wjpmr, 2019,5(5), 106-116

SJIF Impact Factor: 4.639

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

Research Article

ISSN 2455-3301

WJPMR

TO ASSESS THE ROLE OF VARIOUS IMMUNOHISTOCHEMICAL MARKERS IN CLASSIFICATION OF LUNG CARCINOMA ON ENDOBRONCHIAL BIOPSIES

Namita Bhutani[1], Nisha Marwah[2], Dhruv Chaudhary[3], Pradeep Kajal*[4] and Rajeev Sen[5]

[1]Senior Resident, M.B.B.S, M.D, D.N.B. Deptt. of Pathology, PGIMS Rohtak, Haryana.
[2]Professor, M.B.B.S, M.D Deptt. of Pathology, PGIMS Rohtak, Haryana.
[3]Professor, M.B.B.S, M.D. Deptt. of Pulmonary Medicine PGIMS Rohtak, Haryana.
[4]Associate Professor, M.B.B.S, M.S, M.Ch. Deptt. of Pediatric Surgery, PGIMS Rohtak, Haryana.
[5]Sr. Professor & Head, M.B.B.S, M.D. Deptt. of Pathology, PGIMS Rohtak, Haryana.

***Corresponding Author: Pradeep Kajal**
Associate Professor, M.B.B.S, M.S, M.Ch. Deptt. of Pediatric Surgery, PGIMS Rohtak, Haryana.
Email Id: drpradeepkajal@gmail.com, Mob: 8221000060

Article Received on 08/03/2019 **Article Revised on 29/03/2019** **Article Accepted on 19/04/2019**

ABSTRACT

Background: Lung cancer is the most frequently diagnosed cancer and leading cause among cancer mortality worldwide. An accurate classification is difficult in small biopsy specimens due to a variety of reasons. Therefore, there is an increasing need for additional diagnostic techniques such as immunohistochemistry. **Methods**: This study was conducted in Pt. B D Sharma, PGIMS, Rohtak. Endobronchial biopsies of One hundred and sixty patients were subjected to routine H & E and IHC staining. **Results:** The patients were in age group of 25-75 years with a mean of 55.67 years with M: F ratio of 6.61:1. NSCLC constituted the major type, contributing to 83.1% of cases. Amongst the NSCLC, poorly differentiated subtype topped the list, with 53.7% cases. p63 was highly sensitive (98.13%) and specific (100%) for squamous cell carcinoma. Amongst, TTF-1 and napsin-A, the later had higher sensitivity (96.15%) as compared to TTF-1 (92.30%) for diagnosing adenocarcinoma. **Conclusion:** CK and p63 served as highly sensitive markers for diagnosis of squamous cell carcinoma and TTF-1 and napsin A for adenocarcinoma, forming an important diagnostic algorithm for subtyping of poorly differentiated NSCLC on small biopsies.

KEYWORDS: Adenocarcinoma; Non small cell lung carcinoma; Small cell lung carcinoma; Thyroid transcription factor-1.

BACKGROUND

Lung carcinoma is the leading cause of cancer deaths in developed countries and is rising at alarming rates in the developing countries. It is the most frequently diagnosed cancer and leading cause among cancer mortality worldwide. The prognosis is poor; with a 5 year survival rate of 14%. This is partly attributable to relatively ineffective methods for early detection and lack of curative treatment for advanced disease.[1]

Routine sections stained with hematoxylin-eosin (H&E) remain the most common method by which lung cancers are classified. However, typing of Non small cell lung carcinoma (NSCLC) and the more poorly differentiated cancers is often hard to achieve by H&E alone. Moreover, an accurate classification can be difficult in small biopsy specimens due to a variety of reasons, such as scant tumor cells, lack of characteristic architecture in small biopsies, artifacts in specimen prepration, and differentiation and heterogenicity of tumor. The diagnosis of Small cell lung carcinoma (SCLC) remains problematic when biopsies are crushed and small, because of fragile nature of the tumor cells. Therefore, there is an increasing need for additional diagnostic techniques such as immunohistochemistry (IHC).[2]

IHC has emerged as a powerful, adjunctive tool for the differential diagnosis of lung cancer, whether primary or secondary to lung. The limit of small specimen size and need to conserve tissue for additional molecular studies necessitates the use of sensitive and specific markers panels. Conventionally, the most commonly used markers for identification of lung carcinoma are cytokeratin (CK), synaptophysin, chromogranin A, leucocyte common antigen (LCA), napsin-A, Thyroid transcription factor-1 (TTF-1) and p63. Primary panel of CK, LCA, synaptophysin and chromogranin differentiates SCLC, NSCLC and lymphoma, while napsin A, TTF-1 and p63 are used for further categorisation of NSCLC.[3]

The combined test of TTF-1 and napsin A has been considered as a promising attractive tool to sub-classify NSCLC in clinical practice. We planned to carry out this

study to differentiate between primary squamous cell carcinoma and adenocarcinoma with the help of specific IHC markers and compare the cocktails of napsin A, TTF-1, and p63 in the diagnosis of NSCLC and to identify a small, accurate and cost effective IHC panel for further classification of NSCLC.

MATERIALS AND METHODS

This study was conducted in department of Pathology, Pt. B D Sharma, PGIMS, Rohtak. One hundred and sixty patients suspected of having lung cancer on basis of clinical features, radiological imaging and confirmed on histopathological examination of endobronchial biopsy, formed the study material.

Patients with lung malignancy other than primary tumor such as lymphoma, sarcoma, stromal tumor and metastasis were excluded from the study. Histopathological diagnosis was established on the routine heamatoxylin and eosin stain, IHC, and special histochemical stains like PAS and others as applicable for further classification of lung tumors.

Hematoxylin and eosin staining for routine paraffin sections was carried out as per the standard procedure.

Immunohistochemical profile was assessed by subjecting one section from the block to various immunostains. Immunohistochemical stain was performed using standard technique. Immunohistochemical profile of the tumor was assessed by subjecting one section each from a block of tumor to CK, p63, TTF-1, napsin-A, synaptophysin, chromogranin a, NSE, CD 56 and EGFR and results were assesed. Positive and negative controls were run with each batch of IHC stain.

The whole data was subjected to statistical analysis using SPSS 20.0 software. Chi-square test was used to calculate p value and appropriate statistics were applied.

