

Original Research Article

DIAGNOSTIC UTILITY OF TTF-1, NAPSIN-A & P40 IN THE SUBCLASSIFICATION OF NON SMALL CELL LUNG CARCINOMA

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ABSTRACT

Background: Lung carcinoma remains the leading cause of cancer-related deaths globally, with increasing incidence in India. Subclassification of Non-Small Cell Lung Carcinoma (NSCLC) is essential due to its therapeutic implications. However, this is challenging in poorly differentiated tumors and small biopsies. Immunohistochemistry (IHC) using markers like TTF-1, Napsin-A, and p40 enhances diagnostic accuracy. This study evaluates the combined role of these markers in reliably subclassifying NSCLC, especially in morphologically ambiguous cases. **Aim and Objectives:** 1. To study the histopathological spectrum of lung carcinoma. 2. To study the expression of IHC markers TTF-1, Napsin-A, and p40 & to evaluate their role in subclassification of NSCLC.

Materials and Methods: The study was conducted in the Department of Pathology on 50 NSCLC cases. Tissue samples were fixed in 10% buffered formalin, grossed, and stained with H&E. IHC was performed using TTF-1, Napsin-A, and p40. Final diagnosis was made after histopathological and IHC correlation.

Results: TTF-1 was positive in 25 cases of adenocarcinoma (ADC) and 7 cases of squamous cell carcinoma (SCC) cases out of total 50 NSCLC cases. Napsin-A showed positivity in 35 ADC and 1 SCC case. p40 was positive in 13/14 SCC and negative in all ADC. Sensitivities were: TTF-1 – 69.44%, Napsin-A – 97.22%, p40 – 92.86%. IHC enabled subclassification in 12 morphologically unclassifiable cases. Post-IHC, ADC cases increased to 36 and SCC to 14.

Conclusion: IHC significantly improved NSCLC subclassification. Napsin-A emerged as the most reliable marker for adenocarcinoma, aiding in diagnosis of poorly differentiated cases.

Keywords: Non-Small Cell Lung Carcinoma, Adenocarcinoma, Squamous cell carcinoma, TTF-1, Napsin-A, p40.

INTRODUCTION

Lung carcinoma ranks as the most prevalent malignancy globally and remains a leading cause of cancer-related deaths, particularly in developed nations. The World Health Organization (WHO) categorizes primary malignant lung tumors into two main types: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). NSCLC constitutes approximately 80% of all lung tumors.^[1] Adenocarcinoma (ADC) and squamous cell

carcinoma (SCC) are the two major subtypes of non small cell lung carcinoma (NSCLC), differing in their histological patterns, immunohistochemical profiles, key genetic mutations, and treatment approaches.^[2] The 2015 World Health Organization (WHO) Classification of Tumors of the Lung, Pleura, Thymus, and Heart integrated immunohistochemistry (IHC) into the classification for lung cancer and emphasized the significance of molecular characterization in 2021.^[3,4] Tissue biopsy has been deemed the gold standard of lung cancer

confirmation. Immunohistochemistry (IHC) is a widely employed technique for detecting specific proteins within biological tissues, significantly enhancing diagnostic precision, especially in subclassifying lung cancers. It has become an integral component of routine clinical evaluation, particularly useful in small biopsy samples, cytology specimens, and poorly differentiated neoplasms.^[5] Most commonly used marker combination for subtyping non-small cell carcinoma (NSCLC) is TTF-1 and p40, especially in cases which lacks glandular, squamous and neuroendocrine (NE) features on morphology.^[6] Napsin- A can distinguish between patients with SCC and those with ADC in NSCLC.^[7]

Aim and Objectives

1. To study the histopathological spectrum of lung carcinoma
2. To study the expression of IHC markers TTF-1, Napsin-A, and p40 & to evaluate their role in subclassification of NSCLC

MATERIALS AND METHODS

This was a cross-sectional study conducted in the Department of Pathology at our institute between July 2023 and February 2025. A total of 68 biopsies were received, of which 50 were NSCLC cases included in the study. The remaining comprised 9 cases of small cell carcinoma, 1 case of non-Hodgkin lymphoma, and 8 cases of inflammatory lesions. Informed consent and ethical clearance was obtained for the study. Requisition forms were submitted with the formalin-fixed tissue specimens that were received by the pathology department in order to acquire clinical data. Biopsy samples were subjected to routine histopathology processing, embedding, sectioning & slides were stained with H&E stain and examined under a microscope for histological diagnosis. IHC application with the markers TTF-1, Napsin-A, & p40 was done using Pathsite rabbit monoclonal antibody. Sections obtained were examined at 4x, 10x and 40x under light microscope for histopathological and immunohistochemical findings. Images of the same were taken. All cases of lung carcinoma (NSCLC) biopsies with adequate material for further evaluation were included in the study. Biopsies with inadequate representative area, metastatic deposits in lung, autolysed tissue & history of presurgical neo adjuvant therapy cases were not taken.

