Review Article

Immunohistochemistry in Respiratory diseases

Jayaprakash.B

Additional Professor, Department of Pulmonary Medicine,
Govt.Medical college, Trivandrum

Correspondence : Dr.Jayaprakash.B, Additional Professor, Department of Pulmonary Medicine, Govt. Medical college, Trivandrum, Tel : +919447658148,Email:jayansindhu@yahoo.com

Introduction:

Immunohistochemistry (IHC) or Immunocyto chemistry is a method for localizing specific antigens in tissues or cells based on antigen-antibody reaction. It seeks to exploit the specificity provided by the binding of an antibody with its antigen at the light microscopic level[1]. Over the last several decades, an impressive array of antibodies has become commercially available and many of the antibodies have become integrated in to the routine practice of pathology. Pathologic classification of lung cancer remains a critical cornerstone of decision-making in managing patients with lung cancer. Newer targeted therapies further underscore the need for correct classification, both to select patients likely to benefit from these therapies and to avoid potential adverse effects of individual agents when used in patients with specific histologic types of cancers. The increasingly common use of bronchoscopic samples, core-needle biopsies and fine-needle aspiration samples have posed challenges to the pathologist in achieving accurate and definitive diagnosis[2]. IHC is widely used by the pathologist in modern pathology practice. The awareness of IHC and its specific use will be very useful for the pulmonologist for making a correct and more specific diagnosis. IHC is a commonly used effective adjuvant technique in diagnosing primary and metastatic neoplasms of the lung and pleura. Because of its relative ease of use and specificity, IHC has largely replaced histochemistry and electron microscopy in diagnosing pulmonary and pleural neoplasms. Even though most primary lung cancers can be diagnosed by histologic criteria alone, it becomes difficult when they are poorly differentiated or clinical situations become complicated. IHC techniques are often used to confirm or eliminate a pathological diagnosis. In addition, many metastatic tumors are morphologically similar to primary lung and pleural tumors and IHC is an effective way to distinguish them. Even though IHC has come in to wide use in diagnostic pathology since 1990s, the knowledge about different immunostains and its specific role for the accurate diagnosis is poor among practicing pulmonologists. Unless the physician who is evaluating a patient with pulmonary disease gives the detailed clinical history and diagnostic possibilities the pathologist may not be able to do the specific immunomarker. IHC can be performed on a recut section of a paraffin embedded or frozen tissue block or on a cytologic preparation such as a smear or cytospin.

Role of Inmmunohistochemistry in Respiratory diseases

1. Small cell carcinoma Vs Non small cell lung cancer
2. Squamous cell carcinoma Vs Adeno carcinoma
3. Primary pulmonary or Metastatic adenocarcinoma
4. Epitheloid Mesothelioma or Adenocarcinoma
5. Mesothelioma or Squamous cell carcinoma
6. Spindle cell neoplasms
7. Diagnosis of Lymphoma
8. Detection of primary in metastatic carcinoma
9. Pulmonary neuroendocrine tumors
10. Non neoplastic pulmonary diseases

Common Immunohistochemical Markers for lung neoplasms

Thyroid Transcription Factor-1 (TTF-1)

Adenocarcinoma is the most common epithelial malignancy in lung. Adenocarcinoma account for 38%of all lung cancers in the United States[9,10]. Sub classification of adenocarcinomas according to primary site can be a challenging task. Thyroid transcription factor-1 (TTF-1) has been the most widely used antibody to identify pulmonary origin. Greater than 80% of adenocarcinoma express this protein.TTF 1 is a nuclear transcription factor that is expressed in normal lung, in thyroid and in their neoplasms. In normal lung tissues, TTF-1 is expressed in the nuclei of epithelial cells in the distal lung parenchyma, type 2 pneumocytes, and nonciliated bronchiolar cells (Claracells)[6]. Expression of TTF-1 by a tumor in the lung usually indicates a pulmonary or thyroid carcinoma. These two alternatives can usually be differentiated based on additional staining for the surfactant protein B (positive in many lung adenocarcinomas) and thyroglobulin (positive in many thyroid cancers). TTF-1 is a highly specific immunomarker for adenocarcinoma of the lung and malignant effusion[3]. Su YC etal found that 73% of primary lung adenocarcinomas expressed TTF-1, whereas all nonpulmonary adenocarcinomas except thyroid lacked TTF-1 staining.

Metastatic tumors are more common than primary tumors in the lung, and the most frequent histological type is adenocarcinoma[4]. Microscopic comparison of the pulmonary tumor with slides of the previous tumor can frequently help to differentiate primary from metastatic tumor[5].In other cases judicious use of other immunostains will help to identify the primary site.