The tissue biopsies submitted for histopathological study were used up in preparing wax blocks and slides. All the biomedical waste generated during the study was discarded as per the bio-medical waste (management and handling) rules 2011 guidelines.

RESULTS

In the present study, a total of 160 cases of primary lung carcinoma constituted the study group, during the period of 2014-2016, with the age of patients ranging from 25 to 75 years. Mean age at presentation was 55.67 years. Lung Carcinoma was most frequent for the age group 41-60 (89 cases – 55.6%) and most of them were men (139 cases – 86.8%). M: F Ratio in our study was 6.61:1.

The most common presentation was chest pain (53.1%) followed by cough (50%). Dyspnoea, hemoptysis, fever and weight loss were other common symptoms. Most of the patients presented with two or more of the above clinical features. The majority of patients presented within 3 to 6 months of onset of symptoms (53.75%). Our study showed that the symptomatology given by the syndrome of malignant impregnation was prevalent, as majority of the patients present with symptoms due to intrathoracic local extension or extrathoracic metastases.

Analysing the role of smoking in the etiology of lung carcinoma, we have tracked the age of beginning of smoking, the duration of smoking, the number and type of smoked cigarettes, the way of inhalation, the existence and duration of status of the ex-smoker. Smokers and Non-smokers were 86.25% and 13.75% respectively in the present study. Pre-existent occupational hazards were present in 23.75% of patients. Relating to the distribution of patients depending on the presence of active smoking as risk factor, it has been found out that many of the patients included in the study were smokers, both women and men. All retrospective studies found that the risk of lung cancer is higher if the smoker began to smoke at a young age, if the duration of smoking was longer, if the number of smoked cigarettes/day was large, if the number of cigarette package/year was big and if the inhaling way was profound. Fifteen cases (9.4%) had a positive history of lung carcinoma in the family.

Standard radiological examination was carried out as a screening procedure in all the patients. Mass lesion was the most common radiological finding (73.1%) followed by collapse (20.62%), both in X-Ray chest and CT scan. Mediastinal or hilar lymphadenopathy was better picked up on CT scan (9.4%) as compared to X-ray chest (6.25%). High resolution CT was done in 94 (58.75%) patients. Lymphovascular invasion was seen in 15% of cases, pleural involvement in 18.1% and mediastinal lymph nodes involvement in 25.6% of cases.

In our study, 55.6% of the cases were detected at a localized stage and the patients with distant metastatic involvement comprised 16.3%. This may be due to improvement of means of diagnosis due to technical progress, increase of addressability, the echo of the anti-smoking campaign in the last years, as well as the careful follow-up of patients with increased risk of lung cancer. The most common location of tumor was in right upper lobe (28.1%) followed by left upper lobe (18.8%). Right middle lobe was the least common site of involvement in our study.

All the suspicious endobronchial biopsies for lung malignancy, which were received in our department were categorized into lymphomatous and non-lymphomatous type. Lymphomas were excluded from our study. Non-lymphomatous were further categorized into small cell lung carcinoma (SCLC) and non-small cell lung carcinoma (NSCLC). On the basis of histopathological features the cases were first segregated into Small cell carcinoma and Non-small cell carcinoma and confirmed by primary immunohistochemistry panel (Figure 1A-E). Non-small cell carcinomas were further categorized

based on their histological features. Squamous cell carcinomas were diagnosed on the basis of keratinisation and/ or intercellular bridging. Gland formation and / or mucin production were taken as morphological criteria for adenocarcinoma. The cases which lacked the classical histological features of squamous or adenocarcinoma on H&E stained smears were grouped as poorly differentiated non-small cell carcinoma (PDC-NSCLC). These cases were finally classified according to their immunoprofile. All the cases of lung carcinoma, small cell as well as non-small cell carcinoma were positive for CK. A panel of IHC markers including CD 56, Synaptophysin, Chromogranin A and NSE were applied for non-small cell lung carcinoma. However, all were specific but CD 56 was the most sensitive marker for diagnosis of small cell lung carcinoma (Table I). Although differentiated non small cell carcinoma did not require these stains for diagnosis, but their results served as gold standard. CK was positive in both squamous cell carcinoma and adenocarcinoma. p63 served as highly sensitive marker for diagnosis of squamous cell carcinoma and TTF-1 and Napsin A for adenocarcinoma (Figure 2A-C, 3).

Table I: Expression of Immunohistochemistry Markers In Small Cell Lung Carcinoma.

	ADC	SQCC	SCLC	Subtype	Sensitivity	Specificity	PPV	NPV	p value (chi-square)
CK	26/26	105/105	27/27	ALL	100%	0%	100%	0%	<0.001 (18.48)
CD 56	0/26	0/105	24/27	SCLC	88.89%	100%	100%	97.79%	<0.001 (139.08)
SYNAPTO	0/26	0/105	22/27	SCLC	81.48%	100%	100%	96.37%	<0.001 (125.6)
CHROMO	0/26	0/105	21/27	SCLC	77.78%	100%	100%	95.68%	<0.001 (119.07)
NSE	0/26	0/105	20/27	SCLC	74.07%	100%	100%	95%	<0.001 (112.59)

Ck: Cytokeratin Synapto: Synaptophysin Chromo: Chromogranin A Nse: Neuron Specific Enolase.

Ppv: Positive Predective Value Npv: Negative Predective Value Adc: Adenocarcinoma Sqcc: Squamous Cell Carcinoma Sclc: Small Cell Lung Carcinoma.

Based on these results, 86 cases of poorly differentiated non-small cell carcinoma (Non classifiable on histology) were again subtyped according on their immunoprofile into squamous cell carcinoma and adennocarcinoma as far as possible. In 86 cases of poorly differentiated non small cell lung carcinoma, which could not be subtyped on hematoxylin and eosin staining alone, the results of these immunohistochemistry markers i.e. CK, p63, TTF-1 and Napsin A were evaluated for further classification. On the basis of immunoprofile, 84 of the poorly differentiated cases could be categorised further but two cases were negative for all the three immunomarkers, thus, were subtyped as poorly differentiated carcinoma-NOS (Table II).

Table II: Immunoprofile of Poorly Differentiated Non Small Cell Carcinoma (n=86).

TTF-1	NAPSIN-A	p63	NO. OF CASES	Final diagnosis
-	-	+	67	Squamous cell carcinoma
+	+	-	14	Adenocarcinoma
-	+	-	02	Adenocarcinoma
-	-	-	02	Pdc-nos/undifferntiated
+	-	-	01	Adenocarcinoma

Ttf-1: Thyroid Transcription Factor- 1 Pdc-Nos: Poorly Differntiated Carcinoma-Not Otherwise Specified.