Statistical Analysis

The Statistical Package for Social Sciences (SPSS) software, version 25.0, was used to do the final analysis after the data was entered into a Microsoft Excel spreadsheet. The Chi-Square test was used to

analyze the relationship between the qualitative variables. Fisher's exact test was applied if the expected value of any cell was less than 5. TTF-1 and Napsin-A were used to predict adenocarcinoma, while TTF-1 and p40 were used to predict squamous cell carcinoma. Sensitivity, specificity, positive predictive value, and negative predictive value were computed.

RESULTS

The cross-sectional study examined 50 cases of NSCLC with histopathological examination & IHC application. The median age of the patients was 62.2 years, with males preponderance. Tumors located in right side of lung were 25 cases and left side of lung were 11 cases & in the remaining cases, site was not mentioned. The predominant site of involvement was observed in the upper lobe in 11 patients (22%) followed by lower lobe which affected 06 cases (12%). Minimal sites of involvement were Apex (8%), middle lobe (4%) and hilum (2%). According to WHO classification, 5th edition 2021, tumors were classified. Adenocarcinoma emerged as the most prevalent histological subtype, identified in 29 cases (58%). Squamous cell carcinoma was the second most common diagnosis, observed in 9 cases (18%). A total of 12 cases (24%) were categorized as poorly differentiated- NSCLC, indicating a high grade malignancy with limited morphological distinction. (Table- 1). IHC markers TTF-1, Napsin-A & p40 were applied. Initially there were 29 (54%) cases of ADC which increased to 36 (72%) and 9 (18%) cases of SCC which increased to 13 (26%). IHC application improved the histopathological subtyping, as 12 cases of poorly differentiated carcinoma NSCLC on histopathology were subclassified as 7 cases of ADC & 4 cases of SCC. In one case napsin-A & p40 were inconclusive. (Table- 2) The application of IHC significantly enhanced the diagnostic accuracy by enabling precise characterization of tumor subtypes. In the present study, one case was initially diagnosed as poorly differentiated SCC on histopathology which could not be further subtyped using IHC, as only TTF-1 positivity was observed. Consequently, the final diagnosis in this case was made based on histopathological evaluation. Furthermore, there was one case where discordance was noted between the initial histopathological diagnosis and IHC findings. In this case, the final diagnosis was established based on the IHC. Initially diagnosed as poorly differentiated SCC, which after IHC application was classified as Adenocarcinoma. This highlights the critical role of IHC in refining the subclassification of morphologically ambiguous cases.

Table 1: Histopathological diagnosis before & after IHC distribution

	Histopathological diagnosis before IHC	Histopathological diagnosis after IHC
Adenocarcinoma	29/50	36/50
Squamous cell carcinoma	9/50	14/50
Poorly differentiated type of NSCLC	12	- (7 Adeno + 5 SCC)
Total	50	50

ASSOCIATION OF TTF-1 WITH HISTOPATHOLOGICAL DIAGNOSIS AFTER IHC

Table 2: Association of TTF-, Napsin-A & p40 with histopathological diagnosis

	Adenocarcinoma(n=29)		Squamous cell carcinoma(n=9)		Poorly differentiated type of NSCLC (n=12)	
	Positive	Negative	Positive	Negative	Positive	Negative
TTF-1	21	08	04	05	06	06
Napsin-A	29	00	01*	08	06	06
P40	00	29	08	01*	06	06

*Diagnosed as Poorly differentiated Squamous cell carcinoma on histopathology

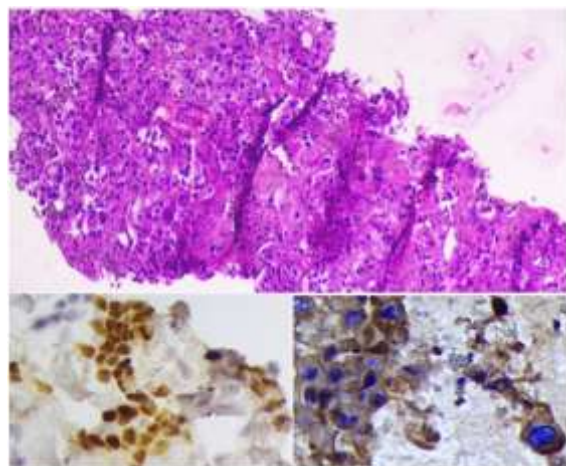


Image 1a: Histopathology Section- Poorly differentiated carcinoma- Invasive carcinoma arranged in sheets with marked cellular pleomorphism and bizarre forms (H&E,10X)