Cytokeratin(CK)

Carcinoma demonstrate epithelial characteristics including production of cytokeratins and epithelial differentiation is therefore usually reflected by staining for one or more CKs. Cytokeratin7 (CK7) and Cytokeratin 20 (CK20) are the most commonly used cytokeratin markers. CK7 is expressed by almost all primary lung adenocarcinomas and CK 20 is usually negative, even though it is co expressed in less than 10%primary mucinous adenocarcinomas[11]. Metastatic adenocarcinoma from an unknown primary site is a common clinical problem. The use of CK20 and CK7 was proposed to identify the primary sites in this situation[8]. If the primary site of malignancy is lung, breast, ovary or thyroid CK 7 will be positive and CK20 negative. Colorectal cancers will be negative for CK7 and positive for CK20. Mesotheliomas are also CK positive, but they stain positive for other mesothelial markers such as Calretinin, WT-1 and Vimentin also. Melanoma and lymphoma are negative for CK. Some sarcomas especially synovial sarcoma and rarely angiosarcoma and leiomyosarcoma can also be positive for CK. Small cell carcinomas show paranuclear dot positivity for cytokeratin.

Other immunomarkers

Surfactant protein B - adenocarcinoma

p63,CK 5/6 - squamous cell carcinoma

WT-1,Calretinin, Vimentin, Mesothelin - Mesothelioma

S 100 - Nerve sheath tumors, Melanoma

CDX2 - Colorectal carcinoma

PE 10 - Bronchoalveolar carcinoma

CD34- Solitary fibrous tumor

Synaptophysin, CD56, Neuron specific Enolase (NSE) and Chromogranin A - Neuroendocrine tumors including small cell carcinoma

CD45 (LCA) - lymphoma

Application of IHC in diagnosis of lung cancer

1. Small cell lung cancer (SCLC) Vs Non small cell lung cancer (NSCLC)

In most cases, SCLC can be differentiated from NSCLC by morphologic criteria. However, biopsy crush artifact, tumor necrosis, poor fixation, and limited tumor representation can occasionally result in suboptimal morphology, making definitive diagnosis more difficult[2]. Cytokeratin (CK) immunostaining show paranuclear dot positivity in most of the cases of SCLC[12]. Commonly used immunostains for detecting neuroendocrine differentiation are chromogranin, synaptophysin and CD56[13]. Recognizing the fact that an individual tumor may stain with one, several, or none of these markers, many pathologists order two or three of the neuroendocrine markers to increase the probability of detecting neuroendocrine differentiation.

Distinguishing SCLC from large cell neuroendocrine carcinoma (LCNEC) on occasion can be difficult, since SCLC and LCNEC overlap in their histologic and immunophenotypic characteristics. Hiroshima K etal reported more frequent CD56 expression in SCLC (96%) than LCNEC (53%), whereas synaptophysin was expressed in 77% of LCNECs and 57% of SCLCs, and chromogranin A in 59% of LCNECs and 36% of SCLCs[14]

2. Squamous cell carcinoma Vs Adeno carcinoma

The differentiation in to Squamous cell carcinoma or Adeno carcinoma is important for targeted therapies and further genetic evaluation. TTF-1 and napsin-A will be positive in 90% of pulmonary adenocarcinoma. Squamous cell carcinoma is almost always negative for TTF-1. P63 and CK5/6 are valuable in confirming squamous cell carcinoma.[15]

3. Primary Pulmonary Vs Metastatic Adenocarcinoma

Metastatic neoplasms are more common than primary tumors of lung. Detailed history with image findings must be provided to the pathologist along with the specimen and any past history of malignancy and its reports must be communicated. In difficult situation IHC is of immense value in differentiating primary pulmonary or metastatic tumors. Thyroid transcription factor-1 (TTF-1) has been the most widely used antibody to identify pulmonary origin. More than 80% of pulmonary adenocarcinoma express this protein. Extra pulmonary adenocarcinomas except thyroid are usually negative for TTF-1. Although TTF-1 immunohistochemistry has clearly advanced the diagnosis of primary and metastatic adenocarcinomas in the lung, antibodies to napsin-A and surfactant protein B have also demonstrated its usefulness for this purpose. 80% of primary adenocarcinoma stain positive for napsin-A. The combined use of napsin A or surfactant protein B and TTF-1 may improve the likelihood of confirming pulmonary origin of an adenocarcinoma[16].