P63 was 100% sensitive and specific for squamous cell carcinoma. Amongst, TTF-1 and napsin-A, the latter was found more sensitive for adenocarcinoma as compared to TTF-1 with sensitivity and specificity of 96.15% and 100% respectively (Table III). Napsin-A was found to be positive only in adenocarcinoma cases. But TTF-1 was also expressed in 48.14% cases of small cell carcinoma, so overall sensitivity of napsin-A is higher as compared to TTF-1. Seventy six percent of adenocarcinoma and 60% of squamous cell carcinoma were positive for EGFR (Figure 4).

Table III: Ihc Staining Of Different Markers In Nsclc.

IHC Marker	Adeno-Carcinoma (26)	Squamous Cell Carcinoma (105)	Sensitivity	Specificity	PPV	NPV	p value (chi-square)
P^{63} (+/T) (-/T)	0/26 26/26	105/105 0/105	100%	100%	1	0.92	<0.001
TTF-1 (+/T) (-/T)	24/26 2/26	0/105 105/105	92.30%	100%	1	0.98	<0.001

NAP-A (+/T) (-/T)	25/26 1/26	0/105 105/105	96.15%	100%	1	0.99	<0.001

Ttf-1: Thyroid Transcription Factor- 1 Nap-A: Napsin-A

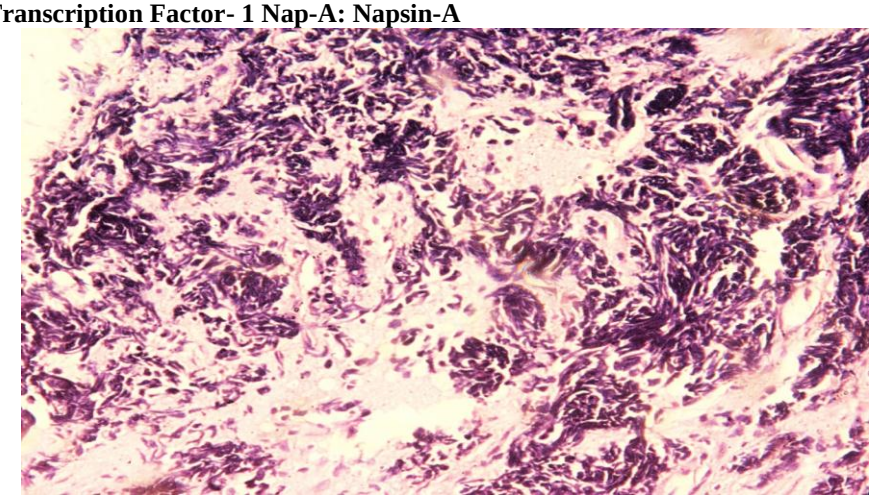

Figure 1 a: Tumor Cells contain scant amount of cytoplasm and oval to spindle hyperchromatic nuclei. Nucleoli are inconspicous. (h&e,100 x).

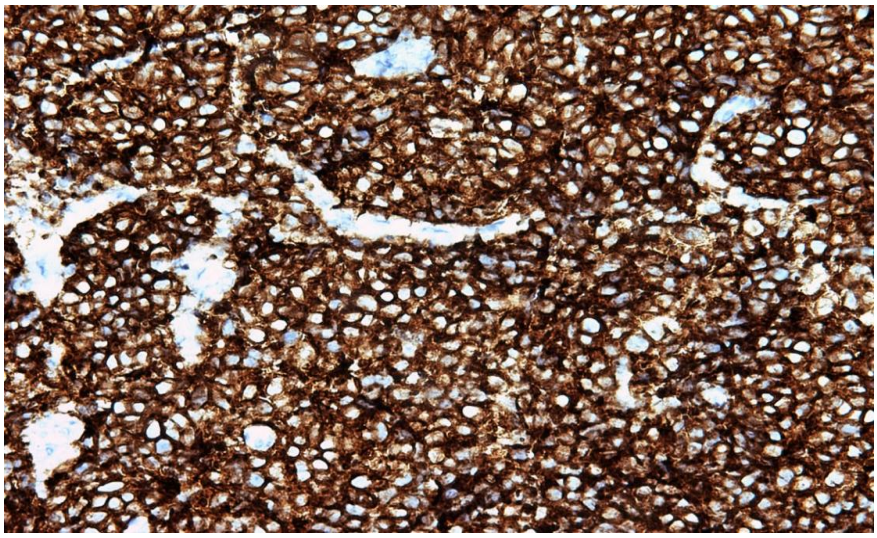

Figure 1 B: Strong Membranous Positivity Of Cd 56 In Sclc (100x)

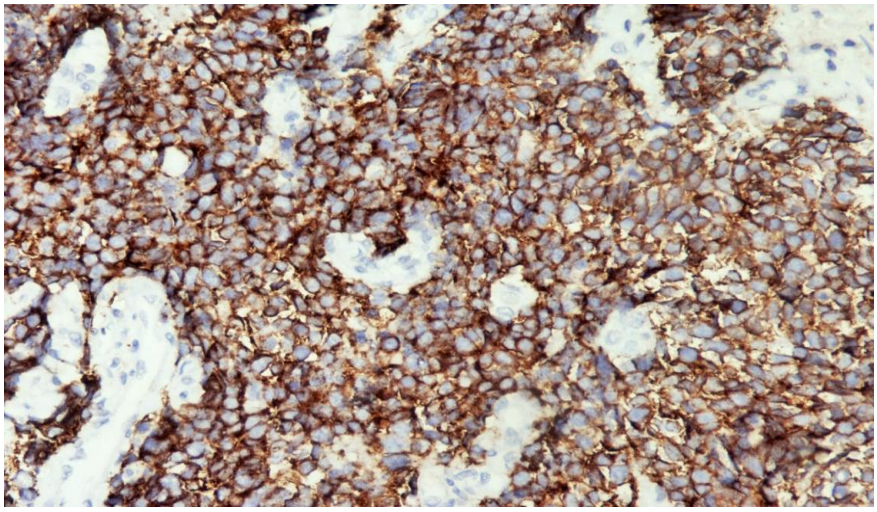

Figure 1c: Strong Membranous Positivity of Chromogranin In Tumor Cells (100x).