Image- 1b & 1c: Immunohistochemical expression of TTF1 and Napsin A showed positivity in tumor cells, hence Poorly differentiated carcinoma was finally diagnosed as Poorly differentiated Adenocarcinoma (IHC,40X)

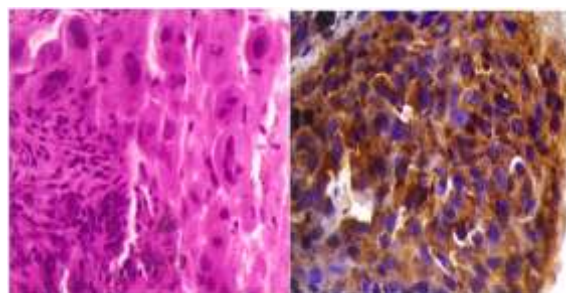


Image 2a: Histopathology Section showing Poorly differentiated SCC (H&E,40X)

Image 2b: Immunohistochemical expression of Napsin-A in a case of histopathologically diagnosed as Poorly differentiated SCC

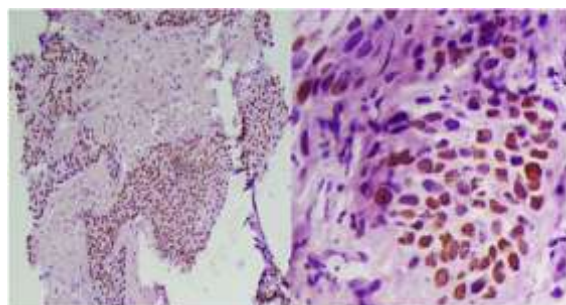


Image 3: Immunohistochemical expression of p40 in SCC

DISCUSSION

Lung cancer stands as the foremost cause of cancer related mortality worldwide. Its silent progression often eludes early detection.^[8] In our study we seek to classify Lung Carcinoma Using Immunohistochemical Markers- TTF-1, Napsin-A & p40 on lung biopsy which aids in accurate sub-classification of Non small cell lung carcinoma.

Adenocarcinoma consistently emerged as the most common NSCLC subtype across multiple studies. Asokan et al. reported adenocarcinoma in 31 and squamous cell carcinoma in 27 out of 64 cases.^[9] Sharma et al. (2022) identified adenocarcinoma in 32.6% and SCC in 23.9%, highlighting the role of immunocytochemistry in accurate subclassification.¹ Hassan et al. (2022) observed adenocarcinoma in 66%, SCC in 26%, and poorly differentiated carcinoma in 8% of 50 cases, emphasizing the diagnostic value of immunohistochemistry, especially in small biopsies.^[10] Ranjan et al. (2022) found that among 47 cases, 80% were NSCLC, with 34% adenocarcinoma, 21% SCC, and 21% NSCLC favoring adenocarcinoma, while a few remained unclassified.^[8] Similarly, Walia et al. reported adenocarcinoma in 31.1%, SCC in 20.5%, and adenosquamous carcinoma in 1.5%, with 46.7% labelled NSCLC-NOS due to inconclusive morphology, underscoring the need for ancillary techniques.^[11] In concordance with these findings, our study also found adenocarcinoma to be the predominant subtype, with 29 of 50 cases diagnosed on histopathology.

Multiple studies have demonstrated the high sensitivity and specificity of TTF-1 in diagnosing adenocarcinoma, particularly in poorly differentiated and small biopsy specimens. Grover et al. reported a sensitivity of 97.56% and specificity of 96.77% for TTF-1 in poorly differentiated adenocarcinomas, with expected negativity in mucinous subtypes.^[12] Sharma et al. (2023) found 100% sensitivity of TTF-1 among 46 NSCLC cases, reinforcing its reliability as a marker for adenocarcinoma.¹ Khan et al. (2019), in a study of 147 NSCLC biopsies, reported TTF-1 sensitivity of 87.5% and Napsin-A sensitivity of 90% for adenocarcinoma, while p40 showed 93.2% sensitivity for SCC.^[13] Pelosi et al. (2012) observed TTF-1 positivity in nearly all 30 adenocarcinoma cases, further supporting its diagnostic utility.^[14] In concordance with these findings, our study observed an increase in adenocarcinoma cases from 29 (54%)

