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Diagnostic Contribution of Mucicarmine Staining in TTF-1- and p40-negative Non-small Cell Lung Carcinoma Diagnosed on Small Biopsy Specimens

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ABSTRACT

Aim: Accurate subclassification of non-small cell lung carcinoma (NSCLC) in small biopsy specimens is essential for appropriate therapeutic decision-making and molecular testing. However, accurate subclassification remains challenging in a subset of tumors, particularly in those lacking both thyroid transcription factor-1 (TTF-1) and p40 expression. This study aimed to evaluate the diagnostic contribution of histochemical mucicarmine positivity in the adenocarcinoma-oriented subclassification of TTF-1 and p40-negative NSCLC cases diagnosed from small biopsy specimens.

Methods: This retrospective study included 120 NSCLC cases that were negative for TTF-1 and p40, diagnosed from small biopsy specimens. All cases underwent histochemical mucicarmine staining. Clinicopathological variables, including patient age, sex, biopsy type, mucin staining pattern, initial diagnostic category, and method of final diagnostic confirmation, were evaluated. Statistical analyses were performed using the chi-square and Fisher's exact tests.

Results: The mean age of the patients was 67.75±9.82 years, and 81.7% of the patients were male. Histochemical mucin positivity was observed in 116 of 120 cases (96.7%): focal in 45 cases (37.5%), diffuse in 41 cases (34.2%), and of unspecified extent in 30 cases (25.0%). Only four cases (3.3%) were mucin- negative and remained classified as NSCLC-not otherwise specified (NSCLC-NOS). A statistically significant association was observed between mucin positivity and adenocarcinoma-oriented subclassification ($p<0.001$). No significant association was identified between biopsy modality and mucin positivity ($p=0.296$).

Conclusion: Histochemical mucicarmine positivity provides valuable diagnostic support for adenocarcinoma-oriented subclassification in TTF-1- and p40-negative NSCLC cases diagnosed on small biopsy specimens. Mucicarmine staining is a practical, inexpensive, tissue-preserving adjunctive technique that may help reduce the frequency of NSCLC-NOS diagnoses in diagnostically challenging cases.

Keywords: Adenocarcinoma, biopsy, lung cancer, mucin, pathology

Introduction

Lung cancer remains the leading cause of cancer-related mortality worldwide, and approximately 70% of patients present with advanced-stage disease at the time of diagnosis. In this setting, small biopsy and cytology specimens frequently constitute the only available diagnostic material for histopathological evaluation and molecular testing. Accordingly, accurate subclassification of non-small cell lung carcinoma (NSCLC) in limited tissue samples has become increasingly important in the era of personalized therapy and predictive biomarker testing [1-4].

Current World Health Organization (WHO) and International Association for the Study of Lung Cancer (IASLC) recommendations emphasize minimizing use of the NSCLC-

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not otherwise specified (NSCLC-NOS) category and encourage subclassification as adenocarcinoma or squamous cell carcinoma whenever possible. Preservation of tissue for molecular testing has become a critical component of modern thoracic pathology practice. Therefore, current diagnostic algorithms advocate the use of limited immunohistochemical panels capable of achieving accurate subclassification while minimizing tissue consumption for downstream molecular analyses, including EGFR, ALK, ROS1, KRAS, and PD-L1 testing [1-6].

In routine practice, thyroid transcription factor-1 (TTF-1) and p40 represent the most widely accepted minimal immunohistochemical panel for subclassification of NSCLC in small biopsy specimens. However, a subset of tumors remains difficult to classify, particularly poorly differentiated carcinomas that lack glandular morphology and are negative for both markers. This challenge is particularly relevant in mucinous and poorly differentiated adenocarcinomas, in which TTF-1 expression may be absent or significantly reduced. Previous studies have demonstrated that invasive mucinous adenocarcinomas frequently exhibit low rates of TTF-1 and Napsin A expression, highlighting the limitations of relying solely on immunohistochemistry in this subgroup [7,8].