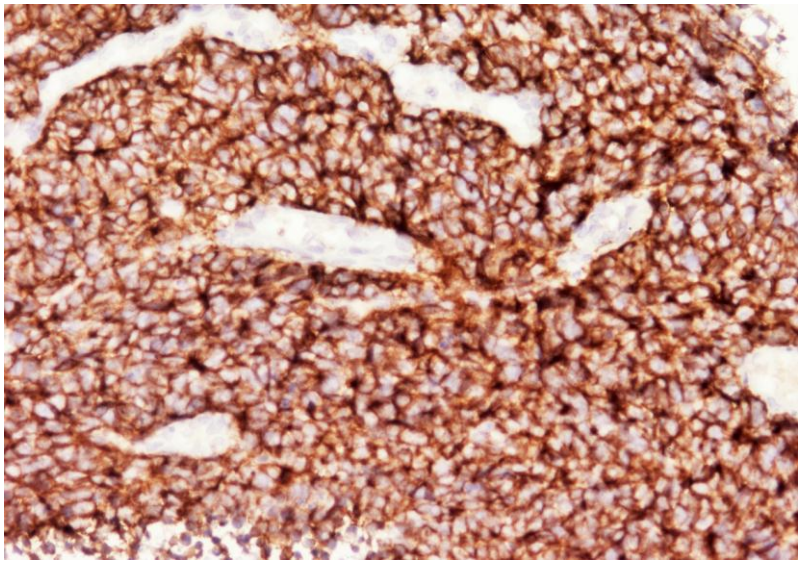

Figure 1d: Synaptophysin Showing Membranous Positivity (100x).

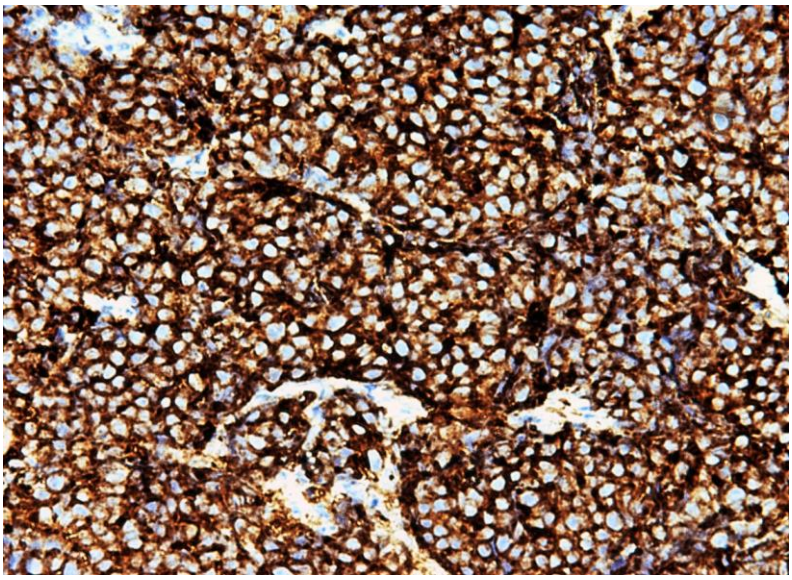

Figure 1e: Neuron Specific Enolase Showingstrong Membranous Positivity In Tumor Cells (100x).

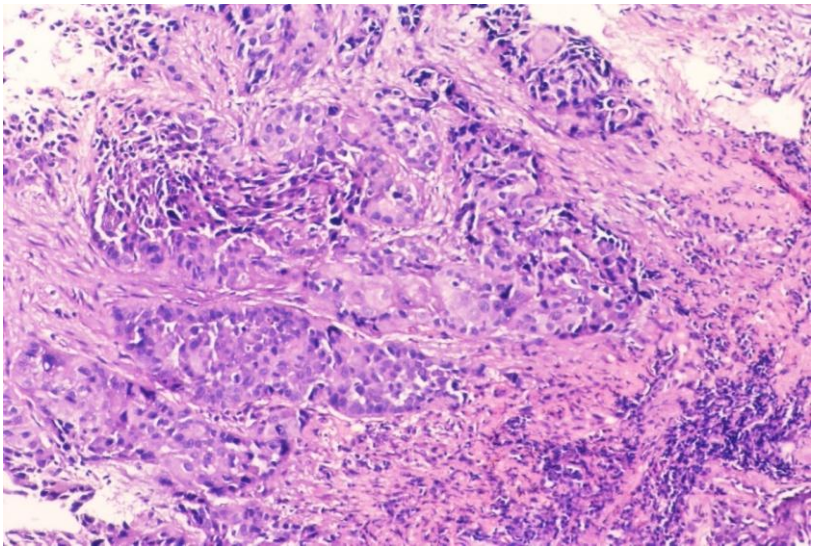

Figure 2a: Poorly Differentiated Tumor Cells Present In Nests And Sheets (H&E, 40 X).

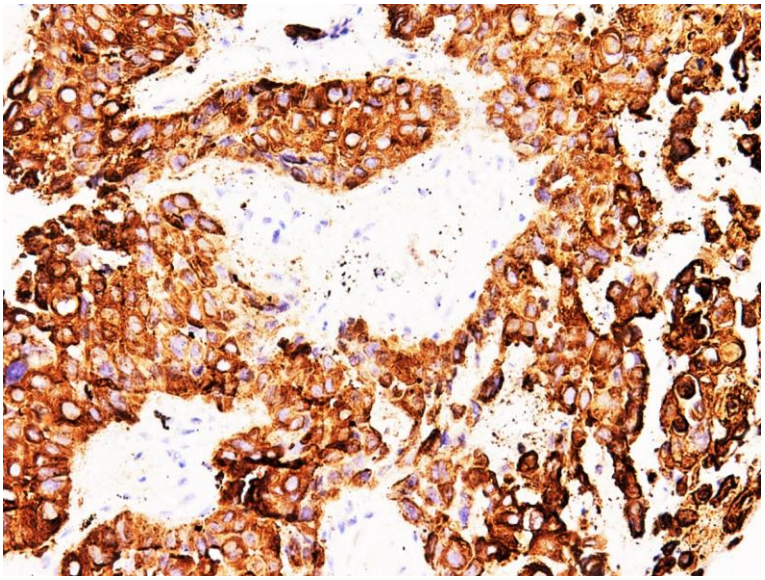

Figure 2b: Cytokeratin Is Positive In Tumor Cells (100x).

