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Evaluating the Efficacy of a Limited 2 - Marker IHC Panel (TTF-1 and p40) in Sub typing poorly Differentiated Lung Masses on Small Core Biopsies

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Abstract

Background: Accurate sub typing of non-small cell lung carcinoma (NSCLC) on small biopsy specimens is essential for selecting appropriate targeted therapies while preserving tissue for molecular testing. Extensive immuno histochemical (IHC) panels may exhaust limited tissue samples, highlighting the need for a simplified diagnostic approach. This study evaluated the diagnostic utility of a limited two-marker IHC panel comprising thyroid transcription factor-1 (TTF-1) and p40 in the sub typing of poorly differentiated lung masses.

Materials and Methods: A retrospective analysis was conducted on 20 representative cases of lung masses and metastatic lesions diagnosed on small tissue samples, including CT-guided core biopsies, Tru - Cut biopsies, bronchoscopic biopsies, cellblocks from malignant effusions, and metastatic tumour specimens. Immuno histochemical staining with TTF-1 and p40 was performed in all cases. Selected cases underwent additional evaluation with CK7, Naps in A, Ki-67, and other ancillary markers for diagnostic confirmation where required.

Results: The limited two-marker panel successfully classified all cases into Adenocarcinoma and Squamous cell carcinoma phenotypes. Nine cases demonstrated a TTF-1-positive/p40-negative Immuno profile consistent with Adenocarcinoma, while ten cases showed a p40-

positive/ TTF-1-negative profile diagnostic of Squamous cell carcinoma. One poorly differentiated Squamous cell carcinoma was negative for both TTF-1 and p40 but was accurately diagnosed based on morphology and supportive ancillary Immunohistochemical findings. The panel was equally effective in metastatic lesions involving the brain, pericardial fluid, Supraclavicular lymph node, and proximal femur. In poorly differentiated tumours, the complementary expression pattern of TTF-1 and p40 provided reliable lineage determination while Minimising tissue consumption and preserving specimens for subsequent molecular biomarker testing.

Conclusion: A limited Immunohistochemical panel consisting of TTF-1 and p40 provides a simple, reliable, cost-effective, and tissue-conserving strategy for sub typing poorly differentiated NSCLC on small biopsy specimens. The approach achieves accurate differentiation between Adenocarcinoma and squamous cell carcinoma while preserving valuable tissue for essential downstream molecular studies, making it an effective first-line diagnostic strategy in routine thoracic pathology practice.

Keywords: Non-Small Cell Lung Carcinoma, Immunohistochemistry, TTF-1, P40, Adenocarcinoma, Squamous Cell Carcinoma, Small Biopsy, Tissue Conservation.

Introduction

Lung cancer remains the leading cause of cancer-related mortality worldwide, with non-small cell lung carcinoma (NSCLC) accounting for approximately 85% of all lung cancers. Accurate subclassification of NSCLC into adenocarcinoma and squamous cell carcinoma has become increasingly important because therapeutic decisions, including targeted therapy and immunotherapy, are guided by tumour histology and molecular characteristics.[1,2]

Most patients with NSCLC are diagnosed on small core needle biopsies or cytology specimens, where the available tissue is often limited. Therefore, pathologists must establish an accurate diagnosis while preserving sufficient tissue for essential molecular testing, including EGFR, ALK, ROS1, and PD-L1 analysis.[2,3]

Immunohistochemistry (IHC) is an indispensable adjunct in the evaluation of poorly differentiated NSCLC. Among the available markers, thyroid transcription factor-1 (TTF-1) is a reliable marker for pulmonary Adenocarcinoma, whereas p40 is regarded as the most specific marker for squamous cell carcinoma. Several studies have demonstrated that a limited panel comprising TTF-1 and p40 provides high diagnostic accuracy while conserving tissue for subsequent molecular investigations.[4–6] The present study was undertaken to evaluate the diagnostic efficacy of a limited two-marker IHC panel (TTF-1 and p40) in sub typing poorly differentiated lung masses on small biopsy specimens and metastatic deposits.

