Original Article

Percutaneous Core Needle Biopsy in the Diagnosis of Lung Lesions: An Experience on 280 Consecutive Cases from a University Hospital in Southern India

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ABSTRACT

Context: Percutaneous needle biopsy of lung (PCNBL) is advantageous over bronchoscopic biopsies to obtain adequate sample for peripheral lung lesions. **Objective:** The objective was to evaluate the diagnostic yield of image-guided PCNBL in the diagnosis of lung lesions and to classify lung carcinomas as per the recently proposed International Association for the Study of Lung Cancer (IASLC)/American Thoracic Society/European Respiratory Society classification for small biopsies modified and adopted by the World Health Organization, 2015. **Materials and Methods:** A total of 280 image-guided PCNBL were analyzed. The radiological findings and routine hematoxylin and eosin (H&E)-stained sections along with immunohistochemistry (IHC) were analyzed in all the cases. Molecular testing was done depending on tissue diagnosis and availability. **Results:** Majority (81%) were diagnosed as malignant lesions, with adenocarcinoma (ADC) being the most common. More than 70% were diagnosed on H&E morphology alone, with thirty cases requiring IHC to categorize as ADC. Nearly 60% were categorized as squamous cell carcinoma on morphology alone and the rest required IHC. Though TTF1 showed higher sensitivity than napsin A, the latter is more specific. Both p63 and p40 were found to be highly sensitive for squamous cell carcinoma, but p40 was more specific than p63. Epidermal growth factor receptor could be evaluated on 94.4% of ADC samples, indicating good yield for molecular testing. **Conclusion:** PCNBL yields adequate sampling for tissue diagnosis and ancillary testing with minimal complications. The use of IHC markers reduces the number of non-small-cell not otherwise specified cases significantly.

KEY WORDS: Immunohistochemistry, molecular tests, percutaneous needle biopsy of lung

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INTRODUCTION

Image-guided percutaneous needle biopsy of lung (PNBL) is an established and cost-effective procedure for selected patients with suspected pathology. With

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advances in imaging technology, biopsy equipment, and technique, the yield, efficacy, and sensitivity of PNBL have also improved.[1,2] It offers advantages over bronchoscopic biopsy particularly when lesions are peripheral and <2 cm in diameter. There is considerable inter-institutional variability in the technical performance of PNBL, with some performing computed tomography (CT)-guided fine-needle aspiration biopsy (FNAB) for cytologic evaluation, whereas others performing PCNBL, or a combination of both.[3] On comparison with FNAB, PCNBL has achieved a superior diagnostic accuracy for benign and malignant lung tumors.[4-6] At present, the role of pathologists in the management of lung carcinomas includes accurate diagnosis, especially categorization of non-small-cell carcinoma (NSCC) into squamous cell carcinoma (SQCC) or adenocarcinoma (ADC) and utilize the tissue in a way that immunohistochemical (IHC) and/or molecular studies can be performed to obtain predictive and prognostic data.

The purpose of this study is to evaluate the diagnostic yield of image-guided percutaneous needle biopsy in lung lesions and to classify lung carcinomas as per the recently proposed International Association for the Study of Lung cancer (IASLC)/American Thoracic Society (ATS)/European Respiratory Society (ERS) classification for small biopsies modified and adopted by the World Health Organization (WHO), 2015.[7]

MATERIALS AND METHODS

Study design

This is a combined retrospective and prospective study including all consecutive cases of image-guided PCNBL received from January 2012 to June 2015. The study was approved by the institutional ethics committee. The relevant clinical and other information was retrieved from medical records.

Imaging and biopsy procedure

Majority of the biopsies were done by radiologists of our hospital, mostly under CT guidance. A few of them which were done outside including tissue or slides or formalin-fixed paraffin-embedded tissue were submitted for opinion. Prior to the procedure, recent contrast-enhanced CT chest images of the patients were reviewed by the radiologist in order to plan the biopsy. Most central lesions accessible to bronchoscopic biopsies were excluded from the study. Peripheral and large central lesions amenable to CT-guided biopsy were included in the study. The biopsy access site and path were decided based on the location of the lesion and its proximity to vascular structures, especially subclavian, internal mammary, intercostal, and intrapulmonary vessels. Patient position for biopsy was chosen depending on the location and size of the lesion and also based on patient's ability to tolerate positioning during the procedure. In patients with emphysematous changes in the lung, passing the needle through bullous lesions and transgressing fissures was avoided as far as possible to minimize the risk of pneumothorax. The shortest possible safe route to target the lesion was selected and percutaneous CT-guided lung biopsy was performed during quiet breathing under aseptic precautions using an 18- or 20G Bard Maxcore biopsy gun through a 17- or 19G coaxial needle under local anesthesia. Details of procedure-related complications, if any, were noted. The radiological findings were reviewed by the radiologist with expertise in lung imaging, wherever images were available for review. For the cases where radiology images were unavailable for review, the findings were collected from the medical records.

Morphological analysis

Hematoxylin and eosin (H and E)-stained slides of all the cases were reviewed by two pathologists to confirm the diagnosis. The initial morphological diagnosis was made with blinding the results of special stains and IHC. IHC results were reviewed wherever performed. All IHCs were done on a fully automated immunostainer (Xmatrx; Biogenex, CA, USA) by poly-HRP (Horse Radish Peroxidase) technique. The primary antibody panel used was determined by initial morphological impression. WIn general, the antibodies used are provided in Table 1.

The number of cases with inadequate biopsies was noted and the reason for inadequacy was analyzed. The rest of the biopsies were initially broadly categorized as either benign or malignant. The former were further classified as neoplastic or inflammatory/infective. Among the nonneoplastic lesions, any specific pathology if identified was also noted. In cases of lung carcinomas, they were categorized as per the recently proposed IASLC/ATS/ERS classification for small biopsies modified and adopted by the WHO, 2015.[7]

Molecular testing

Molecular testing for treatment selection was done in some of the cases. The cases selected for testing depended on the pathology diagnosis, availability of tissue, and affordability of treatment. The molecular analysis was performed in an outside referral lab by sending formalin-fixed paraffin-embedded tissue after initial morphological diagnosis. All the available molecular testing results were collected and analyzed. The treatment and follow-up details were collected wherever available.

RESULTS

During the study period, a total of 280 image-guided PCNBL from 273 patients were received. Seven patients had second biopsy with the reasons being follow-up biopsy to know disease status, inadequate initial biopsy, and discrepancy between clinico-radiological and initial biopsy findings in two patients each. In the remaining one patient, biopsy was repeated to obtain an additional tissue for IHC.