4. Mesothelioma Vs Adenocarcinoma

The important differential diagnosis of pleural malignant epithelial tumors are malignant mesothelioma and metastasis. IHC is now an integral part of the diagnosis of malignant mesothelioma. Unfortunately, despite extensive investigation, there is no immunostain that can reliably differentiate mesothelial hyperplasia from malignant mesothelioma, and this remains a topic of continued interest[17]. Currently, the most commonly used mesothelial markers include antibodies to CK5/6, Calretinin and Vimentin. Anti-podoplanin (D240) is also gaining popularity and is an emerging marker that is highly sensitive and specific for epitheliod mesothelioma[18]. Epithelioid mesotheliomas are more likely to stain for these markers than sarcomatoid mesotheliomas[19]. Adenocarcinomas, on the other hand, are usually positive for one or more of the following antibodies: Carcino embryonic antigen (CEA), Leu-M1, B72.3 20.

5. Mesothelioma vs Squamous cell carcinoma

Squamous cell carcinoma can also present as pleural or subpleural mass and may mimic a mesothelioma both clinically and radiologically. True keratinisation and p63 staining is highly suggestive of squamous cell carcinoma. Negative p63 and positive calretinin are diagnostic of mesothelioma

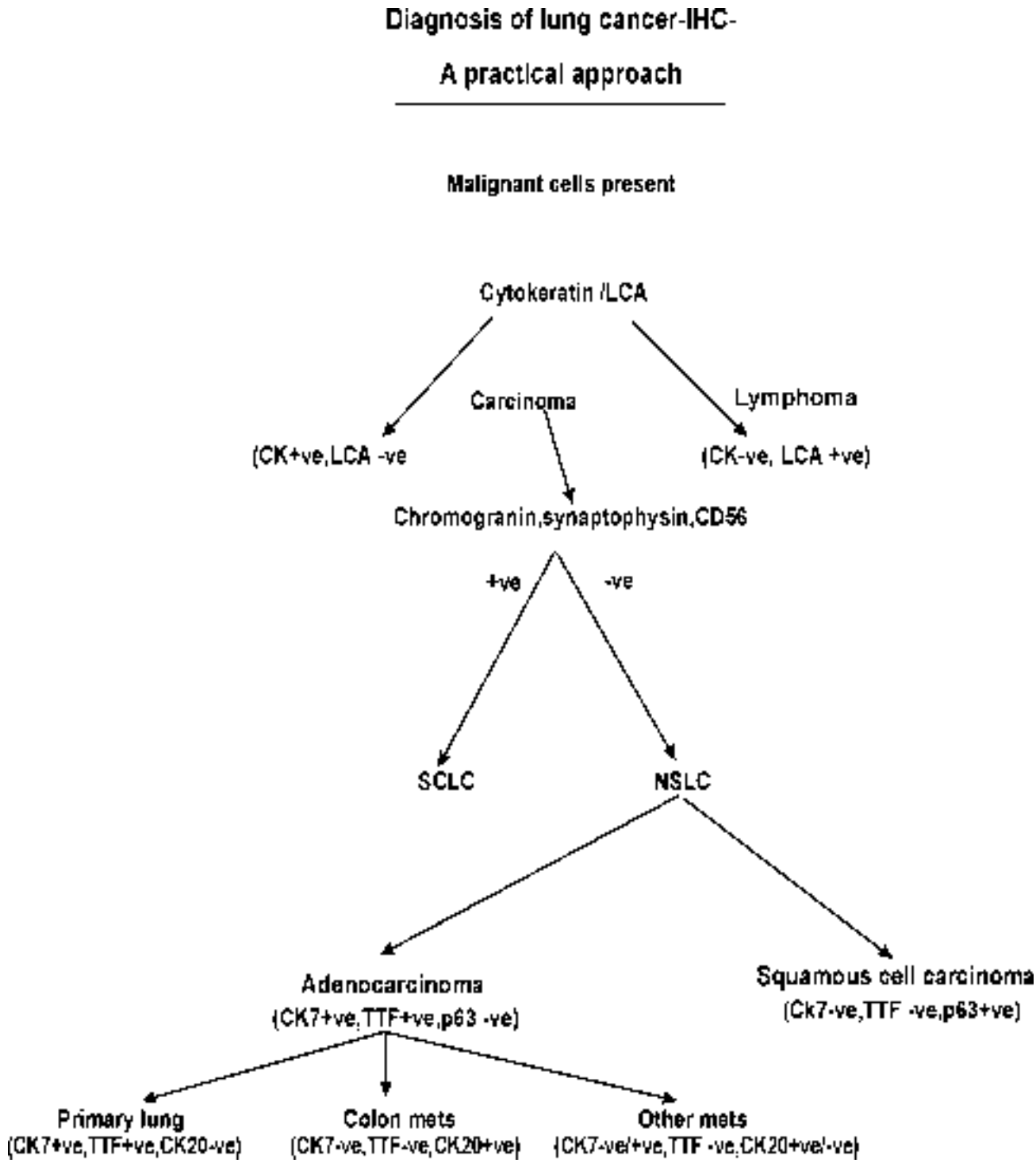
6. Solitary fibrous tumor of pleura(SFT)

