The Significant Impact of Immunohistochemistry in the Classification of Lung Carcinoma on Small Biopsies

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Abstract

Background : There are limitations of histomorphology in the appropriate categorization of lung carcinoma where immunohistochemistry can confirm the morphological diagnosis and may add value in the poorly differentiated and undifferentiated tumors. The aim of the study was to assess the role of immunohistochemistry in classifying lung carcinoma on small biopsy samples.

Methods and Material: A retrospective hospital based, observational study was conducted on cases of lung carcinoma diagnosed by core needle or bronchoscopic biopsies over a 3-year period. After evaluation of clinical findings and H&E sections, all biopsies were evaluated by immunohistochemical staining. The immunohistochemistry panel included cytokeratin cocktail, CK7, CK 20, TTF1, Napsin A, CK5/6, p40, synaptophysin, chromogranin, CD 56.

Result: Out of 78 cases, the mean age was 58 +/- 11 years. Most prevalent malignancy type was adenocarcinoma (30, 38.1%). Adenocarcinoma cases were positive for CK7 (25/26, 96%), Napsin A (24/26, 92%), TTF1 (15/30, 50%) and negative for CK 20. Squamous cell carcinoma cases showed positivity for p40(18/22, 82%) and CK 5/6 (17/22.71%).Small cell carcinoma showed positivity for neuroendocrine markers synaptophysin (5/7,71%) and chromogranin(4/7, 57%) and CD 56 (6/7cases (85%).88% of small cell carcinomas,75% of adenocarcinomas and 72 % of squamous cell carcinomas were accurately diagnosed by morphology. Morphologic prediction was poor in the NSCC NOS group (0%) and poorly differentiated carcinomas (64%), which were finally, diagnosed by immunohistochemistry.In the morphologically diagnosed cases, immunohistochemistry confirmed the diagnosis.

Conclusion: Thus, morphology added with immunohisto chemistry provides a crisp diagnosis thereby improving therapeutic efficacy.

Keywords: immunohistochemistry, lung carcinoma, morphology, small biopsy.

INTRODUCTION

Lung carcinoma has become a major public health problem. Worldwide lung carcinoma is the most common cancer. It causes 1.76 million cancer related deaths per year.[1]However in India, the incidence of lung carcinoma is lower than the West. The cancer related death is ranked 4th following breast, cervix, lip and oral cavity.[2] Histopathological diagnosis is still the main modality of classifying lung carcinoma on small biopsies. The introduction of immunohistochemistry has proven beneficial role in sub classification of lung carcinomas and in the areas of diagnostic dilemmas on morphological diagnosis. In this era of targeted therapy the morphology added with immunohistochemistry provides a crisp diagnosis thereby improving therapeutic efficacy.[3]The challenge in accurately diagnosing lung biopsy is due to the overlapping histopathological features especially in poorly differentiated malignant neoplasm, artifacts and also the lack of availability of discriminating biomarkers. Here lays the importance of immunohistochemistry which provides solution in majority of cases, specially poorly differentiated neoplasms. 4Immunohistochemistry confirms the diagnosis in morphologically diagnosed cases as well as contributes immensely in poorly differentiated neoplasms where morphology alone is incomplete for the final diagnosis. The present study highlights the importance of immunohisto chemistry to add further information in classification of lung tumors. The World Health Organization (WHO) Classificationof LungTumors 2015 provides a new classification for small biopsies similar to that proposed in the 2011 Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society in contrary to WHO 2004 classifications of lung cancer classification which was based on resection specimens. These guidelines of small biopsy are important for diagnosis in advanced cases, cancer screening of patients, in early stages of the disease giving scope for molecular testing.5

The aim of the present study was to evaluate the limitations of histomorphology in the appropriate categorization of lung carcinoma and to assess the role of immunohistochemistry in classification of lung carcinoma on small biopsy samples.