Table 1: Primary antibody panel used in the study

	Antibody panels	Remarks
Squamous cell carcinoma markers	P63, P40, CK5/6	These antibodies were used either alone or in combination
Adenocarcinoma markers	TTF-1, napsin A, CK7, CK20	These antibodies were used either alone or in combination
Neuroendocrine markers	Chromogranin, synaptophysin	
Lymphoma markers	LCA, CD3, CD20, CD10, CD5, CD23, Cyclin D1, CD30, Alk, Bcl2, Pax-5	These antibodies were used in variable combinations
Mesenchymal markers	SMA, desmin, S-100, vimentin, CD34, Alk	These antibodies were used in variable combinations
Mesothelial markers	Calretinin, WT1, D240	These antibodies were used in variable combinations
Metastatic tumors	CK7, CK20, CDX2, ER, PR, GCDFP-15, EMA, thyroglobulin, TTF-1	The antibodies used varied based on known/unknown primary
Proliferation markers	Ki-67	

*The IHC panel used varied in each case based on initial morphological impression and availability of antibody. The combination included one or more antibodies both within and across the groups mentioned above. IHC: Immunohistochemistry

Demographic and clinical findings

Of the 273 patients, there were 194 males and 79 females with a M: F of 2.5:1. The age of the patients ranged from 10 to 90 years (median, 55 years). A history of smoking was elicited in 51 patients. These patients most commonly presented with cough with or without expectoration followed by breathlessness, fever, and chest pain.

Biopsy procedure and radiological findings

Of the total 273 patients, 253 (92.7%) underwent biopsy in our hospital and the remaining 20 (7.3%) had biopsies done outside. Of these 253 patients, 49 (19.3%) had procedure-related complications, which included pneumothorax in 38 (33 – minimal and 5 – significant), hemorrhage in 6 (5 – minimal and 1 – significant), and combined pneumothorax and hemorrhage in 5 (all minimal). There was an underlying emphysematous change in 12 patients. Among the patients who had complications, three of them were smokers and none of the patients had emphysema. All cases of minimal pneumothorax resolved spontaneously, but the remaining five patients who had significant pneumothorax required chest tube insertion and intercostal chest drainage. All patients with hemorrhage were managed conservatively.

In 193 patients, images were available for review by the radiologist with expertise in lung imaging and were blinded to the final pathology diagnosis. Of the total 148 cases which were radiologically considered to be primary malignant tumors, 13 (8.7%) turned out be benign lesions after pathological examination and 4/29 (13.8%) lesions categorized as benign on radiology turned out to be malignant after histological examination (sensitivity – 97.12%, specificity – 61.76%, positive predictive value (PPV) – 91.22%, and negative predictive value [NPV] – 84.00%). Of the seven patients who were radiologically diagnosed as having metastatic malignancy, two each turned out to be primary malignancy and benign (infective) on pathology. In the nine radiologically indeterminate cases, four each were diagnosed as primary malignant tumors and infective lesion and the remaining one as inflammatory myofibroblastic tumor on pathology. In 125/193 patients, the lesions had nodular/lobulated pattern, but rest showed ill-defined opacities or consolidation pattern. The size of the lesions ranged from 2.5 cm to 13 cm with a mean diameter of 6 cm. Except for five cases, all others had lesions >3 cm in diameter, with 70 of them being >5 cm with a mean diameter of 6 cm.

Pathology findings

Of the 280 biopsies, 22 (7.8%) were considered inadequate/nondiagnostic. Of these, 12 biopsies showed no lung tissue and 10 showed normal lung parenchyma and were considered nonrepresentative. Of the remaining 258 biopsies, 209 (81%) were malignant and 49 (19%) were benign.

Benign lesions

More than half of the nonneoplastic lesions showed nonspecific pathological findings such as pneumonia (15 cases), interstitial pneumonia (6 cases), organizing pneumonia (1 case), and alveolar hemorrhage (3 cases). Among the specific lesions as depicted in Figure 1, majority were granulomatous inflammation (15 cases), of which 10 showed caseating granulomas consistent with tuberculosis. Three cases showed fungal hyphae which were highlighted on special stains in the background of necrotizing inflammation. Two of these had broad, aseptate, nonacute, angle branching hyphae of *Zygomycetes* species and the remaining one showed narrow, septate, acute angle branching hyphae suggestive of *Aspergillus* species. No culture confirmation was available in any of these cases. One of the patients diagnosed with *Zygomycetes* infection was a known case of Wegener's granulomatosis and the other was a known diabetic. The patient diagnosed with *Aspergillus* infection did not have any obvious evidence of immunosuppression. There was one case each of vasculitides and pulmonary alveolar proteinosis.

Of the two carcinoids diagnosed, one was typical carcinoid and the other was a recurrent tumor in a previously diagnosed case of atypical carcinoid with a Ki67 of 14%. There was a single case of inflammatory myofibroblastic tumor presenting as a right upper lobe mass comprising of spindle cells showing positivity for smooth muscle actin (SMA) and anaplastic lymphoma kinase (ALK). Apart from these, there was one case of pulmonary (chondroid) hamartoma presenting as a space-occupying lesion in the left upper lobe with foci of calcification.

