ORIGINAL ARTICLE

Role of stratification of immunohistochemical markers in primary lung carcinomas: experience in a south Indian tertiary care center

Swathi Prabhu[1], Anitha Mathews[2], Sindhu Nair[2], Anuradha Calicut Kini Rao[1], Rari Mony[2], Jayasree Kattoor[2]*

[1]Department of Pathology, Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal-576104 (Karnataka) India, [2]Department of Pathology, Regional Cancer Center, Thiruvananthapuram-695001 (Kerala) India

Abstract

Background: Accurate subtyping of lung carcinomas is essential owing to therapeutic implications. *Aim and Objectives:* To assess the role of immunohistochemical markers in the classification of primary lung carcinomas in resource-poor setting. *Material and Methods:* This was a 6-month retrospective study from January 2019 to June 2019 carried out in a tertiary care cancer centre. Biopsy confirmed cases of lung carcinoma were retrieved. Immunohistochemistry (IHC), wherever performed, was reviewed and the cases were typed. IHC markers were used in a tiered manner comprising TTF-1, Napsin A, p40, p63, Synaptophysin and Chromogranin A. CD56, Cytokeratin 7 (CK7), Pancytokeratin (CK) were performed in selective cases. *Results:* Two hundred and sixty-four (264) cases of primary lung carcinomas comprising of seven lobectomies and 257 small biopsies were studied. Prior to IHC, the distribution of cases was as follows: Small Cell Carcinoma (SCLC) (6.8%, n=18), non-small Cell Lung Carcinoma (non-SCLC) (89.4%, n=236) further divided into Adenocarcinoma (ADC) (35.9%, n=95), Squamous Cell Carcinoma (SCC) (19.8%, n=52) and non-SCLC, unclassifiable (33.7%, n=89). Non-SCLC, unclassifiable after use of IHC was non-SCLC favoring ADC (20%, n=53), non-SCLC favoring SCC (7.9%, n=21). One case of non-SCLC (adenocarcinoma) with SCLC (0.4%) on biopsy; one case of non-SCLC on biopsy showed both squamous and adenocarcinoma components (0.3%, n=1). Two cases were of adenosquamous (1.1%, n=2) and three cases of large cell neuroendocrine carcinomas (n=3,1.1%). Salivary gland type tumors comprised 6 (2.2%) cases. Eight cases (3%) could not be reclassified despite adequate IHC, and hence were retained in non-SCLC, NOS category. *Conclusion*: IHC panel of TTF-1, Napsin A, p40, p63, Synaptophysin, Chromogranin A, CK, CK7 used judiciously and in tiered manner aids in accurately subtyping most primary lung carcinomas.

Keywords: Lung; Cancer; Immunohistochemistry; Adenocarcinoma; Squamous cell carcinoma

Introduction

Lung cancer is the second most frequently diagnosed cancer following breast and is the one of the major causes of cancer-related deaths world-wide. According to Global Cancer Observatory (GLOBOCON) 2020 statistics, the incidence of lung cancer in India was 5.5%, causing 7.8% of cancer-related deaths [1]. Lung cancer has been observed to impact individuals across all socio-economic groups. This factor likely highlights the delayed presentation and the absence of early detection, especially when compared to oral Squamous Cell Carcinoma (SCC) [1, 2].

Significant changes made in the 2015 World Health Organization (WHO) classification of lung

carcinomas from 2004 portend remarkable advances in the molecular and therapeutic aspects. The 2021 WHO classification stressed molecular aspects of lung tumours and its therapeutic implications. A separate chapter with specific guidelines was established regarding the reporting of malignancies in small biopsies and cytology. This is necessary as most lung cancers present at an advanced stage; diagnosis is usually rendered on small biopsies or cytology specimens, followed by molecular testing [2]. India, where most laboratories run on histopathology alone with no Immunohistochemical (IHC) aid, the distinction becomes quite difficult, as approximately 30% of the cases fall into poorly differentiated categories. In small biopsies, the standard morphology cannot specifically subtype tumors in all cases. IHC staining is a valuable tool for accurately subtyping lung cancer [3].