to 36 (72%) following IHC application, with TTF-1 showing a sensitivity of 69.44% for adenocarcinoma. Several studies have highlighted the high sensitivity and diagnostic utility of Napsin-A in identifying adenocarcinoma of the lung. Grover et al. reported a sensitivity of 90.24% and specificity of 81.08%, noting its superior performance in mucinous adenocarcinomas where TTF-1 may be negative.^[12] Sharma et al. (2023) observed a sensitivity of 93.1%, emphasizing its complementary role to TTF-1.^[11] Singh et al., in a prospective study of 210 small biopsies, found Napsin-A positive in 90% of adenocarcinoma cases, confirming its effectiveness in subtyping.^[15] Ranjan et al. reported 85.7% positivity⁸, while Khan et al. observed 90% sensitivity with complete negativity in SCC, further reinforcing its specificity.^[13] Ezzat and Touhan similarly documented 89.3% positivity in ADC cases, underscoring Napsin-A's reliability.^[16] In our study, Napsin-A was positive in 36 of 50 NSCLC cases, including 35 of 36 adenocarcinomas, resulting in a sensitivity of 97.22%. One case initially diagnosed as SCC was reclassified as ADC based on Napsin-A positivity. It remained negative in all other SCCs and one ADC case, confirming its strong association with glandular differentiation and high diagnostic accuracy. Multiple studies have established p40 as a highly sensitive and specific marker for squamous differentiation in NSCLC. Grover et al. (2024) reported 100% sensitivity (30/30) for p40 in SCC, with complete absence of expression in adenocarcinomas, underscoring its superiority over p63, especially in small biopsies.^[12] Singh et al. noted that the combined use of p40 and Napsin-A enabled accurate subtyping in 71.9% of NSCLC cases, with p40 showing a sensitivity of 80%.^[15] Khan et al., in their study of 147 NSCLC cases, demonstrated that p40 outperformed p63 in identifying SCC, leading to reclassification of 80 cases (54.4%) as SCC based on its enhanced specificity and diagnostic reliability.^[13] Similarly, Pelosi et al. reported strong p40 positivity in all 10 SCC cases, with expression in over 50% of tumor cells.^[14] In the present study, p40 expression was detected in 13 out of 14 SCC cases, yielding a sensitivity of 92.86%, reaffirming its value as a reliable marker for squamous cell carcinoma.

Limitation

The use of immunohistochemistry (IHC) alone in diagnosing non-small cell lung carcinoma (NSCLC) presents several inherent limitations. A major concern is the potential overlap in marker expression, as markers like TTF-1 and p40 may occasionally be expressed in more than one NSCLC subtype, particularly in poorly differentiated tumors, leading to diagnostic ambiguity. In small biopsies or inadequately preserved samples, compromised antigenicity can result in weak or false-negative staining. Moreover, IHC is unable to detect actionable molecular alterations which are vital for guiding targeted therapy. Variability in interpretation among pathologists and the subjective nature of

staining assessment can further impact diagnostic consistency. Diagnostic accuracy also relies heavily on the selection of an appropriate and comprehensive marker panel; incomplete or suboptimal panels may contribute to misclassification. Thus, while IHC serves as an important adjunct to morphological assessment, it should not be used in isolation for definitive diagnosis or subtyping of NSCLC. The correlation of histopathological features with IHC findings is crucial for accurate subtyping of NSCLC, particularly in limited biopsy material and poorly differentiated tumors. Histopathological evaluation guides optimal marker selection, enhances diagnostic reliability, and ensures the efficient use of limited tissue for downstream molecular testing. This integrated diagnostic approach is essential for accurate tumor classification, which directly influences prognosis and therapeutic decision-making in NSCLC.

CONCLUSION

The immunohistochemical expression of TTF-1, Napsin-A, and p40 proved valuable in the histological subclassification of NSCLC. Napsin-A emerged as the most reliable marker for adenocarcinoma, with an overall sensitivity of 97.22%. The findings highlight the value of IHC in refining diagnosis, particularly in poorly differentiated tumors, while also emphasizing that IHC may not be necessary in well and moderately differentiated NSCLC cases where morphology alone is often diagnostic.

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