Material and methods

A retrospective, hospital based, observational study was conducted on cases of lung carcinoma diagnosed by core needle or bronchoscopic biopsies over a 3-year period. Inclusion criteria was all cases of lung carcinoma who underwent small biopsy procedure (core needle or bronchoscopic) for diagnosis in the stipulated period. Cases with history of previous chemotherapy or radiation therapy and cases where diagnostic materials were inadequate were excluded from the study. Data were collected for age, sex, clinical features and radiological findings. As per institutional protocol all the specimens were fixed with 10% neutral phosphate-buffered formalin. Paraffin-embedded, 4 µm-thick sections of the tumors were stained by Haematoxylin and Eosin stain were studied. All the blocks were reanalyzed by two independent pathologists as per the diagnostic criteria in WHO 2015 classification of lung tumors of small biopsy specimens similar to that proposed in the 2011 Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society.5,6Immunohistochemistry was done in all the biopsy blocks with monoclonal antibodies for cytokeratin cocktail, CK7,CK 20, CK 5/6, thyroid transcription factor1(TTF1), NapsinA,p40, synaptophysin, chromogranin and CD 56.This was done to find the significance of IHC in sub classifying the lung tumours over and above the morphological diagnosis. It was especially relevant in cases of dual differentiation which were cryptic on morphology and poorly differentiated cases. Data was tabulated and analyzed statistically using MS Office Word Excel software and SAS software. Descriptive statistics and hypothesis testing was performed to find statistical significance.

Result

A total of 83 samples were initially considered for inclusion in the study. However, after applying the exclusion criteria, five cases were rejected (3 for inadequate or unsatisfactory tissue and 2 for previous therapy).Out of 78 cases the mean age was 58 +/- 11 years. Overall male female ratio was 2.5:1. Analysis of the data revealed that the majority of adenocarcinoma cases occurred a decade earlier than squamous cell carcinoma cases. The probability of occurring of adenocarcinoma in the age group 41 to 50 years was 40% higher than squamous cell carcinoma.(Table 1)All the subtypes of lung carcinoma showed male preponderance except adenocarcinoma .Female preponderance in adenocarcinoma is higher than in squamous cell carcinoma and the difference was statistically significant by T test (p value <0.001).(Table 1)

In the present study, the most prevalent malignancy type was adenocarcinoma (30, 38.1%), followed by squamous cell carcinoma (22, 28.2%) and Non-small cell carcinoma (not otherwise specified) (NSCC NOS) (11, 14.1%). Others were small cell carcinoma (7, 9 %), indeterminate (4, 5%), adenosquamous carcinoma (2, 2.6%), other neuroendocrine carcinomas (2, 2.6%) and respectively. (Table 1)

Overall, immunohistochemistry provided major support to morphology. It helped in confirming the morphologically diagnosed cases and in cases where there was confusion in the opinion of two pathologists. In poorly diagnosed cases it was of paramount importance to arrive at a final diagnosis. The results of immunohistochemistry panel in the different subtypes in the present study are as follows:

Adenocarcinoma cases were positive for CK7 (25/26, 96%), Napsin A (24/26, 92%), TTF1 (15/30,50%)and negative for CK 20. Squamous cell carcinoma cases showed positivity for p40 (18/22, 82%) and CK 5/6 (17/22.71%). NSCC NOS group showed mixed positivity for CK 7, Napsin A, TTF1, p63 and CK 5/6. (Table 2). Two cases of adenosquamous carcinoma show positivity for CK7(2/2,100%) and p40 (2/2, 100%) and negative for CK 20, CK 5/6, Napsin A , TTF 1,synaptophysin, chromogranin, CD 56.Small cell carcinomashowed positivity for neuroendocrine markers synaptophysin (5/7,71%) and chromogranin(4/7,57%) and 6/7cases (85%) showed positivity for CD 56.Two cases of neuroendocrine carcinomas other than small cell carcinoma showed positivity for synaptophysin (1/2,50%),chromogranin (1/2,50%) and CD 56 (2/2 , 100%). Indeterminate group (4 cases) was positive for Pan CK and negative for most of the markers.

Diagnostic accuracy of histopathology diagnosis was compared for different lung carcinomas with their final diagnosis after immunohistochemistry confirmation.88% small cell carcinomas were accurately diagnosed by morphology, followed by 75% of adenocarcinomas and72 % of squamous cell carcinomas respectively. However, morphologic prediction was poor in the NSCC NOS group (0%).The diagnostic pitfall of histopathology was in the poorly differentiated cases, which showed no clear differentiation for adenocarcinoma, squamous cell carcinoma or neuroendocrine pattern. All these cases were finally diagnosed by immunohistochemistry. There were 11 poorly differentiated carcinomas which were later diagnosed after immunohistochemistry as adenocarcinomas (3, 27%), squamous cell carcinomas (3, 27%), adenosquamous (1, 9%) and indeterminate (4, 36%). The indeterminate groups were negative for most of the markers, thereby giving inconclusive results. (Table3).