Pathologists play a very important role and face the challenge of accurate diagnoses and subtyping with limited available tissue, as it is required for molecular studies. Our objective was to broadly classify primary lung carcinomas based on histomorphologic features and evaluate the role of IHC in tumor type confirmation and categorization of lung carcinomas.

Material and Methods

This was a 6-month retrospective study of primary lung carcinomas from Jan 1, 2019, to June 30, 2019. Two hundred and sixty-four cases of primary lung carcinomas diagnosed on histopathology in our department over a period of six months were retrieved. Our hospital is one of the referral cancer centers catering to population of South India. Patients' age and sex were retrieved from the electronic medical records. The cases were typed according to the WHO 2021 classification of lung tumors. They were divided initially into Small Cell Lung Cancer (SCLC) and non-SCLC groups. Non-SCLCs were further categorized as SCC and Adenocarcinoma (ADC). Histomorphological subclassification of ADC into histological subtypes (lepidic, acinar etc.) was not performed because the morphology in most of the cases was compromised due to tissue processing and scant tissue might lead to incorrect interpretation. IHC was not performed in cases showing definite squamous or glandular differentiation. An IHC panel comprising of TTF-1, Napsin A, p40, p63, Chromogranin A, Synaptophysin, CK, CK7, CK20 was used in appropriate combination and in a stratified manner to accurately subtype the tumor, for diagnosing adenosquamous cases and for tumors showing poor differentiation. Forty-five cases (45) of metastatic carcinoma were excluded from our study after relevant IHC and clinico-radiological correlation. The study was approved by the institutional review board. Formal written informed consent was waived off by the board owing to retrospective nature of the study and strict anonymity of the patient was respected throughout.

Results

Two hundred sixty-four (264) cases of primary lung carcinomas were studied, comprising seven cases of lobectomy and 257 small biopsies.The men to women ratio was 3.9:1. The patients' age ranged from 18 to 85 years (mean age of 60.21 years). The division of cases after histopathological examination, according to WHO 2021 classification is given in Table 1.

Diagnosis was rendered on histopathology alone without the aid of IHC in 11 cases of which eight cases comprised well-differentiated SCC, one case

of ADC, and 2 cases of mucoepidermoid carcinoma. The rest of the cases, though typed, were confirmed by specific IHC for accurate diagnosis. Lobectomy comprised of two cases of adenosquamous carcinoma, one case of mucoepidermoid carcinoma, one case of carcinoid, three cases of large cell neuroendocrine carcinoma.

The most common histological type of lung carcinoma was ADC (35.9%, n = 95), followed by SCC (19.8%, n = 52), SCLC (6.8%, n = 18), non-SCLC unclassifiable (33.7%, n = 89), salivary gland tumors (2.2%, n=6) and neuroendocrine tumors (1.4%, n=4).

Within the unclassifiable category of non-SCLC, IHC was performed in all cases; eight cases could not be classified even after IHC and were retained under non-SCLC, NOS category. The remaining 81 cases comprised of ADC (20%, n = 53), SCC (7.9%, n = 21), adenosquamous carcinoma (1.1%, n = 2), non-SCLC (ADC) with SCLC (0.4%, n=1), large cell neuroendocrine carcinoma (1.4%, n = 3) and one case of non-SCLC with both squamous and adenocarcinoma component n biopsy (0.3%, n=1).

Of 52 cases of SCC, diagnosis was rendered in eight cases directly on histopathology without IHC; Remaining 44 cases were confirmed by a 2-panel IHC comprising p40 and or p63 and TTF-1 (Figure 2 D, E). In 22 doubtful cases of ADC, TTF1 performed was positive in all cases and Napsin A was positive in 19 cases (86%) (Figure 2 B, C). Salivary gland type tumors comprised of six cases (2.2%): four cases of mucoepidermoid carcinoma, of which one was diagnosed on lobectomy and rest two cases of adenoid cystic carcinoma.