Materials and Methods

A retrospective evaluation of ten representative Immunohistochemistry reports from clinical lung mass biopsies was performed. Tissue specimens included:

- CT-guided lung core biopsies
- Tru-Cut needle biopsies
- Bronchoscopy biopsies
- Cell blocks derived from malignant effusions
- Excised metastatic tumor tissue

All specimens were fixed in formal-saline or standard formalin fixative within the recommended 1-hour cold ischemia window to optimize antigen preservation. Antigen retrieval was uniformly executed using the

Biogenex EZ retriever system V.3, followed by visual detection via the Biogenex Sensitive Polymer HRP IHC system. The primary IHC panel evaluated consisted of TTF-1 and p40. Select cases utilized secondary confirmatory markers such as Napsin A and CK7 (for Adenocarcinoma) or morphological evaluation to cross-validate findings. Proliferative indexes were assessed via Ki-67 expression.

Results and Diagnostic Profiling

The limited 2-marker panel cleanly separated the cohort into two distinct Immunophenotypic categories, which correlated directly with final histological diagnoses:

The Adenocarcinoma Profile (TTF-1 Positive / p40 Negative)

Six cases demonstrated a definitive Immunophenotype Favouring or consistent with lung Adenocarcinoma.

- TTF-1 Reactivity: Ranged from focal positive to diffuse, strong positive expression.
- p40 Reactivity: Consistently and completely negative across all samples.
- Confirmatory Overlap: These cases showed co-expression of CK7 and Napsin A, confirming primary pulmonary origin.
- Application to Metastases: This exact Immunophenotypic signature effectively identified primary lung Adenocarcinoma when presenting as metastatic deposits in distant fluid cell blocks (pericardial effusion) and central nervous system biopsies (temporal/occipital mass).

The Squamous Cell Carcinoma Profile (p40 Positive / TTF-1 Negative)

Four cases demonstrated a definitive Immunophenotype favouring squamous cell carcinoma.

- p40 Reactivity: Strong nuclear positivity was observed across primary bronchogenic tumors and poorly differentiated cohorts.

Figure 1: [IHC 40x hpf; TTF1 positive: Adenocarcinoma]

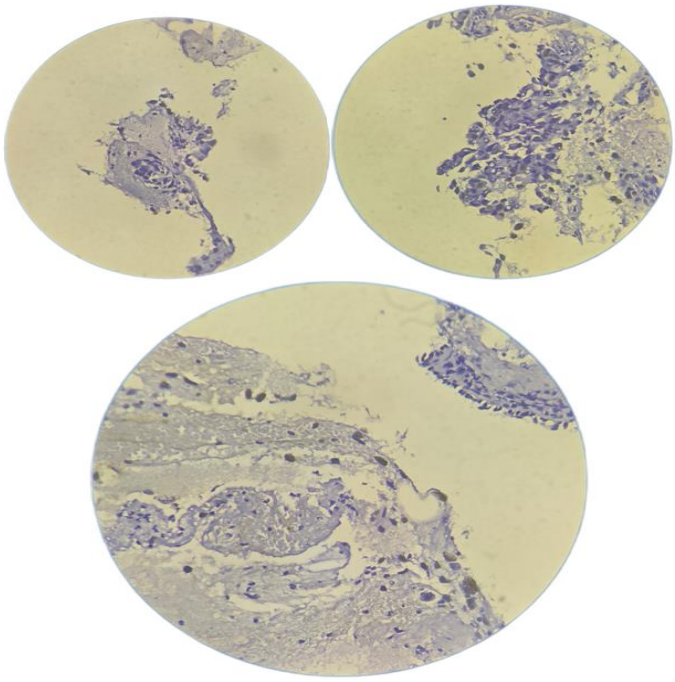
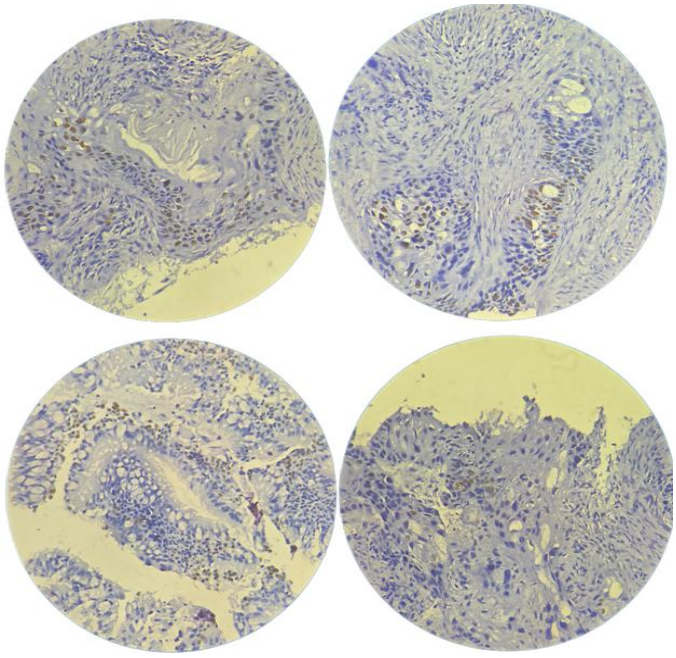


