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HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL EVALUATION OF NON-SMALL CELL LUNG CARCINOMA IN SMALL LIMITED LUNG BIOPSY SPECIMENS WITH EMPHASIS ON PREDICTIVE BIOMARKERS

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ABSTRACT

Background: Non-small cell lung carcinoma (NSCLC) constitutes the majority of lung cancers and accurate classification is particularly challenging in small, limited lung biopsy specimens. Histopathological examination supported by immunohistochemistry is essential for subclassification, while assessment of predictive biomarkers provides additional information relevant to therapeutic planning. **Objectives:** To evaluate the histopathological and immunohistochemical features of NSCLC in small limited lung biopsy specimens and to assess the expression and distribution of TTF-1, Napsin A, p40, ALK, ROS1 and PD-L1, with special emphasis on predictive biomarkers.

Materials and Methods: This institution-based observational cross-sectional study was conducted in the Department of Pathology, North Bengal Medical College and Hospital, Darjeeling, from July 2023 to December 2024. A total of 30 cases of NSCLC diagnosed in small limited lung biopsy specimens were included. Cases with malignancies other than NSCLC, benign or non-neoplastic lesions, inadequate samples, patient refusal, previously treated NSCLC and secondary lung carcinoma were excluded. Histopathological examination was performed using hematoxylin and eosin staining. Immunohistochemical evaluation included TTF-1, Napsin A and p40 for tumour characterization, while ALK, ROS1 and PD-L1 were assessed as predictive biomarkers. Data were compiled in a master chart and analyzed using Jamovi version 2.5.1.

Results: The mean age of the study population was 63.0±10.4 years, with a median age of 62 years. Males constituted 80.0% of cases and females 20.0%. Smoking was reported in 63.3% of patients, while 36.7% were non-smokers. The most common tumour location was the right upper lung (36.7%), and 73.3% of tumours were peripheral. Histopathologically, NSCLC-NOS accounted for 46.7%, adenocarcinoma for 43.3%, and squamous cell carcinoma for 10.0%. TTF-1 was positive in 96.7%, Napsin A in 63.3%, and p40 in 43.3% of cases. ALK was negative in all cases, whereas ROS1 was positive in 56.7%. PD-L1 expression was positive in 73.3% of cases.

Conclusion: Histopathological evaluation combined with focused immunohistochemistry is important for characterization of NSCLC in small limited lung biopsy specimens. The observed expression patterns of TTF-1, Napsin A, p40 and predictive biomarkers including ALK, ROS1 and PD-L1 demonstrate the value of integrated pathological assessment for accurate tumour classification and biomarker characterization.

Keywords: Non-small cell lung carcinoma; Small lung biopsy; Histopathology; Immunohistochemistry; TTF-1; Napsin A; ALK; ROS1; PD-L1.

INTRODUCTION

Lung cancer remains one of the leading causes of cancer-related morbidity and mortality worldwide and contributes substantially to the global cancer burden. The high mortality associated with lung cancer is largely related to late-stage diagnosis and limited curative treatment options. The epidemiology of lung cancer varies between populations, with differences related to environmental exposure, genetic susceptibility and lifestyle factors. In India, distinct epidemiological patterns have been reported between smokers and non-smokers, emphasizing the heterogeneous nature of lung cancer.[1-3]

Lung cancer is broadly classified into small cell lung carcinoma and non-small cell lung carcinoma (NSCLC), with NSCLC accounting for approximately 85% of lung cancer cases. NSCLC comprises major histological subtypes including adenocarcinoma, squamous cell carcinoma and large cell carcinoma. The classification of lung tumors has progressively evolved from being predominantly morphology-based to incorporating immunohistochemical and molecular characteristics. This evolution has been important for accurate tumor classification and for the development of personalized treatment strategies.[5]