Although immunohistochemistry has become the cornerstone of NSCLC subclassification, histochemical mucin stains continue to represent a practical and widely available ancillary diagnostic tool. Current WHO- and IASLC-based recommendations recognize mucin positivity as supportive evidence of glandular differentiation, particularly in small biopsy specimens lacking definitive morphologic or immunophenotypic features. Several studies have demonstrated that histochemical mucin staining may improve subclassification accuracy and reduce the frequency of NSCLC-NOS diagnoses in limited specimens. In particular, the combined use of TTF-1 and mucin staining has been shown to improve adenocarcinoma-oriented subclassification in poorly differentiated NSCLC [2-4,9-11]. Recent review articles have also emphasized that histochemical mucin stains remain valuable ancillary diagnostic tools in selected morphologically and immunophenotypically challenging cases of NSCLC, despite major advances in immunohistochemistry and molecular pathology [12].

Despite these observations, studies specifically evaluating the diagnostic contribution of histochemical mucin positivity in TTF-1- and p40-negative NSCLC diagnosed on small biopsy specimens remain limited. Accordingly, the present study was undertaken to evaluate the diagnostic contribution of histochemical mucicarmine positivity in the adenocarcinoma-oriented subclassification of TTF-1- and p40-negative NSCLC diagnosed on small biopsy specimens and to determine whether this approach could reduce the frequency of NSCLC-NOS diagnoses in routine practice.

Methods

Ethical Statement

This study was approved by the Scientific Studies Ethics Committee of University of Health Sciences Türkiye, Ankara Atatürk Sanatorium Training and Research Hospital (approval no: 2024-BÇEK/559, date: 06.05.2026). All procedures were performed in accordance with the ethical principles of the Declaration of Helsinki. Due to the retrospective nature of the study, informed consent was waived.

Study Design and Case Selection

This retrospective, single-center, observational study included consecutive patients diagnosed with NSCLC from small biopsy specimens between January 2023 and January 2026. Cases were identified by a retrospective search of the pathology archives in the Department of Pathology at University of Health Sciences Türkiye, Ankara Atatürk Sanatorium Training and Research Hospital.

Inclusion criteria were as follows:

1. Evaluation of small biopsy material, including bronchoscopic biopsy or transthoracic needle biopsy,
2. Absence of TTF-1 and p40 expression on immunohistochemical examination,
3. Availability of histochemical mucin staining,
4. Initial diagnosis rendered as "NSCLC, favor adenocarcinoma" or "NSCLC-NOS,"
5. Final diagnosis of adenocarcinoma established either by examination of resection specimens or clinicoradiologic correlation.

Cases diagnosed with small cell lung carcinoma or squamous cell carcinoma, and those with insufficient biopsy material or technically inadequate mucicarmine staining, were excluded. The study workflow is summarized as follows: eligible TTF-1/p40-negative NSCLC cases diagnosed on small biopsies were identified retrospectively. Histochemical mucicarmine staining results were reviewed, and the final diagnosis was confirmed by resection specimens or by clinicoradiologic correlation, when available.

Clinicopathological Parameters

The following clinicopathological variables were recorded from pathology reports and hospital records:

- patient age and sex,
- biopsy type (bronchoscopic biopsy or transthoracic needle biopsy),
- histochemical mucin status,
- initial diagnostic category,
- method of final diagnosis confirmation.

Initial diagnostic categories were defined as "NSCLC, favor adenocarcinoma" and "NSCLC-NOS."

The final diagnosis was established either by evaluation of the resection specimens or by clinicoradiologic correlation.

Histochemical Evaluation

Histochemical evaluation of mucin production was performed on formalin-fixed, paraffin-embedded tissue sections using mucicarmine staining as part of the routine diagnostic workup. All stained slides were reviewed by experienced pulmonary pathologists. Distinct intracellular and/or luminal mucin staining within tumor cells was considered positive.

Based on the extent of staining described in the pathology reports, mucin positivity was categorized as focally positive, diffusely positive, or positive with unspecified extent. Cases lacking detectable staining were classified as mucin negative.

Immunohistochemistry

All cases included in the study showed a complete absence of TTF-1 and p40 expression by routine diagnostic immunohistochemistry. Representative histopathological, immunohistochemical, and histochemical findings are shown in Figure 1.

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics for Windows, version 27.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were expressed as mean ± standard deviation for continuous variables and as frequency and percentage for categorical variables. Associations between mucin positivity and clinicopathological variables, including initial diagnostic category, biopsy type, and final diagnostic method, were evaluated using the chi-square test or Fisher's exact test, where appropriate. A p value <0.05 was considered statistically significant.

Results

A total of 120 patients with NSCLC negative for TTF-1 and p40, diagnosed on small biopsy specimens were included in the study. All cases underwent histochemical mucin staining, and a final diagnosis of adenocarcinoma was established either by resection specimens or clinicoradiologic correlation. The clinicopathological characteristics of the study cohort are summarized in Table 1.