Pleuropulmonary fibrous tumor of pleura is a rare mesenchymal cell tumor which has gained importance during the last two decades. SFT originate from the submesothelial tissue of pleura. Fibrous tumor of pleura is positive for CD34 in most of the cases and some of the tumors are also positive for Bcl-2, smooth muscle actin, Desmin, Leu 7 and Vimentin[21].

7. Spindle Cell Neoplasms

The lung and pleural neoplasm with spindle cell morphology include sarcomatoid and pleomorphic carcinomas, sarcomatoid and biphasic mesotheliomas, true sarcomas and melanoma. A neoplasm arising in lung and consisting of spindled cells is most often a sarcomatoid carcinoma. But sarcomatoid carcinoma of the lung is rare, and its incidence is estimated as 0.3-1.3% of all lung malignancies [22, 23,.]

IHC by itself may not always be definitive in resolving

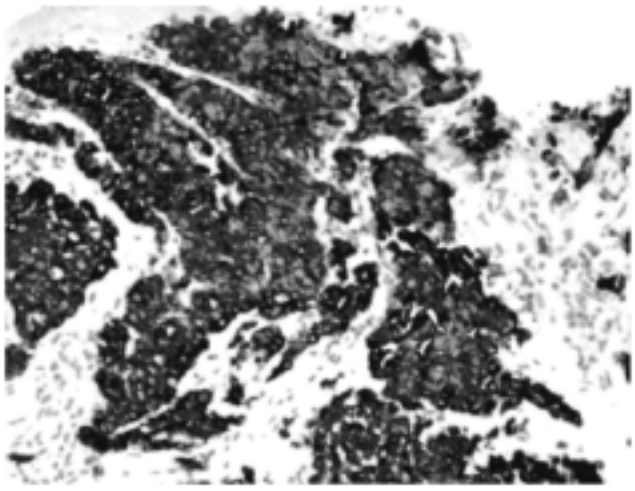

Diagnosis of lung cancer-IHC-
A practical approach
Malignant cells present
Cytokeratin /LCA
Carcinoma
Lymphoma
(CK+ve,LCA -ve
(CK-ve, LCA +ve)
Chromogranin,synaptophysin,CD56
+ve
-ve
SCLC
NSLC
Adenocarcinoma
(CK7+ve,TTF+ve,p63 -ve)
Squamous cell carcinoma
(Ck7-ve,TTF -ve,p63+ve)
Primary lung
(CK7+ve,TTF+ve,CK20-ve)
Colon mets
(CK7-ve,TTF-ve,CK20+ve)
Other mets
(CK7-ve/+ve,TTF -ve,CK20+ve/-ve)

the exact differential diagnosis in case of spindle cell neoplasm. In pleura, mesothelioma, metastases from other carcinomas, synovial sarcoma, angiosarcoma and leiomyosarcoma are other possibilities. Cytokeratin is positive for 90% of sarcomatoid carcinoma. Cytokeratin (65%) and Bcl2 are used for synovial sarcoma. Desmin, Smooth muscle actin (SMA) and Muscle specific actin (MSA) are the markers for leiomyosarcoma. CD34 and CD31 are useful for angiosarcoma. If melanoma is suspected S100, HMB 45 and melan-A is advisable.

EGFR (Epidermal Growth Factor Receptor Gene)

Among the targeted therapies the most widely used for the treatment of lung cancers are currently the EGFR tyrosine kinase inhibitors. Epidermal growth factor pathway has been found to be activated in a significant percentage of lung cancers, particularly adenocarcinomas. Mutations that lead to EGFR over expression or over activity have been associated with a number of cancers, including lung cancer[24].