Adenosquamous carcinoma constituted three cases (1.2%) of which two were diagnosed on lobectomy specimens with definite squamous and glandular areas. One case of adenosquamous was diagnosed on IHC with different areas showing TTF1, Napsin A and p40 positivity (Figure 2A).

The neuroendocrine lesions included four cases of neuroendocrine tumors; three cases of typical carcinoids, and one case of atypical carcinoid. Synaptophysin, Chromogranin A, and CK were positive in all cases. One of the cases showed focal TTF1 positivity.

SCLC was confirmed using a combination of CK, synaptophysin and chromogranin A. CD56 was used only in two cases where morphology was strongly suggestive of small cell carcinoma, and synaptophysin was negative and chromogranin A weakly positive. Doubtful cases of ADC and SCC were confirmed by TTF-1 and p40 respectively. Napsin A and p63 were used as secondary line IHC only in those cases where TTF-1 and p40 exhibited weak or negative staining respectively and thus helped us in accurately subtyping the tumor in difficult cases.

Tumors which did not show positivity for any lineage except CK were placed into non-SCLC, NOS category. Immunohistochemistry applied included TTF-1, Napsin A, p63, p40, CK, CK7. The non-SCLC unclassifiable was reclassified after applying appropriate IHC markers (Table 2), thereby reducing the category from 33.7% to only 3%. Figure 1 demonstrates algorithmic approach of lung carcinomas using IHC.

Table 1: Histologic subtype of lung cancer among the studied cases according to WHO 2021 classification of lung cancer (morphological diagnosis by histopathological examination) (n=264)

Types	Subtype	N	%
SCLC**		18	6.8
Non-SCLC	**Adenocarcinoma**	95	35.9
	Squamous cell carcinoma	52	19.8
	Non-SCLC unclassifiable	89	33.7
Salivary gland type tumors	**Mucoepidermoid carcinoma***	4	1.5
	Adenoid cystic carcinoma	2	0.7
Neuroendocrine tumor*		4	1.6

**One case was diagnosed on lobectomy **small cell lung carcinoma*

Table 2: Division of cases in non-SCLC unclassifiable after applying TTF1, Napsin A, p40, p63 synaptophysin, chromogranin A, CK (AE1/AE3), CK7 (n=89 cases) in a tiered manner

Types	N=89	% of the total 264 cases
Primary adenocarcinomas	53	20
Squamous cell carcinomas	21	7.9
Non-small cell carcinoma with both squamous and adenocarcinoma component	1	0.3
Adenosquamous*	2	1.1
Non-small cell lung carcinomas (adenocarcinoma) with small cell carcinoma	1	0.3
Non-small cell carcinoma, NOS	8	3
Large cell neuroendocrine carcinoma*	3	1.1