Age group	Adenocarcinoma (N=30)	Squamous cell carcinoma (N=22)	Adeno squamous(N=2)	Small cell carcinoma(N=7)	Non Small Cell Carcinoma (Not Otherwise Specified) (N=11)	Other Neuro endocrine carcinoma(N=2)	Indeterminate Group (N=4)
<=30	0	0	0	0	0	0	0
31-40	1	1	0	0	0	0	0
41-50	5	3	1	1	0	1	0
51-60	13	8	0	0	6	0	1
61-70	8	9	0	0	3	0	3
71-80	2	0	1	1	1	1	0
>=81	1	1	0	0	1	0	0
Total (%)	30 (38.1%)	22 (28.2%)	2 (2.6%)	2 (2.6%)	11 (14.1%)	2 (2.6%)	4 (5%)
M:F ratio	1:1.4	1:0.1	1:1	1:1	1:0	1:1	1:0.3

Table 1: Age and gender distribution of lung carcinomas in the study group

IHC Marker	Adeno carcinoma	Squamous cell carcinoma	Adeno squamous	Small cell carcinoma	Non Small Cell Carcinoma (Not Otherwise Specified)	Other Neuroendocrine Carcinomas
CK 7	29/30 (96%)	0/22 (0%)	2/2(100%)	0/7 (0%)	5/11 (45%)	0/2(0%)
CK 20	0/30(0%)	0/22(0%)	0/2(0%)	0/7(0%)	0/11(0%)	0/2(0%)
TTF-1	15/30 (50%)	1/22 (4%)	0/2(0%)	5/7 (71%)	1/11 (9%)	0/2(0%)
Napsin A	24/30 (80%)	0/22 (0%)	0/2(0%)	0/7 (0%)	0/11 (0%)	0/2(0%)
CK 5/6	0/30 (0%)	17/22(77%)	0/2(0%)	5/7 (71%)	2/11 (18%)	0/2(0%)
P40	11/30 (36%)	18/22 (81%)	2/2(100%)	5/7 (71%)	0/11 (0%)	0/2(0%)
synaptophysin	2/30 (6%)	0/22 (0%)	0/2(0%)	5/7(71%)	1/11 (9%)	1/2(50%)
chromogranin	2/30 (6%)	0/22 (0%)	0/2(0%)	4/7(57%)	0/11 (0%)	1/2(50%)
CD 56	0/30 (0%)	0/22 (0%)	0/2(0%)	6/7(85%)	0/11 (0%)	2/2(100%)

Table 2: Immunohistochemical profile of different histological types of lung carcinoma

		IHC Confirmation								Diagnostic Accuracy of histopathology diagnosis(%)
		Adeno carcinoma	Squamous cell carcinoma	Non Small Cell Carcinoma (Not Otherwise Specified)	Adenosquamous	Small cell carcinoma	Other Neuroendocrine carcinomas	indeterminate	Total	
	Adeno carcinoma	21	2	2	1	1	1	0	28	75%

		IHC Confirmation								Diagnostic Accuracy of histopathology diagnosis(%)
		Adeno carcinoma	Squamous cell carcinoma	Non Small Cell Carcinoma (Not Otherwise Specified)	Adenosquamous	Small cell carcinoma	Other Neuroendocrine carcinomas	indeterminate	Total	75%
	Non Small Cell Carcinoma (Not Otherwise Specified)	2	0	0	0	0	0	0	2	0%
	Squamous cell carcinoma	4	21	3	0	1	0	0	29	72%
	Small cell carcinoma	0	0	0	0	7	1	0	8	88%
	Poorly differentiated carcinoma	0	0	0	0	7	1	0	8	64%
	TOTAL	30	26	5	2	9	2	4	78	

Table 3: Comparison diagnostic accuracy of lung tumours based on initial histopathological diagnosis and Immunohistochemicalevaluation thereafter.

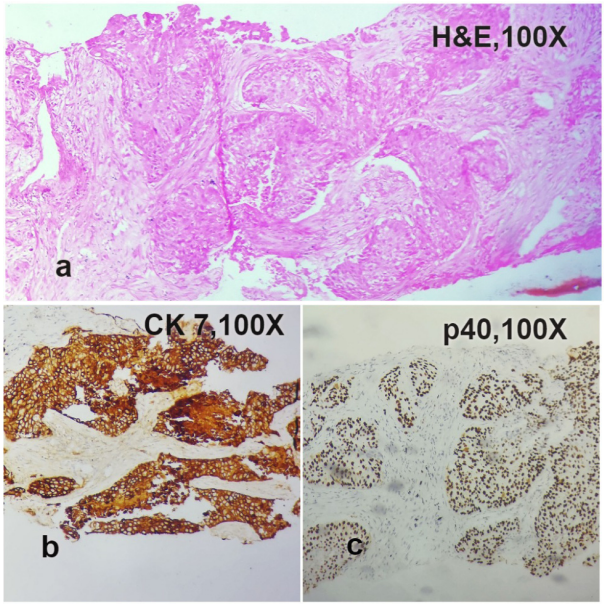

Figure 1

1a,Photomicrograph showing sheets of poorly differentiated cells mostly in sheets in a case of poorly differentiated carcinoma of lung (H & E, 100 X).

