

Correlative Study of Imprint and Crush Cytology with Histopathology in Endobronchial Growths

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Abstract

Objective: This study was undertaken to study the correlation between imprint cytology, crush smears and histopathology in endobronchial growths using fiberoptic bronchoscopy.

Methods: The study was conducted in the Department of Internal Medicine and Pathology at Sher-i-Kashmir Institute of Medical Sciences. Eighty-one patients with clinical and radiological evidences of lung lesions were enrolled. From all patients five pieces of tissue were obtained during fiberoptic bronchoscopy which revealed an endobronchial growth. From each bit of tissue an imprint and crush smear were prepared by imprinting the tissue on a clean surface of glass slide without compressing the tissue while as crush smear was made by gently crushing the tissue between two glass slides. The tissue specimens were then placed in formaldehyde solution for histological examination. Standard statistical methods were used to analyze the data (chi-square (χ^2) analysis; a Fisher's exact test was used to evaluate the data if more than 25% of the expected values were <5). The level of significance selected was $P < 0.05$.

Results: Overall 49 cases (60.49%) were diagnosed as malignant and 31 cases (38.2%) as benign on histopathology. In one case with active bleed, smears were taken from blood clots and bronchoalveolar lavage was done, both of which revealed malignancy. Forty histopathologically proven malignant cases were positive for malignancy (81.6%) on crush smear cytology, while nine cases were not. Imprint smears were positive for malignant cells in 41 cases out of 49 histologically proven malignant cases (83.6%) and eight cases did not show malignant cells on imprint. The sensitivity, specificity, positive predictive value and negative predictive value of crush smear results were 81.6%, 100%, 100% and 77.5% respectively.

Conclusion: Imprint and crush cytology are rapid, reliable and accurate techniques. The technique can improve the diagnostic accuracy in endobronchial lesions, as it is faster and more cost effective.

Keywords: Imprint and crush; Endobronchial growth; Bronchoscopy; Histopathology

Introduction

Lung cancer has emerged as the most common form of malignant disease and leading cause of cancer deaths in developed as well as in developing countries [1,2]. In Kashmir, lung cancer is the second most common cancer with high mortality [3]. Smoking remains the leading cause of lung cancer; other risk factors include air pollution, occupational exposures, radiation and genetic factors [4,5]. Prompt and early diagnosis of lung cancer plays a pivotal role in early treatment, planning, management and outcome of patients. Although various investigations like computerized tomography (CT) scan are routinely adopted, imprint cytology has revolutionized and facilitated the diagnosis of tumors [6,7] including those of breast [8], gastrointestinal tract [9], lymph nodes [10] and bone marrow [11]. Imprint cytology can also be used as an intraoperative tool for deciding the extent of surgery a patient has to undergo [12].

The first realization that cancer of the lung could be accurately diagnosed and typed by the microscopic study of expectorated cells is generally attributed to Dudgeon and Patrick [13], Dudgeon and Barret [14]. Other authors have stressed the utility of this technique on malignant and non-malignant lesions. Most of the studies have been confined to lesions in lymph nodes and breast [8,10]. In the case series of thyroid lesions reported by Sakai et al. [15] an accuracy of 95.5% was achieved. Staniszevski et al. [16,17] have done intraoperative cytology during pulmonary surgery with an accuracy of 84%. Pulmonary cytology and histopathology are valuable tools in the diagnosis of

lung malignancies. Fibreoptic bronchoscopy was introduced in 1968 as a diagnostic procedure. Since then, apart from sputum, different methods for obtaining satisfactory specimens have become available like bronchial brushings, imprint and crush smears. Although the examination of sputum can provide evidence of malignancy, its sensitivity for accurate diagnosis is only 65% [18,19] as compared to the specimens collected by fiberoptic bronchoscope which yield a higher positivity rate of 74%, thus revolutionizing the diagnosis of lung cancers. Moreover, specimens obtained by bronchoscopy can be used for cytological examination [20,21]. It should, however, be emphasized here that fiberoptic bronchoscopy when combined with pulmonary cytology enhances the sensitivity significantly to 88% [22]. Okubo et al. [23] showed that touch imprint cytology could be superior to conventional histopathology in the identification of a small proportion of cancer cells against a background of non-malignancy.

