Original Research Article

Analysis of histopathological patterns of lung and pleural biopsy in correlation with immunohistochemistry

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Abstract

Introduction: Lungs are the most exposed organs to different risk factors like pollution, smoke, infections, tuberculosis and allergens. Lungs are covered by parietal and visceral layers of pleura within which pleural fluid is present. Aim of the study was to evaluate various histopathological patterns of lung and pleural biopsy in correlation with age, sex and immunohistochemistry examination findings.**Material and methods:** This is a retrospective study of three year three months done at Pathology Department, S.S.G. Hospital and Medical College, Baroda from October 2016 to December 2019. In present study, total 169 cases were received for histopathological examination, out of which 151 cases were of lung biopsy and 18 cases were of pleural biopsy. Immunohistochemical examination was done as and when required.**Results**:Lung biopsy of 151 cases were examined. Out of which, 88 cases (58.3%) were neoplastic, 54(35.8%) cases were non-neoplastic and 9 cases(5.9%) were inconclusive. The commonest malignancy was squamous cell carcinoma. Commonest non-neoplastic lesion was interstitial inflammation (6.6%). Malignancy was seen more common than inflammatory conditions in patients presented withlung masses in our institute. While out of 18 cases of pleural biopsy, 6 cases(33.3%) were neoplastic and 12 cases (66.7%) were non-neoplastic. Adenocarcinoma was the most common neoplastic lesion while tuberculosis was the most common non-neoplastic pleural lesion.**Conclusion:**Histopathological examination plays an important role in making a correct and accurate diagnosis of various lesions of lung and pleura. Although histopathological examination is gold standard, immunohistochemistry can enhance the accuracy of such diagnosis.

Keywords: Lung biopsy, pleural biopsy, histopathological examination.

Introduction

Lung cancer is the most commonly diagnosed and fatal cancer annually since 1985 in the world. Worldwide,there are 1.61 million new cases of lung cancer per year, with 1.38 million deaths, making lung cancer the leading cause (28%) of cancer-related mortality[1]. In India, approximately 63,000 new cases of lung cancer are reported each year[2].Most cases of squamous cell carcinoma start in the centre of the lungs. These tumors may cause some symptoms such as coughing up blood at an earlier stage than tumors on the edges of the lungs such as adenocarcinomas[3]. Despite the modest improvements in treatments during the last few decades, the prognosis of lung cancer is still poor. Pleural diseases involve the parietal and visceral pleura and may be of infectious, inflammatory, or malignant origin, often resulting in pleural effusions. Primary pleural malignancies tend to originate from the parietal pleura and spread to the visceral pleura, while metastatic disease (i.e. bronchogenic carcinoma) starts on the visceral pleura and spreads to the parietal pleura [4]. Pleural biopsy have higher diagnostic yield and provide better diagnostic sensitivity. In addition, the use of immunohistochemistry provides increased diagnostic

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accuracy[5]. Biopsy not only distinguishes between benign and malignant lesions but also helps in typing of tumor, so initiation of specific therapy like chemotherapy or surgery is possible without unnecessary delay.

Material and Methods

This retrospective study was done for the period of three years and three months at Department of Pathology, S.S.G.Hospital and Medical College,Baroda. Here 169 cases of lung and pleural biopsy specimen received in formalin and processed in Yorko automated tissue processor. The biopsies were subjected to histopathological examination (H & E stain) and IHC panel of stains Pankeratin, CK 5/6, P63,TTF-1, Napsin, CK-7, CK-20, Synaptophysin, Chromogranin, CD 45, CD 20, CD 5, Ki 67, CD 99, BCL2, Calretinin and WT1 wereused for further subtyping whenever required. Detailed case history and clinical examination data were studied in correlation with age, gender and histopathological morphology.