The mean age of the patients was 67.75±9.82 years (range, 30-87 years). The cohort was predominantly male, comprising 98 men (81.7%) and 22 women (18.3%).

Regarding biopsy type, 78 cases (65.0%) were obtained by transthoracic needle biopsy, whereas 42 cases (35.0%) were obtained by bronchoscopic biopsy.

Histochemical mucin positivity was observed in 116 of 120 cases (96.7%). Among mucin-positive cases, focal positivity was observed in 45 cases (37.5%), diffuse positivity in 41 cases (34.2%), and positivity of unspecified extent in 30 cases (25.0%). Only 4 cases (3.3%) were completely negative for mucin staining.

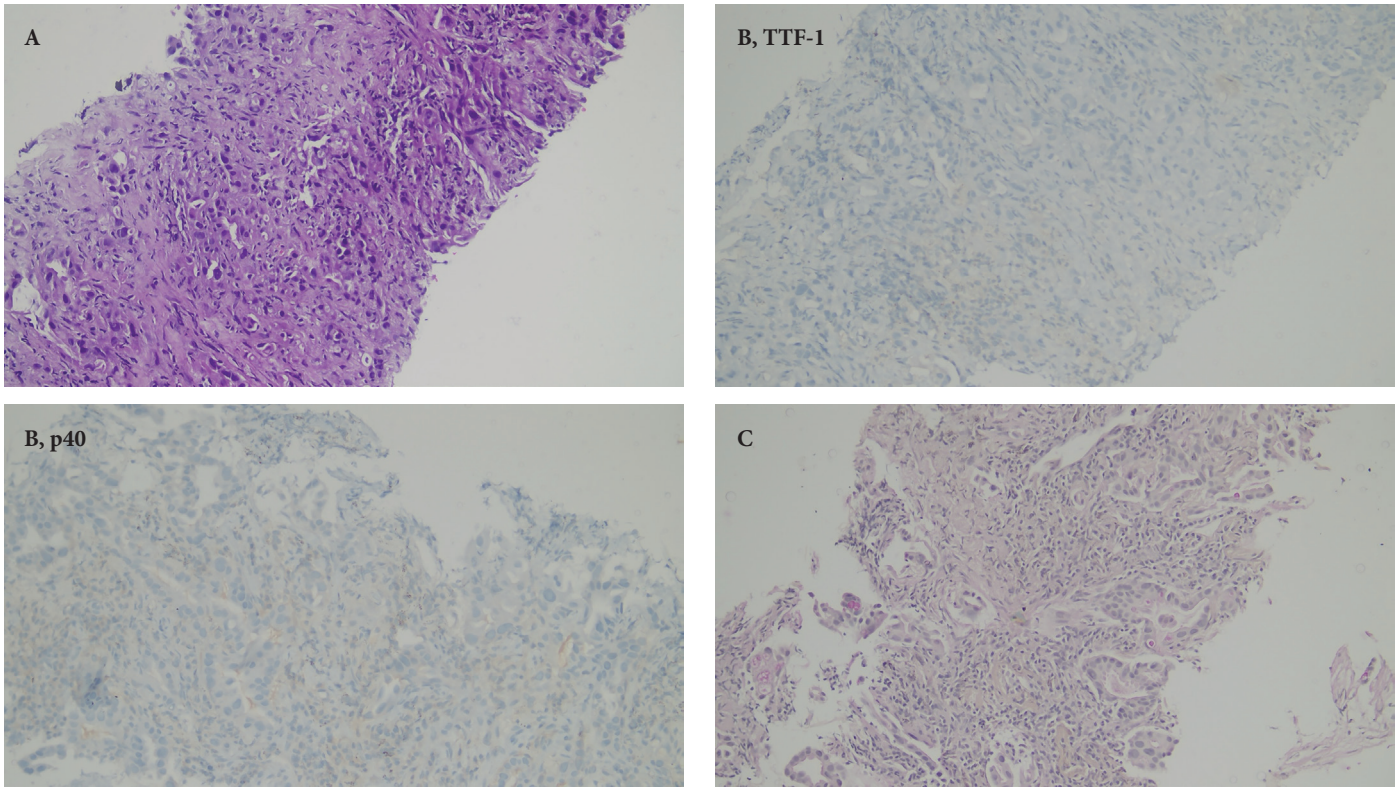

Figure 1. Representative TTF-1-negative and p40-negative NSCLC diagnosed on transthoracic needle biopsy. **(A)** H&E section showing a poorly differentiated NSCLC lacking definitive glandular or squamous differentiation, **(B)** Tumor cells are negative for TTF-1 and p40 immunohistochemical staining, **(C)** Mucicarmine stain demonstrates intracytoplasmic mucin positivity supporting adenocarcinoma-oriented subclassification