The development of lung cancer is multifactorial and is influenced by genetic, environmental and lifestyle factors. Smoking remains the most well-established risk factor for lung cancer and accounts for a substantial proportion of cases. However, an increasing proportion of lung cancers occurs among non-smokers. Air pollution, occupational exposure to carcinogens and other environmental factors have been implicated in lung carcinogenesis, while genetic predisposition and molecular alterations also contribute to tumor development.[2,3] Molecular alterations such as epidermal growth factor receptor mutations and anaplastic lymphoma kinase rearrangements have important implications in lung carcinogenesis and treatment.[6]

Histopathological examination remains fundamental to the diagnosis and classification of NSCLC. In small limited lung biopsy specimens, the amount of available tissue may restrict morphological assessment and the extent of ancillary investigations that can be performed. Hematoxylin and eosin staining provides the initial morphological assessment, while immunohistochemistry plays an important role in differentiating adenocarcinoma from squamous cell carcinoma, particularly in poorly differentiated tumors. Thyroid transcription factor-1 and Napsin A are used in the evaluation of adenocarcinomatous differentiation, whereas p40, p63 and cytokeratin 5/6 are associated with squamous differentiation.[11,12]

The distinction between the major histological subtypes of NSCLC is clinically important. Adenocarcinoma is characterized by glandular differentiation and is frequently encountered in the peripheral lung, whereas squamous cell carcinoma is strongly associated with smoking and may arise centrally in the bronchial epithelium. In cases where morphological assessment is limited or differentiation is poor, immunohistochemical markers provide important assistance in establishing the appropriate histological classification.[5,11,12]

The classification of lung tumors has undergone substantial refinement with successive World Health Organization classifications. The 2015 World Health Organization classification incorporated molecular pathology and emphasized the integration of morphological, immunohistochemical and molecular characteristics in the evaluation of lung tumors. Large cell carcinoma, in particular, represents a diagnosis of exclusion when glandular or squamous differentiation cannot be established, and molecular analysis has demonstrated that some tumors previously categorized as large cell carcinoma can be reclassified into more specific histological categories.[5,13]

Advances in molecular oncology have resulted in the increasing importance of predictive biomarkers in NSCLC. Epidermal growth factor receptor mutations, predominantly described in adenocarcinoma, have predictive significance for response to tyrosine kinase inhibitors. Anaplastic lymphoma kinase and ROS1 rearrangements occur in a subset of lung adenocarcinomas and identify patients who may be candidates for targeted therapies. Anaplastic lymphoma kinase-rearranged lung cancer has been described particularly among younger and non-smoking patients.[6]

Programmed death-ligand 1 expression has also emerged as an important predictive biomarker in NSCLC. Its expression is relevant to the assessment of response to immune checkpoint inhibitor therapy, including pembrolizumab. Thus, evaluation of predictive biomarkers has become an important component of the pathological work-up of NSCLC and has contributed to the transition from a predominantly histology-based treatment approach towards personalized treatment.[4,6]

Despite these advances, the evaluation of NSCLC in small limited lung biopsy specimens remains challenging. Limited tissue availability creates the need to balance accurate histological classification with preservation of tissue for immunohistochemical and predictive biomarker assessment. Tumor heterogeneity and the increasing requirement for molecular characterization further emphasize the importance of an appropriate and focused diagnostic approach. The integration of histopathology and immunohistochemistry is therefore particularly relevant in cases where the initial biopsy provides limited material for diagnosis and subsequent therapeutic decision-making.[5,13]

The present study was undertaken to perform a histopathological and immunohistochemical analysis of non-small cell carcinoma in small limited lung biopsy specimens, with special emphasis on predictive biomarkers. The study evaluated the

morphological features of NSCLC on hematoxylin and eosin staining, assessed the utility of TTF-1, Napsin A and p40 for histological classification, and evaluated ALK, ROS1 and PD-L1 as predictive biomarkers by immunohistochemistry. The study also aimed to determine the frequency and distribution of these biomarkers and assess their relationship with relevant clinical characteristics including age, gender, smoking history and clinicoradiological tumor characteristics.

