Original Article

Histopathological Spectrum and Immunohistochemical Profile of Lung Carcinomas: A 9-Year Study from a Tertiary Hospital in North India

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Abstract

Background: Lung cancer is the most common cancer worldwide and the leading cause of cancer-related death. Diagnostic bronchoscopic or percutaneous biopsies are usually small. However, judicious use of immunohistochemistry (IHC) helps in accurate subtyping, which forms the basis for molecular tests and treatment. **Aim:** The aim was to study the role of IHC in the diagnosis of various histological subtypes of lung cancer. **Methods:** This 9-year study from 2009 to 2017 included all cases diagnosed as lung carcinoma on tissue biopsies. IHC markers were selected based on histopathology, from a panel comprising CK7, CK20, CK5/6, p63, thyroid transcription factor 1 (TTF-1), napsin A, synaptophysin, chromogranin A, neuron-specific enolase, CD56, and CDX2. Metastatic cancers to the lung were excluded from the study. **Results:** There were 199 cases of lung carcinoma comprising squamous cell carcinoma (37.7% [*n* = 75]), adenocarcinoma (26.1% [*n* = 52]), small cell carcinoma (20.6% [*n* = 41]), non-small cell lung carcinoma-unclassified (10.1% [*n* = 20]), adenosquamous carcinoma (2.5% [*n* = 5]), and others (3% [*n* = 6]). IHC was done on 47.7% (95/199) of cases. Squamous cell carcinomas showed CK5/6 and p63 positivity in 13/13 (100%) and 12/13 (92.3%) cases, respectively. Adenocarcinomas were positive for napsin A in 12/13 (92.3%) and TTF-1 in 35/41 (85.4%) cases. Neuroendocrine markers were positive in all small cell carcinomas. **Conclusion:** Squamous cell carcinoma was the most common primary lung malignancy in the North Indian population, followed by adenocarcinoma and small cell carcinoma. IHC panel of TTF-1, napsin A, CK5/6, and p63 is very helpful to classify most non-small cell lung carcinomas.

Keywords: *Cancer, immunohistochemistry, lung, North India*

Introduction

Lung cancer is a major public health problem. It is the most common cancer worldwide and the leading cause of cancer-related mortality, amounting to 1.76 million cancer deaths per year.[1] It is almost twice as common in males than in females.

In India, the incidence of lung cancer is lower than that in the west. It is the second most common cancer in males, while in females, it is ranked 6th. The mortality rate is ranked 4th after breast, cervix, and lip-oral cavity cancer.[2]

Diagnostic biopsies for detecting lung cancer are usually small and are obtained by bronchoscopy, by percutaneous route under image guidance, or from metastatic sites. Cell block preparations from malignant pleural effusions can also be used.

It is a challenge for a pathologist to accurately diagnose and classify lung cancer in small biopsies. However, judicious use of immunohistochemistry (IHC) assists in accurate histological categorization, which forms the basis for deciding further molecular tests and planning treatment.[3] This cross-sectional study from a tertiary care hospital in North India aimed to study the distribution of various histological subtypes of lung cancer in the Indian population and role of IHC in their categorization.

Methods

This 9-year study from 2009 to 2017 included all cases diagnosed as lung carcinoma, whether biopsied from lung or metastatic sites. Histopathology of all cases and IHC, wherever done, were reviewed. The IHC markers were chosen based on histopathological findings, from a panel comprising CK7 (Leica, clone OV-TL 12/30, prediluted), CK20 (Dako, clone K$_s$20.8, prediluted), CK5/6 (Dako, clone D5/16 B4, prediluted), p63 (Biogenex,

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clone 4A4, prediluted), TTF-1 (Biogenex, clone 8G7G3, prediluted), napsin A (Leica, clone IP64, dilution 1:400), synaptophysin (Biogenex, clone Snp88, prediluted), chromogranin A (Dako, clone DAK-A3), neuron-specific enolase (NSE, Biogenex, clone MIG-N3, prediluted), CD56 (Leica, clone 1B6, prediluted), and CDX2 (Biogenex, clone CDX2-88, prediluted). IHC was performed using the Novolink HRP-linked Polymer Detection System with DAB chromogen from Leica Biosystems.