Results

In present study, 151 cases of lung biopsy and 18 cases of pleural biopsy were received.Among 151 cases of lung biopsy, 112 cases (73.6%) were males while 39 cases (26.4%) were females with male to female ratio of 2.8:1. Age ranged from 13-83 years with majority of cases were in fifth and sixth decade.(Table 1). Mean age was 57.4 year.Transthoracic and bronchoscopy guided biopsy were performed where 88 cases were neoplastic (58.3%), 63 cases were non-neoplastic(35.8%), 9 cases(5.9%) were inconclusive. There were 26 cases that did not show malignancy in suspected clinical and radiological conditions. The most common malignancy of lung was squamous cell carcinoma i.e. 29 cases (19%) (Figure 1 and Figure 2) and the second most common malignancy was Adenocarcinoma 27 cases (17.7%)(Figure 3). Both malignancy were predominant in male patients. Nine cases of small cell carcinoma were also more common in male patients(Figure 4),which were confirmed immunohistochemically(Figure5).

Among non neoplastic lung lesions, interstitial inflammation was the commonest lesion consisting of 10 cases (6.6%). In present study, immune-histochemistry was performed in sixty nine lung biopsy and six pleural biopsy. Among 18 cases of pleural biopsy, the age range of the patients were 30-75 years with a mean age of 51.4 year. 11 cases (61.1%) were males and 7 cases(38.9%) were females. Tuberculosis was the most common non neoplastic lesion (Figure 6) followed by chronic non specific pleuritis among nonneoplastic cases. Majority (33.3%) of malignancy were in the age group of 50-70 years. Adenocarcinoma was found to be the commonest malignant neoplasm in the pleurae present in all three females followed by Non small cell lung carcinoma were all encountered in male patients. The pleural malignancy was extension of underlying lung malignancy which was confirmed by immunohistochemical examination.

Statistical Analysis: Data analysis was done by Microsoft Excel worksheet. Descriptive statistics evaluated number of cases for percentage of different types of lung and pleural lesions and distributed them by age and sex.

Table 1: Distribution of lung and pleural lesions as per different age groups

Age group	Lung lesions	Pleural lesions
1 to 10 year	0	-
11 to 20 year	1	-
21to 30 year	8	2
31 to 40 year	9	4
41 to 50 year	24	2
51 to 60 year	45	5
61 to 70 year	44	4
71 to 80 year	19	1
81 to 90 year	1	-

Table 2 : Distribution of various lesions of lung and pleural biopsy

	Lung lesions				**Pleural lesions**			
	Cases	**Percentage**	**Male**	**Female**	**Number of cases**	**Percentage**	**Male**	**Female**
Neoplastic	**88**	**58.3%**	**63**	**25**	**6**	**(33.3%)**	**2**	**4**
Adenocarcinoma	27	17.7%	17	10	3	16.6%	**0**	**3**
squamous cell carcinoma	29	19%	23	6	-	-	-	-
Non small cell carcinoma	14	9.2%	11	3	2	1.3%	**2**	**0**
Non small cell carcinoma, SCC	3	1.9%	3	0	-	-	-	-
Small cell carcinoma	9	5.9%	6	3	-	-	-	-
Lymphoblatic lymphoma	2	1.3%	1	1	-	-	-	-
Adenosquamous carcinoma	1	0.65%	0	1	-	-	-	-
Metastatic	1(MGCT*)	0.65%	1	0	1(Breast)	6.6%	**0**	**1**
Carcinoid tumor	1	0.65%	0	1	-	-	-	-
Synovial sarcoma	1	0.65%	1	0	-	-	-	-
Non-neoplastic	**54**	**35.8%**	**41**	**13**	**12**	**66.7%**	**9**	**3**
Tuberculous inflammation	3	1.9%	2	1	3	16.6%	2	1
granulomatous inflammation	2	1.3%	2	0	-	-	-	-
Pneumonia	2	1.3%	1	1	-	-	-	-
Interstitial Inflammation	10	6.6%%	7	3	2	1.3%	2	0
Fibrosis with inflammation	3	1.9%	2	1	-	-	-	-
Fibrous tissue only	6	3.9%	6	0	-	-	-	-
No evidence of malignancy	26	17.1%	20	6	**7**	38.8%	5	2
No evidence of Tb	2	1.3%	1	1	-	-	-	-
Inconclusive	**9**	**5.9%**	**8**	**1**	-	-	-	-
Total	**151**		112	39	**18**		**11**	**7**
Total lung and pleural biopsy					**169**			