Figure 2: [IHC 40x hpfP40 positive: Squamous cell carcinoma]



- TTF-1 Reactivity: Completely negative.

- Application to Metastases: In patients with metastatic spread to regional lymph nodes (e.g., Supraclavicular node), a strong p40 expression coupled with a negative TTF-1 and negative Naps in profile successfully localized the source to a primary lung squamous cell carcinoma.

Table 1: Summary of Patient Marker Expression and Diagnoses

No.	Age/Sex	Specimen Type	TTF-1	p40	Secondary Markers (CK7/Napsin/Ki-67)	Final Diagnosis / Impression
1	46 / M	Right Lung Core Biopsy	Focal Positive	Negative	CK7 (+), Napsin (+), Ki-67: 8%	Poorly Differentiated Adenocarcinoma
2	68 / M	Lung Tru-Cut Biopsy	Focal Positive	Negative	CK7 (+), Napsin A (+)	Moderately Differentiated Adenocarcinoma
3	49 / F	Pericardial Fluid Cell Block	Positive	Negative	CK7 (+), Napsin A (+), Ki-67: 6%	Deposits of Moderately Differentiated Adenocarcinoma
4	52 / M	Lung Tru-Cut Biopsy	Negative	Positive	CK7 (Focal +), Napsin A (-), Ki-67: 34%	Moderately Differentiated Squamous Cell Carcinoma
5	67 / M	RML Lung Tru-Cut Biopsy	Focal Positive	Negative	CK7 (+), Napsin A (+), Ki-67: 54%	Moderately Differentiated Adenocarcinoma
6	68 / M	Upper Lobe Core Biopsy	Negative	Positive	CK7 (Focal +), Napsin A (-), Ki-67: 8%	Poorly Differentiated Squamous Cell Carcinoma
7	33 / M	Brain Metastasis Excision	Positive	Negative	CK7 (+), Napsin A (+), Ki-67: 22%	Metastatic Deposits of Moderately Differentiated Adenocarcinoma
8	65 / F	Lung Tru-Cut Biopsy	Focal Positive	Negative	CK7 (+), Napsin A (+), Ki-67: 12%	Moderately Differentiated Adenocarcinoma
9	64 / M	Supraclavicular Node Biopsy	Negative	Positive	CK7 (Dim +), Napsin (-)	Metastatic Deposits of Squamous Cell Carcinoma