**Was diagnosed on lobectomy specimen*

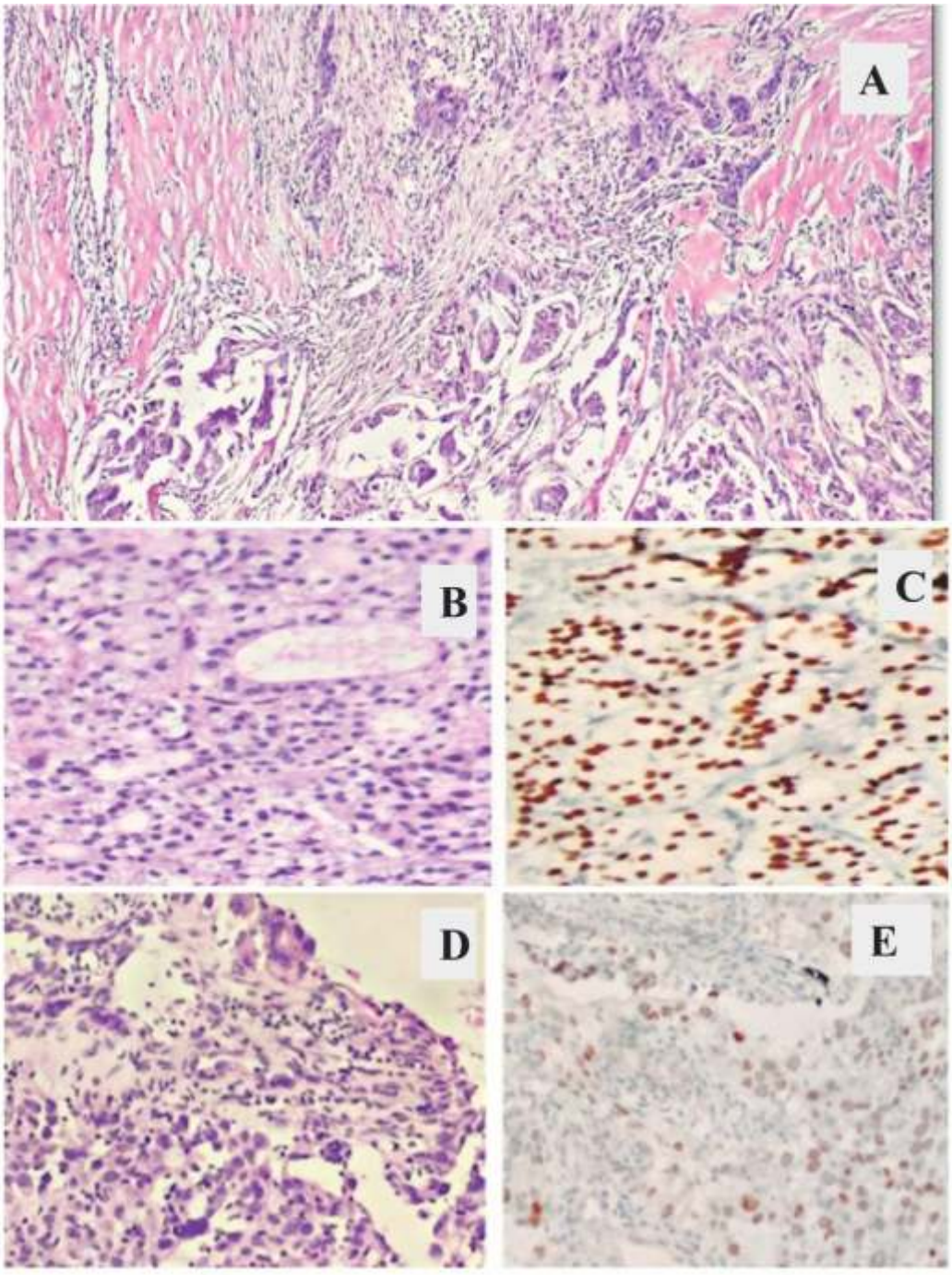

Figure 1: Photomicrograph shows adenosquamous carcinoma with squamous cell carcinoma to the top right and adenocarcinoma below (A). Non-small cell carcinoma (B) categorized as adenocarcinoma after TTF-1(C) showed positivity. Non-small cell carcinoma (D) categorized as squamous cell carcinoma after p40 showed positivity (E).