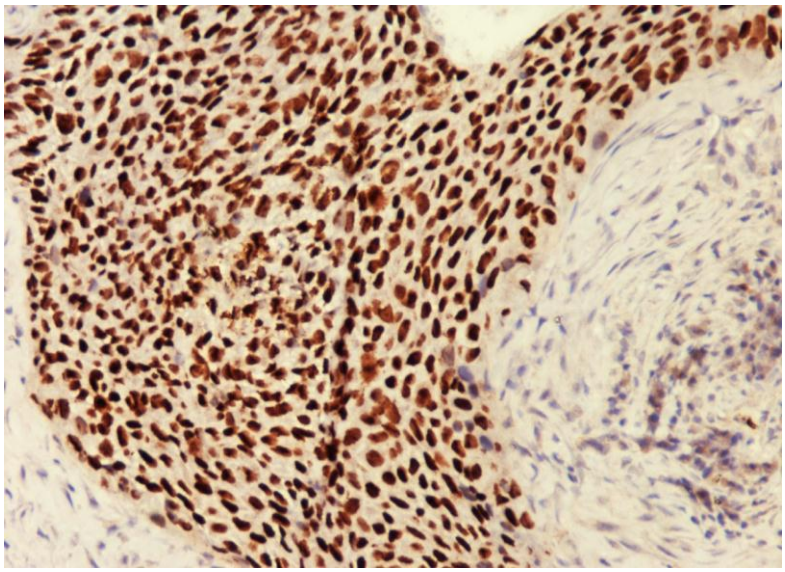

Figure 2c: P63 Shows Strong Nuclear Positivity In Tumor Cells (100x).

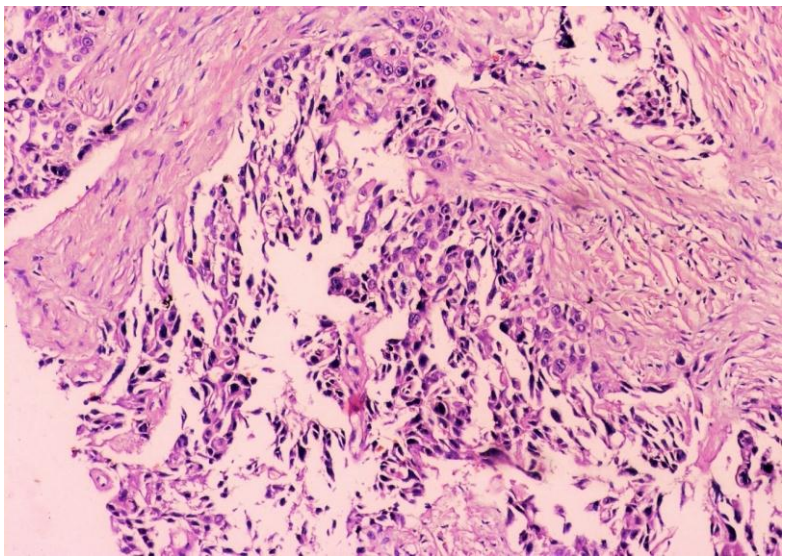

Figure 3 A: Tumor cells are present in clusters, lacking any morphological pattern. (h&e, 100x).

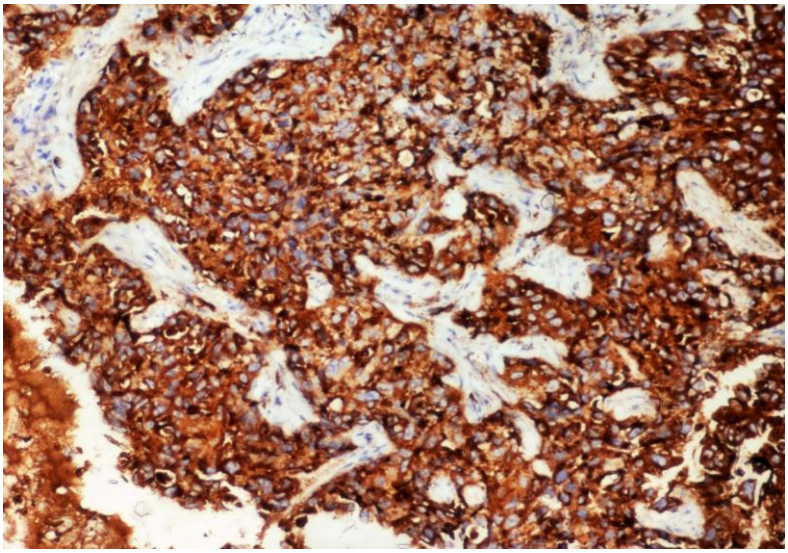

Figure 3b: Napsin- A Showing Strong Granular Membranous Staining In Tumor Cells, Favouring Adenocarcinoma (100x).

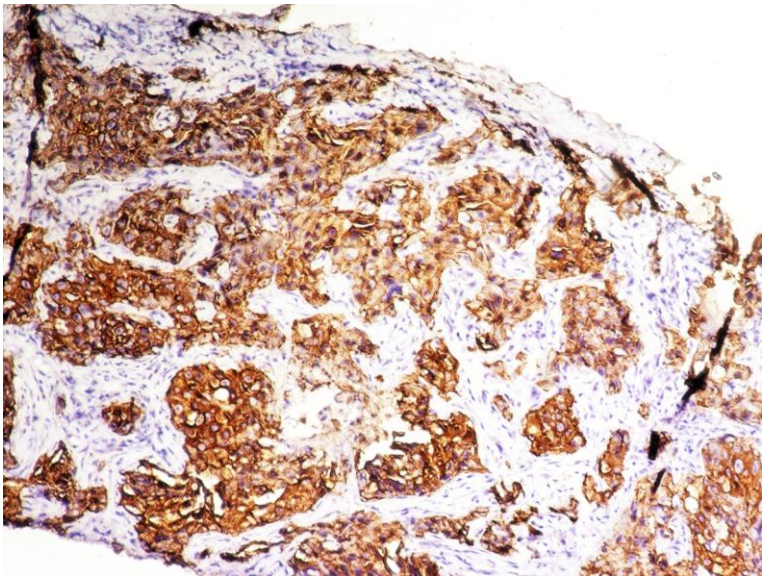

Figure 4: Strong Positivity of Egfr In Poorly Differentiated Adenocarcinoma (100x).