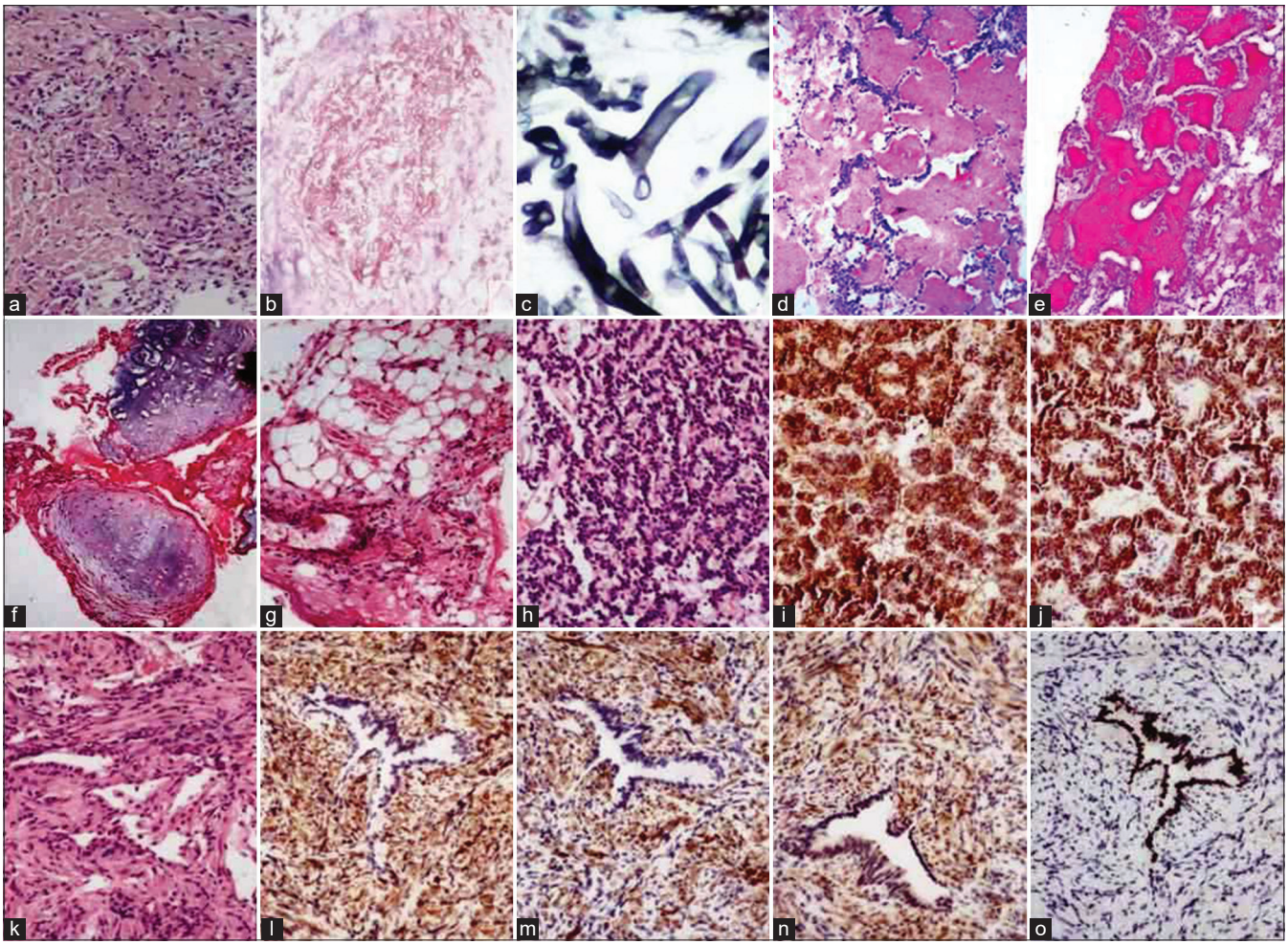

Figure 1: (a) Necrotizing granulomatous inflammation (H and E; ×100). (b) Pulmonary zygomycosis showing infarcted lung tissue with fungal hyphae (H and E; ×400). (c) Broad aseptate hyphae highlighted on GMS stain (GMS; ×400). (d and e) Pulmonary alveolar proteinosis showing alveolar spaces filled with acellular eosinophilic material highlighted on PAS stain (H and E and PAS; ×400). (f and g) Pulmonary chondroid hamartoma showing lobules of hyaline cartilage and mature adipose tissue (H and E; ×100). (h) Typical carcinoid showing interconnecting cords of cells with a uniform round nucleus (H and E; ×100). (i and j) Cells showing diffuse strong positivity for synaptophysin and chromogranin (poly HRP; ×100). (k-o) Inflammatory myofibroblastic tumor showing interlacing fascicles of spindle cells with entrapped alveoli. Cells are positive for vimentin, smooth muscle actin, and anaplastic lymphoma kinase and negative for TTF1 (H and E, ×100 [k] and poly HRP ×100 [l-o])

Malignant lesions

All the malignant lesions were initially categorized on H and E morphology in correlation with the clinical and imaging findings. The distribution of malignant lesions before and after IHC is provided in Table 2 and Figure 2. The IHC results of all the cases initially categorized as malignant epithelial tumors on H and E morphology are provided in Table 3.

Epithelial tumors

Of the total 196 primary epithelial tumors categorized initially on H and E morphology, one each were later categorized as metastatic carcinoma from endometrium and mesothelioma (epithelioid type). In the case categorized as metastatic endometrioid carcinoma, the endometrial primary was detected later. Morphologically, this tumor was ADC, but on IHC, the tumor cells were negative for TTF-1 and showed positive staining for pan-cytokeratin (CK), vimentin, and estrogen receptor. The case of mesothelioma was a pleural-based mass morphologically mimicking papillary ADC of lung. On IHC, the tumor showed negative staining for TTF-1, napsin A, and CD34 with positive staining for WT1 and calretinin.

In the present study, ADC was the most common (109/194; 56.2%) primary malignant epithelial tumor. Majority had predominant acinar pattern (45) followed by lepidic (12), mucinous (8), papillary (7), solid (5), and micropapillary (2) pattern and are depicted in Figure 3. Three of the predominantly acinar tumors showed cribriform pattern and one showed focal micropapillary areas. Four cases of lepidic ADCs and two mucinous tumors did not show invasive component in the biopsies submitted. Of these, tumor dimensions were available in two cases of lepidic carcinoma and both were >3 cm in the greatest dimension (6.5 cm in one and 3.5 cm in other).

Nearly 60% of the cases (32/54) were categorized as SQCC on H and E morphology alone and the remaining 22

Table 2: The distribution of malignant lesions before and after immunohistochemistry

H and E diagnosis	*n*	Final diagnosis after IHC*	*n*
Epithelial tumors		Epithelial tumors	
ADC	81	ADC	79
SQCC	32	QCC	32
NSCC-NOS	71	NSCC favor ADC	30
		NSCC favor SQCC	22
		NSCC-NOS	19
NSCC with spindle cells	2	NSCC with spindle cells	2
NSCC with NE morphology	3	NSCC possibly LNEC	3
Small-cell carcinoma	7	Small-cell carcinoma	7
Mesenchymal tumors		Mesenchymal tumors	
Spindle cell	3	Leiomyosarcoma	1
		Spindle cell sarcoma morphology and IHC favor monophasic (fibrous) synovial sarcoma	1
		Spindle cell sarcoma, unclassified	1
Round cell	2	Wings sarcoma	1
		Unclassified	1
Lymphohistiocytic tumors		Lymphohistiocytic tumors	
Lymphoma	2	Lymphohistiocytic tumors	
		Low-grade B-cell NHL (MALT Type)	1
		ALCL	1
Metastatic tumors		Metastatic tumors	
Breast	1	Breast	1
Colon	1	Colon	1
Papillary thyroid carcinoma	1	Papillary thyroid carcinoma	1
Endometrioid adenocarcinoma	1	Endometrioid adenocarcinoma	2
Meningioma	1	Meningioma	1
Germ cell tumor	1	Germ cell tumor	1
		Mesothelioma	1

*IHC was not done in all cases. In such cases where IHC was not done, initial morphological diagnosis was considered as final diagnosis. H and E: Hematoxylin and eosin, IHC: Immunohistochemistry, ADC: Adenocarcinoma, SQCC: Squamous cell carcinoma, NSCC: Non-small-cell carcinoma, NE: Neuroendocrine, LNEC: Large-cell neuroendocrine carcinoma, NHL: Non-Hodgkin's lymphoma, MALT: Mucosa-associated lymphoid tissue, ALCL: Anaplastic large-cell lymphoma

(about 40%) required IHC to categorize them as NSCC favor SQCC. Seventy-one tumors (36.6%) were morphologically undifferentiated carcinoma. The sub-categorization of these tumors after IHC is provided in Table 4. Even after IHC, 19 cases (26.7%) could not be categorized as either ADC or SQCC and these were designated as NSCC- not otherwise specified (NOS). There were ten tumors which showed neuroendocrine morphology; of these, seven were categorized as small-cell carcinomas. Six of these cases showed positivity for neuroendocrine markers, whereas no IHC was done in the remaining one case. Three cases with neuroendocrine morphology had large cells and both showed positivity for neuroendocrine markers, hence were categorized as NSCC, possibly large-cell neuroendocrine carcinoma.

