

Immunohistochemical Profile of Lung Tumors in Image Guided Biopsies

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ABSTRACT

Background: Lung cancer is the leading cause of cancer related mortality in both men and women worldwide. Establishing the histological type and grade of pulmonary carcinoma is very important especially for the therapy and prognosis. Study design: cross-sectional descriptive study. The study analyses various histomorphological patterns of lung tumors in correlation with immunohistochemical profile.

Methods: All the bronchoscopic and CT guided needle biopsy specimens (50 biopsy specimens) received in the Pathology department of Coimbatore medical college hospital over a period of one year were analysed. Both H&E and immunohistochemical sections were studied with panel of markers- CK7, CK20, TTF-1, chromogranin, synaptophysin, CD45, vimentin, smooth muscle actin.

Result: The most common histological type was squamous cell carcinoma (48%), followed by adenocarcinoma (28%) and small cell lung carcinoma (18%). Large cell neuroendocrine carcinoma and metastatic deposit constituted 2% each. Out of 50 cases, 24 cases were squamous cell carcinoma which showed positivity with HMWCK (20 cases) and P63 (22 cases) ($p < 0.001$). Fourteen cases reported as adenocarcinoma showed positivity with CK7 (14 cases) and TTF-1 (13 cases) ($p < 0.001$). All the nine cases of small cell carcinomas showed positivity with both TTF-1 and Ki 67. One case of large cell carcinoma with neuroendocrine features showed immunopositivity with neuroendocrine markers.

Conclusion: Integration of conventional histomorphological diagnosis with panel of immunohistochemical markers allows more accurate identification of histological type, which has significant treatment implications.

Keywords: Lung Tumor, Non-small Cell Lung Carcinoma, Small Cell Lung Carcinoma, Cytokeratin, TTF-1, p63.

Introduction

Lung cancer is one of the most deadly cancers with increased morbidity and mortality in the world leading to 1.58 million deaths in 2016.^[1] Clinically, lung carcinomas are classified as non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC), as the therapy and prognosis varies. The most common histological pattern is squamous cell carcinoma, but recently the incidence of adenocarcinoma has increased significantly and now it is the leading malignancy in both sexes.^[2]

Despite new diagnostic techniques, the overall 5-year survival rate is only 14% in males and 9% in females.^[3] Oncogenes c-myc, k-ras, EGFR, c-kit are involved in lung cancers.^[4] Presence of EGFR mutation may predict response to therapy.^[5]

Histomorphological assessment on hematoxylin–eosin (H&E) stained sections remains the most important diagnostic tool for classifying lung carcinomas.

Immunohistochemistry (IHC) can be used for the diagnosis and classification of lung tumors as NSCLC or SCLC. IHC

can also be used to interpret neuroendocrine differentiation of a neoplasm. Panel of immunohistochemical markers have been developed and used both as diagnostic and prognostic markers.

The purpose of this study was to analyze the histomorphological patterns of lung tumors and to study the immunohistochemical profile using panel of markers- Cytokeratin (CK)7, CK20, thyroid transcription factor (TTF-1), high molecular weight cytokeratin (HMWCK), P63, CD45/ leukocyte common antigen (LCA), chromogranin, synaptophysin, vimentin and smooth muscle actin.

Materials and Methods

This was a cross-sectional study conducted in the Department of Pathology, Coimbatore medical college Hospital, spanning over a period of 1 year. A total of 50 cases of lung biopsy specimens including bronchoscopic and needle biopsies received were studied. Treated patients and inadequate biopsies were excluded from the study. Detailed history regarding age, sex, clinical findings,

history of primary tumor elsewhere in the body and radiological investigations were reviewed in all the cases.

Ethical clearance for the study was obtained from the Ethics Committee of Coimbatore Medical College, Coimbatore.

