

# Role of immunohistochemistry in cell block sections for categorization of non-small cell carcinoma of lung

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## Abstract

**Background:** Computed tomography (CT) guided fine needle aspiration and cytology (FNAC) is a useful modality which aids in early and fairly accurate diagnosis of mass lesions of lungs specially the malignant ones. Previously in patients with primary lung cancer the most important consideration was to discriminate between small cell and non-small cell carcinoma (NSCC) of the lung. With advancement of cancer therapy, sub-typing of NSCC has become very important. The aims of the study were to evaluate the pathological spectrum of the pulmonary diseases in CT guided FNAC of lung mass lesions and utility of limited immunohistochemistry (IHC) in cell block sections to subcategorize NSCC.

**Methods:** The study was carried out at Department of Histopathology in Armed Forces Institute of Pathology from 1<sup>st</sup> January 2018 to 31<sup>st</sup> December 2018. It involved total 140 patients who underwent CT guided FNAC in Combined Military Hospital, Dhaka for lung mass lesions. Aspiration was done by pathologist and obtained material was used to make slide smears and cell blocks. Both FNAC smears and cell block sections were examined by histopathologists. The entities diagnosed as NSCC and poorly differentiated carcinomas in FNAC were treated with limited IHC panel in cell block sections.

**Results:** Out of 140 cases 110 were male and 30 were female patients. Age range was from 18 to 85 years, (mean age 60±13.5). Total 83(59%) malignant neoplasms and 57(41%) benign lesions were diagnosed. Among the benign lesions, 28 were diagnosed as granulomatous lesion suggestive of tuberculosis and rests were non-specific inflammatory lesion. In FNAC smear, small cell carcinoma was diagnosed in 16.9% (14/83) of all malignant lesions, squamous cell carcinoma (SCC) in 26.5% (22/83) patients and adenocarcinoma in 22.9% (19/83) patients. Twenty-five patients were categorized as NSCC as they could not be further subcategorized in FNAC as SCC/adenocarcinoma. Three patients were categorized as poorly differentiated carcinoma as their cytological features could not rule in/out small cell carcinoma. NSCC and poorly differentiated carcinoma diagnosed by FNAC were 33.7% (28/83). Cell blocks of all of them were examined, three of NSCC cell blocks were found inadequate for evaluation. All of the rest cases underwent limited IHC panel in cell block sections and were further subcategorized; 13 as SCC and 12 as adenocarcinoma. Total cases of SCC diagnosed in the study after IHC on cell block sections were 35/83(42%) followed by adenocarcinoma 31/83, (33.35%).

**Conclusion:** CT guided FNAC is an effective tool and can be used for fairly accurate and early diagnosis of lung mass lesions. Immunohistochemistry on cell block sections can be used as an effective tool for categorization of NSCC.

**Key words:** cell block, immunohistochemistry, non-small cell carcinoma.

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## Introduction

Fine-needle aspiration cytology (FNAC) is a well-established modality for cytological diagnosis of lung mass lesions, especially with the guide of computed tomography (CT) scan. FNAC was first used by Martin and Ellis as a diagnostic tool.<sup>1</sup> Transthoracic FNAC has been recognized since the 1970's as a critically important diagnostic technique. In 1976, Hagg and Alfy reported CT guided FNAC to be more effective and accurate for diagnosis of lung mass lesions as more accurate localization and site of needle could be seen at the time of aspiration.<sup>2</sup>

The great advantage of FNAC is that it is relatively easy, less invasive and can spare a more invasive surgical intervention. Previously management of lung cancer patients was decided basing on classification of small cell or non-small cell carcinoma (NSCC) type. This distinction is possible in more than 95% of cases in FNAC smears.<sup>3</sup>

With advent of newer therapeutic approach classification of non-small cell carcinoma has become utmost important and new guide line advocates to subclassify this entity as squamous cell carcinoma/adenocarcinoma.<sup>4</sup> The present 2015 World Health Organization (WHO) classification of lung cancer recommends use of immunohistochemistry (IHC) to subtype all NSCC cases that cannot be classified based on morphology alone.<sup>5</sup>