Metastatic carcinoma

Of the seven metastatic carcinomas, there was a prior history of primary tumor elsewhere in six patients. Biopsies were done in these cases to rule out the possibility of second primary in the lung. IHC studies were done to confirm the primary in all cases except for one case of metastatic germ cell tumor which had classical morphology. The combination of CK7 and CK20 expression along with the organ-restricted/differentiation cell-specific markers such as GCDFP-15, ER, PR, CDX2, and thyroglobulin facilitated in confirming the origin of the primary tumor.

Lymphohistiocytic tumors

Of the two cases diagnosed as lymphomas, one was previously diagnosed and treated for anaplastic large-cell lymphoma who presented with two pulmonary nodules. Biopsy of one of these nodules had classical morphology and showed positive staining for LCA and CD30 and was negative for pan-CK and TTF-1, as shown in Figure 4. The other patient presented as nonresolving pneumonia with consolidation in the left lower lobe. Morphologically, there was diffuse infiltration by small round cells which showed positive staining with CD20, CD5, Pax5, and bcl2. These cells were negative for CD3, CD10, CD23, and pan CK with a Ki67 LI (Labeling Index) of 15%.

Mesenchymal tumors

Of three spindle cell sarcomas diagnosed on initial morphology, one was categorized as leiomyosarcoma after IHC. The tumor cells were positive for SMA with negative staining for desmin, S100, CD34, pan-CK, ALK, and WT-1. The Ki67 LI was 12%. Another case showed morphology of monophasic (fibrous) synovial sarcoma and on IHC tumor cells showed positive staining with Bcl2, EMA, CK, and Ki67 with LI of 5% and were negative for SMA, S100, CD34 and ALK. However, molecular confirmation was not available. The third case remained unclassified even after IHC with SMA, pan-CK, ALK, and CD34. None of these cases had any prior diagnosis of extrapulmonary sarcomas.

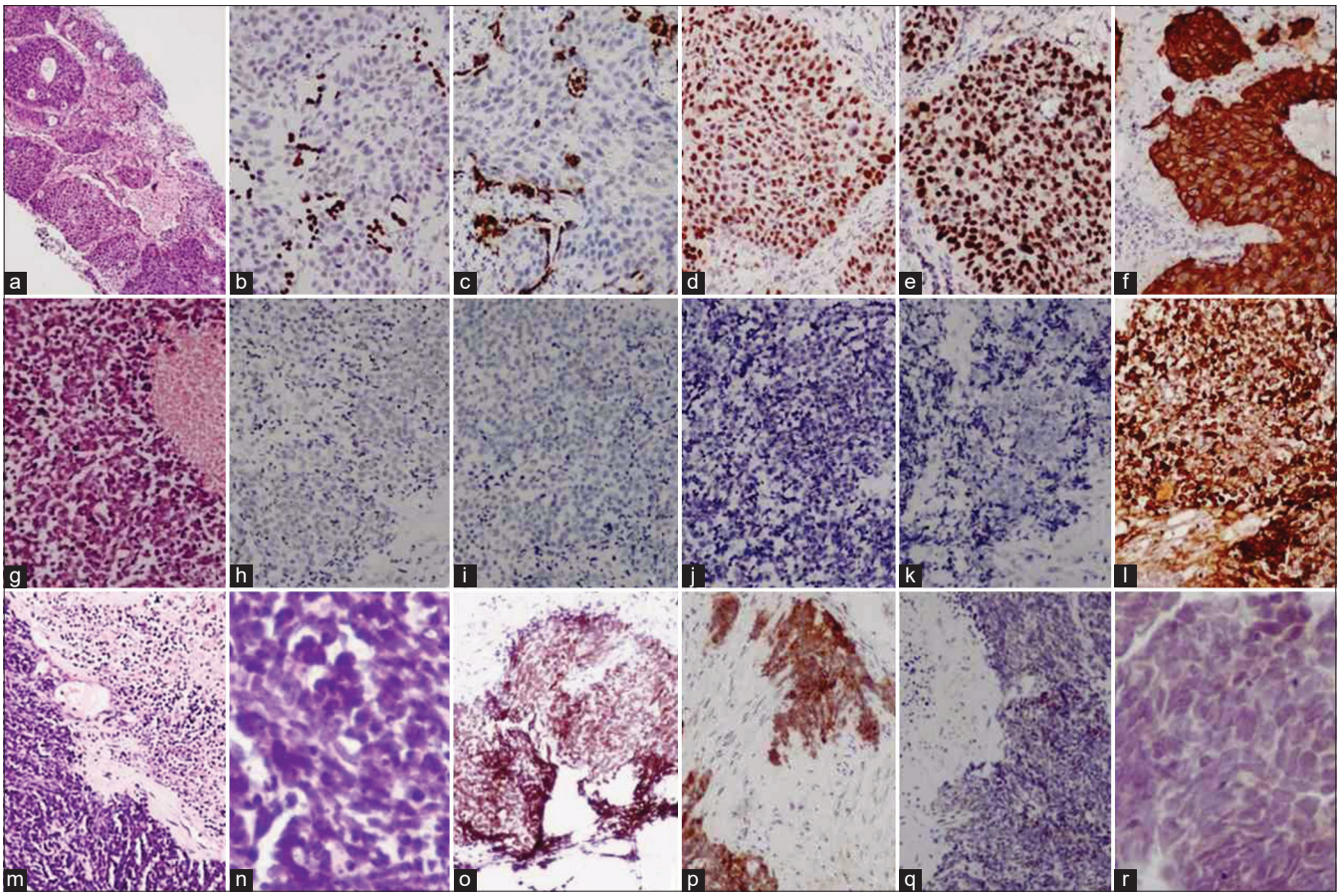

Figure 2: (a-f) Squamous cell carcinoma: (a) Nests of polygonal cells with nuclear pleomorphism and hyperchromasia (H and E; ×100). (b-f) On immunohistochemistry, cells are negative for TTF1 and napsin A but positive for p63, p40, and CK5/6 (poly HRP; ×100). (g-l) Non-small-cell carcinoma NOS: (g) Sheets of undifferentiated cells with foci of necrosis (H and E; ×100). (h-l) On immunohistochemistry, the tumor cells are negative for TTF1, Napsin-A, p-40, and CK5/6 but positive for pan-CK (poly HRP; ×100). (m-r) Small-cell carcinoma: (m) Sheets of small cells with hyperchromatic nucleus and inconspicuous nucleoli (H and E; ×100). (n-r) On immunohistochemistry, the tumor cells are positive for chromogranin, synaptophysin, and TTF1 but negative for P63 (poly HRP; ×100)

Of the two round cell sarcomas, one showed positivity for CD99 and LCA, but desmin and synaptophysin were negative and this case was diagnosed as Ewings/PNET. No molecular confirmation was available. The other case of round cell sarcoma remained unclassified even after staining with LCA, CD99, and chromogranin.