Histomorphological patterns and immunohistochemical profiles of the lung tumors were analysed. Because of the availability of very limited tissue in the lung biopsy specimens, panel of markers limited to the particular histological type diagnosed in H&E were used. Panel included markers of squamous differentiation- p63, HMWCK, cytokeratin specific for primary pulmonary origin- CK7, TTF-1, cytokeratin for primary gastrointestinal tract origin- CK20, markers of neuroendocrine differentiation- chromogranin, synaptophysin, proliferation antigen- Ki67, LCA and vimentin. Tumour cells were scored positive based on the pattern and intensity of staining in the neoplastic cells.

Statistical data analysis of various histomorphological patterns and percentage positivity of various immunohistochemical markers were studied and compared with those in the literature.

Result

In our study, it was observed that the peak incidence of lung malignancies occurred in the age group of 51-60 years (46%) with a male preponderance (84%). 43 out of 50 cases could be diagnosed and subtyped precisely using routine Hematoxylin & Eosin stained sections and confirmed by IHC. Remaining seven cases were diagnosed as NSCLC and could not be subtyped as SCC or adenocarcinoma requiring the aid of IHC. With the IHC findings, four cases were concluded as SCC and two cases as adenocarcinomas.

Only one case showed inconclusive result with IHC and reported as NSCLC. (Table 1)

In our study, we observed 24 cases were squamous cell carcinoma (48%), 14 cases of adenocarcinoma (28%) and 9 cases of small cell lung carcinoma (18%). Large cell neuroendocrine carcinoma and metastatic deposit constituted 2% each. One case was reported as NSCLC alone. (2%). It was observed that among the 20 cases reported as squamous cell carcinomas and 4 cases as NSCLCs histologically, HMWCK was expressed in 20 cases with a sensitivity of 83.3%. P63 was expressed in 22 out of 24 cases with a sensitivity of 91.7%. (Table 2) P63 was not expressed in any of the adenocarcinoma cases with 100% specificity (Table 3). It was observed that both HMWCK and p63 were equally good immunohistochemical markers for the diagnosis of squamous cell carcinomas.

All the 12 cases reported as adenocarcinomas in H&E stained sections and 2 cases as NSCLCs, showed immunopositivity with CK7, with a sensitivity of 100%. 13 out of 14 cases of adenocarcinomas showed nuclear immunoreactivity with TTF-1. TTF-1 was not expressed in any of the squamous cell carcinomas; thus the sensitivity of TTF-1 in this study was found to be 92.8% and specificity 100%. (Table 3). One case reported as NSCLC alone histologically, gave inconclusive results with IHC markers of both squamous and glandular differentiation.

In the present study, 9 cases reported as small cell lung carcinomas histomorphologically showed positivity with both TTF-1 and Ki 67 immunohistochemically and negative staining with LCA. (Table 4).

Table 1: Interpretation of Results with H&E Sections and Immunohistochemistry.

Total cases (n=50)	RESULTS CONCLUSIVE	RESULTS INCONCLUSIVE
H&E SECTIONS	43	7
IHC	49	1

(H&E- haematoxylin and eosin, IHC- immunohistochemistry)

Table 2: Expression of IHC Markers in Scc (N=24).

S. NO	IHC MARKERS	POSITIVITY	POSITIVE PERCENTAGE (%)	NEGATIVITY
1.	HMWK	20	83.3%	4
2.	P63	22	91.7%	2
3.	TTF 1	0	0%	24

(HMWK-high molecular weight cytokeratin, TTF-1- thyroid transcription factor)

Table 3: Expression of Ck-7, Ck-20, TTF-1 and P63 in Lung Adenocarcinoma (N=14).

S.NO	IHC MARKERS	POSITIVITY	POSITIVEPERCENTAGE(%)	NEGATIVITY
1.	CK-7	14	100%	0
2.	CK-20	0	0%	15
3.	TTF-1	13	92.8%	1
4.	P63	0	0%	15

(CK-Cytokeratin, TTF-1- thyroid transcription factor)

Table 4: Expression of TTF-1, KI 67 and LCA in Sclc (N=9).