IHC No.	Age/Sex	Specimen Type	TTF-1	p40	Secondary Markers (CK7/Napsin/Ki-67)	Final Diagnosis / Impression
10	73 / M	Left Main Bronchus Mass	Negative	Negative	Napsin (-), Synaptophysin (-)	Poorly Differentiated Squamous Cell Carcinoma
11	74 / M	CT Guided Biopsy from Left Apex Lung Mass	Negative	Positive [Nuclear]	CK7 (Diffuse +), Napsin (-), CK20 (-), Synaptophysin (-)	Squamous Cell Carcinoma [Poorly Differentiated]
12	59 / M	Biopsy from Right Bronchus Lesion	Positive [Nuclear]	Negative	CK7 (+), Napsin A (+), Chromogranin (-), Synaptophysin (-)	Adenocarcinoma, Lung
13	54 / F	CT Guided Biopsy from Right Hilar Lung Mass	Positive	Negative	CK7 (+), Napsin A (+), HMB45 (-), PAX-8 (-)	Adenocarcinoma Lung [Acinar Type, Moderately Differentiated]
14	74 / M	USG Guided Biopsy from Lung Mass	Negative	Focal Positive	CK7 (Diffuse +), Napsin (-), Ki-67: 22%, Synaptophysin (-)	Squamous Cell Carcinoma [Poorly Differentiated]
15	55 / M	Endobronchial Biopsy from Right Upper Lobe	Negative	Positive	Napsin (-), Ki-67: 48%, Synaptophysin (-)	Squamous Cell Carcinoma [Moderately Differentiated]
16	75 / M	USG Guided Tru-cut Biopsy from Left Lung Mass	Negative	Positive	CK7 (Focal +), Napsin (-), Synaptophysin (-)	Squamous Cell Carcinoma [Poorly Differentiated]
17	74 / M	CT Guided Tru-cut Biopsy from Right Upper Lobe Mass	Negative	Positive	CK7 (+), Napsin (-), Synaptophysin (-)	Squamous Cell Carcinoma [Moderately Differentiated]
18	65 / M	CT Guided Tru-cut Biopsy from Right Upper Lobe Mass	Negative	Positive	Napsin (-)	Squamous Cell Carcinoma [Moderately Differentiated]

IHC No.	Age/Sex	Specimen Type	TTF-1	p40	Secondary Markers (CK7/Napsin/Ki-67)	Final Diagnosis / Impression
19	62 / M	Biopsy from Right Upper Lobe Lung Mass	Negative	Positive [Nuclear]	Napsin (-), Ki-67: 34%, Synaptophysin (-)	Poorly Differentiated Squamous Cell Carcinoma, Lung
20	72 / F	Left Proximal Femur Pathological Fracture Tissue	Focal Positive	Negative	Napsin A (+), Synaptophysin (-)	Metastatic Deposits of Adenocarcinoma, Lung

Resolving "Poorly Differentiated" Morphologies

When an initial morphological examination reveals a "Poorly Differentiated Carcinoma" or generic "Non-Small Cell Lung Carcinoma (NSCLC)," light microscopy alone cannot determine cellular lineage. In this study, cases initially categorized as poorly differentiated or indeterminate NSCLC were immediately stratified into distinct therapeutic pathways utilizing the 2-marker panel. For instance, one patient's morphologically poorly differentiated tumor was confirmed as Adenocarcinoma due to positive focal TTF-1 and negative p40, while another patient's poorly differentiated lung mass was cleanly resolved as squamous cell carcinoma by showing positive p40 and negative TTF-1.

The Diagnostic Utility of Minimalist Marker Combinations

While larger panels incorporating CK7, CK20, Napsin A, and Calretinin offer comprehensive phenotypic mapping, our data indicates that TTF-1 and p40 alone provide the core diagnostic weight necessary for sub typing. Because p40 displays superior sensitivity and specificity for squamous lineage over older markers (like CK5/6) and TTF-1 reliably highlights respiratory epithelium, an inverse expression profile between the two is highly diagnostic. Mutually exclusive results — where one marker is positive and the other is negative — obviate the need for broader screening panels in the majority of cases.