Most patients with lung carcinoma are elderly and present with clinically advanced disease. In most of them surgical approach is difficult and FNAC may be the only available diagnostic specimen for therapeutic decision making.<sup>6</sup>

Cell blocks prepared from fine-needle aspiration sample can be useful adjunct to smears for establishing a more definitive cytopathological diagnosis. Ancillary studies can be done using cell blocks including IHC which can aid in sub-typing of NSCC.<sup>7</sup> The objectives of the present study were to evaluate the pathological spectrum of the lung mass lesion from CT guided FNAC sample in a group of Bangladeshi patients and to evaluate the efficacy of limited IHC panel in cell block sections to subcategorize NSCC.

## Methods

The cross-sectional study was carried out from 1<sup>st</sup> January 2018 to 31<sup>st</sup> December 2018 in the Department of Histopathology in Armed Forces Institute of Pathology (AFIP) in association with the Department of Radiology, Combined Military Hospital (CMH) Dhaka on 140 patients. The patients who underwent CT guided FNAC of lung mass lesions were included in the study. Smear inadequate cases were excluded. FNAC was done by the pathologist; the aspirates was smeared on 5-6 slides and were fixed in 95% ethyl alcohol. Rest of the material in the needle bud was used to make cell block which was taken in 10% formalin. Smears were stained by H&E and Papanicolaou stain and were categorized on the basis of presence or absence of anaplastic cells

as malignant or benign. The benign entities were looked into for presence of granulomas and caseous necrotic material which suggested tuberculosis. The rest of the benign lesions without granuloma containing mixed inflammatory cells were categorized as nonspecific inflammatory lesion.

Diagnosis of small cell carcinoma was based on findings of cellular smear with round to ovoid cells having scanty cytoplasm and granular, salt pepper chromatin pattern of nuclei with nuclear molding. Squamous cell carcinoma was diagnosed upon finding of clusters of anaplastic squamous cells with extra/intracytoplasmic keratin and hyperchromatic pleomorphic nuclei. Adenocarcinoma was suggested upon presence of glandular or papillary arrangement of anaplastic cells with/or intra-cytoplasmic mucin, vesicular nuclei with prominent nucleoli.

The malignant smears in which the anaplastic cells having moderate amount of cytoplasm without characteristic salt pepper chromatin pattern of small cell carcinoma, showing neither any squamous nor adenocarcinoma differentiation were grouped in NSCC. The malignant smears in which the cellular features were not decisive of small/ NSCC were categorized as poorly differentiated carcinoma.

The cell blocks were processed; sections were stained and examined by histopathologist. The cell blocks with cyto-diagnosis of NSCC carcinoma were treated with immunohistochemical panel (TTF-1, p63) to sub-categorize them as Squamous cell carcinoma (SCC) or adenocarcinoma. Three cases with cyto-diagnosis of poorly differentiated carcinoma were dealt with CD56 in addition to TTF-1 and p63 to rule in/out small cell carcinoma.

## Results

Total 140 patients with lung mass lesion who underwent CT Guided FNAC were included in the study. Among them 110 were male and 30 were female. Age range was from 18 to 85 years, with mean age  $60.13 \pm 13.49$  years. Total 57 lesions were diagnosed as benign entities among them 28 were suggested as tuberculosis. Number of malignant lesions were 83.

Age and sex distribution of different benign and malignant entities are shown in Table I.

**Table I** Age and sex distribution of patients with benign and malignant lung mass lesions (N=140)

Age limit	Benign				Malignant		Total
	Nonspecific		Tuberculosis		M	F	
	M	F	M	F			
0-20	0	0	1	0	0	0	01
21-40	2	2	3	1	1	1	10
41-50	3	1	1	1	4	1	11
51-60	8	0	6	4	10	3	31
61-70	8	3	7	2	29	7	56
71-80	0	0	2	0	16	4	22
>80	2	0	0	0	7	0	09
Total	23	6	20	8	67	16	140

In FNAC smears, 22 cases showed anaplastic cells having clear cut squamous differentiation, and 19 cases showed features of adenocarcinoma. Features of small cell carcinoma was discernable in FNAC smears in 14 cases. Twenty-five cases were categorized as NSCC as definitive differentiation of squamous or adenocarcinoma was not evident in FNAC smear. Three cases were categorized as poorly differentiated carcinoma as small cell carcinoma could not be ruled in/out from FNAC smear.