MATERIALS AND METHODS

This observational cross-sectional study was conducted in the Department of Pathology, North Bengal Medical College and Hospital (NBMCH), Darjeeling, over a period from 1 July 2023 to 31 December 2024. The study population comprised all clinicoradiologically suspected cases of lung neoplasm who were referred to the Department of Pathology for CT-guided trucut biopsy during the study period. Based on the data collected from the concerned department, a total sample size of 30 cases was included for the specified study period. The study was undertaken after obtaining approval from the Institutional Ethical Committee of North Bengal Medical College and Hospital. Written informed consent was obtained from all patients before participation in the study.

Cases were selected according to predefined eligibility criteria. All cases diagnosed as non-small cell lung carcinoma were eligible for inclusion. Patients with malignancies other than non-small cell lung carcinoma, benign lung tumours, non-neoplastic lung lesions, inadequate samples or refusal to participate were excluded. Previously treated cases of non-small cell lung carcinoma and cases of secondary lung carcinoma were also excluded. Confidentiality of participant information was maintained throughout the study. Patient identifiers, including names, were not recorded on the study proforma, and a case record form was completed by the investigator for each participant. Routine investigations required for conducting the study were performed in all enrolled patients.

The variables recorded for each participant included demographic, clinical, radiological, histopathological, immunohistochemical and molecular biomarker parameters. Demographic variables comprised age, sex, smoking status and pack-years of smoking. Clinical variables included the available radiological findings. Histopathological variables included the type of tissue sample and tumour classification. Tissue sample types considered in the study included core biopsy, bronchial biopsy, CT-guided biopsy and other specified samples. Histopathological classification included adenocarcinoma, squamous cell carcinoma, large cell carcinoma and non-small cell carcinoma, not otherwise specified. These variables were recorded to characterize the clinicopathological spectrum of non-small cell lung carcinoma in the study population.

Histopathological examination was performed using routine tissue processing, microtomy, hematoxylin and eosin staining and light microscopy. The laboratory was equipped with an automated tissue processor, microtome and standard hematoxylin and eosin staining setup with the required equipment and reagents. Immunohistochemical evaluation was performed using an automated immunohistochemistry stainer and light microscope. Thyroid transcription factor-1 and Napsin A were used for morphological confirmation of adenocarcinoma, while p40 was used for confirmation of squamous cell carcinoma. The immunohistochemical variables were recorded as positive or negative according to the expression identified in the biopsy specimens. The study also evaluated predictive biomarkers, including anaplastic lymphoma kinase, ROS1 and programmed death ligand-1. ALK and ROS1 expression were assessed as positive or negative by fluorescence in situ hybridization or immunohistochemistry, as applicable. Programmed death ligand-1 expression was assessed using the tumour proportion score, categorized as less than 1%, 1–49% and 50% or greater.

The study tools therefore covered both routine histopathology and immunohistochemistry. The histopathology laboratory facilities included an automated tissue processor, microtome, hematoxylin and eosin staining equipment and reagents, and light microscope. For immunohistochemistry, an automated stainer, the selected antibodies and light microscopy were used. The biomarker panel was selected to support morphological confirmation of the major non-small cell lung carcinoma categories and to document the status of clinically relevant predictive biomarkers within the available biopsy material. Findings obtained from these investigations were recorded systematically in the case record form and incorporated into the master chart for analysis.

The primary outcome measures comprised histopathological diagnosis, immunohistochemical biomarker expression patterns and predictive biomarker expression relevant to targeted therapy. Histopathological assessment included the proportion of cases classified into adenocarcinoma, squamous cell carcinoma, large cell carcinoma or non-small cell carcinoma, not otherwise specified, with concordance between biopsy and resection specimens considered where applicable. Immunohistochemical outcomes included the frequency and intensity of expression of thyroid transcription factor-1, Napsin A and p40, together with the concordance between immunohistochemistry-based subtyping and histopathology-based subtyping. Predictive biomarker outcomes included the expression of ALK, ROS1 and programmed death ligand-1 and their relationship with histological subtypes. The study also included assessment of the prognostic

implications of biomarker expression as a secondary outcome, defined in relation to disease aggressiveness.