Results

There were 199 cases of lung carcinoma, of which 148 (74.4%) were diagnosed on biopsies from primary site in the lung and the rest 51 (25.6%) from metastatic sites. The overall male-to-female (M:F) ratio was 2.8:1, and the age ranged from 29 to 89 years with a mean of 60.9 ± 11 years. The mean age for males and females was 61.6 and 59.1 years, respectively. The age group of 51–70 years included 131 (65.9%) patients. The age and gender distribution of lung carcinomas are shown in Table 1.

The most common histological type of lung carcinoma was squamous cell carcinoma comprising 37.7% (n = 75), followed by adenocarcinoma (26.1% [n = 52]), small cell carcinoma (20.6% [n = 41]), and non-small cell lung carcinoma-unclassifiable (10.1% [n = 20]). Within the unclassifiable category of non-small cell lung carcinoma, IHC was not done in 15 cases, while five cases could not be classified even after IHC. Rest of the 11 cases comprised adenosquamous carcinoma (2.5% [n = 5]), spindle cell carcinoma (1% [n = 2]), adenoid cystic carcinoma (1% [n = 2]), combined small cell carcinoma (0.5% [n = 1]), and large cell neuroendocrine carcinoma (0.5% [n = 1]).

Squamous cell carcinoma and small cell carcinoma showed a marked male predominance with a sex ratio of 9.7:1 and 4.1:1, respectively, while adenocarcinoma was more common in females with a M:F ratio of 1:1.4.

Of the 51 cases which were diagnosed on biopsies from metastatic sites, the distribution was as follows: regional lymph nodes (47.1% [n = 24]), liver (17.7% [n = 9]), bone (11.8% [n = 6]), contiguous soft-tissue extension (9.8% [n = 5]), pleura (5.9% [n = 3]), brain (5.9% [n = 3]), and pericardium (2% [n = 1]).

IHC was done on 47.7% (95/199) cases. The IHC profile of various histological types is shown in Table 2.

Of 75 cases diagnosed with squamous cell carcinoma, IHC was done on 13 cases. CK5/6 was positive in all

Table 1: Age and gender distribution of lung carcinomas

Age range	Squamous cell carcinoma	Adenocarcinoma	Small cell carcinoma	Non-small cell lung carcinoma-unclassified	Others	Total (%)
≤30	0	1	0	0	0	1 (0.5)
31-40	2	2	1	0	1	6 (3.0)
41-50	12	9	6	2	1	30 (15.1)
51-60	24	18	12	9	3	66 (33.2)
61-70	25	14	18	4	4	65 (32.7)
71-80	7	6	4	4	2	23 (11.5)
≥81	5	2	0	1	0	8 (4.0)
Total	75	52	41	20	11	199
Male: Female ratio	9.7:1	1:1.4	4.1:1	9:1	1.2:1	2.8:1

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

IHC marker	Squamous cell carcinoma ($n^{\dagger}$=13/75) (%)	Adenocarcinoma (n=41/52) (%)	Small cell carcinoma (n=28/41) (%)	Non-small cell lung carcinoma - unclassifiable (n=5/20) (%)
CK7	7/13 (53.9)	37/37 (100)	14/22 (63.6)	3/4 (75)
CK20	1/13 (7.7)	6/39 (15.4)	4/22 (18.2)	1/4 (25)
CK5/6	13/13 (100)	7/15 (46)	6/15 (40)	3/5 (60)
p63	12/13 (92.3)	6/15 (40)	14/17 (82.4)	3/5 (60)
TTF-1	3/13 (23.1)	35/41 (85.4)	25/28 (89.3)	4/5 (80)
Napsin A	0/9 (0)	12/13 (92.3)	0/5 (0)	2/3 (66.7)
Synaptophysin	0/1 (0)	0/1 (0)	27/27 (100)	-
Chromogranin A	0/2 (0)	-	13/13 (100)	-
NSE	-	-	9/9 (100)	-
CD56	0/1 (0)	0/1 (0)	12/12 (100)	-

†(n) number of cases on which IHC was done; (-): IHC marker not done. NSE: Neuron- specific enolase; IHC: Immunohistochemistry; TTF-1: Thyroid transcription factor 1

the 13 cases [Figure 1a and b] followed by p63, which was positive in 92.3% (12/13). Positivity for CK7 in 53.9% (7/13) cases and thyroid transcription factor 1 (TTF-1) in 23.1% (3/13) cases was also observed. The staining intensity of TTF-1 was, however, weak.