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The aim of this study was to evaluate the diagnostic accuracy of imprint and crush smear cytology being faster and cost effective in a low resource setting in the diagnosis of lung cancer. The cytological diagnosis was verified against the histological diagnosis which was considered as the gold standard. The imprint and crush smear technique is simple, cost-effective and appear reliable as other methods such as frozen section. Since the cytological reports are available same day while as histopathology takes 7-8 days, the treatment can be planned earlier.

Material and Methods

The study was conducted in the Department of Medicine and Pathology at Sheri-Kashmir Institute of Medical Sciences from October 2006 to February 2007. Patients were selected from those referred for bronchoscopy with radiological and clinical evidence of lung lesions to the Department of Medicine. Patients were considered for bronchoscopy who presented with shortness of breath, hemoptysis, cough, chest pain and a radiological evidence of mass on chest radiograph, which was confirmed on a computed tomography scan (CT) with contrast enhancement. A platelet count of <40,000/cubic millimetre and an oxygen saturation of >85% were regarded as contraindications for performing bronchoscopy. An informed consent was routinely obtained from the enrolled patients. Fiberoptic bronchoscopy was done with PENTAX-15 introduced transnasally under 2% topical xylocaine. The patient was kept on continuous cardiorespiratory monitoring during the procedure.

Eighty-one patients with endobronchial growths seen on bronchoscopy were biopsied with the biopsy forceps of PENTAX-15; no radiological guidance was used. Five to six bits of tissue were obtained from each patient. From each bit of tissue an imprint and crush smear were prepared and rest of the tissue was sent for histopathology. The imprint smear was prepared by imprinting the tissue on clean surface of a glass slide without compressing the tissue while as crush smear was made by gently crushing the tissue between the two glass slides to generate a uniformly spread smear. The smears (imprint and crush) were fixed in 95% alcohol and later stained by Papanicolaou stain. The tissue specimens were then placed in formaldehyde solution (10% formalin) for histological examination.

The smears were read by a pathologist and categorized as negative and positive for malignant cells. Definite differentiation of type of tumor cells was not done based on smears. Two independent pathologists reported the histopathology and cytology and subsequently the results of the study were statistically analyzed.

Standard statistical methods were used to describe and analyze the data. Categorical data consisting of more than two categories were evaluated by chi-square (χ^2) analysis; a Fisher's exact test was used to evaluate the data if >25% of the expected values were <5. For calculating sensitivity, specificity and related parameters, methods of Galen and Gambino were used, as follows: sensitivity= $TP/(TP+FN) \times 100$; specificity= $TN/(TN+FP) \times 100$; false positive rate (FP)= $FP/(FP+TN) \times 100$; false negative rate (FN)= $FN/(FN+TP) \times 100$; PV of a positive result= $TP/(TP+FP) \times 100$; PV of a negative result= $TN/(TN+FN) \times 100$; prevalence rate= $(TP+FN)/(TP+TN+FP+FN) \times 100$. The overall diagnostic accuracy is the probability of the patients being correctly identified as true positive and true negative by the cytological test. The level of significance selected was $P < 0.05$.

Results

Of the 81 patients biopsied, 64 were males and 17 were females with

a mean age of 59 years (range 35-76 years). Sixty-six (81.5%) patients were smokers and in 15 patients (18.5%) the smoking history was not available. The biopsy specimens were diagnosed as malignant in 49 (60.49%) and benign in 31 (38.2%) cases on histopathology. Of these, 40 (81.6%) were positive for malignancy on crush smear cytology while 9 (18.4%) cases were not picked up on the crush smear (Figures 3 and 4). Imprint smears were positive for malignant cells in 41 (83.6%) of 49 histologically proved malignant cases; 8 cases did not show malignant cells on imprint smear (Figures 1 and 2). The sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of crush smear results were 81.6%, 100%, 100% and 77.5% respectively (Table 1). As shown in Table 2, the difference between the crush positive and negative in the two groups and Histopathological examination (HPE) positive and negative were statistically significant ($p < 0.0005$).