Twenty-five cell block sections of cytologically diagnosed NSCC were examined. Three (3/25, 12%) of them were found inadequate for further evaluation by IHC and were reported as NSCC-not otherwise specified (NOS).

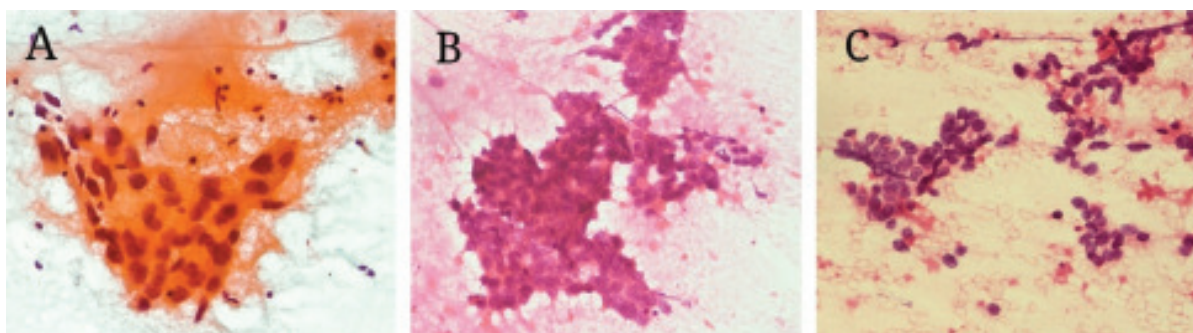
**Table II** Summary of distribution of malignant entities categorized in FNAC smear (N=83)

Diagnosis	Sex		Total
	Male	Female	
Squamous cell carcinoma	21	1	22
Adenocarcinoma	14	5	19
NSCC	21	04	25
Small cell carcinoma	10	4	14
Poorly differentiated carcinoma	03	0	03
Total	69	14	83

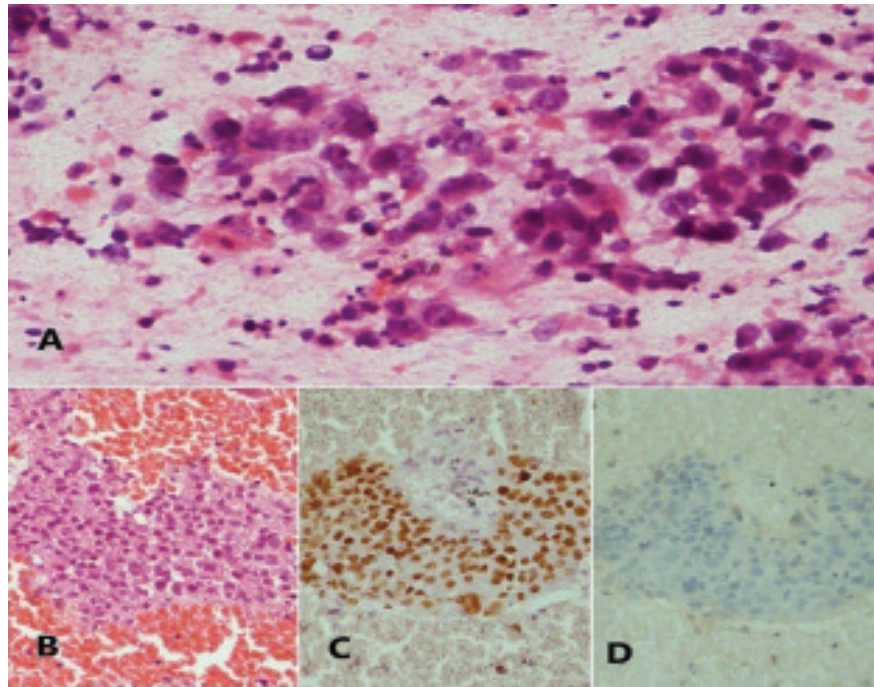
IHC of cell block sections of the rest 22 (88%) NSCC entity could subcategorize them; 10 as SCC (P63 positive, TTF-1 negative) and 12 as adenocarcinoma (TTF-1 positive, P63 negative). After IHC of cell block sections of the entity poorly differentiated carcinoma, all three were labeled as SCC (TTF-negative, p63 positive, CD56 negative). Summary of malignant entities after IHC in cell block section is shown in Table III.