Of 52 cases diagnosed with adenocarcinoma, IHC was done in 41 cases. CK7 was positive in all the cases on which it was done (37/37), followed by napsin A in 92.3% (12/13) and TTF-1 in 85.4% (35/41) [Figure 1c and d]. Positivity for p63 in 40% (6/15), CK5/6 in 46% (7/15), and CK20 in 15.4% (6/39) cases was also observed although staining was focal and/or weak in most cases.

Histologically, ten cases were invasive mucinous adenocarcinomas. Seven cases, on which IHC was done, showed positivity for CK7 (6/6), TTF-1 (4/7), CK20 (1/6), CDX2 (1/6), and CK5/6 (1/1). They were negative for p63 (0/1) and napsin A (0/1).

Three cases were poorly differentiated adenocarcinomas with signet ring cells. Two of them on IHC were positive for CK7 and TTF-1, whereas they were negative for CK20 and CDX2. One case showed positivity for CK7, CK20, and CDX2 whereas being negative for TTF-1. Clinical and radiological correlation to rule out metastasis from the gastrointestinal tract was necessary in this case.

There were 41 cases of small cell carcinoma, of which IHC was done on 28 cases. Neuroendocrine markers were positive in all cases tested as shown in Table 2 and Figure 1e, f. TTF-1, p63, and CK7 were positive in 89.3% (25/28), 82.4% (14/17), and 63.6% (14/22) cases, respectively. In addition, there was a single case of large cell neuroendocrine carcinoma diagnosed on a metastatic axillary lymph node, which was diffusely positive for TTF-1 and synaptophysin, focally for CK7 and was negative for p63, CK5/6, and chromogranin A. A single case was diagnosed with metastatic combined small cell carcinoma, showing components of small cell and spindle cell carcinoma.

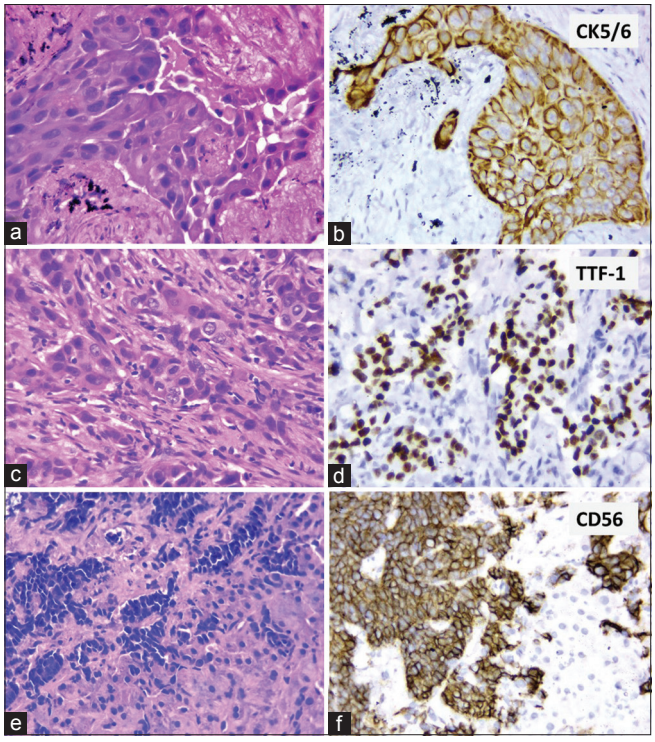

Figure 1: (a and b) Squamous cell carcinoma H and E and CK5/6 immunohistochemistry × 400; (c and d) primary lung adenocarcinoma H and E and thyroid transcription factor 1 immunohistochemistry × 400; (e and f) liver metastasis of small cell lung carcinoma H and E and CD56 immunohistochemistry × 400

Five cases of non-small cell lung carcinoma-unclassifiable showed variable expression of CK5/6, p63, TTF-1, napsin A, CK7, and CK20 and hence could not be further classified even after IHC.

There were five cases of adenosquamous carcinoma. In one case, the two components were obvious on histopathology. In rest of the four cases, IHC showed positivity for CK5/6, p63, TTF-1, napsin A, and CK7 in different cell populations.

There were two cases of spindle cell carcinoma, showing malignant spindle cells positive for cytokeratin and vimentin.