Imprint cytology showed a better positive yield with sensitivity, specificity, PPV and NPV of 83.6%, 100%, 100% and 79.4% respectively (Table 3). Of the 49 cases of malignancy diagnosed on histopathology,

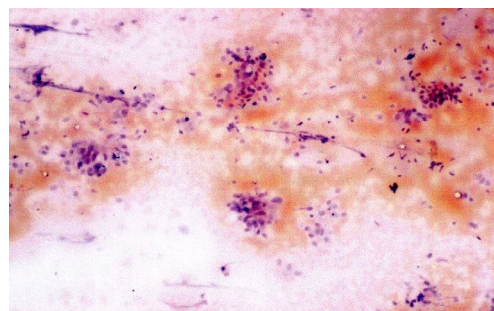


Figure 1: Scanning view of imprint smear of bronchial tissue showing groups of epithelial cells and few inflammatory cells. Pap stain (x- 100).

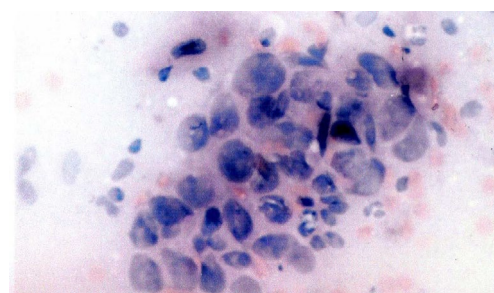


Figure 2: Imprint smear showing groups of malignant cells with marked nuclear pleomorphism Pap stain (x- 400).

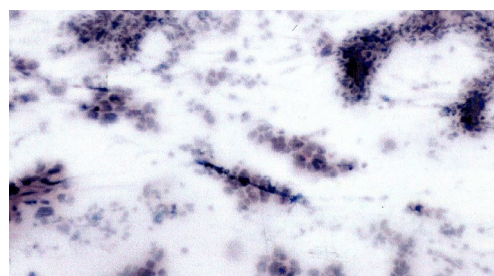


Figure 3: Scanning view of crush smear showing malignant bronchial epithelial cells and few inflammatory cells Pap stain (x- 100).

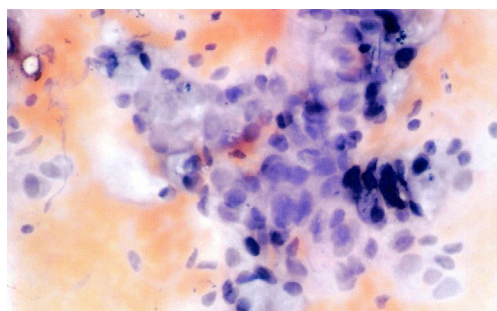


Figure 4: High power view of crush smear showing malignant cells and few inflammatory cells Pap stain (x- 400).

	HPE +ve	HPE -ve (31)
Crush +ve	40	0
Crush -ve	9	31
TOTAL	49	31

HPE- Histopathology, +ve-positive, -ve-negative
 Sensitivity- 81.6%
 Specificity- 100%
 Positive predictive value- 100%
 Negative predictive value- 77.5%

Table 1: Crush smear results in HPE +ve cases.

	HPE +ve (n=49)	HPE -ve (n=31)
Crush +ve	40 81.6%	0
Crush -ve	9 (18.4%)	31 (100%)

P value (Yates χ^2)= <0.0005 ; Yates χ^2 =47.4

Table 2: Crush smear results in HPE +ve cases.

	HPE +ve (49)	HPE -ve (31)
Imprint +ve	41	0
Imprint -ve	8	31
TOTAL	49	31

Table 3: Imprint smear result in HPE +ve cases.

	HPE +ve (n=49)	HPE -ve (n=31)
Imprint +ve	41 (83.7%)	0
Imprint -ve	8 (16.3%)	31 (100%)

P value (Yates χ^2)= <0.0005 ; Yates χ^2 =49.9

Table 4: Imprint smear result in HPE +ve cases.

