was positive for acid fast bacilli in 35% of cases. Similarly, Purohit et al.6,1 and Kulpati et al.7, 1 demonstrated acid fast bacilli in 42% and 40% resply(1).

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There were one each case of hydatid cyst, Loefflers syndrome (FIG I) and Alveolar proteinosis (FIG II). Both bronchial washings and brushings was normal but biopsy showed hydatid cyst with laminated membrane and hooklets. A patient, 22 yrs old came with the history of cough and cold since five days which was serous initially but later turned out to be mucoid. Her bal/bw specimen consist of pink, eosinophilic, granular material where as brush only showed inflammation with bronchial epithelial cells. Later biopsy was done which confirmed alveolar proteinosis which consists of PAS positive pink lipoproteinaceous, granular and eosinophilic material. Eosinophilia was seen both in wash and brush which was diagnosed as Loefflers syndrome.

NEOPLASTIC lesions
There were 49 malignant neoplastic lesions. The diagnosis of the patients were confirmed by cytological and histological examinations. Male patients accounted for 73.46% and female patients for 26.53% with male:female ratio was 2.8:1. There was a male preponderance in the present study.

In similar studies done by Andrews et al(8), M:F ratio was 6.8:1 where as Mc Duffie et al(9) reported M:F ratio of 3.7:1. The age of the clinically confirmed lung cancer patients ranged from 29 to 85 years, with mean age of 58.48±11.21. The youngest patient was a male aged 29 years and the oldest was also male aged 85 years.

In a study by Tuladhar et al(2) (2011), the mean age of the patients was 59±11 yrs. A study done in Kathmandu, showed similar features with age range from 40 yrs and 78 yrs, with mean age of 59 yrs. Vital statistics of the United States (1997) reported that majority of bronchogenic carcinoma are between the age groups 55 – 74 years10. In our study the majority of the patients were in the age group of 61-70 years. Malhotra et al
(1986) reported that majority of the lung tumours occurred in fifth, sixth and seventh decades with the peak incidence in sixth decade\(^\text{11, 10}\). Similarly in the recent studies done by Al Rawi f. a. z. (2004)\(^\text{12}\) and Gaurds et al (2007)\(^\text{13}\), majority of the cases were in the age group of 61-70 yrs. In a study by Vivekanand et al (2014), the majority of the cases were in fifth and sixth decade and the peak incidence was seen in sixth and seventh decade\(^\text{10}\).

In the present study there were total of 73 smokers all were male patients with 27 male patients having smoking history of >40 pack years with p-value of 0.0001. In SPORE (specialized program of research excellence) study with a high risk cohort of patients with chronic obstructive pulmonary disease and >40 pack year smoking history, dysplasia was present in 25% of cases and subsequent follow up study showed malignancy in 36% of cases. So smoking history of >40 pack years is very often associated with risk of carcinoma. There were 8 male patients and all the female patients were non-smokers. A. Vigg and associates reported male to female ratio of about 6:1 and 62% ex-smokers, 10% current smokers and 28% non-smokers amongst males\(^\text{10}\). This high incidence in males could be due to the higher prevalence of smoking in males.

The most common clinical manifestation of lung cancer in present study is cough followed by breathlessness. In a similar study by Arora et al\(^\text{14}\) (1990), the common clinical manifestation reported was cough (92%), hemoptysis (29%), chest pain (52%), breathlessness (40%). Similarly in a study by Dr. Sridhiva et al\(^\text{15}\), the common clinical manifestation was cough (100%) followed by fever (75%). Vivekananda et al\(^\text{1}\) presented with common symptoms of cough of long duration and chest pain, and very few cases presented with hemoptysis as seen in our study where hemoptysis was presented by only 7 cases of lung cancer.\(^\text{16, 10}\)

In the current study, right lung is more affected than left lung in which right upper lobe was commonly affected. In a study by Vivekananda et al\(^\text{10}\), lung cancer mostly occurred in the right lung (56.6%) as compared to the left lung (43.30%) with a right lung, left lung ratio of 1.3:1. Again in a study by Dr. Sridhiva et al\(^\text{15}\), right lung was more affected than left. In the right lung it was the right main bronchus that was largely affected.

In the diagnosis of 49 malignant lesions, bronchial washings yielded 17 positive cases and the remaining 32 cases showed dysplasia (11 cases), atypia (2 cases) and inflammatory smear (19 cases) where as bb gave positive results in 42 cases and dysplasia was seen in 4 cases, inflammation was seen in (4 cases). There was a case in which squamous cell carcinoma was diagnosed by both bal/wash and bb but only necrotic tissue was seen. One case of squamous cell carcinoma also had aspergillus. It was included in both the neoplastic and non-neoplastic study group. In a case of adenosquamous carcinoma which was confirmed by biopsy both bal/wash and bb specimens showed adencarcinoma.