**Table III** Distribution of malignant entities of lung mass lesion after IHC categorization of NSCC and poorly differentiated carcinoma from cell block sections (N=83)

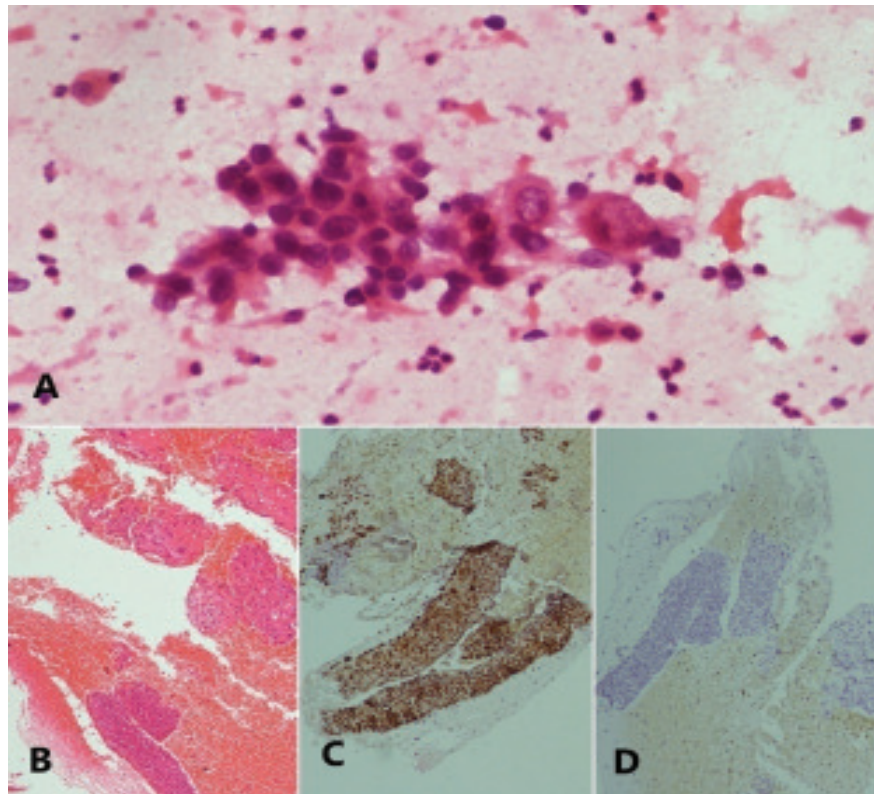
Types	Number n=83	Percentage
Squamous cell carcinoma	35	42%
Adenocarcinoma	31	37.35%
Small cell carcinoma	14	16.87%
Non-small cell carcinoma (NOS) (could not further categorized)	3	3.6%



**Figure 1** A. SCC- Papanicolau stained smear showing cytoplasmic keratinization and hyperchromatic pleomorphic nuclei. B. Adenocarcinoma- H&E stained smear showing cells having vesicular nuclei arranged in clusters and in glandular pattern. C. Small cell carcinoma-H&E stained smear shows small cells with scanty cytoplasm and “Salt and pepper chromatin pattern.