DISCUSSION

Although there has been a long-standing quest to identify a ‘‘lung-specific tumor marker,’’ these efforts have, until recently, largely been directed at distinguishing primary from metastatic lesions.[4] Given the important therapeutic and prognostic information, identification of a ‘‘histologic specific tumor marker’’ has recently emerged as a valuable goal, and a number of markers have been studied. Given the inherent difficulties of trying to rely on a single antibody, panels of immunohistochemical markers have been used to improve sensitivity and specificity.

Although the ideal classification of lung tumors is based on resection specimens that allow inspection of the entire tumor, this is often not the case for pathologists who are increasingly faced with small biopsies. In well differentiated cases, the distinction of adenocarcinoma and squamous cell carcinoma is readily achieved based on standard morphologic criteria. However, difficulty arises in some poorly differentiated tumors and is further amplified in small specimens (small biopsies and cytology) where focal evidence of morphologic differentiation may not be represented as a result of scant cellularity, crush artifact, or cell dispersal. Until recently, a non-committal diagnosis of non-small cell lung carcinoma-not otherwise specified was widely advocated as a general approach to small specimens.

Molecular studies of lung cancers have led to the development of personalized/ targeted therapy.[5] An important example is the discovery of epidermal growth factor receptor gene (EGFR) alterations, and the successful administration of EGFR tyrosine kinase inhibitors (TKIs) in lung cancer patients whose tumor harbors EGFR alterations.[6] Another therapeutic target, the echinoderm microtubule associated protein like 4

(EML4)-anaplastic lymphoma kinase (ALK) fusion protein, has also been uniquely detected in a subset of adenocarcinomas. The recombinant antibody bevacizumab, targeting the VEGF protein, has been shown to be effective when used in combination with standard first-line chemotherapy. However in patients with squamous cell carcinomas, in particular the cavitating variant, it is associated with fatal pulmonary hemorrhage. It has thus been recommended for use only in lung carcinoma patients with non-squamous cell histology. Recently, more targeted therapies aimed at specific pathways and/ or cell types have been developed and are in clinical trials. Taken together, subclassification of NSCLC plays a critical role in the clinical management of NSCLC patients.[7]

In well differentiated NSCLC, morphological features are sufficient for subtyping in most of the cases. However, in poorly differentiated NSCLC subtyping is a challenging task based on H&E alone. These cases lack the histological hallmarks of specific differentiation. It is rather acceptable to classify a case as NSCLC than to incorrectly subtype it, since in this case the patient is deprived of the targeted therapy and genetic studies. In such cases, IHC can be used as a powerful adjuvant tool.

The panel of immunomarkers used comprised p^{63}, TTF-1 and napsin-A. After the application of IHC markers, out of 86 cases of poorly differentiated non small cell carcinoma, 17 were subytped as adenocarcinoma, 67 as squamous cell carcinoma and 2 cases were NSCLC-NOS subtype, since these two cases were negative for all the three immunomarkers.

Cytokeratin was positive in all 160 cases of lung carcinoma. But it was not specific for any particular subtype. Thus, it only helped to identify tumors of epithelial origin and excluding out lymphomas and others.

Among the IHC markers, we evaluated the role of p^{63} in squamous cell carcinoma. The immunoexpression of p^{63} was positive in all the cases of squamous cell carcinoma, while none of adenocarcinoma was positive, thus having 100% sensitivity and specificity.

The reported positivity of p^{63} by IHC is usually over 80% in most of the series. However, in study by Conde et al[8] the sensitivity and specificity of p^{63} was 76% and 74% respectively. They attributed the lower positivity of p^{63} to better differentiated areas and even well differentiated tumors which may be negative for p.[63]

In the current study, amongst the poorly differentiated adenocarcinoma, TTF-1 was found to have sensitivity of 92.3%. Prior studies have reported the sensitivity in the range of 60-86% (Table IV).

The sensitivity of napsin-A was 96.15% in our study while the specificity was 100%. None of the other subtype of lung carcinoma had positive immunoexpression for it. The sensitivity of napsin-A varies over a wide range from 65% to 92%, while the specificity varies from 83% to 100% in the literature.[9,10,11] Previous studies using surgical resected specimens indicated that napsin A has a better sensitivity and specificity than TTF-1 in well to moderately differentiated lung ADCs.[9,11] Therefore, it has been used with TTF-1 together in the differential diagnosis of lung adenocarcinomas. Napsin A may be particularly useful in poorly differentiated ADCs, which may lose TTF-1 expression.[12]

When the diagnostic role of TTF-1 and napsin-A are compared, we found that sensitivity of napsin-A is more than TTF-1(96.15% vs 92.3%). The specificity of both in categorization of NSCLC was 100%. However, TTF-1 was also found positive in 48.14% of cases of small cell carcinoma. Similar to our study, many studies in literature have reported a higher sensitivity and specificity for napsin-A as compared to TTF-1 in subtyping of primary lung adenocarcinoma.[9,11,12] However, Ming hui et al have reported that sensitivity of napsin-A was lesser than TTF-1 (65% vs 81%).

Table IV: Immunohistochemical Staining Of Various Markers.

S. No.	Study (YEAR)	Markers	SQCC	ADC	SCLC	Sensitivity (%)	Specificity (%)
1.	UENO ET AL (2005)[13]	CK	-	-	-	-	-
		P63	-	-	-	-	-
		TTF-1	-	-	-	84.6	76.7
		NAPSIN-A	-	-	-	84.9	94.3
2.	ZHANG ET AL (2010)[14]	CK	-	-	-	-	-
		P63	-	-	-	-	-
		TTF-1	2.4	84.4	66.7	84.4	83.9
		NAPSIN-A	0.0	84.9	0.0	84.9	93.8
3.	Rekhtman ET AL (2011)[15]	CK	-	-	-	90	97
		P63	-	-	-	99	96
		TTF-1	-	-	-	84	100
		NAPSIN A	-	-	-	-	-
4.	TURNER ET AL	CK	-	-	-	-	-