High EGFR expression by IHC can identify patients who may benefit from tyrosine kinase inhibitors[25]. Identification of those patients with lung cancer who have EGFR mutations in advance can not only determine which patients will benefit from EGFR TKIs but also identify those patients who will not benefit from standard cytotoxic chemotherapy and also spare them from the potential complications of these drugs.



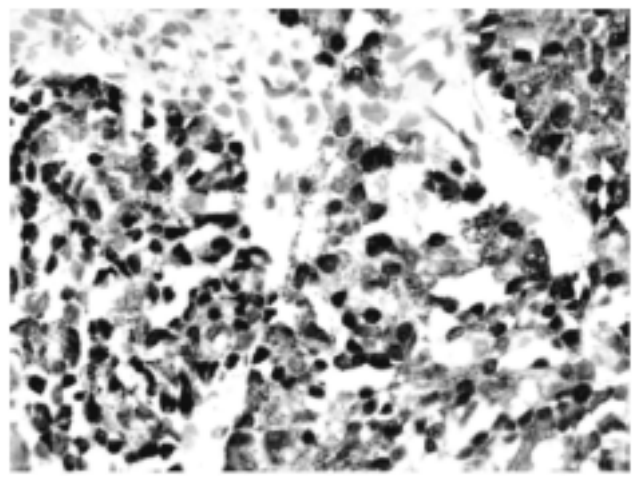
CK 7

8. IHC for non neoplastic Pulmonary diseases

The role of immunohistochemistry in the diagnosis of non neoplastic respiratory diseases is limited. A number of studies are available in the literature looking to the utility of IHC in non neoplastic respiratory diseases. Musthafa et al reported that Immuno histochemistry with anti-MPT64 antiserum is a rapid, sensitive, and specific method for establishing an etiological diagnosis of tuberculosis in histologic specimens[26]. Ki67 monoclonal antibody for sarcoidosis[27], Cathepsin-K for detection of micro-granulomas in hypersensitivity pneumonitis [28], Langerin and CD1a markers in distinguishing LCH from other interstitial and inflammatory processes[29]. N-cadherin, Ki-67, PI3K and p110 marker expression in IPF[30]. are few immunomarkers identified.

Conclusion.

Immunohistochemistry represents an important complementary tool for the routine diagnosis of lung cancer and for the identification of the different histological types and prognostic factors. Targeted therapy for lung cancer has emerged as an important component for the treatment of defined groups of patients and immunohistochemistry is among the techniques that have been investigated for their usefulness in identifying patients whose tumors are likely to respond to individual agents. IHC is an invaluable tool for the pathologist in resolving the diagnostic issues in lung tumors. The awareness among the practising pulmonologists is vital because proper clinical details and discussion will direct the pathologist to choose the correct IHC.