Discussion

Our study of 199 lung cancer patients diagnosed over 9 years gives a glimpse of its occurrence in the North Indian population.

The mean age at the diagnosis was 60.9 years, which is slightly higher as compared to other Indian studies, which have shown a median or mean age ranging between 47.4 and 56 years [Table 3].[4-8] In this study, males had a higher mean age (61.6 years) as compared to females (59.1 years), an observation reflected in other Indian studies also.[5,7]

Lung cancer has always been more common in males. The gender ratio in our study was 2.8:1, which is higher as compared to that in the world population, in whom the ratio is 1.9:1.[1] In developed countries, the incidence rates between men and women have started converging, partly attributed to changing patterns of cigarette smoking.[9] However, studies on Indian population are still showing a high M:F ratio ranging from 3.5 to 4.6:1 [Table 3].[4-8]

Histological types

Epidemiological studies have shown a changing trend in the histological subtypes of lung cancer over time. In the Western countries and most of the Asian countries, adenocarcinoma has surpassed squamous cell carcinoma as the most common histologic variant of lung cancer. The Surveillance, Epidemiology, and End Results cancer statistics 2011–2015 covering US population shows percentage distribution of various histological subtypes to be adenocarcinoma (47.9%), squamous cell carcinoma (23.2%), small cell

Table 3: Demographic profile and distribution of histological subtypes of lung carcinoma in various Indian studies

Study	Duration (period)	Number of cases	Mean/Median age	Male:Female ratio	Squamous cell carcinoma (%)	Adenocarcinoma (%)	Small cell carcinoma (%)	Other (%)
Present study	9 years (2009-2017)	199	60.9	2.8:1	37.7	26.1	20.6	15.6
Malik *et al*.[4]	3 years (2008-2011)	434	55	4.6:1	25.1	41.0	14.8	19.1
Dey *et al*.[5]	4 years (2006-2009)	607	47.4	4.14:1	35.1	30.8	16.5	17.6
Noronha *et al*.[6]	1 year (2008)	489	56	3.5:1	24.1	40.3	8.0	27.6
Krishnamurthy *et al*.[7]	5 years (2003-2007)	258	56	3.5:1	15.9	42.6	13.2	28.3
Singh *et al*.[8]	1.5 years (2007-2009)	250	57.9	4.4:1	34.8	26	18.4	20.8

carcinoma (12.9%), and others (15.9%).[10] However, in this regard, the Indian population is heterogenous. While some Indian studies are reflecting the trend of increasing incidence of lung adenocarcinoma as seen in the west,[4,6,7] some studies continue to report squamous cell carcinoma as the most prevalent subtype of lung cancer[5,8] [Table 3]. In this study, squamous cell carcinoma was the most common, followed by adenocarcinoma and small cell carcinoma. Since cigarette smoking is strongly associated with squamous cell carcinoma and small cell carcinoma, the declining incidence of these two subtypes is probably related to declining prevalence of smoking. The increase in the incidence of adenocarcinoma could be related to change in design of cigarettes, composition of tobacco and inhalation patterns, or other unknown causes.[9]

Although evaluation of hematoxylin and eosin (H and E) sections is sufficient to classify a large proportion of lung cancers, IHC plays an important role in their evaluation. Since lung is the most common site for metastatic tumors, it is essential to rule out metastasis before considering a primary lung malignancy.[11]

IHC is also required in poorly differentiated primary lung cancers, showing no definite squamous or glandular differentiation on H and E morphology. In this context, differentiation between squamous cell carcinoma and adenocarcinoma, erstwhile clubbed into non-small cell lung carcinoma category for therapeutic purposes, is very crucial in the present-day oncology practice. This is because the anti-folate chemotherapeutic drug, pemetrexed, has shown efficacy in only adenocarcinomas of the lung. Moreover, anti-vascular endothelial growth factor drug, bevacizumab, is contraindicated in squamous cell carcinoma of lung due to the risk of fatal pulmonary hemorrhage.[12,13] If the histology is adenocarcinoma, molecular tests play an important role due to the availability of targeted therapy.[3]

Most small cell carcinomas can be recognized by their distinct morphology. The tumour comprises of densely packed small cells with scant cytoplasm, showing nuclear molding, finely dispersed chromatin, and inconspicuous nucleoli. IHC can subsequently be done to confirm the neuroendocrine nature of the small cells.