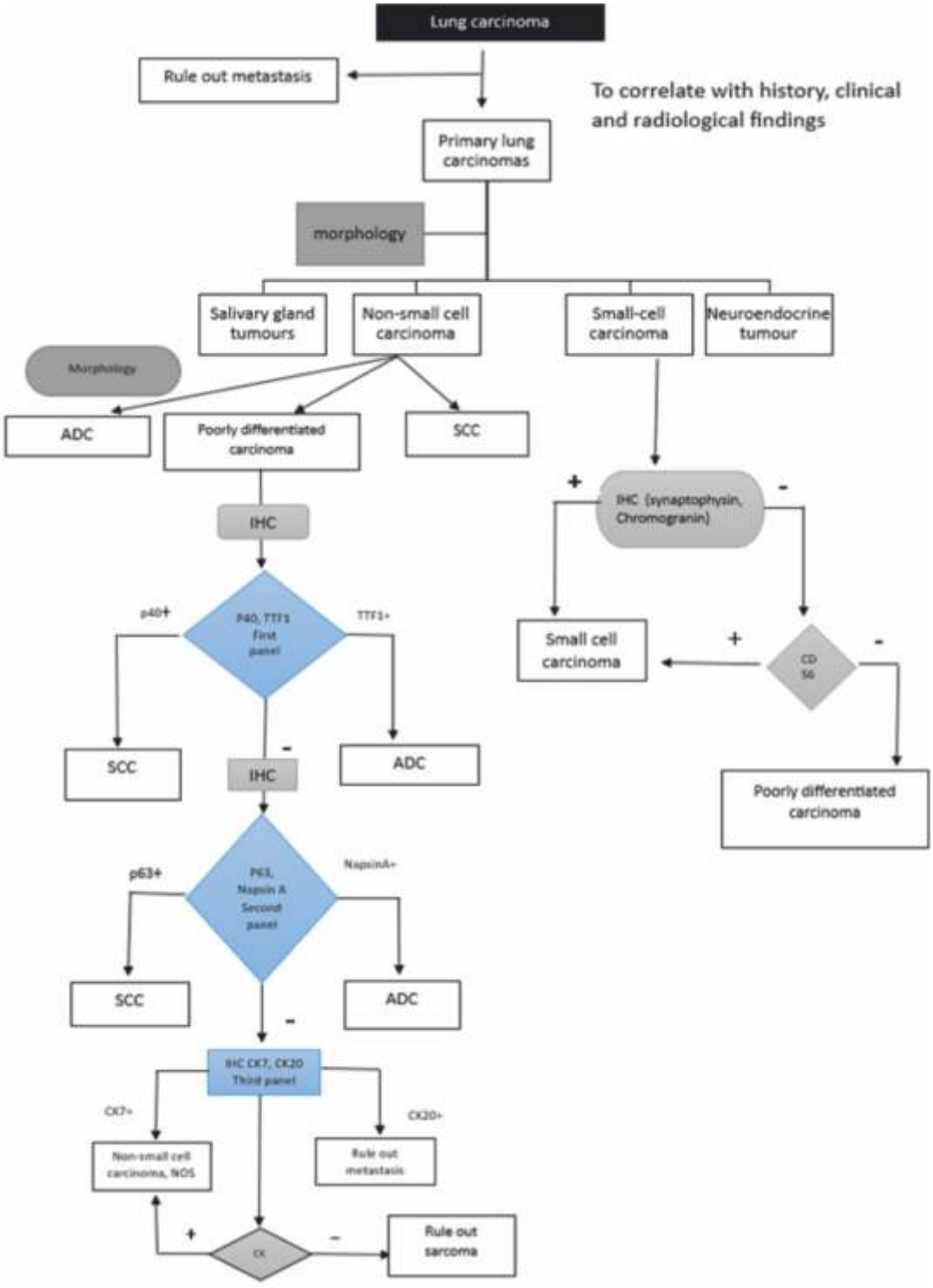

Figure 2: The algorithm provides a stepwise approach to the diagnosis of primary lung carcinomas based on histomorphology and targeted immunohistochemical markers

Discussion

This study highlights the incidence of different types of primary lung carcinomas and stresses the importance of a stratified approach for subtyping primary lung carcinomas. Lung cancer is most common in men, ranging from the highest ratio of 5:1 in North Africa and Western Asia to the lowest of 1.2:1 in Oceania [2]. Gender ratio in our study was 3.9:1. The study that supports our findings includes that of Dev *et al*., where the sex ratio was 4.14:1 [4].

Tobacco consumption is a major contributor to the development of lung cancer [5]. Symptoms often include difficulty breathing, persistent cough, chest discomfort or tightness, voice changes such as hoarseness, and coughing up blood. In certain cases, the lesion may invade the esophagus, creating a clinical presentation similar to esophageal cancer. About 10% of cases exhibit paraneoplastic syndromes, which can manifest as elevated calcium levels, neurological abnormalities, or signs of neuroendocrine activity [2, 6].