Immunohistochemistry results in lung carcinomas

The sensitivity, specificity, PPV, and NPV of lung ADC markers (TTF-1, napsin A, and CK7) and SQCC markers (P63, P40, and CK5/6) are provided in Table 5.

On comparing TTF-1 and napsin A, TTF-1 showed higher sensitivity but lower specificity than napsin A for lung ADC. Apart from lung ADC, TTF-1 showed positivity in 5/6 small-cell carcinomas and 1 case of metastatic papillary thyroid carcinoma. TTF-1 was also positive in two cases categorized as NSCC favor SQCC. These cases showed only weak focal staining for TTF-1, but showed diffuse strong staining for P63 and P40. Napsin A was 100% specific and did not stain any tumor other than lung ADC. There were 38 cases of lung ADCs, in which both TTF-1 and napsin A staining was performed. Of these, 26 showed positivity for both markers, 4 only TTF-1 positivity, 2 only napsin A positivity, and 6 were negative for both markers. Of these six cases which were negative for both markers, four were histologically mucinous ADCs, one was cribriform carcinoma, and the other was NSCC favor ADC. The case of cribriform carcinoma was positive for CK7 and also showed epidermal growth factor receptor (EGFR) mutation.

CK7 showed higher sensitivity than TTF-1 and napsin A for lung ADCs, but had poor specificity due to staining of SQCC, neuroendocrine carcinomas, and metastatic carcinomas.

Both P63 and P40 showed 100% sensitivity for SQCCs, but P63 showed poor specificity with positive staining in good number of ADCs, neuroendocrine carcinomas, and metastatic carcinomas. Of the total 40 cases of lung ADCs (ADC – 26 and NSCC favor ADC – 14) positive for P63, 31 (77.5%) showed staining in >25% of nuclei, with 28 of these showing moderate-to-strong intensity of staining. Both P63 and P40 staining had been performed in eight

Table 3: Immunohistochemistry results of all the cases initially categorized as malignant epithelial tumors on hematoxylin and eosin morphology

H and E diagnosis	*n*	IHC findings							
		CK7 (%)	CK20 (%)	TTF1 (%)	Nap A (%)	P63 (%)	P40 (%)	CK5/6 (%)	Chr (%)
Epithelial tumors									
ADC	81	47/48 (97.9)	5/44 (11.4)	49/57*,# (85.9)	19/23# (82.6)	27/35* (77.1)	0/9 (0)	1/3* (33.3)	0/4 (0)
SQCC	32	4/8 (50)	0/2 (0)	0/22 (0)	0/4 (0)	22/22 (100)	5/5 (100)	15/16 (93.7)	0/1 (0)
NSCC-NOS	71	31/34 (91.1)	2/20 (10)	30/61 (49.1)	12/29 (41.3)	37/54 (68.5)	3/9 (33.3)	16/20 (80.0)	0/14 (0)
NSCC with spindle cells	2	1/1 (100)	0/1 (0)	0/1 (0)	-	0/1 (0)	-	-	-
NSCC with NE morphology	3	1/3 (33.3)	0/1 (0)	0/3 (0)	0/1 (0)	1/3 (33.3)	-	0/1 (0)	3/3 (100)
Small-cell carcinoma	7	3/3 (100)	-	5/6 (83.3)	-	4/6 (66.6)	-	0/1 (100)	4/6 (66.6)

H and E diagnosis	*n*	IHC findings									
		Syn (%)	NSE (%)	WT1 (%)	Calr (%)	Ki67 (%)	Cdx2 (%)	Pan-CK (%)	Vim (%)	CD34 (%)	ER (%)
Epithelial tumors											
ADC	81	-	-	1/1# (100)	1/1# (100)	-	0/1 (0)	-	1/1* (100)	0/1# (0)	1/1* (100)
SQCC	32	-	-	-	-	-	-	-			
NSCC-NOS	71	1/1 (100)	-	0/2 (0)	0/1 (0)	-	0/2 (0)	3/3 (100)			
NSCC with spindle cells	2	-	-	-	-	-	-	-			
NSCC with NE morphology	3	-	-	-	-	1/1 (100)	-	-			
Small-cell carcinoma	7	3/3 (100)	1/1 (100)	-	-	2/2 (100)	-	-			

*Includes one case which was later categorized as metastatic endometrioid adenocarcinoma; #Includes one case later categorized as mesothelioma. H and E: Hematoxylin and eosin, IHC: Immunohistochemistry, ADC: Adenocarcinoma, SQCC: Squamous cell carcinoma, NSCC: Non-small-cell carcinoma, NE: Neuroendocrine, NapA: Napsin A, Chr: Chromogranin, Syn: Synaptophysin, Calr: Calretinin, Pan-CK: Pancytokeratin, Vim: Vimentin, ER: Estrogen receptor

cases of SQCC and both these markers were positive in all the eight cases. CK5/6 showed slightly lower sensitivity than P63 and P40 for SQCC, but it had better specificity than P63.

CK20 was positive in six cases of lung ADCs, of which four were histologically mucinous ADCs.

Molecular testing in non-small-cell carcinoma of lung

Epidermal growth factor receptor mutation testing

A total of 54 cases of lung carcinoma (ADC/NSCC favor ADC) were sent for EGFR mutation testing, of which 3 were found to be inadequate. Of the remaining 51, 18 (35.3%) were positive for EGFR mutation. There were ten females and eight males, with their age ranging from 29 to 77 years (mean: 51.2, median: 52). All except two were nonsmokers. Majority showed acinar ADC on morphology. Exon 19 deletions and L858 mutations were found in 14 cases and 4 cases, respectively. On correlating EGFR mutation-positive cases with IHC results of TTF-1 and Napsin A, it was found that TTF-1 was positive in 6/10 cases tested and napsin A was positive in 3/5 cases.

EML4-anaplastic lymphoma kinase rearrangements

Of the total 34 cases sent for ALK testing by FISH, 3 were found to be inadequate. Only 2/31 (6.4%) remaining cases showed ALK rearrangement. Both these patients were male and nonsmokers having acinar type of ADCs with TTF-1 positivity on IHC.