Data collected during the study were entered into a master chart and statistically analyzed using Jamovi version 2.5.1. The analysis was primarily directed towards establishing the prevalence and distribution of the observed histopathological and biomarker patterns and evaluating relevant clinical patterns. Demographic, clinical, radiological, histopathological and immunohistochemical findings were summarized from the collected data, and the results were subsequently compared with observations reported in other studies. All study procedures were performed after ethical clearance from the Institutional Ethical Committee of North Bengal Medical College and Hospital, with appropriate measures taken to maintain participant confidentiality and informed consent.

RESULTS

A total of 30 cases of non-small cell lung carcinoma (NSCLC) diagnosed on small limited lung biopsy specimens were included in the study. The demographic, clinical, radiological, histopathological and immunohistochemical findings were analysed descriptively. The study population had a mean age of 63.0 ± 10.4 years, with a median age of 62 years and an age range of 39–86 years.

Table 1: Demographic and smoking profile of the study participants (N = 30)

Variable	Category	Frequency	%
Age group (years) Mean age: 63.0 years; SD: 10.4 years; Median: 62.0 years; Range: 39–86 years.	30–39	1	3.3
	40–49	1	3.3
	50–59	8	26.7
	60–69	14	46.7
	70–79	3	10.0
	80–89	3	10.0
Sex	Male	24	80.0
	Female	6	20.0
Smoking status	Smoker	19	63.3
	Non-smoker	11	36.7
Type of smoking	Cigarette	12	40.0
	Bidi	7	23.3
	Not applicable	11	36.7
Duration of smoking	<20 years	10	33.3
	≥20 years	9	30.0
	Not applicable	11	36.7
Habit of smoking	Everyday smoker	6	20.0
	Someday smoker	6	20.0
	Former smoker	7	23.3
	Not applicable	11	36.7

The majority of participants were in the 60–69-year age group (46.7%), followed by those aged 50–59 years (26.7%). Overall, 80.0% of patients were male and 20.0% were female. Smoking was reported in 63.3% of cases, with cigarette smoking being the commonest type (40.0%), followed by bidi smoking (23.3%). A smoking duration of ≥20 years was present in 30.0% of the total study population.

Table 2: Clinical and radiological profile of the study participants (N = 30)

Variable	Category	n	%
Comorbidities	Nil	1	3.3
	Hypertension	12	40.0
	Obesity/overweight + hypertension	4	13.3
	Hypertension + diabetes mellitus	7	23.3
	Obesity/overweight + hypertension + diabetes mellitus	6	20.0
Tumor location	Right upper lung	11	36.7
	Right lower lung	7	23.3
	Left lower lung	7	23.3
	Left upper lung	2	6.7
	Right hilar region	2	6.7
	Left hilar mass	1	3.3
Site of mass	Peripheral	22	73.3
	Central	8	26.7

Hypertension was the most frequently recorded comorbidity, occurring in 40.0% of patients, followed by hypertension with diabetes mellitus in 23.3% and hypertension with overweight/obesity in 13.3%. Only one patient (3.3%) had no documented comorbidity.

Radiologically, the right upper lung was the most frequent tumor location (36.7%), followed by the right lower lung and left lower lung, each accounting for 23.3% of cases. Most masses were peripheral (73.3%), whereas 26.7% were centrally located.

Table 3: Histopathological profile of non-small cell lung carcinoma (N = 30)

Histopathological parameter	Category	n	%
Histopathological diagnosis	Adenocarcinoma	13	43.3
	Squamous cell carcinoma	3	10.0
	NSCLC, NOS	14	46.7
Adenocarcinoma pattern	Glandular	8	26.7
	Lepidic	3	10.0
	Papillary	2	6.7
	Not applicable	17	56.7

On histopathological examination, NSCLC-NOS constituted the largest category, accounting for 46.7% of cases, followed by adenocarcinoma (43.3%) and squamous cell carcinoma (10.0%). The relatively high proportion of NSCLC-NOS reflects the diagnostic limitation imposed by small biopsy specimens, in which complete morphological assessment may be difficult.