S.NO.	IHC MARKERS	POSITIVITY	POSITIVE PERCENTAGE %
1.	Ki 67	9	100%
2.	TTF-1	9	100%
3.	LCA	0	0%

(SCLC- small cell lung carcinoma, TTF-1- thyroid transcription factor, LCA- leukocyte common antigen)

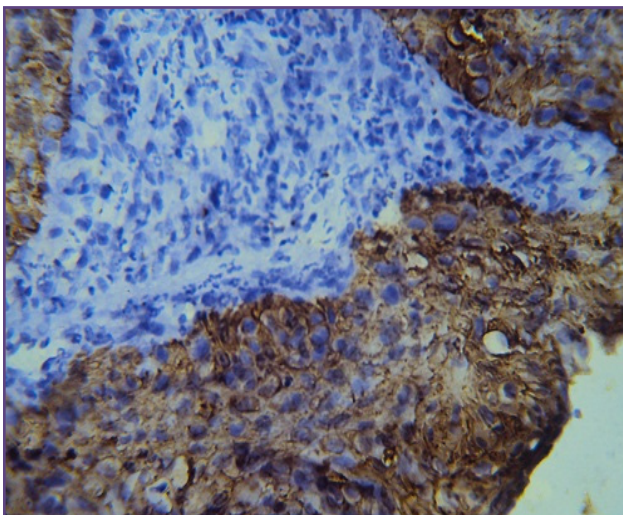


Fig. 1: SCC showing cytoplasmic positivity with HMWCK.

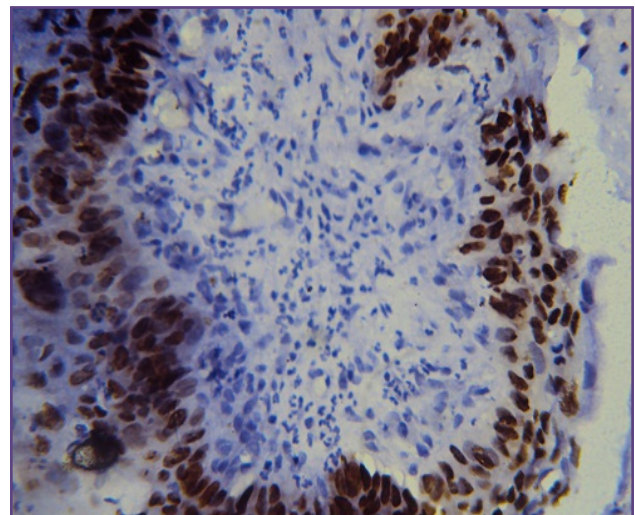


Fig. 2: SCC showing strong nuclear immunostaining with P63.

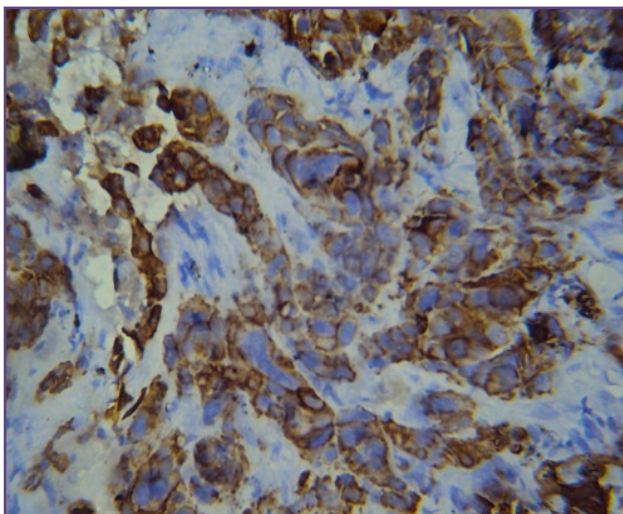


Fig. 3: Adenocarcinoma- CK7 cytoplasmic positivity.