Treatment and follow-up

The treatment details were available in 99 patients. Immediately after histological diagnosis of ADCs, majority of the patients were started on initial therapy with combination of pemetrexed and platins (carboplatin/cisplatin) for four cycles. Some patients received paclitaxel/abraxane and carboplatin combination as initial therapy. Those individuals who did not tolerate or had poor performance status were started directly on gefitinib/erlotinib. Those individuals with ADCs/NSCC favor ADCs who were started initially on non-tyrosine kinase inhibitor (TKI) agents were switched to TKIs (gefitinib/erlotinib) if they were found positive for EGFR mutation. Those patients who showed progression on TKIs were given second-line therapy with pemetrexed/abraxane. EGFR mutation-negative/nontested patients were continued on maintenance with pemetrexed/abraxane. If these patients were found intolerant to these drugs, they were also switched to gefitinib/erlotinib therapy. Patients with ADC who were found positive for EML4-ALK rearrangements were treated with crizotinib. Follow-up data showed a median survival of 12 months for patients treated with pemetrexed and platins. The EGFR mutation-positive patients treated with TKIs showed a median survival of 15 months.

DISCUSSION

The diagnostic accuracy of PCNBL is high especially for sampling of peripheral and small lung lesions to obtain a

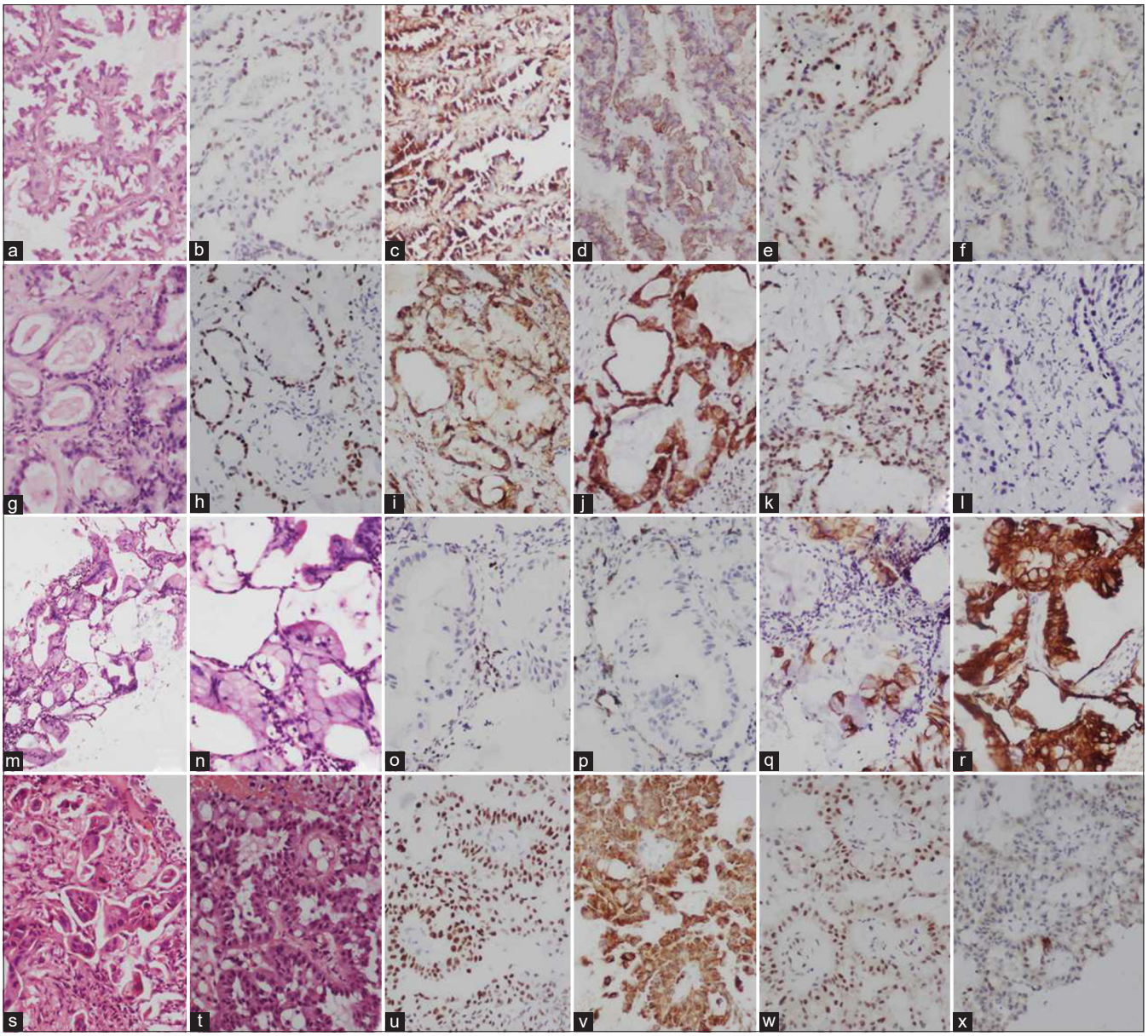

Figure 3: (a-f) Lepidic adenocarcinoma: (a) Cuboidal cells lining the preexisting alveolar spaces (H and E; ×100). (b-f) Tumor cells are positive for TTF1, napsin A, CK7, and p63 and negative for p40 (poly HRP; ×100). (g-l) Invasive mucinous adenocarcinoma: (g) Acinar structures filled with mucin (H and E; ×100). (h-l) On immunohistochemistry, cells are positive for TTF1, napsin A, and CK7 and negative for CK20. P63 shows positive staining in the tumor cells (poly HRP; ×100). (m-r) Mucinous adenocarcinoma with lepidic pattern: (m and n) Columnar cells with basally located nuclei and intracytoplasmic mucin lining the preexisting alveolar spaces (H and E; ×40 and ×100). (o-r) On immunohistochemistry, cells are negative for TTF1 and napsin A and positive for CK20 (focal) and CK7 (poly HRP; ×100). (s) Micropapillary adenocarcinoma showing micropapillae without central fibrovascular core (H and E; ×100). (t-x) Papillary adenocarcinoma: (t) Papillae lined with cuboidal-to-columnar cells lining the papillae (H and E; ×100). (u-x) Cells show positivity for TTF1 and napsin A and p63 but negative for p40 (poly HRP; ×100)

tissue diagnosis.[8] Beslic *et al*. reported that FNAB yielded adequate samples for definitive diagnosis in nearly 80%, whereas it was much higher for PCBL accounting for nearly 97%.[9] Yuan *et al*. in a large study involving 1014 patients reported diagnostic efficiency of CT-guided lung biopsy to be 94.8%, which is close to that of the present study (92.2%).[10] Despite observing all precautions complication may occur during or after the procedure. In the study by Yuan *et al*., the overall complication rate was 18.5%.[10] The main complications that occur are pneumothorax and pulmonary hemorrhage. The other complications include hemoptysis, subcutaneous hematoma, infection, air embolism, and chest pain. The reported incidence of pneumothorax varies from 9% to 54%,[8,11-16] with an average of around 20%.[17] The reported incidence of pulmonary hemorrhage has varied from 1.6% to 28.6%.[10,12]

In the present study, the overall complication rate was 19.3%, with pneumothorax occurring in 17% of patients and pulmonary hemorrhage in 4.3%. The majority of