Among the 13 cases classified as adenocarcinoma, the glandular pattern was most frequent (8/30; 26.7%), followed by lepidic (3/30; 10.0%) and papillary patterns (2/30; 6.7%). The remaining 17 cases were non-applicable because they were not classified as adenocarcinoma.

Table 4: Immunohistochemical expression of diagnostic markers in NSCLC (N = 30)

Immunohistochemical marker	Expression	n	%
TTF-1	Positive	29	96.7
	Negative	1	3.3
Napsin A	Positive	19	63.3
	Negative	11	36.7
p40	Positive	13	43.3
	Negative	17	56.7

TTF-1 demonstrated the highest positivity among the diagnostic immunohistochemical markers, with 29 of 30 cases (96.7%) showing positive expression. Only one case (3.3%) was negative. Napsin A was positive in 19 cases (63.3%) and negative in 11 cases (36.7%). p40 was positive in 13 cases (43.3%) and negative in 17 cases (56.7%).

The high frequency of TTF-1 expression, together with Napsin A positivity, supported the identification of pulmonary adenocarcinoma in the appropriate morphological setting. p40 expression was observed in 43.3% of cases and served as a marker of squamous differentiation. These findings demonstrate the importance of a focused immunohistochemical panel in small limited biopsy specimens, particularly in cases initially categorized as NSCLC-NOS.

Table 5: Expression of predictive biomarkers in NSCLC (N = 30)

Predictive biomarker	Result	n	%
ALK rearrangement	Negative	30	100.0
	Positive	0	0.0
ROS1 rearrangement	Positive	17	56.7
	Negative	13	43.3
PD-L1 expression	Positive	22	73.3
	Negative	8	26.7

Among the predictive biomarkers evaluated, ALK rearrangement was not detected in any of the 30 cases. In contrast, ROS1 rearrangement was reported in 17 cases (56.7%), while 13 cases (43.3%) were negative.

PD-L1 expression was positive in 22 cases (73.3%) and negative in 8 cases (26.7%). Thus, PD-L1 was the most frequently positive predictive biomarker evaluated in the study, followed by ROS1 rearrangement.

Overall, the immunohistochemical analysis demonstrated a high frequency of TTF-1 expression (96.7%), moderate Napsin A expression (63.3%) and p40 expression (43.3%). Among the predictive biomarkers, ALK rearrangement was absent, whereas ROS1 rearrangement and PD-L1 expression were reported in 56.7% and 73.3% of cases, respectively. These findings emphasize the role of immunohistochemistry in both histological classification and biomarker assessment in small limited lung biopsy specimens.

DISCUSSION

In the present study, the mean age of patients with non-small cell lung carcinoma was 63.0 years, with a median age of 62 years and a standard deviation of 10.4 years. The age distribution showed a predominance of patients in the older age groups, with the largest proportion belonging to the 60–69-year age group. This finding is consistent with the established observation that lung cancer predominantly affects older adults, reflecting the cumulative effect of tobacco exposure, environmental carcinogens and other genetic and

cellular alterations occurring over several decades. In the present cohort, the relatively advanced age at presentation may also reflect the tendency for lung malignancy to be diagnosed after clinically significant disease has developed.

A study by Du et al. (1996),[14] in China reported a comparable age profile, with lung cancer occurring predominantly among individuals in the sixth and seventh decades of life. The authors also emphasized the contribution of environmental and lifestyle-related exposures to the development of lung cancer. In the present study, the concentration of cases around the sixth and seventh decades therefore appears broadly comparable with previously reported epidemiological observations. The relatively wide age range of 39–86 years, however, also indicates that NSCLC can occur considerably earlier and should not be regarded exclusively as a disease of the elderly.