**Figure 2** Adenocarcinoma; after IHC categorization of cell block sections of NSCC. A. H&E stained smears of NSCC. B. H&E stained cell block section. C. TTF-1 positive D. P63 Negative



**Figure 3** Squamous cell carcinoma; after IHC categorization of cell block sections of NSCC. A. H&E stained smears of NSCC. B. H&E stained cell block section C. P63 positive D. TTF-1 Negative

## Discussion

NSCC has become a historically diagnosed entity after introduction of WHO classification of lung cancer in 2015. For appropriate therapy, sub-typing of NSCC is mandatory and is usually done in biopsy specimens, whenever possible by use of IHC. But in majority of lung cancer patients, the initial diagnosis is made from FNAC smears and obtaining biopsy of lung mass lesions is relatively difficult. Cell blocks prepared from FNAC samples can be very useful adjunct to smears. These paraffin embedded cyto-blocks can be handled like any other histologic specimen. IHC can be carried out in cell block sections like any biopsy specimen; therefore subtyping of NSCC can be confidently done.

The study involved total 140 patients who underwent CT Guided FNAC in CMH Dhaka for lung mass lesions. Majority of the patients were male. The male predominance of lung mass lesion is similar to other study findings in our subcontinent.<sup>8,9</sup> The major age group affected with both benign and malignant diseases is seventh decade followed by sixth decade. Majority of the lesions in the study were malignant. Similar higher prevalence of malignancy in lung mass lesions is found in others studies conducted in our country and south Asia.<sup>8,9</sup> Among benign diseases, the most common entity was tuberculosis accounting 20% of all the patients. The finding is similar to other studies conducted in Bangladesh and India.<sup>8,9</sup>

In FNAC smear, confident categorization of small cell carcinoma (14/83, 16.9%), SCC (22/83, 26.5%), and adenocarcinoma (19/83, 22.9%) was possible in 66% patients. In 34% patients (28/83) cyto-diagnosis was made under a broad umbrella term as NSCC/ poorly differentiated carcinoma as definite evidence of specific diagnosis was lacking in the smears. Similar proportion of malignancy (36%) was reported as NSCC in a study conducted by Luissella Righi in Rome.<sup>10</sup> All the NSCC/ poorly differentiated carcinoma cases were dealt with IHC in cell block sections for specific categorization. Three cell blocks (3/25, 12%) of NSCC were found to have inadequate material. Sensitivity of cell block varies from 60% to 86% depending on sampling size and type.<sup>11-14</sup> In our study it was a bit higher (88%). The rest NSCC cell blocks (22/25) were further subcategorized; 10 as SCC (p63 positive, TTF-1 negative) and 12 as adenocarcinomas (TTF-1 positive, p63 negative). For sub-typing the entity poorly

differentiated carcinoma CD56 was used in addition; all the three cases came out to be SCC (P63 positive, TTF-1 and CD56 negative). Similar study was conducted by Dong Z and Busra Al-Ayadhy with use of limited IHC panel in cell block section, where they could further sub-classify all the entities.<sup>15,16</sup>

The most frequent malignant entity in our study was SCC constituting 42%, followed by adenocarcinoma. Singh R conducted similar studies on FNAC of lung mass and found adenocarcinoma as the most common malignant entity.<sup>18</sup> But similar to our finding squamous cell carcinoma was the most frequent malignancy in a number of studies in India and study conducted by Ahmed Z et al in Feni district of Bangladesh.<sup>18,19</sup>

This variation in findings of FNAC can be explained by the existence of the entity non-small carcinoma in all these studies where subcategorization was not done by biopsy or IHC.

In our study we could subcategorize the entity non-small cell carcinoma and poorly differentiated carcinoma by IHC in cell block sections in most the cases. NSCC could not be further sub-categorized in 12% patients due to inadequacy of material in cell block and they were reported as NSCC- NOS.

In conclusion, in most of the patients of lung mass lesion FNAC can provide an accurate diagnosis. With use of cell block and IHC, diagnosis can reach to almost pin point in most of the cases. It can yield information as good as a needle core biopsy if the material is adequate. Our study is of short term and confined in a specific group of people. Large group study with incorporation of FNAC, cell block and tissue core biopsy with use of IHC can focus lights on accuracy of this diagnostic approach of lung mass lesions.

**Conflict of interest:** Nothing to declare.

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