Table 4: Further sub-categorization of non-small-cell carcinoma based on immunohistochemistry results

NSCC-NOS (*n*=71)	*n*	IHC findings											
		CK7 (%)	CK20 (%)	TTF1 (%)	Nap A (%)	P63 (%)	P40 (%)	CK5/6 (%)	Chr (%)	Syn (%)	*WT1* (%)	*Calr* (%)	*Cdx2* (%)	*Pan-CK* (%)
NSCC favor ADC	30	17/19 (89.4)	1/14 (71.4)	28/30 (93.3)	12/15 (80)	14/25 (56)	0/5 (0)	3/6 (50)	0/3 (0)	-	0/1 (0)	-	0/2 (0)	1/1 (100)
NSCC favor SQCC	22	9/9 (100)	0/2 (0)	2/22 (9)	0/11 (0)	22/22 (100)	3/3 (100)	12/12 (100)	0/7 (0)	1/1 (100)	0/1 (0)	-	-	1/1 (100)
NSCC-NOS	19	5/6 (83.3)	1/4 (25)	0/9 (0)	0/3 (0)	1/7 (0)	0/1 (0)	1/2 (50)	0/4 (0)	-	-	-	-	1/1 (100)
Total	71	31/34 (91.1)	2/20 (10)	30/61 (49.1)	12/29 (41.3)	37/54 (68.5)	3/9 (33.3)	16/20 (80)	0/14 (0)	1/1 (100)	0/2 (0)	0/1 (0)	0/2 (0)	3/3 (100)

IHC: Immunohistochemistry, ADC: Adenocarcinoma, SQCC: Squamous cell carcinoma, NSCC: Non-small-cell carcinoma, NE: Neuroendocrine, NapA: Napsin A, Chr: Chromogranin, Syn: Synaptophysin, Calr: Calretinin, Vim: Vimentin, ER: Estrogen receptor

cases of pneumothorax developing after needle biopsy resolve spontaneously, but a few intractable cases require chest tube placement. The reported frequency of chest tube placement has ranged from 1.5% to 15%.[10,12] In the present study, there was need for chest insertion in only 5 patients (2%).

Akhtar *et al*. reported a sensitivity of 94%, a specificity of 33.3%, a PPV of 64.4%, and a NPV of 81.2%.[18] Our results show significantly better specificity and PPV than that of their study.

In a study of 311 patients by Yang *et al*., 217 (69.8%) were malignant and 94 (30.2%) were benign lesions.[19] The relatively higher percentage of malignant lesions in the present study (89.7%) may be due to fact that in majority of the cases, biopsies were performed to confirm the malignancy. However, the distribution of the nonneoplastic lesions was similar in both the studies. The distribution of primary epithelial tumors in the present study was comparable with the results of other recently published studies.[19,20]

As discussed by Travis *et al*., no well-established grading system for ADC exists; however, grading according to predominant histologic architecture seems to be of prognostic importance and is a simple and reproducible method.[21] We applied this approach in the present study and classified ADCs based on the presence of predominant histomorphological pattern. The results were comparable with those of Travis *et al*. except for a lower prevalence of solid pattern (37.7% vs. 6.3%).[21]

IHC had been performed in both morphologically differentiated and undifferentiated tumors. The present study showed that the combination of TTF-1 and napsin A marginally increased sensitivity but specificity was significantly improved. Similar results were seen by Tacha *et al*. except for a lower sensitivity of TTF1 compared to that of the present study (90.59% vs. 69%).[22]

Sterlacci *et al*. followed the guidelines of IASLC/ATS/ERS for classification of lung cancers. The IHC panel used by them to categorize SQCC and ADC included TTF-1, CK7, P63, and CK5/6.[23] A comparison of results of IHC markers between the present study and those reported by Sterlacci *et al*. and Mukhopadhyay and Katzenstein is provided in Table 6.[24,25] The present study showed better sensitivity of TTF1 (90.59%) than that reported by other two studies (83.5% and 80%).[24,25] Our specificity results are similar to that reported by Mukhopadhyay and Katzenstein (89%), but lower than that reported by Sterlacci *et al*. (96.6%).[24,25] In the present study, positivity for either TTF-1 or napsin A was considered diagnostic of ADC irrespective of p63 or CK5/6 staining.

Tumors positive for p63 but negative for CK5/6 were diagnosed as SCC only if they were diffusely and strongly positive for p63 and negative for TTF-1 and napsin A.