A marked male predominance was observed in the present study, with males constituting 80% of the cases and females 20%. This finding is compatible with the historically higher prevalence of tobacco consumption among men and the established association between smoking and lung cancer. However, the increasing recognition of lung cancer among women, particularly among never-smokers, indicates that factors other than active tobacco consumption may also contribute to disease development.

Wu et al. (1990),[15] in their observations from northeastern China, reported a lower male-to-female ratio than traditionally observed in many populations and highlighted the increasing occurrence of lung cancer among women. Environmental carcinogens, passive smoking and genetic susceptibility may partly account for this changing epidemiological pattern. Thus, although the present study demonstrated a clear male predominance, the presence of 20% female patients emphasizes the importance of considering lung cancer in women irrespective of their smoking history.

Smoking was an important exposure characteristic in the present cohort. Overall, 63.3% of patients were smokers, whereas 36.7% were non-smokers. The predominance of smokers supports the established relationship between tobacco exposure and lung carcinoma. The classical work of Doll and Hill,[16] demonstrated a strong association between cigarette smoking and carcinoma of the lung and provided important epidemiological evidence for the role of tobacco in lung carcinogenesis.

Nevertheless, the substantial proportion of non-smokers in the present study is noteworthy. It suggests that active smoking alone cannot account for all cases of NSCLC. Hirayama,[17] reported an increased risk of lung cancer among non-smoking women exposed to cigarette smoke from their spouses, highlighting the potential importance of passive smoking. Similarly, other epidemiological investigations have examined the association between environmental tobacco smoke and lung cancer in non-smokers. Therefore, exposure to secondhand smoke, indoor air pollution, occupational carcinogens and other environmental or genetic factors may have contributed to disease occurrence among the non-smoking patients in the present cohort. These associations should, however, be interpreted cautiously because the present study was not designed to quantify individual environmental exposures.

Histopathological examination in the present study demonstrated adenocarcinoma in 43.3% of cases, squamous cell carcinoma in 10.0%, and NSCLC-NOS in 46.7%. The relatively high proportion of adenocarcinoma is consistent with the changing histological profile of lung carcinoma observed in many populations. Adenocarcinoma has become an important histological subtype, particularly among non-smokers and women, and its recognition has gained further importance because of the availability of molecularly targeted therapies.

Gao et al,[18] reported adenocarcinoma as an important histological type among non-smokers, supporting the observation that adenocarcinoma may occur independently of heavy tobacco exposure. Conversely, older studies such as that of Minna et al.19 reported a greater contribution of squamous cell carcinoma, reflecting differences in the populations, smoking patterns and pathological classification systems used during earlier periods. The relatively high proportion of NSCLC-NOS in the present study may also be explained by the limited biopsy material available for histological assessment. Small biopsy specimens may not always contain sufficient architectural or cytological features to permit definitive subclassification, thereby increasing the requirement for immunohistochemical evaluation.

The right upper lobe was the most frequent tumour location in the present study, accounting for 36.7% of cases, followed by the right lower lobe and left lower lobe, each accounting for 23.3%. The predominance of upper-lobe involvement has been described in relation to smoking-associated lung carcinogenesis. Buchagen,[20] reported a greater frequency of upper-lobe involvement among smokers, which may be related to patterns of inhaled carcinogen deposition and regional differences in airway exposure.

The predominance of smokers in the present cohort provides a possible context for the observed distribution. However, because the present study was based on a relatively small sample of 30 patients, the observed anatomical distribution should be regarded as descriptive rather than definitive evidence of a preferential site of tumour development.

Immunohistochemistry provided important additional information for characterization of NSCLC in the present study. TTF-1 was positive in 96.7% of cases, while Napsin A was positive in 63.3%. p40 was positive in 43.3% of cases. TTF-1 and Napsin A are particularly useful markers in the evaluation of pulmonary adenocarcinoma, whereas p40 is an important marker for squamous differentiation. The high frequency of TTF-1

expression observed in the present study supports its usefulness in the characterization of pulmonary adenocarcinomatous differentiation.