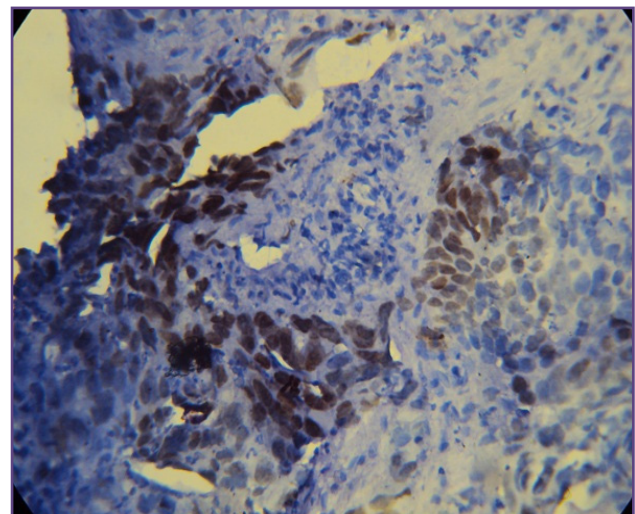


Fig. 4: Immunostaining with TTF-1 showing nuclear positivity.

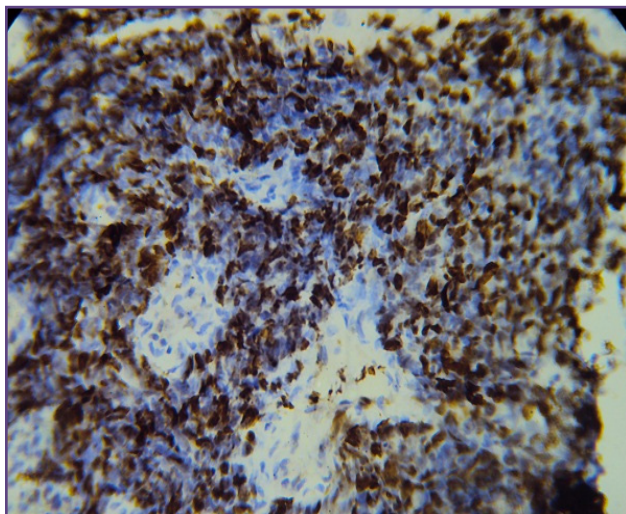


Fig. 5: Tumor cells of SCLC show strong nuclear immunostaining with Ki67.

Discussion

Lung cancer is the leading cause of cancer related morbidity and mortality. The primary intent of histopathological study is to classify lung tumors as primary pulmonary tumors or metastatic lesions. Primary lung carcinomas are classified as small cell lung carcinomas (SCLC) and non-small cell lung carcinomas (NSCLC). NSCLC accounts for 75-80% of all the lung carcinomas and further subtyped as squamous cell carcinoma and adenocarcinoma.^[6]

The objective of this study is to analyze the histomorphological patterns and immunohistochemical profile of lung tumors in bronchoscopic and needle biopsy specimens. Panel of immunohistochemical markers were used to confirm the histopathological diagnosis and correctly classify the lung tumors. It is a global observation that lung cancer has a higher incidence in males than in females with a male to female ratio of 2.7:1.^[7] These tumors commonly affect individuals in the 6th to 7th decades of life. In our study also, males are more commonly affected (84%) and the peak incidence occurred in the age group of 51-60 years.