Mitigating Challenges in Diminutive Tissue Samples

A persistent issue in modern thoracic pathology is managing "diminutive" tumor samples. Biopsies showing low-volume tumor tissue present a high risk of tissue exhaustion. Utilizing a broad 5- to 7-marker screening approach can completely deplete the paraffin block, leaving no room for mandatory predictive molecular pathology (e.g., EGFR mutations or PD-L1 expression scoring). Applying a strict 2-marker (TTF-1/p40) panel successfully establishes the diagnosis while maximizing tissue conservation. If the tissue is so sparse that even restricted IHC compromises molecular study, a repeat biopsy remains the recommended backup strategy.

Discussion

Accurate sub classification of poorly differentiated non-small cell lung carcinoma (NSCLC) on limited biopsy specimens remains a major challenge in thoracic pathology, particularly because tissue preservation has become increasingly important for molecular testing. In the present study, the limited two-marker Immuno histochemical (IHC) panel comprising TTF-1 and p40 successfully classified the vast majority of cases into

Adenocarcinoma and squamous cell carcinoma, demonstrating its practical utility in routine diagnostic practice.

Our findings are consistent with the study by Walia et al**.**, who demonstrated that the combined use of TTF-1 and p40 reliably sub typed NSCLC-not otherwise specified (NSCLC-NOS) with high diagnostic accuracy while minimizing the need for additional Immuno histochemical markers. The authors concluded that this limited panel is particularly useful for conserving tissue for subsequent molecular testing, a finding that closely parallels the observations of the present study.[4]

Similarly, Bishop et al. reported that p40 exhibits significantly greater specificity than p63 for pulmonary squamous cell carcinoma while maintaining excellent sensitivity. Their study established p40 as the preferred squamous marker because it substantially reduces false-positive staining in adenocarcinomas. Our findings support these observations, as all squamous cell carcinomas demonstrated p40 positivity except one poorly differentiated case in which the final diagnosis was established using morphology and ancillary markers.[6]

The present study also corroborates the work of Mukhopadhyay and Katzenstein, who demonstrated that a limited Immuno histochemical panel centered on TTF-1 and squamous markers accurately sub classifies poorly differentiated NSCLC on small biopsy specimens. They emphasized that extensive Immuno histochemical panels are often unnecessary when morphology is carefully interpreted together with selected lineage-specific markers.[5]

The importance of tissue stewardship has been highlighted in the recommendations of the IASLC/ ATS/ERS classification of lung Adenocarcinoma, which advocates minimizing Immuno histochemical staining on small biopsies to preserve tumour tissue for predictive biomarker analysis, including EGFR, ALK, ROS1, and PD-L1 testing. Our study further supports this approach by demonstrating that the TTF-1/p40 panel provided sufficient diagnostic information in nearly all cases while avoiding unnecessary depletion of limited biopsy material.[3]

A recent study by **Grover et al.** also demonstrated that TTF-1 and p40 constitute an optimal first-line Immuno histochemical combination in resource-constrained settings. The authors reported excellent concordance between the two-marker panel and the final Histopathological diagnosis while reducing investigation costs and preserving tissue for molecular studies. These findings closely mirror the results of the present study and reinforce the clinical applicability of a minimalist Immuno histochemical approach.[7]

Overall, the present study demonstrates that a limited two-marker panel consisting of TTF-1 and p40 provides a simple, accurate, economical, and tissue-conserving strategy for sub classifying poorly differentiated NSCLC in routine practice. The findings are in agreement with current international evidence supporting the use of focused Immuno histochemical algorithms before resorting to extended marker panels.

Conclusion

A limited 2-marker IHC panel consisting of TTF-1 and p40 is a highly effective, robust, and economically sound strategy for sub typing poorly differentiated lung masses on small core needle biopsies. It provides unequivocal differentiation between Adenocarcinoma and squamous cell carcinoma lines while proactively preserving essential tumor tissue for mandatory downstream molecular profiling. Pathologists should adopt this

tissue-conserving diagnostic model as a standard initial reflex tier when evaluating small-volume non-small cell lung carcinomas.

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