Histologic types

The most common histological type of lung carcinoma was ADC comprising of 35.9% cases (n = 95), followed by SCC (19.8%, n = 52), SCLC (6.8%, n = 18), and non-SCLC unclassifiable (33.7%, n = 89). This correlates with the findings of Zhang et al. wherein 39% and 57% were SCC and ADC, respectively. In their study, SCC constituted 25% and 12% in men and women, respectively. The third most common was SCLC (men=11%, women=9%) followed by least common being large cell neuroendocrine carcinoma (men=8%, women=6%) [7]. Indian studies vary in the incidence, with Noronha *et al*. and Krishnamurthy *et al*. documenting ADC as the most common malignancy with 40.3% and 42.6%, respectively [8-9]. The increased incidence of ADC is suspected to be due to changes in the composition of tobacco and its design and is related to inhalation patterns or unknown reasons [2].

The WHO does not recommend the use of IHC in those cases exhibiting clear differentiation on histopathology. However, in the slightest of difficulty, IHC has to be used to confirm whether it's squamous or adenocarcinoma as the treatment modality completely differs, besides preventing missing out of adenosquamous cases. IHC with TTF-1, Napsin A can be used to demonstrate ADC differentiation and p40, p63 can be used to demonstrate squamous differentiation [2].

In present-day therapeutic practices, differentiation of SCC and ADC is crucial because of their role in targeted therapies. These subtypes respond differently to various drugs. Accurate subtyping helps carrying out further molecular analysis, including programmed death ligand (PDL1) and selecting panel for genetic analysis such as *EGFR, KRAS, ALK* and *ROS1* mutations, which are known to occur in ADC [10-11].

The SCLC can be recognized by their unique and distinct morphology. The tumor comprises of small cells, densely packed with nuclear molding, crush artifacts and streaking due to fragile nuclei. Sometimes, morphology can be completely obscured by streaking artifacts. IHC can subsequently be done to confirm the neuroendocrine nature of the cells [12]. Since most cases can be accurately typed on histopathology alone, it is non-SCLC unclassifiable category with no histopathological differentiation is the one that requires maximum immunohistochemical aid.

Immunohistochemistry

Several studies have recommended the use of various markers for lung carcinoma. The WHO recommends TTF-1 and Napsin A which are the pneumocyte markers as helpful in detecting ADC. Squamous differentiation markers would be p40 and p63 [2].

Role of immunohistochemistry in differentiating ADC from SCC in non-SCLC

TTF-1 and Napsin A showed high sensitivity and specificity for lung ADC while p40 and p63 for squamous differentiation and were routinely used for diagnosing difficult cases. This is supported by various studies. Kim *et al.* demonstrated that TTF1 in ADC have 70% sensitivity and 98% specificity, while Napsin-A showed sensitivity and specificity of 81% and 100% cases respectively. IHC p63 was 90% sensitive and 91% specific in SCC [13]. In our study, TTF-1 was performed in 68 cases and was positive in all the ADC cases (100%) whereas Napsin A was performed only in 24 cases of which 22 cases (91.6%) showed positivity, and two cases were negative. This contrasts with the findings of Kim, likely because Napsin A was not uniformly applied across all cases in our study.

CK7 is positive in ADC and must be interpreted with caution, as SCC can be CK7 positive. In our study, seven out of 15 (46%) cases showed CK7 positivity in SCC which is a pitfall and must be remembered before labelling the tumor as ADC. This is supported by Bhatti *et al.* and Camilio *et al.*, where 53.9% and 8.1% of SCC showed CK7 positivity [3, 14]. In the present study, p40 was performed in 30 cases and all 30 cases showed positivity (100%); p63 was performed in three cases where p40 was weakly positive for confirmation. Thus, p40 is an excellent marker for squamous differentiation.

Role of immunohistochemistry in SCLC

Neuroendocrine markers such as chromogranin A, synaptophysin and CD56 are expressed in SCLC [12]. In our study, synaptophysin, chromogranin A were done in all suspected cases of SCLC. CD56 was performed in two cases in SCLC with poorly differentiated morphology, with weak expression of synaptophysin and chromogranin A, and was positive in both cases. CK and CK7 typically show dot-like cytoplasmic staining in small cell carcinoma. It should be kept in mind that non-SCLC markers such as TTF-1 are expressed in SCLC (90% of the cases), which can potentially lead to incorrect interpretation. However, can be used as lineage marker in metastatic settings [15].