TTF1

References

1. David J Dabbs, Diagnostic Immunohistochemistry ;Churchil livingstone: 2002;3-4
2 ManiH, Zander DS; Immunohistochemisrty, Application to the evaluation of lung and pleural neoplasms :Part1, CHEST 2012:142(5);1316-1323
3. Gomez Fernandez,Jorda M,Delqada PI,Ganjei AzarP.Cancer 2002 oct 25;96(5):269-93
4. Crow J , Slavin G , Kreel L . Pulmonary metastasis: a pathologic and radiologic study . Cancer . 1981 ; 47 (11): 2595 - 2602 .
5. Mani H,ZanderDS ; Immunohistochemisrty, Application to the evaluation of lung and pleural neoplasms :Part2,CHEST 2012:142(5);1324-1333
6. Yatabe Y , Mitsudomi T , Takahashi T . TTF-1 expression in pulmonary adenocarcinomas . Am J Surg Pathol . 2002 ; 26 (6): 767 - 773
7. Su YC, Hsu YC, Chai CY . Kaohsiung J Med Sci. 2006 Jan;22(1):14-9
8. Tot T .Eur J Cancer. 2002 Apr;38(6):758-63
9. Travis WD,Travis LB,Devesa SS.Lung cancer.Cancer 1995:75;191-202
10. Altekruse SF, Kosary CL, Krapcho M etal.SEER cancer statistics review 1975-2007.National cancer Institute; 2010.
11. Yatabe Y , Koga T , Mitsudomi T , Takahashi T . CK20 expression,CDX2 expression, K-ras mutation, and goblet cell morphology in a subset of lung adenocarcinomas . J Pathol . 2004 ; 203 (2):645 - 652 .
12. Nicholson SA, Beasley MB, Brambilla E, et al. Small cell lung carcinoma (SCLC): a clinicopathologic study of 100 cases with surgical specimens . Am J Surg Pathol . 2002 ; 26 (9): 1184 - 1197 .
13. Travis WD . Lung tumours with neuroendocrine differentiation . Eur J Cancer . 2009 ; 45 (suppl 1): 251 - 266 .
14. Hiroshima K , Iyoda A , Shida T , et al . Distinction of pulmonary large cell neuroendocrine carcinoma from small cell lung carcinoma: a morphological, immunohistochemical, and molecular analysis . Mod Pathol. 2006 Oct;19(10):1358-68.
15. Rekhtman N , Ang DC , Sima CS , Travis WD , Moreira AL .Immunohistochemical algorithm for differentiation of lung and squamous cell carcinoma based on large series of whole-tissue sections with validation in small specimens. Mod Pathol. 2011 Oct; 24(10):1348-59.
16. Ye J , Findeis-Hosey JJ , Yang Q , et al . Combination of napsin A and TTF-1 immunohistochemistry helps in differentiating primary lung adenocarcinoma from metastatic carcinoma in the lung . Appl Immunohistochem Mol Morphol . 2011 ;19 (4): 313 - 317 .
17. Anani W , Bruggeman R , Zander DS . b -catenin expression in benign and malignant pleural disorders . Int J Clin Exp Pathol .2011 ; 4 (8): 742 - 747 .
18. Tan D , Zander DS . Immunohistochemistry for assessment of pulmonary and pleural neoplasms: a review and update .Int J Clin Exp Pathol . 2008 ; 1 (1): 19 - 31
19. Marchevsky AM . Application of immunohisto chemistry to the diagnosis of malignant mesothelioma . Arch Pathol LabMed . 2008 ; 132 (3): 397 - 401
20. Hammar SP . Lung and pleural neoplasms. In: Dabbs DJ, ed. Diagnostic Immunohistochemistry . New York: Churchill Livingstone ; 2002 : 267 - 312 .
21. Vanderijn M,Lombard CM,rouse RV.Expression of CD34 by solitary fibrous tumors of pleura,mediastinum nad lung.Am J Surg Path.1994;18:814-20
22. N.F. Fishback, W.D. Travis, C.A. Moran, D.G. Guinee Jr., W.F. McCarthy, C.A. Koss. Pleomorphic (spindle/giant cell) carcinoma of the lung: a clinicopathologic correlation of 78 cases.Cancer, 73 (1994), pp. 2936-2945
23. Wick MR , Ritter JH , Humphrey PA . Sarcomatoid carcinomas of the lung: a clinicopathologic review . Am J Clin Pathol .1997 ; 108 (1): 40 - 53
24. Sterman DH . Point: should epidermal growth factor receptor mutations be routinely tested for in patients with lung cancer?Yes. Chest . 2013 ; 143 (3): 597 - 600
25. Pirker R , Pereira JR , von Pawel J , et al . EGFR expression as a predictor of survival for first-line chemotherapy plus cetuximab in patients with advanced non-small-cell lung cancer:analysis of data from the phase 3 FLEX study . Lancet Oncol .2012 ; 13 (1): 33 - 42 .

26. Mustafa T, Wiker HG, Mfinanga SG, Mørkve O, Sviland L ;Immunohistochemistry using a Mycobacterium tuberculosis complex specific antibody for improved diagnosis of tuberculous lymphadenitis, Mod Pathol. 2006; Dec;19(12):1606-14.

27. Marco Chilosi, Fabio Menestrina, Paola Capelli, Licia Montagna,etal .Immunohistochemical Analysis of Sarcoid Granulomas Evaluation of Ki67+ and Interleukin-1+ Cells; Am J Pathol. 1988 May; 131(2): 191-198

28. Reghellin D, Poletti V, Tomassett S, Dubini A etal .Cathepsin-K is a sensitive immunohistochemical marker for detection of micro-granulomas in hypersensitivity pneumonitis. Sarcoidosis Vasc Diffuse Lung Dis. 2010 Jul;27(1):57-63

29. Sholl LM, Hornick JL, Pinkus JL, Pinkus GS, Padera RF .Immunohistochemical analysis of langerin in langerhans cell histiocytosis and pulmonary inflammatory and infectious diseases; Am J Surg Pathol. 2007; Jun;31(6):947-52

30. Lomas NJ, Watts KL, Akram KM, Forsyth NR, Spiteri MA; Idiopathic pulmonary fibrosis: immunohistochemical analysis provides fresh insights into lung tissue remodelling with implications for novel prognostic markers. Int J Clin Exp Pathol. 2012;5(1):58-71