	(2012)[10]	P63	-	-	-	-	-
		TTF-1	-	-	-	64	90
		NAPSIN-A	-	-	-	87	97
5.	**Whithaus ET AL (2012)[16]**	CK	-	-	-	53	96
		P63	-	-	-	95	86
		TTF-1	-	-	-	60	98
		NAPSIN-A	-	-	-	83	98
6.	**Tacha ET AL (2012)[11]**	CK	100	100	-	86.4	100
		P63	88.4	11.3	-	88.4	100
		TTF-1	5.3	70	-	69	94.7
		NAPSIN-A	0.0	86	-	87	100
7.	**Brown ET AL (2013)[9]**	CK	-	-	-	100	100
		P63	-	-	-	-	-
		TTF-1	-	-	-	77.4	100
		Napsin-A	-	-	-	86	100
8.	**Ming HUI ET AL (2014)[17]**	CK	-	-	-	89.6	80
		P63	-	-	-	93.5	80
		TTF-1	-	-	-	85.7	75
		Napsin-A	-	-	-	89.6	90
9.	**ZHAO ET AL (2014)[18]**	CK	81.25	100	71.4	100	35
		P63	100	16	0.0	100	88
		TTF-1	-	80	42.8	80	87
		Napsin-A	-	64	0.0	64	100
10.	**MA Y ET AL (2014)[19]**	CK	-	-	-	78.9	97.7
		P63	-	-	-	87	81
		TTF-1	-	-	-	-	-
		Napsin-A	-	-	-	-	-
11.	**GUARDA ET AL (2015)[20]**	CK	100	93.8	-	100	77.8
		P63	91.7	21.7	-	91.7	78.3
		TTF-1	03.6	84.5	-	84.5	96.4
		Napsin-A	0.0	92.0	-	92	100
12.	**Present Study (2016)**	CK	100	100	100	100	0
		P63	100	0.0	0.0	100	100
		TTF-1	0.0	92.3	48.1	92.3	100
		Napsin-A	0.0	96.1	0.0	96.15	100

-=number not mentioned in the study

EGFR testing is recommended for all locally advanced or metastatic adenocarcinoma lungs but recommendation in squamous histology is uncertain. The potential use of EGFR expression as a marker has been widely investigated, with conflicting results.

EGFR mutations are more common in never-smokers, in patients with Asian ethnicity, and in patients with adenocarcinoma histology.[75] However, solely on the basis of histology, EGFR testing should not be excluded in patients with squamous cell cancer, especially females, never smokers and Asian ethinicity.[21]

In the current study, 76% of adenocarcinomas and 60% of squamous cell carcinomas had positive immunoexpression for EGFR. While its expression is rare in small cell carcinoma, but one case was documented in the present study. In our study, we found a total rate of 60% for the EGFR in patients with SQCLC. This rate was higher than some other studies[21] and the possible explanation may be owing to the difference in the sex ratio of the enrolled patients. As a result, we speculate that the total rate of mutation in SQCLC might increase as the number of female cases increases. Another explanation may involve differences among races and regions, as the factors that drive genomic alteration between races are consequential.[21] Similarly, Asian patients have a higher incidence of the EGFR gene mutation than Caucasian patients.

Similar to our study, various other studies document the increased EGFR overexpression in between 40% to 89% of NSCLC, with highest rates seen in squamous tumours (89%) and lowest in adenocarcinomas (41%).[21]

IHC for EGFR mutation has been shown to correlate poorly or not at all with presence of EGFR mutation. Positivity of EGFR on IHC has no therapeutic role. Traditionally the gold standard for EGFR mutation testing requires direct sequencing of extracted tumor DNA, a time consuming methodology with low sensitivity (high levels of tumor DNA required). Newer validated methods for EGFR mutation testing provides increased sensitivity (fewer tumor cells required),

improved turnaround time allow for testing on a greater variety of clinical samples. Therefore surgical pathologists must be aware of the available tests and specific tissue requirements for their local molecular laboratory. Unfortunately, we could not perform the same because of lack of molecular diagnostics at our institute.

The use of a minimum panel of antibodies is critical for specimens with reduced cellularity. Moreover, the limitation of the sample with reduced size is also imposed by the necessity to preserve the tissue for additional molecular studies.

Our study demonstrated that overall efficacy using p63 and napsin-A was 96.5% in poorly differentiated NSCLC. When TTF-1 was used instead of napsin-A the efficacy was 95.4%. Using both TTF-1 and napsin-A 97.6% of cases could be diagnosed, which is only a marginal increase over limited panel of TTF-1/napsin-A and p63. While TTF-1 being a nuclear stain is easier to interpret, napsin-A serves as a more specific marker for differentiation of primary lung adenocarcinoma (Table V).

Table V: Subtyping On The Basis Of Limited Panel.

Markers	ADC (26)	SQCC (105)	PDC-NOS (02)	Sensitivity	Specificity	PPV	NPV	P Value
TTF-1+P63	24	105	0	95.4%	100%	94.86%	98.37%	<0.001
NAPSIN A+P63	25	105	0	96.5%	100%	100%	80%	<0.001

Ttf-1: Thyroid Transcription Factor- 1 Sqcc: Squamous Cell Carcinoma.

Adc: Adenocarcinoma Ppv: Positive Predectiove Value Npv: Negative Predictive Value.

Based on the findings that both TTF-1 and Napsin A have a high sensitivity and specificity for the diagnosis of primary lung ADCs, and p63 stain is highly sensitive and specific for squamous differentiation, we outlined an algorithmic approach in the subclassification of NSCLC. In the algorithm, the evaluation of morphology in the conjunction of immunostaining patterns is necessary for the final diagnosis of the tumor and further decision-making steps (Table VI). Adenocarcinoma should be favoured for cases with both napsin A and TTF-1 positivity; alternatively, either TTF-1 or napsin A positivity, alongside p63 negativity, while Squamous cell carcinoma should be favoured for cases with p63 positivity alongside napsin A and TTF-1 negativity.

Table VI: Algorithm For Subtyping Of Poorly Differentiated Nsclc According To Ihc Staining On Endobronchial Biopsies.

p63	CK	TTF-1	Napsin A	Diagnosis
+	+	-	-	**SQCC**
-	+	-	-	**SQCC/ADENO**
-	+	+	+	**ADENO CA**
-	+	-	+	**ADENO CA**

Ck: Cytokeratin Ttf-1: Thyroid Transcription Factor- 1 Sqcc: Squamous Cell Carcinoma.

ADC: Adenocarcinoma

We also emphasise that the vast majority of specimens can be classified by TTF-1/p63 and napsin-A/p63, with a third marker being needed in only a small subset of cases.