In our study, NSCLC accounted for 78%; Squamous cell carcinoma was the most common histological pattern (48%), followed by adenocarcinoma (28%). Small cell lung carcinoma constituted 18%, large cell neuroendocrine carcinoma and metastatic deposit of sarcoma constituted 2% each. In a study by S. Sheikh, A. Shah et al., (2010), they observed squamous cell carcinoma (71.3%) as the most common histological pattern, followed by small cell carcinoma (20.8%), adenocarcinoma (2.6%), bronchoalveolar carcinoma (1.8%) while other tumors constituted 3.6%.^[7] Because of its relative ease of use

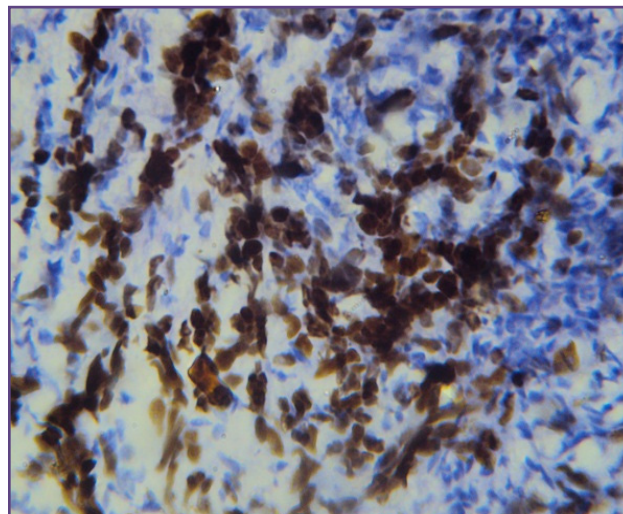


Fig. 6: SCLC showing nuclear immunoreactivity with TTF-1.

and specificity, immunohistochemistry has largely replaced mucin histochemistry and electron microscopy in diagnosing pulmonary neoplasms. Many studies have proposed panel of markers for lung tumors.

In our study, panel of markers were restricted according to the histological type of tumor. For NSCLC, panel of markers included- P63, HMWCK for squamous cell differentiation and CK7, CK20 and TTF-1 for glandular differentiation. In case of SCLC, panel included CK, TTF-1, Ki67, neuroendocrine markers and LCA (CD 45), to confirm the diagnosis and to differentiate it from lymphoma.

HMWCK is usually expressed in SCC and does not show reactivity in adenocarcinomas and SCLCs.^[8] P63 is also expressed only in SCCs. Both HMWCK and P63 are useful to differentiate SCC from adenocarcinoma and poorly differentiated SCC from SCLC and large cell neuroendocrine carcinoma.^[9] In our study, out of 20 cases reported as SCC and 4 cases as NSCLC in H&E, HMWCK was expressed in 20 cases (sensitivity 83.3%) (fig.1) and P63 showed immunopositivity in 22 cases (sensitivity 91.7%) (fig.2). P63 was not expressed in any of the adenocarcinoma cases with 100% specificity. (p value<0.001) (Table-2).

This observation was similar to the study by N.Kalhor, D.S.Zander et al., 2006. According to their study, P63 expression was found in all 13 cases of squamous cell carcinomas.^[10] In a study by R.Ocque, N.Tochigi et al., 2011, P63 expression was found in all the 30 cases of SCCs with 100% sensitivity.^[9]

Recent studies have proposed a panel of markers for pulmonary adenocarcinomas- CK-7, CK-20, TTF-1.

Primary lung adenocarcinoma shows strong and diffuse positivity with CK-7. CK-20 is expressed in metastatic deposits from colonic carcinomas. CK-7 is used in combination with CK-20, in differentiating primary pulmonary carcinoma from metastatic colonic carcinoma.^[11] 75% of primary pulmonary adenocarcinomas express TTF-1.^[12,13] Napsin A is also a sensitive marker for adenocarcinoma that has a stronger intensity than TTF-1.^[14]

Y.Su, Y.Hsu et al., used a panel of markers CK-7, CK-20, TTF-1 to differentiate primary from metastatic lung adenocarcinomas. They observed that 73% of primary pulmonary adenocarcinomas expressed TTF-1, whereas all the metastatic adenocarcinomas lacked TTF-1 staining. They concluded in their study that TTF-1 has high sensitivity and specificity for primary pulmonary adenocarcinomas. CK-7 expression was present in 75% of pulmonary adenocarcinomas and none of the cases expressed CK-20. In their study they found combination of CK-7+/CK-20- along with TTF-1 immunoreactivity was highly specific for primary pulmonary adenocarcinoma.^[15]