*MGCT-Mixed germ cell tumor

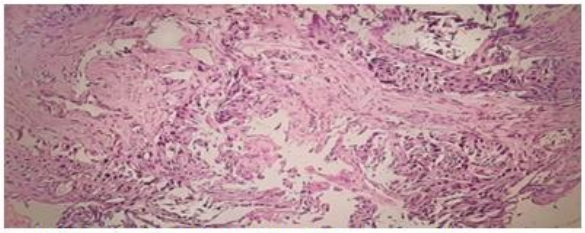

Fig 1: Infiltration of malignant squamous epithelial cells in desmoplastic stroma diagnosed as squamous cell carcinoma

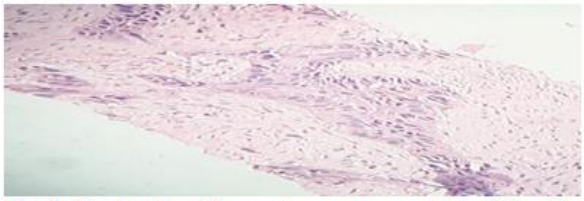

Fig 2: Nests of malignant squamous epithelial cells having hyperchromatic nuclei and moderate amount of cytoplasm

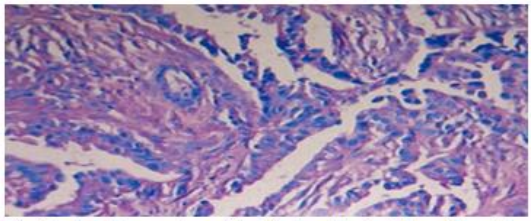

Fig 3: Malignant epithelial cells infiltrating the stroma. There is architectural complexity and focally glands formation. Inset figure shows positivity for TTF 1 confirmed diagnosis of adenocarcinoma

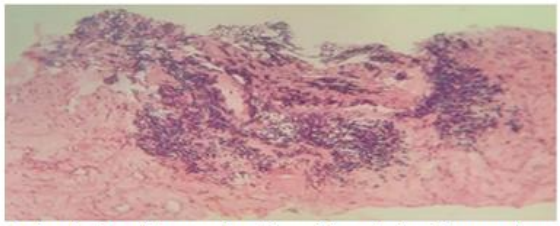

Fig 4: Small round cells with minimal cytoplasm having finely dispersed nuclear chromatin and indistinct nucleoli showing characteristic crushing artifact diagnosed as Small cell carcinoma

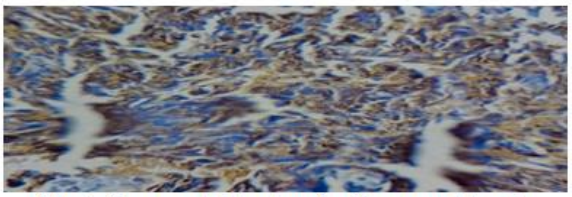

Fig 5: Immunohistochemically,synaptohysin positivity confirmed small cell carcinoma

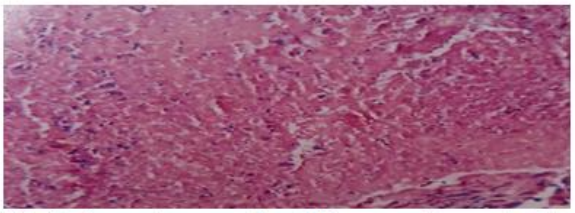

Fig 6: Amorphous eosinophilic granular material surrounded by epithelioid cells suggestive of caseous necrosis and diagnosed as tuberculosis