An accurate classification of poorly differentiated NSCLC becomes very difficult in endobronchial biopsies. In such circumstances, IHC markers are of great help and the use of a minimum panel of antibodies is critical. Since, newer protocols incorporate the molecular analysis of the tumor and plan targeted therapy for the patient. Thus, our study was aimed at subclassification of NSCLC using minimum tumor material and limited IHC panel in order to be cost effective and also to preserve tumor tissue for further molecular studies.

CONCLUSION

An accurate classification of NSCLC is essential to plan targeted therapy for the patient. However, the classification of poorly differentiated NSCLC becomes very difficult in small biopsies due to scant tissue. In such circumstances, IHC markers are of great help. At the same time, minimal panel should be used in view of small biopsies, scant material and to save the tissue for further molecular studies. p63 and napsin-A/TTF-1 should be used as first line panel, only a marginal proportion of cases require an expanded panel for subtyping.

REFERENCES

1. Jemal A, Siegal R, Xu J, Ward E. Cancer statistics, CA Cancer J clin, 2010; 60(5): 277-300.
2. Stang A, Pohlaben H, Muller KM, Jahn I, Giersiepien K. Diagnostic aggrement in the histopathological evaluation of lung cancer tissue in a population based case control study. Lung cancer, 2006; 52(1): b 29-36.
3. Mukhopadhyay S, Katzenstein AL. Subclassification of non small cell lung carcinoma lacking morphological differentiation on biopsy specimens: Utility of an immunohistochemical panel containing TTF-1, Napsin A, p^{63} and CK5/6. Am J Surg Pathol. 2011; 35: 15-25.

4. Jagirdar J. Application of immunohistochemistry to the diagnosis of primary and metastatic carcinoma to the lung. Arch Pathol Lab Med, 2008; 132(3): 384–96.
5. Zakowski MF. Lung cancer in the era of targeted therapy: a cytologist's perspective. Arch Pathol Lab Med, 2013; 137: 1816–21.
6. Munfus-McCray D, Cui M, Zhang Z, Gabrielson E, Askin F, Li QK. Comparison of EGFR and KRAS mutations in primary and unpaired metastatic lung adenocarcinoma with potential chemotherapy effect. Hum Pathol, 2013; 44: 1286–92.
7. Rivera MP, Mehta AC. Initial diagnosis of lung cancer: ACCP evidence-based clinical practice guidelines (2nd edition). Chest, 2007; 132: 131S–48S.
8. Conde E, Angulo B, Redondo P, Toldos O, Gracia E, Gauthier A et al. The use of p63 Immunohistochemistry for the identification of squamous cell carcinoma of the lung. PLoS ONE, 5(8): e12209. doi:10.1371/journal.pone.0012209.
9. Brown AF, Sirohi D, Fukoka J, Cagle PT, Nicolas MP, Tacha D. Tissue preserving antibody cocktails to differentiate primary squamous cell carcinoma, adenocarcinoma and small cell carcinoma of lung. Arch Pathol Lab Med, 2013; 137(9): 1274-81.
10. Turner BM, Cagle PT, Sainz IM, Fukoka J, Steven SS, Jgirdar R. Napsin A, A new marker for lung adenocarcinoma is complementary and more sensitive and specific than TTF-1 in differential diagnosis of primary pulmonay carcinoma.: Evaluation of 1674 cases by tissue microarray. Arch Pathol Lab Med, 2012; 136(2): 163-71.
11. Tacha D, Yu C, Bremer R, Qi, W, Ha T. A 6-Antibody Panel for the Classification of Lung Adenocarcinoma Versus Squamous Cell Carcinoma. Appl Immunohistochem Mol Morphol, 2012; 20: 201–7.
12. Stoll LM, Johnson MW, Gabrielson E, Askin F, Clark DP, Li QK. The utility of napsin-A in the identification of primary and metastatic lung adenocarcinoma among cytologically poorly differentiated carcinomas. Cancer Cytopathol, 2010; 118: 441–9.
13. Ueno T, Linder S, Elemberger G. Aspartic proteinase napsin is a useful marker for diagnosis of primary lung adenocarcinoma. Br J Cancer, 2003; 88: 1299-33.
14. Zhang P, Han YP, Huang L, Li Q, Ma DL. Value of napsin A and thyroid transcription factor-1 in the identification of primary lung adenocarcinoma. Oncol Lett, 2010; 1(5): 899-903.
15. Rekhtman N, Ang DC, Sima CS, Travis WD, Moreira AL. Immunohistochemical algorithm for differentiation of lung adenocarcinoma and squamous cell carcinoma based on large series of whole-tissue sections with validation in small specimens. Mod Pathol, 2011; 24(10): 1348–59.
16. Whithaus K, Fukuoka J, Prihoda TJ, Jagirdar. Evaluation of Napsin A, Cytokeratin 5/6, p63, and Thyroid Transcription Factor 1 in Adenocarcinoma Versus Squamous Cell Carcinoma of the Lung. Arch Pathol Lab Med, 2012; 136: 155–62.
17. Ao MH, Zhang H, Sakowski L, Illei PB, Gabrielson E et al. The utility of triple novel marker (combination of TTF-1, napsin A and p40) in the subclassification of non-small cell lung cancer. Hum Pathol, 2014; 45(5): 926-34.
18. Zhao W, Wang H, Peng Y, Tian B, Peng L, Zhang DC. p63, CK5/6, TTF-1 and napsin A, a reliable panel to subtype non small cell cancer in biopsy specimens. Int J Clin Exp Pathol, 2014; 7(7): 4247-53.
19. Ma Y, Fan M, Dai L, Kang X, Liu Y, Sun Yet al. Expression of p63 and CK5/6 in early-stage lung squamous cell carcinoma is not only an early diagnostic indicator but also correlates with a good prognosis. Thoracic Cancer, 2015; 6: 288–95.
20. Gurda GT, Zhang L, Wang W, Chen L, Geddes S, Cho WC et al. Utility of five commonly used immunohistochemical markers TTF-1, Napsin A, CK7, CK5/6 and P63 in primary and metastatic adenocarcinoma and squamous cell carcinoma of the lung: a retrospective study of 246 fine needle aspiration cases. Clinical and Translational Medicine, 2015; 4: 16. DOI 10.1186/s40169-015-0057-2.
21. Ellison G, Zhu G, Moulis A, et al. EGFR mutation testing in lung cancer: a review of available methods and their use for analysis of tumor tissue and cytology samples. J Clin Pathol, 2013; 66: 79-89.