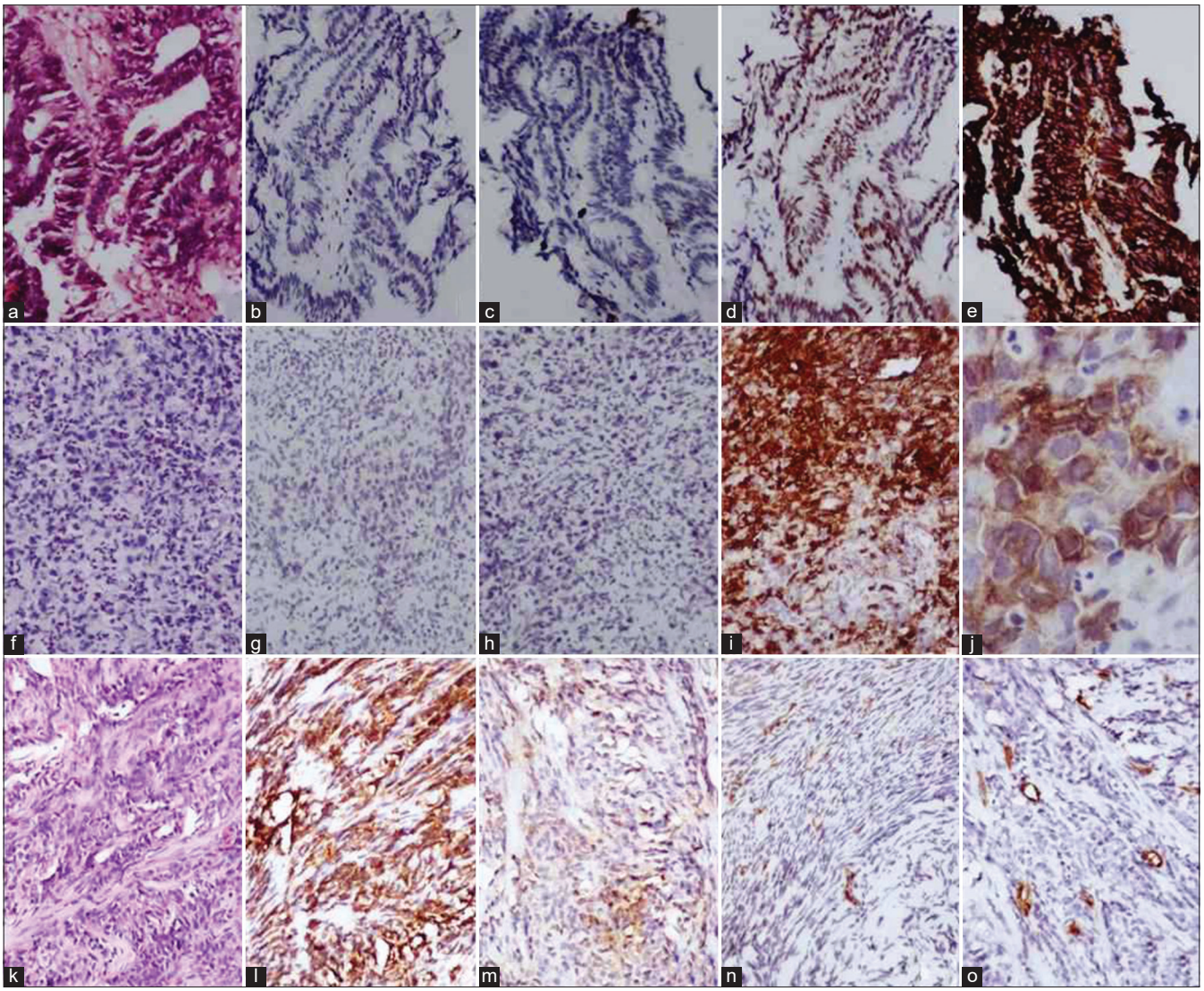

Figure 4: (a-e) Metastatic endometrioid carcinoma: (a) Infiltrating tubular glands lined by tall columnar cells (H and E; ×100). (b-e) On immunohistochemistry, the tumor cells are negative for TTF1 and napsin A and positive for estrogen receptor and vimentin (poly HRP; ×100). (f-j) Anaplastic large-cell lymphoma: (f) Diffuse sheets of small and large lymphoid cells admixed with plasma cells and eosinophils (H and E; ×100). (g-j) Cells are negative for pan CK and TTF1 and positive for LCA and CD30 (poly HRP; ×100 [g-i] and ×400 [j]). (k-o) Synovial sarcoma: (k) Sheets and fascicles of spindle cells (H and E; ×100). (l-o) Cells are positive for Bcl2, pan-CK, and EMA and negative for CD34 (poly HRP; ×100)

Table 5: Sensitivity, specificity, positive predictive value, and negative predictive value of adenocarcinoma and squamous cell carcinoma markers

IHC marker	Sensitivity		Specificity		PPV		NPV	
	Value (%)	95% CI	Value (%)	95% CI	Value (%)	95% CI	Value (%)	95% CI
TTF-1	90.59	82.29-95.85	89.74	80.79-95.47	90.59	82.29-95.85	89.74	80.79-95.47
Napsin A	83.78	67.99-93.81	100.00	86.28-100.00	100.00	88.78-100.00	80.65	62.53-92.55
CK7	95.52	87.47-99.07	24.24	11.09-42.26	71.91	61.38-80.93	72.73	39.03-93.98
P63	100.00	91.96-100.00	37.50	26.92-49.04	46.81	36.44-57.39	100.0	88.43-100.00
P40	100.00	63.06-100.00	100.00	88.43-100.00	100.00	63.06-100.00	100.0	88.43-100.00
CK5/6	96.43	81.65-99.91	61.54	31.58-86.14	84.38	67.21-94.72	88.89	51.75-99.72

IHC: Immunohistochemistry, CI: Confidence interval, NPV: Negative predictive value, PPV: Positive predictive value

Mukhopadhyay and Katzenstein and Sterlacci *et al*. also followed similar criteria in their study.[24,25] In the present study, we also analyzed SQCC marker P40 in some of the cases and found it to be highly specific for SQCC. The sensitivity of P40 was similar to that of P63 in the cases tested for both. Bishop *et al*. in their study showed similar results for P40.[26]

In the present study, using IHC, it was possible to accurately subtype 52 of 71 (73.2%) cases categorized as

Table 6: Comparison of immunohistochemistry results of adenocarcinoma and squamous cell carcinoma markers of the present study with those of Sterlacci *et al*.[24] and Mukhopadhyay *et al*.[25]

Immunohistochemical stains	Sterlacci *et al*.[24]				Mukhopadhyay *et al*.[25]				Present study			
	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
TTF-1	83.5s	96.6	97.7	76.9	80	89	89	81	90.59	89.74	90.59	89.74
Napsin A	-	-	-	-	58	100	100	70	83.78	100	71.91	80.65
CK 7	97.2	76.5	88.4	93.6	100	37	61	100	95.52	24.24	71.91	72.73
P63	99.2	93	88.9	99.5	100	92	88	100	100	37.50	46.81	100
CK 5/6	99.2	90.2	84.9	99.5	73	100	100	86	96.43	61.54	84.38	88.89

NPV: Negative predictive value, PPV: Positive predictive value

NSCC-NOS on H and E morphology alone. Of these, 30 were subtyped as NSCC favor ADC and 22 as NSCC favor SQCC. Twelve tumors remained unclassified even on IHC. These along with seven other undifferentiated cases on morphology where IHC was not done were categorized as NSCC-NOS. Mukhopadhyay and Katzenstein were able to subcategorize 30 of 39 (77%) poorly differentiated NSCLCs on biopsy specimens after IHC, 16 as ADCs, and 14 as SQCCs. In their study as well, nine cases remained unclassified even after IHC.[25]

The potential information derived from molecular tests is enormous, and evaluation for EGFR mutations and ALK rearrangements is now considered to be the standard of care in advanced-stage pulmonary ADCs. IHC stains can aid in sub-classifying NSCLC, but performing these ancillary studies can significantly reduce the quantity of tissue available for molecular tests, requiring careful balancing of these two needs.[27]

The EGFR mutation rate was much higher in never-smokers than in smokers. The rate of EGFR mutation in the study by Zhu *et al*. was 63/131 (48.1%), including exon 19 deletion mutations in 31 (23.7%) and exon 21 point mutations in 32 (24.4%) samples.[28] There were significantly higher mutation rates in women and nonsmokers. Similar finding was observed in our study as well as in the study by Gervas *et al*.[29]

In the present study, EML4-ALK rearrangement was detected in 2/31 (6.5%) patients tested. In the study by Zhu *et al*., ALK rearrangement was detected in 9/131 patients (6.9%).[28] ALK rearrangement occurred more frequently in young patients (8/9) and nonsmokers (8/9) in their study.

CONCLUSION

PCNBL is a safe and efficient method for obtaining samples tissue diagnosis of lung lesions. The IASLC/ATS/ERS classification was found to be easily applicable on these biopsies. The tissue samples obtained are adequate for ancillary tests such as IHC and molecular studies. The use of IHC significantly reduces the number of NSCC-NOS cases. TTF-1 and napsin A are the most sensitive and specific markers for lung ADC. P40 is more specific marker than p63 while being equally sensitive for the diagnosis of SQCC.

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Conflicts of interest
There are no conflicts of interest.

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