The results also illustrate the complementary nature of immunohistochemical markers. No single marker should be interpreted in isolation, particularly in small biopsy specimens in which tumour architecture may be limited. The combined interpretation of morphology with TTF-1, Napsin A and p40 can therefore assist in narrowing the histological classification of NSCLC and reducing the proportion of cases that remain unclassified.

In the present study, ALK expression was negative in all cases, whereas ROS1 expression was reported as positive in 56.7% of cases. Earlier literature has demonstrated that molecular alterations such as ALK rearrangements occur in a subset of NSCLC, particularly in selected clinical and histological groups. Minna et al,[19] described the importance of genetic alterations in lung carcinogenesis, emphasizing the molecular heterogeneity of lung cancer. The absence of ALK positivity in the present cohort may reflect the limited sample size and the characteristics of the study population rather than the absence of ALK alterations in the wider population.

PD-L1 expression was positive in 73.3% of cases in the present study. This relatively high proportion indicates that assessment of PD-L1 may provide clinically relevant information when evaluating patients for immunotherapeutic approaches. However, the present analysis did not establish treatment response or survival outcomes, and therefore PD-L1 positivity should be interpreted as a biomarker finding rather than direct evidence of therapeutic benefit.

The findings of the present study emphasize the importance of integrating clinical information, radiological findings, histopathological examination and immunohistochemistry in the evaluation of NSCLC. The predominance of older male patients and smokers was consistent with the established epidemiological profile of lung cancer, while the substantial proportion of non-smokers demonstrated the importance of considering additional risk factors. The predominance of adenocarcinoma and frequent TTF-1 and Napsin A expression further emphasize the value of focused immunohistochemical evaluation in small biopsy specimens.

The assessment of ALK, ROS1 and PD-L1 additionally highlights the increasing importance of predictive biomarker testing in contemporary lung cancer diagnosis. Overall, the study supports a multidisciplinary diagnostic approach in which morphology remains the foundation, while immunohistochemical and molecular biomarker assessment provides additional information required for accurate classification and appropriate therapeutic planning. Given the relatively small sample size and single-centre design, the findings should be interpreted cautiously and validated in larger cohorts.

CONCLUSION

The present study evaluated the histopathological and immunohistochemical profile of non-small cell lung carcinoma in 30 limited lung biopsy specimens, with particular emphasis on predictive biomarkers. The patients were predominantly older males, with a mean age of 63.0 years, and 63.3% had a history of smoking. Adenocarcinoma was the most frequent defined histological subtype (43.3%), followed by squamous cell carcinoma (10.0%), while 46.7% were classified as NSCLC-NOS. TTF-1 showed positivity in 96.7% of cases and Napsin A in 63.3%, supporting their utility in histopathological characterization. p40 was positive in 43.3% of cases. ALK was negative in all cases, whereas ROS1 positivity was observed in 56.7% and PD-L1 positivity in 73.3%. These findings highlight the importance of integrating morphology with focused immunohistochemistry and predictive biomarker assessment in limited biopsy specimens. Current guidance similarly emphasizes tissue conservation and judicious IHC because small biopsy material may be required for subsequent molecular testing.

Recommendations

Histopathological examination should remain the primary diagnostic approach, supplemented by a focused immunohistochemical panel when morphology alone is insufficient. TTF-1, Napsin A and p40 should be appropriately utilized for histological characterization of NSCLC in limited biopsy specimens, while avoiding unnecessary IHC that may exhaust available tissue. Predictive biomarker assessment, including ALK, ROS1 and PD-L1, should be incorporated into the diagnostic workflow where clinically indicated, as biomarker status can influence therapeutic decision-making. Larger, multicentric studies with standardized molecular testing and clinical follow-up are recommended to validate the biomarker patterns observed in the present study. Adequate tissue preservation and multidisciplinary coordination between pathologists, radiologists and clinicians should be emphasized to optimize diagnosis and subsequent personalized management.

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