In a study by R.Ocque, N.Tochigi et al., 2011, CK-7 expression was found in all the cases of adenocarcinomas (100%) and 86.2% of cases expressed TTF-1. They also observed in their study the immunoreactivity of TTF-1 in 9 out of 43 cases of SCCs with a sensitivity of 86% and specificity of 73%.^[9]

In our study, all the 12 cases reported as adenocarcinomas and 2 cases reported as NSCLC, showed positivity with CK7 immunostaining with a sensitivity of 100% and negative immunoreactivity with CK-20. (fig.3) TTF-1 immunoreactivity was positive in 13 out of 14 cases of adenocarcinomas. (fig4) TTF-1 was not expressed in any of the squamous cell carcinoma cases with a sensitivity of 92.8% and specificity 100%. (p value <0.001) (Table-3). The results were consistent with the prior studies.

In our study, 9 cases were reported as SCLCs in H&E stained sections with crush artefacts. Crush artifact can also occur in carcinoids, lymphomas, poorly differentiated non-small cell carcinomas and lymphocytes of inflammation.^[16] Mitotic activity is impossible to assess in small biopsy specimens with crush artifacts. In such cases, Ki67 labelling can be more reliable.

In a study by D. L. Aslan, H. E. Gulbahce et al., 2005 they found all the 34 cases of SCLC showed immunoreactivity with Ki-67 even in the crushed areas, with diffuse staining in more than 80% of all tumor cells.^[17] Small cell carcinoma is positive for TTF-1 in 90% of cases.^[18]

In our study, all the 9 cases of SCLC showed positivity with both TTF-1 (100%) and Ki67 (100%)

immunohistochemically. (fig 5&6) Immunostaining with Ki67 showed reactivity in more than 80% of the tumor cells. (fig5) Immunostaining with LCA (CD 45) showed negative results and hence lymphoma was ruled out.

In our study, one case of large cell carcinoma with neuroendocrine features was reported in H&E. Immunohistochemistry showed strong cytoplasmic positivity with neuroendocrine markers- chromogranin and synaptophysin. HMWCK and p63 expression, largely restricted to NSCLCs showed negative results.^[19] CK-7 showed focal cytoplasmic positivity. Thus the possibility of poorly differentiated NSCLC was excluded. With the combined H&E and immunohistochemical profile, it was concluded as 'Large cell neuroendocrine carcinoma.'^[20]

Large cell carcinoma with neuroendocrine architecture but without immunoreactivity for neuroendocrine markers should be concluded as 'Large cell carcinoma with neuroendocrine architecture'.^[20]

With the clinical history, radiological findings, H&E and IHC reports with vimentin positivity, one case was concluded as metastatic high grade spindle cell sarcomatous deposit.

We observed in our study that 43 out of 50 cases could be diagnosed and subtyped precisely in H&E stained sections histomorphologically and confirmed by IHC. Remaining seven cases were diagnosed as NSCLC alone and proceeded with IHC. With the IHC findings, four cases were concluded as SCC and two cases as adenocarcinomas. Only one case showed inconclusive result with IHC and reported as NSCLC. (Table 1) This negative result may be due to reaction bias like specimen fixation, tissue processing and antigen retrieval.

Integration of conventional histomorphological diagnosis with immunohistochemistry increases the refinement of diagnosis, so that a diagnosis of NSCLC can be avoided. Subclassification of NSCLC has significant treatment implications, especially for advanced stage tumors for which chemotherapy is being considered.^[18]

Conclusion

From this study it is concluded that immunohistochemistry should be done in all the small lung biopsy specimens to confirm the histomorphological diagnosis as well as in cases where histological subtyping is difficult with H&E sections. Also, panel of markers can be restricted to the histological type because of the limited availability of tissues in bronchoscopic and CT-guided biopsy specimens.

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