Discussion

Lung and pleural biopsy is widely recognized as a valuable tool for the diagnosis and management of various pathological lesions.Thus tissue sampling is indicated when the diagnosis cannot be obtained by the non-invasive techniques and the diagnosis will modify the stage of the disease or influence the therapeutic strategy. Radiographic diagnosis can be useful, but it cannot accurately predicthistology or whether a lesion is benign or malignant. Lung biopsy can be performed by bronchoscopy guided biopsy, CT guided biopsy or excisional biopsy.Pretreatment biopsy of the primary tumor is essential for most patients presenting with lung masses. A CT-guided percutaneous needle biopsy of the lung is commonly used as an outpatient diagnostic procedure and is relatively safe, sensitive and accurate method of diagnosing benign and malignant lesions as well as suitable for obtaining tissue samples of sufficient quantity and quality for allowing molecular analysis of biomarkers. Image-guided approaches also allow biopsy from areas of the tumour feltmost likely to harbour viable tumour (i.e., avoiding centrally necrotic areas) and representative of whole tumour. Trucut

biopsy is very helpful in early diagnosis and less invasive as compared to excisional biopsy. A large field of research is going on for improving outcome in lung cancer.Lung cancer is the leading cause of cancer-related death worldwide and being increasingly detected in India due to increased awareness about lung carcinoma and improved diagnostic techniques. In the management of lung tumours, accurate diagnosis, using a combination of clinical, radiographic and histological data is critical inoptimizing outcome. In malignant tumour, first approach should be to classify tumour as small cell carcinoma(SCLC) or non- small cell carcinoma (NSCLC). The histological subtyping of non-small cell lung carcinoma in squamous cell carcinoma and adenocarcinoma is also important now because the therapeutic approaches,outcome, prognosis and survival differs greatly. Inadenocarcinoma, several targetable molecular alterations as EGFR mutation, KRAS mutation and EML4-ALK rearrangements are identified. Targeted therapies hold a considerable promise in the treatment of patients with lung cancer.Age range in the present study was 13 to 83 years with the peak in fifth and sixth decade, which was correlated with study by Shah S., Saha A., and Mondal SK et al.[6,7,8]. The preponderance of male patients (69%) with male to female ratio of 2.8:1 in the present study, was comparable to Tan KB et al. i.e. 71.1% male patients [9]. In the present study,88 cases (58.2%) were neoplastic, 54 cases (35.7%) were non-neoplastic and 9 cases (5.9%) were inconclusive.Regina Girones et al. studied clinical, histopathological and epidemiological characteristics of lung cancer over period of 10 years. Among 701 patients91.4% were male with mean age 67.6 year. Squamous cell carcinoma prevailed in men (45.5%) and adenocarcinoma in women (60.3%) [10]. Manoj Kumar Agrawal et al. studied clinical and pathological profile of patients with lung cancer in a tertiary care centre,Bareilly. In this retrospective study, 93 confirmed cases of lung cancer were included. The most common histopathological type was squamous cell carcinomain 69 cases(74.19%) followed by adenocarcinoma in 16 cases(17.20%) and small cell carcinoma in 7 cases(7.52%)[11].The study concluded that squamous cell carcinoma was the most frequent histopathological lesion which also supports results of present study. Histopathologically, Carcinoma arising from squamous epithelial cells, morphologically characterized by proliferation of atypical, often pleomorphic squamous cells; graded as well, moderately, or poorly differentiated; well differentiated carcinomas are usually associated with keratin production and presence of intercellular bridges between adjacent cells; subtypes include basaloid, clear cell type, papillary, small cell nonkeratinizing. Non small cell lung Adenocarcinoma of lung shows histopathological morphology of malignant cells with glandular differentiation, mucin production, or pneumocyte marker expression. It shows lepidic, acinar, papillary, micropapillary and solid pattern.Non-small cell lung cancers comprise 75% of all lung cancers and consist of three major histologic types: squamous cell carcinoma, adenocarcinoma, and large cell carcinoma.