17 were small cell carcinoma, 30 were squamous cell carcinoma and 2 were adenocarcinoma. As shown in Table 4 statistical analysis of imprint smear results of HPE positive and negative cases showed a significant difference between the two approaches ($p=0.0005$).

Discussion

Pulmonary cytology and histopathology are valuable tools in the diagnosis of lung malignancies. Fiberoptic bronchoscopy was introduced in 1968 as a diagnostic procedure. Since then, apart from sputum, different methods for obtaining satisfactory specimens have become available like bronchial brushings, imprint and crush smears. Although the examination of sputum can provide evidence of

malignancy, its sensitivity for accurate diagnosis is only 65% [18,19] as compared to the specimens collected by fiberoptic bronchoscope which yield a higher positivity rate of 74%, thus revolutionizing the diagnosis of lung cancers. Moreover, specimens obtained by bronchoscopy can be used for cytological examination [20,21]. It should, however, be emphasized here that fiberoptic bronchoscopy when combined with pulmonary cytology enhances the sensitivity significantly to 88% [22-27].

In this study, we had 81.6% - 83.6% sensitivity of crush and imprint cytology, respectively. These results were concordant with the studies conducted by Wolfgang et al. [28] and Paulose et al. [33] who showed sensitivity between 84% and 89 %. We found imprint technique quick and easy to perform, however, there were some false-negative results which could be due to factors like poor smear quality, error in fixation drying artifact, bloody smear, necrotic tissue, fatty or tumor tissue that was fibrosed and/or very scanty tumor cells.

There is a need to find rapid and reliable diagnostic methods, which have high yield of positive and accurate results. Techniques like sputum examination and bronchial washings are inappropriate [25,26] since the site of the specimen cannot be properly examined[27]. Screening is very time-consuming and the number of positive cells in early lesions is relatively low. Degenerative processes have occurred in the body long before the tumor cells can be treated with fixative [28]. The lesion can be visualized by bronchoscope, and the location of the specimen can be mapped precisely. Viable cells may be obtained and fixed immediately for detailed cytologic examination. In cytological examinations, the quality of results depends on the skill of the surgeon and the level of cytopathologic expertise [29].

The imprint and crush smear technique is simple, cost-effective and appears as reliable as other methods such as frozen section [7,30]. Moreover, several authors have reported that imprint cytology has superior quality to frozen section histopathology, especially for small specimens where there is a sampling error and potential loss of cryo sectioning of lesion tissue, which is necessary for a permanent histopathological diagnosis [31]. The malignant component of a core biopsy specimen may be lost during the processing of permanent section, but imprint smear preserves the malignant cells regardless of the small specimen[32]. The cytological reports are available either the same day or a day after while as histopathology takes 7-8 days. It, therefore, helps a clinician to plan treatment one week earlier.

Imprint and crush cytology is helpful in diagnosing lung malignancy but we did not attempt the differentiation of type of tumor cells based on smear cytology. The superiority of imprint smear cytology over crush smear cytology may be due to fewer artifacts in preparing the smear. Therefore, imprint and crush cytology when used in conjunction, enable early diagnosis of lung cancer. The technique (especially imprint) is quick, sensitive and highly specific method for detecting lung malignancies.

In conclusion, imprint and crush (especially imprint) cytology are easy and reliable methods that can be used to provide quick diagnosis. The imprint cytology technique is quick, reliable, sensitive and highly specific method of detecting lung malignancies. Diagnosis becomes clear at least 7 to 10 days earlier, which helps in planning management faster.

Conflict of interest: none declared.

Summary

Article focus

The article focuses on the need to incorporate imprint and crush smear cytology in conjunction with histopathology in order to improve the diagnostic yield of bronchoscopy. It also emphasizes the importance of time in planning the management, which ultimately affects the outcome of these patients.

Key messages

- The technique is quite simple and does not need any additional sophisticated equipment.
- The imprint and crush smear techniques are quick, reliable, sensitive and highly specific methods for diagnosing lung malignancy.

Limitations of our study

- On average, the cytology results in this study were available only after approximately 12-18 hours. Availability of imprint cytology results in much shorter intervals (55 minutes) has been reported in literature[34].
- Definite categorization of the lung tumors was not done on smear cytology.

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