1b.and 1c The same tumour diagnosed as poorly differentiated carcinoma after immunohistochemical evaluation, based on positive staining for CK 7 (CK 7, 100X); and p40 (p 40 ,100X)

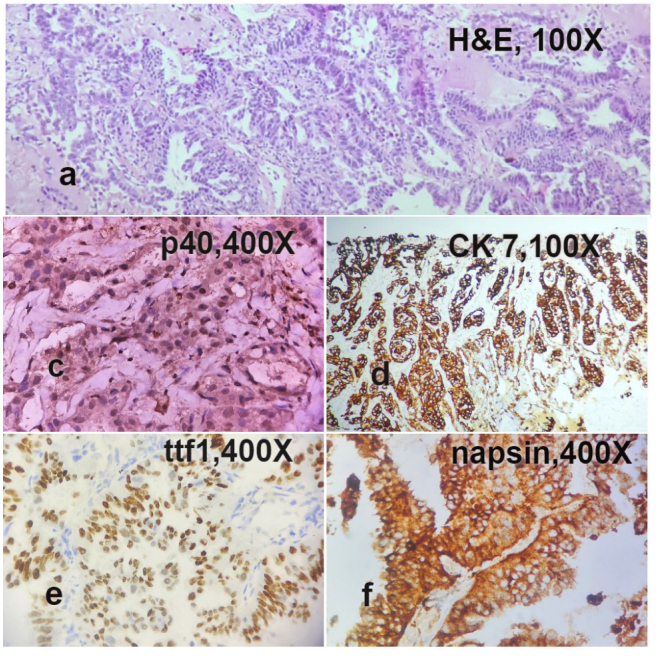

Figure 2

2a.Lung biopsy showing ill defined glands and areas with squamous differentiation in a case of adenosquamous carcinoma lung (H&E, 100X);

2b to 2e. The diagnosed was confirmed by immunohistochemical staining for p40 (p40,400X) in squamaoid areas, diffuse CK 7 positivity (CK 7, 100X) and TTF1 (TTF1,400X),Napsin A (Napsin A, 400X) positivity in glandular areas.

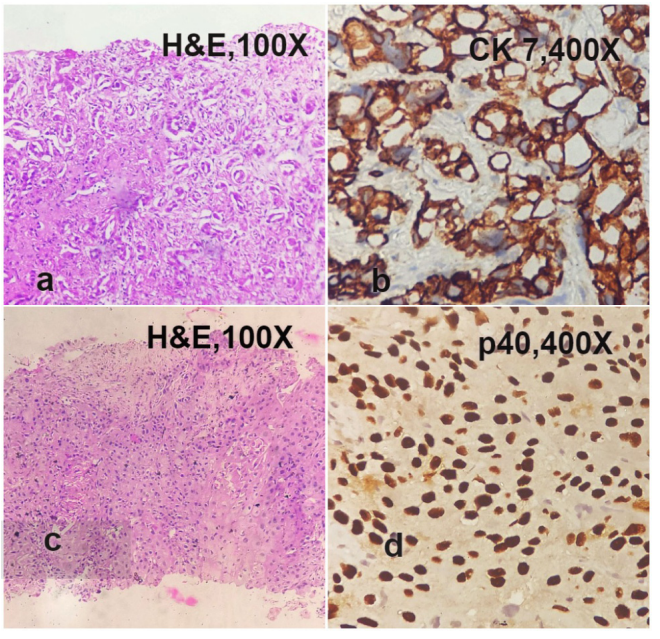

Figure 3

3a, 3b: The photomicrograph showing glands lined by neoplastic cells in a case of adenocarcinoma (H&E, 100X); 3b)which showing cytoplasmic immunostainging for CK 7 (CK7, 400X).