Role of immunohistochemistry in differentiating primary lung carcinoma from metastatic carcinoma

Various IHC markers have been evaluated to differentiate primary lung adenocarcinomas from metastatic adenocarcinomas. TTF-1 and napsin A are pneumocyte markers, and both have shown high sensitivity and specificity for primary lung adenocarcinomas [Table 4].[14-21] CK7 in combination with CK20 and CDX2 is useful to differentiate primary lung adenocarcinoma (CK7+/CK20−/CDX2−) from metastatic colon carcinoma (CK7−/CK20+/CDX2+).[11,23] However, immunoprofile of primary mucinous lung adenocarcinomas (including formerly designated bronchoalveolar carcinomas), colloid carcinomas, and adenocarcinomas with enteric differentiation is different from other adenocarcinomas and overlaps with that of gastric and pancreatobiliary tract carcinomas. These tumors are often positive for CK7, CK20, and CDX2 and can be negative for TTF-1 and napsin A.[11,23] Of the ten cases of mucinous adenocarcinoma in our study, TTF-1 was negative in 42.9% (3/7) cases and CK7 was positive in 100% (6/6), while CK20 and CDX2 were positive in 16.7% (1/6) cases each. Clinical correlation was necessary to rule out metastasis from gastrointestinal tract and pancreatobiliary tract.

TTF-1 has limited value in differentiating pulmonary from extrapulmonary squamous cell carcinomas since most pulmonary squamous cell carcinomas are immunonegative [Table 4].[14,18-20] However, weak and/or focal positivity for TTF-1 can be encountered, especially in poorly differentiated tumors [Table 4].[15-17,21] In this study, TTF-1 was weakly and/or focally positive in 23.1% cases of squamous cell carcinoma, most of which were poorly differentiated.

Table 4: Comparative immunohistochemical profile of pulmonary adenocarcinoma and squamous cell carcinoma

Study	Adenocarcinoma (percentage IHC positivity)					Squamous cell carcinoma (percentage IHC positivity)				
	TTF-1	Napsin A	CK5/6	p63	CK7	TTF-1	Napsin A	CK5/6	p63	CK7
Present study	85.4	92.3	46	40	100	23.1	0	100	92.3	53.9
Xu *et al*.[14]	94.6	-	4.5	9	80.2	0	-	91.9	97	8.1
Kim *et al*.[15]	70	81	4	9	-	2	0	90	91	-
Whithaus *et al*.[16]	60	83	4	14	-	2	2	53	95	-
Rekhtman *et al*.[17]	89	-	18	32	-	3.5	-	97.8	100	-
Pelosi *et al*.[18]	81.9	-	4.5	46.7	100	0	-	100	100	30
Nicholson *et al*.[19]	62.5	-	0	14.3	-	0	-	100	100	-
Johansson[20]	100	-	9.1	-	100	0	-	100	-	33.3
Tan *et al*.[21]	68	-	-	-	-	20.9	-	-	-	-
Camilo *et al*.[22]	-	-	56.2	0	94.1	-	-	47.1	77.8	5.6

IHC: Immunohistochemistry; TTF-1: Thyroid transcription factor 1; (-): IHC marker not done

Role of immunohistochemistry in differentiating poorly differentiated pulmonary adenocarcinoma from squamous cell carcinoma

As already stated, TTF-1 and napsin A show high sensitivity and specificity for lung adenocarcinomas [Table 4].[14-21] Caution needs to be excised in interpreting TTF-1 and napsin A IHC in small lung biopsies as normal alveolar epithelium will also express these markers. CK7 has also been used as a marker for adenocarcinomas; however, sometimes, it can be expressed by squamous cell carcinoma, limiting its utility [Table 4].[14,18,20]

Markers of squamous differentiation commonly evaluated in lung carcinomas include p63, CK5/6, and p40.[14-20,22,24] Most studies have shown high sensitivity of CK5/6 and p63 for the detection of squamous cell carcinoma; however, their expression in adenocarcinomas can be variable [Table 4]. Camilo *et al*.[22] found CK5/6 positivity in 56% of adenocarcinomas, while Pelosi *et al*.[18] found p63 expression in 47.6% of adenocarcinomas, thus limiting the usefulness of these markers. In our study, although CK5/6 and p63 were expressed in 100% and 92.3% cases of squamous cell carcinomas, respectively, focal and/or weak expression of CK5/6 and p63 were found in 46% and 40% cases of adenocarcinoma, respectively. More recently, p40 has been shown to be an excellent marker for squamous differentiation. p40 is equivalent to p63 in sensitivity for squamous cell carcinoma, but it is markedly superior to p63 in specificity.[24]