In our study, TTF-1 was performed in 13 cases of SCLC, of which 7 (53.8%) were positive. CK was done in all cases and found to be positive in all 18 cases (100%). Synaptophysin was positive in 13 of 18 cases (72.2%), but chromogranin A was positive in all cases (100%). CD56 was performed in two cases and was positive in both cases. CK7 was also positive in 3 out of 11 cases (27.2%). This is consistent with the findings of Guinee *et al.* in which synaptophysin was positive in only 19% of the cases compared to chromogranin A, which was positive in 60% of the cases. It also states that cases can be negative for neuroendocrine markers and should not be labelled as non-SCLC [16].

Role of immunohistochemistry in non-SCLC unclassifiable category

The non-SCLC unclassifiable category includes tumors that show no differentiation on histopathology but can be accurately subtyped using relevant IHC markers. In contrast, the non-SCLC NOS category comprises tumors that remain undifferentiated despite an exhaustive IHC panel. This

category can account for up to 30% of cases, posing significant diagnostic challenges, particularly in settings with limited access to IHC facilities [17].

The non-SCLC unclassifiable after the application of IHC decreased from 33.7% to 3%. In this study, we studied a total of 264 cases of which eight (3%) cases could not be categorized as ADC or SCC even after using the relevant IHC markers and were placed into non-SCLC, NOS category. These tumors showed only CK positivity and were negative for the rest of the markers i.e. CK7, CK20, TTF-1, Napsin-A, p63, p40, synaptophysin and chromogranin A. Sarcoma was ruled out owing to CK diffuse positivity and negativity for other markers (SMA, S100, vimentin and, desmin). Thus, most of the cases in the non-SCLC unclassifiable category were easily subtyped using the minimum IHC panel comprising p40 and TTF-1, with addition of Naspin A and p63 as and when required.

Various studies have been conducted regarding the minimal use of IHC markers for appropriate subtyping of lung carcinomas. A recommendation by Hassan *et al.*, who used step wise approach to diagnose lung carcinoma, was that TTF-1, Napsin A, p63, and CK7 can accurately subtype non-SCLC category on small lung biopsies [18]. Rahman *et al.*'s recommended minimal panel that can be used to classify lung primary are Napsin A, CK5/6, CD56 which can effectively divide the primary lung carcinomas. Eighty-four cases were studied, of which 8 (9.5%) could not be specifically categorized and were placed into the non-SCLC category in their study [18]. These studies specifically stress the importance of using combination of several markers in lung carcinomas to accurately classify lung carcinomas.

Conclusion

We conclude that IHC markers comprising of TTF-1, Napsin A, p40, p63, synaptophysin, chromogranin A, CK and CK7 when used judiciously and in tiered manner aids in accurately subtyping primary lung carcinomas and potentially narrow down the cases categorized as non-SCLC unclassifiable on histopathology from 33.7% down to 3%, thereby effectively aiding in appropriate therapy.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, *et al*. Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2021;71(3):209-249.
2. Nicholson AG, Tsao MS, Beasley MB, Borczuk AC, Brambilla E, Cooper WA, *et al*. The 2021 WHO Classification of lung tumors: Impact of advances since 2015. *J Thorac Oncol* 2022;17(3):362-387.
3. Bhatti V, Kwatra KS, Puri S, Calton N. Histopathological spectrum and immunohistochemical profile of lung carcinomas: A 9-year study from a tertiary hospital in north India. *Int J Appl Basic Med Res* 2019; 9(3):169-175.
4. Dey A, Biswas D, Saha SK, Kundu S, Kundu S, Sengupta A. Comparison study of clinicoradiological profile of primary lung cancer cases: an eastern India experience. *Indian J Cancer* 2012; 49(1):89-95.
5. Sinha P, Kapoor S, Kadam S, Sahu N, Jaiswal A. Potential therapeutic modalities in cancer gene therapy. *J Krishna Inst Med Sci Univ* 2017; 6(2): 3-18.
6. Kamakeri NS. Bronchogenic carcinoma mimicking esophageal carcinoma – A case report. *J Krishna Inst Med Sci Univ* 2016; 5(3):131-135.
7. Zhang Y, Vaccarella S, Morgan E, Li M, Etxeberria J, Chokunonga E, *et al*. Global variations in lung cancer incidence by histological subtype in 2020: a population-based study. *Lancet Oncol* 2023; 24(11): 1206-1218.