3c, 3d:The tumour showing neoplastic squamous cells arranged in sheets (H& E, 100X) having strong nuclear positivity for p40 (p40, 400X)

Discussion

The mean age of cases in our study (58 years) was comparable with the study of Malik et al (55 years) which was also conducted in Indian population. 7The gender ratio in the study was 2.5:1 was in concordance with study of Bhatti et al (2.8:1).8

The most prevalent type of malignancy was adenocarcinoma (30,38.1%) in our study, similar to the study of Malik et al. contrary to the study of Dey et al where squamous carcinoma was the commonest malignancy (35.1%).(4). 9

The immunohistochemistry panel used in the present study was similar to the study ofBhatti et alexcept that they used Neuron specific enolase as an additional marker for neuroendocrine differentiation and p63 for squamous cell marker. 8

Adenocarcinoma panel in the present study was CK7 (25/26, 96%), Napsin A(24/26, 92%), TTF1(15/30, 50%).In the study of Mukhopadhyay et al similar panel showed strong reactivity for adenocarcinomas; CK 7 (100%), Napsin A(58%), TTF1 (80%). 10Squamous cell carcinoma cases showed positivity for p40(18/22, 82%) and CK 5/6 (17/22.71%) in the present study.However,Stojsic et al showed absolute positivity (100%) for CK 5/6 and p63 in squamous cell carcinomas. 11

In the present study, the comparison of initial morphological diagnosis with final immunohistochemistry confirmation showed that morphological diagnosis is challenging in cases of NSCC (NOS) and poorly differentiated carcinomas with diagnostic accuracy 0%, 64% respectively.In cases of poorly differentiated carcinomas, 7/11cases could be finally diagnosed with the help of immunohistochemistry resulting in 64% diagnostic accuracy of histopathology. After application of immunohistochemistry, 11 cases of poorly differentiated carcinomas were further categorized into adenocarcinomas (3, 27%), squamous cell carcinomas (3, 27%) and adenosquamous carcinoma (1,9%). This is due to overlapping histological features and immunohistochemistry was of immense helpin categorizing these cases in small biopsy.

Bhatti et al also stated that IHC is crucial in categorizing poorly differentiated casesshowing no definite and overlapping features of squamous or glandular differentiation on morphology. In practice, they are histologically grouped into non-small cell lung carcinoma category.8 but for therapeutic purposes, anti-folate chemotherapeutic drug, pemetrexed, is effective only in lung adenocarcinomas. Whereas targeted drug against anti-vascular endothelial growth factor, bevacizumab, is contraindicated in squamous cell carcinoma of lung due to the risk of fatal pulmonaryhemorrhage. 12, 13

However, there were some cases of an indeterminate group (4,5%) which did not yield definite categorization even with the help of immunohistochemistry. Similarly there were similar unclassified cases (5, 2.5%) in other studies also.8Further extensive study with extended IHC panel and mutational studies are recommended in such cases.

Immunohistochemistry provides a conclusive diagnosis in small cell carcinomas. CD56, chromogranin A, synaptophysin are consistently expressed by small cell carcinomas. However, CD56 is the mostsensitive. In the study of Bhatti et al, all cases of small cell carcinoma showed 100% positivity for all these markers.8The present study showed small cell carcinomas reacting positivity for synaptophysin (5/7,71%) and chromogranin(4/7, 57%) and CD 56 (6/7, 85%).

Biopsy specimens are preferred over cytology specimens and satisfactory biopsy is crucial for accurate histological diagnosis.10In the present study, 3 cases were rejected for unsatisfactory sampling.

Based on the observation of the present study, immunohistochemistry panel can be applied in conjuncture with morphology in a step wise manner for optimal use of tissue and reagents. Initially cytokeratin cocktail, CK 7 and CK 20 immunostains may be done to differentiate primary from secondary deposits of adenocarcinomas. In CK 7 positive CK 20 negative pattern, implying primary lung tumours, the subsequent IHC may be done based on the morphology. For adenocarcinoma, Napsin A and TTF1 and for squamous differentiation, p63 or p40should be done. In cases of neuroendocrine morphology, synaptophysin, chromogranin and CD 56 may be performed. However for poorly differentiated and tumours with dual morphology, all the markers of IHC panel should be evaluated for appropriate classification. Both p40 and p63 exhibits similar sensitivity for pulmonary squamous cell carcinoma. However, p40 shows higher specificity to p63 thereby eliminating misinterpretation of p63 positive adenocarcinoma or unsuspected lymphoma as squamous cell carcinoma. If available, p40 is a better marker for squamous cell carcinoma.14

Conclusion

In this era of targeted therapy, accurate diagnosis of lung carcinoma on small biopsy specimens, histopathologically with help of immunohistochemistry confirming the histopathological diagnosis and further categorizing the problematic cases remains the mainstay of diagnosis and thereby helping in proper patient management.

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