Signet ring cell carcinoma of the lung is rare and regarded as a variant of poorly differentiated adenocarcinoma. IHC is often helpful to rule out metastasis as primary pulmonary adenocarcinomas with signet ring cells frequently express TTF-1 and CK7 but are usually negative for CK20 and CDX-2.[25] Two of three cases in our study were positive for CK7 and TTF-1, whereas they were negative for CK20 and CDX2. The third case showed positivity for CK7, CK20, and CDX2 whereas being negative for TTF-1. Clinical correlation was necessary in this case to rule out metastasis from the gastrointestinal tract.

A small percentage of non-small cell lung cancers cannot be classified even after an elaborate IHC panel.[16,19,26] There were five such cases in our study, which could not be classified and were designated non-small cell lung carcinoma-unclassifiable.

Immunohistochemistry in small cell carcinoma

Neuroendocrine markers such as CD56, chromogranin A, synaptophysin, and NSE are consistently expressed by small cell carcinomas, of which CD56 is the most sensitive. In this study, all cases of small cell carcinoma showed 100% positivity for all these markers. Expression of non-neuroendocrine markers in small cell carcinoma have also been studied [Table 5]. Besides pan-cytokeratin, which is positive, other cytokeratins such as CK7, CK5/6, and CK20 can also show variable positivity.[20,27] CK5/6 expression in our study was higher as compared to other studies.[20,27,28] p63 and TTF-1 are also expressed in a large proportion of cases.[20,27-29] However, caution needs to be excised because TTF-1 expression can be encountered in small cell carcinomas of sites other than lung, such as small cell carcinomas of the bladder, prostate, gastrointestinal tract, and female genital tract.[30]

Metastatic sites comprised 25.6% (51/199) of biopsies in our study. Lymph node was the most common site (47.1%), followed by liver (17.7%), bone (11.8%), contiguous soft-tissue extension (9.8%), pleura (5.9%), brain (5.9%), and pericardium (2%). Singh *et al*. in their study of 607 cases found metastasis in the liver (7.7%), brain (4%), adrenal (3.3%), bone (4.5%), chest wall (5.9%), and opposite lung (1.2%).[8]

Conclusion

Squamous cell carcinoma is still the most prevalent histological type of lung cancer in the North Indian population, followed by adenocarcinoma and small cell carcinoma. The M:F ratio is also higher than the global ratio.

Although morphology alone is sufficient for diagnoses in a large proportion of cases, IHC can help classify problematic cases.

Table 5: Immunohistochemical profile (percentage positivity) of non-neuroendocrine markers in small cell lung carcinoma

Study	CK7	CK20	CK5/6	p63	p40	TTF-1	Napsin A
Present study	63.6	18.2	40	82.4	-	89.3	0
Johansson[20]	84.6	15.4	15.4	-	-	100	-
Masai *et al*.[27]	25	-	1.5	22.1	-	85.5	-
Zhang *et al*.[28]	-	-	0	0	0	71.4	0
Au *et al*.[29]	-	-	-	77	-	-	-

TTF-1: Thyroid transcription factor 1; (-): IHC marker not done

TTF-1 in combination with napsin A can reliably identify primary lung adenocarcinomas. Weak expression of TTF-1 can be encountered in squamous cell carcinomas; however, napsin A is more specific and not expressed by squamous cell carcinomas. Although CK5/6 and p63 will stain most squamous cell carcinomas, they may be focally positive in adenocarcinomas and hence should be interpreted carefully in combination with TTF-1 and napsin-A.

An IHC panel comprising TTF-1, napsin A, CK5/6, and p63 can resolve most of the diagnostic issues related to non-small cell lung carcinoma morphology. p40, which is a promising novel marker for squamous cell carcinoma, was not assessed in our study; however, additional studies can be done to further explore its utility.

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Conflicts of interest

There are no conflicts of interest.

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