8. Noronha V, Dikshit R, Raut N, Joshi A, Pramesh CS, George K, *et al*. Epidemiology of lung cancer in India: focus on the differences between non-smokers and smokers: a single-centre experience. *Indian J Cancer* 2012; 49(1):74-81.
9. Krishnamurthy A, Vijayalakshmi R, Gadigi V, Ranganathan R, Sagar TG. The relevance of "Nonsmoking-associated lung cancer" in India: a single-centre experience. *Indian J Cancer* 2012; 49(1): 82-88.
10. Howlader N, Forjaz G, Mooradian MJ, Meza R, Kong CY, Cronin KA, *et al*. The effect of advances in lung-cancer treatment on population mortality. *N Engl J Med* 2020; 383(7):640-649.
11. Poirier JT, George J, Owonikoko TK, Berns A, Brambilla E, Byers LA, *et al*. New approaches to SCLC therapy: From the laboratory to the clinic. *J Thorac Oncol* 2020;15(4):520-540.
12. Krpina K, Vranić S, Tomić K, Samaržija M, Batičić L. Small cell lung carcinoma: Current diagnosis, biomarkers, and treatment options with future perspectives. *Biomedicines* 2023; 11(7):1982.
13. Kim MJ, Shin HC, Shin KC, Ro JY. Best immuno-histochemical panel in distinguishing adenocarcinoma from squamous cell carcinoma of lung: tissue microarray assay in resected lung cancer specimens. *Ann Diagn Pathol* 2013;17(1):85-90.
14. Camilo R, Capelozzi VL, Siqueira SA, Del Carlo Bernardi F. Expression of p63, keratin 5/6, keratin 7, and surfactant-A in non-small cell lung carcinomas. *Hum Pathol* 2006; 37(5):542-546.
15. Misch D, Blum T, Boch C, Weiss T, Crolow C, Griff S, *et al*. Value of thyroid transcription factor (TTF)-1 for diagnosis and prognosis of patients with locally advanced or metastatic small cell lung cancer. *Diagn Pathol* 2015; 10:21.
16. Guinee DG Jr, Fishback NF, Koss MN, Abbondanzo SL, Travis WD. The spectrum of immunohistochemical staining of small-cell lung carcinoma in specimens from transbronchial and open-lung biopsies. *Am J Clin Pathol* 1994;102(4):406-414.
17. Bishop JA, Teruya-Feldstein J, Westra WH, Pelosi G, Travis WD, Rekhtman N. p40 (ΔNp63) is superior to p63 for the diagnosis of pulmonary squamous cell carcinoma. *Mod Pathol* 2012; 25(3):405-415.
18. Hassan A, Alahmadi S, Waqas O, Waseem H, Abdelrahman AS, Almansouri M, *et al*. Accuracy of classifying lung carcinoma using immunohistochemical markers on limited biopsy material: A two-center study. *Cureus* 2022;14(12): e32956.

Author for Correspondence:

Dr. Anitha Mathews, Regional Cancer Center, Thiruvananthapuram, Kerala-695001

Email: anithamfrancis@gmail.com Cell: 9847919270

How to cite this article:

Prabhu S, Mathews A, Nair S, Rao ACK, Mony R, Kattoor J. Role of stratification of immunohistochemistry markers in primary lung carcinomas: experience in a south Indian tertiary care center. *J Krishna Inst Med Sci Univ* 2024; 13(4):149-158.

Submitted: *21-July-2024* ***Accepted:*** *21-Sep-2024* ***Published:*** *01-October-2024*