Immunohistochemistry is a widely available technique that is less challenging and can provide clinically meaningful results quickly and cost-efficiently in comparison with other techniques. In addition, immunohistochemistry allows for the evaluation of cellular localization of proteins in the context of tumor structure; thus, a greater range of information is provided. In an era of precision medicine, pathologists are required to classify lung cancer into specific subtypes and assess biomarkers relevant to molecular-targeted therapies[12]. Differential diagnosis between adenocarcinoma and squamous cell carcinoma is beneficial because targetable driver genetic alterations are mostly identified in adenocarcinoma, and inappropriate drugs need to be avoided for patients with Squamous cell carcinoma[13].

In the current 2015 WHO classification, a solid carcinoma without glandular structures or mucin production, but with immunohistochemical positivity for “adenocarcinoma markers”, i.e., TTF-1 (NKX2-1) and/or Napsin A, can be diagnosed as an adenocarcinoma. Similarly, a solid carcinoma without keratinization or intercellular bridges, but with immunohistochemical positivity for “Squamous cell carcinoma markers”, such as p40, CK5/6, and p63, can be diagnosed as squamous cell carcinoma. [14].

The neuroendocrine tumors comprise three subtypes: Small cell lung carcinoma (SCLC), large cell neuroendocrine carcinoma (LCNEC), and carcinoid tumor (typical/atypical) as per classification. Although high-grade neuroendocrine tumors (HGNETs), comprising SCLCs and LCNECs, belong to the same category as carcinoid tumors, their clinical characteristics are substantially different. HGNET is an aggressive and deadly subtype characterized by patients with a history of heavy smoking. In contrast, carcinoid tumors usually follow a benign clinical course and frequently occur in patients without a history of smoking. Despite their different clinical characteristics,

these tumors share the features of neuroendocrine differentiation. As the definition of LCNEC in the WHO classification, the diagnosis of LCNEC requires not only neuroendocrine morphology but also immunohistochemical expression of at least one of the three neuroendocrine markers, i.e., CHGA (chromogranin A), SYP (synaptophysin), or NCAM1 (CD56)[15]. The pleural space is a potential space between the visceral and parietal layers of the pleurae. The pleural space normally contains 0.1-0.2 ml/kg body weight of fluid. Pleural effusion is an abnormal accumulation of fluid in the pleural cavity. Pleural effusion remains the most common manifestation of pleural pathology[16]. Although a variety of clinical conditions like heart failure, malignancy, pneumonia, tuberculosis may be the cause of a pleural effusion. The possibility of a malignant involvement of pleural cavity should always be considered in difficult to diagnose cases. Indications for percutaneous pleural biopsy include undiagnosed pleural effusions and pleural thickening or pleural masses[17]. Since percutaneous access of the pleural space is relatively simple, techniques like pleural biopsy have become very popular. Pleural biopsy were considered as tuberculous inflammation when biopsy revealed typical epithelioid cell granuloma formation. In pleura, primary tumor may be pulmonary or extrapulmonary. The common pulmonary tumors infiltrating in to the pleura are squamous cell carcinoma, adenocarcinoma and small cell carcinoma. Adenocarcinoma is the most common malignancy encountered in the pleural biopsy because adenocarcinoma of lung mainly occurs mainly in the periphery of lung so local spread of underlying lung tumoris common in pleura. Pandit S, Gaur DS and Bhattacharya et al also documented adenocarcinoma as the most common neoplastic lesion in pleura which was direct extension from underlying lung parenchyma, the findings are also consistent with present study[17,18,19].Pleural biopsy is a useful and minimally invasive procedure. It is not only cost effective but a reasonably safe procedure, which shows reasonably good sensitivity and specificity in diagnosing primary, as well as metastatic pleural malignancies [20].

Conclusion

Biopsy of lungs and pleurae play an important role for correct and accurate diagnosis and subtyping of various pathological lesions. Immunohistochemistry enhance the accuracy of such analysis and plays a critical role as diagnosing tool. It is strongly indicated when non invasive techniques can not conclude or confirm the diagnosis and to finalise therapeutic strategy.

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