In the present study the diagnostic accuracy of bronchial washings is low when compared to brushings. Which coincides with the study of Miep A, Vander Drift et al. in 2005\(^\text{17}\). The low diagnostic results in washing is due to the following reasons. 1.) Tumors just confined to bronchial wall not infiltrating into lumen. 2) Tumor necrosis 3) Lack of exfoliation due to poor communication of tumor with bronchus secondary to bronchial stenosis. 4) Exclusion of atypical or highly suspicious cell from positive criteria. 5) Inadequate collection of material.

The present sample when analysed by using combination of washing and brushing cytology and confirmed on biopsy the gold standard, adenocarcinoma (FIG III) is the commonest type of lung tumour 42.9% (21 cases), followed by squamous cell carcinoma 38.8% (19 cases), small cell carcinoma 6.1% (3 cases), poorly differentiated carcinoma 4.1% (2 cases) and one each case of carcinoid carcinoma, large cell carcinoma, adenosquamous carcinoma and pleomorphic carcinoma confirmed
by biopsy. This is closely compared with a study by Vivekananda et al\textsuperscript{[10]}, where adenocarcinoma was the most commonly diagnosed tumor at 34.82\% followed by SCC 31.02\%. But in many other studies like in Anupam Sharma et al\textsuperscript{[18]}, Choudhary m et al\textsuperscript{[1]}, dr. Srividhya et al\textsuperscript{[15]} and many more, SCC was the most common carcinoma followed by adenocarcinoma.

Tamboli P and Ro. J.Y (Lung cancers, M.D. Anderson Cancer care series, 2003) stated squamous cell carcinoma used to be the most common type of lung cancer accounting for 25-45\% of all lung tumors but the incidence of adeno-carcinoma has significantly has increased in the last two decades; 25-40\% of lung carcinoma are now classified as adeno-carcinoma and this tumor is now the most common form of lung cancer in women and in many studies, in men as well\textsuperscript{[19]}.

In a study by Tuladhar et al\textsuperscript{[2]}, the most common carcinoma was SCC (51\%), followed by small cell carcinoma (19\%) and then adenocarcinoma (11\%).

In our study, the Sensitivity, Specificity and Accuracy of BAL/bw samples were 59.2\%, 75\% and 63.1\% respectively. The sensitivity of bronchial aspirates in diagnosing lung cancers has been 75 to 88.1 \% at various centres.\textsuperscript{[20,21,22]} Truong et al\textsuperscript{[23,13]} reported Sensitivity of 66.0\%; while Ng. & Horak\textsuperscript{[24]} reported a Sensitivity as high as 74.0\% for BAL. Studies have shown that increasing the number of attempts obtaining BAL/bw sampling can improve its Sensitivity, Specificity and Accuracy.\textsuperscript{[23,20,25]}

The sensitivity, specificity and accuracy of bb were reported as 91.8\%, 87.5\% and 90.7\%. Studies from various authors show the range of sensitivity for bb from 67\% to 97.3\%.\textsuperscript{[26,27,28]}

Similarly various other workers in the past, like Chopra et al\textsuperscript{[36]} (86.3\%); Zavala et al\textsuperscript{[29]} (88.5\%); and Solomon et al\textsuperscript{[30]} (89.1\%), Shroff et al\textsuperscript{[28]} reported the Sensitivity of BB to be as high as 97.3\% in their study. Hence, they reported the Sensitivity of BB to be much higher (98\%) which was comparable to our study.

The specificity of bal/bw in other studies like Choudhary m et al\textsuperscript{[1]} is 47.6\%, Gaur ds et al\textsuperscript{[13]} is 89.6\% and accuracy is 57.1\% and 71.4\% respy comparable to our study. Similarly in bb the specificity and accuracy in the study of Choudhary m et al is 85.7\% and 82.8\% respy. The two studies of Gaur ds et al have reported similar results in which the first study gave specificity and accuracy of 77.8\% and 80.6\% and the second study gave the specificity and accuracy as 97.6\% and 93.9\% respy as seen in our study. In another study by Anupam sarma et al\textsuperscript{[18]} the specificity and accuracy of bal/bw was 96.6\% and 87.3\% respy.

With a good Sensitivity (87.3 \%), Specificity (97.6 \%) and Accuracy (93.9 \%), bronchial brushing promises to be a very convenient cytological technique that can be confidently utilized for screening of doubtful cases and early diagnosis of lung cancer, as it saves the time needed for the processing of biopsy specimens.

**Conclusion**

The advent of fiberoptic bronchoscopy has greatly facilitated the detection and diagnosis of bronchopulmonary lesions. Although it has been shown that the combined use of cytology and biopsy renders the highest probability of detecting malignancy, the merit of routine brush cytology has been questioned since it appears to duplicate biopsy.

With increased experience and adherence to strict cytological criteria for malignancy, it can be effectively detected in early stages and possibly cured.

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