Based on the initial pathological evaluation of the small biopsy specimens, 116 cases (96.7%) were classified as "NSCLC, favor adenocarcinoma," whereas 4 cases (3.3%) remained categorized as "NSCLC-NOS." Notably, all mucin-negative cases remained within the NSCLC-NOS category.

A statistically significant association was observed between mucin positivity and the adenocarcinoma-oriented subclassification (Fisher's exact test, p<0.001). Nearly all mucin-positive cases could be subclassified as NSCLC favor adenocarcinoma, supporting the diagnostic contribution of histochemical mucin staining in this challenging subgroup of tumors.

No statistically significant association was found between transthoracic needle biopsy and bronchoscopic biopsy specimens with respect to mucin positivity (Fisher's exact test, p=0.296), suggesting that the utility of mucin staining does not depend on biopsy type.

Final diagnosis was established by clinicoradiologic correlation in 107 cases (89.2%) and by resection specimens in 13 cases (10.8%). A statistically significant association was observed between mucin positivity and the final diagnostic method (Fisher's exact test, p=0.004). Associations between mucin positivity and clinicopathological parameters are presented in Table 2. Mucin-negative cases more frequently required confirmation on resection specimens, whereas most mucin-positive cases were confirmed by clinicoradiologic correlation.

Table 1. Clinicopathological characteristics of the study cohort

Variable	Value
Age, mean ± SD (range)	67.75±9.82 (30-87)
Sex, n (%)	
Male	98 (81.7)
Female	22 (18.3)
Biopsy type, n (%)	
Bronchoscopic biopsy	42 (35.0)
Transthoracic needle biopsy	78 (65.0)
Mucin status, n (%)	
Negative	4 (3.3)
Focal positive	45 (37.5)
Diffuse positive	41 (34.2)
Positive, extent unspecified	30 (25.0)
Initial diagnosis, n (%)	
NSCLC favor adenocarcinoma	116 (96.7)
NSCLC-NOS	4 (3.3)
Final diagnosis method, n (%)	
Resection specimen	13 (10.8)
Clinicoradiologic correlation	107 (89.2)

SD: Standard deviation, NSCLC-NOS: Non-small cell lung carcinoma-not otherwise specified

Discussion

Accurate subclassification of NSCLC in small biopsy specimens has become increasingly important in contemporary thoracic pathology because treatment selection, predictive biomarker assessment, and molecular testing are largely dependent on histologic subtype. At the same time, preservation of limited tissue material remains a major concern, particularly in patients with advanced-stage disease in whom small biopsies frequently represent the only available diagnostic specimens. Consequently, current WHO- and IASLC-based approaches advocate the use of minimal ancillary testing while aiming to reduce the use of the NSCLC-NOS category whenever possible [1-6]. Recent studies based on the 5th WHO classification have further confirmed that limited immunohistochemical panels can accurately classify most NSCLCs while preserving tissue for molecular testing [4].

In routine practice, TTF-1 and p40 constitute the most widely accepted minimal immunohistochemical panel for subclassification of NSCLC in small biopsies. Nevertheless,

Table 2. Association between mucin positivity and clinicopathological parameters

Variable	Mucin negative n (%)	Mucin positive n (%)	p value
Sex			
Male	4 (100)	94 (81.0)	0.571
Female	0 (0)	22 (19.0)	
Biopsy type			0.296
Bronchoscopic biopsy	0 (0)	42 (36.2)	
Transthoracic needle biopsy	4 (100)	74 (63.8)	
Initial diagnosis			<0.001
NSCLC favor adenocarcinoma	0 (0)	116 (100)	
NSCLC-NOS	4 (100)	0 (0)	
Final diagnosis method			0.004
Resection specimen	3 (75.0)	10 (8.6)	
Clinicoradiologic correlation	1 (25.0)	106 (91.4)	

Statistical analysis was performed using Fisher exact test
NSCLC-NOS: Non-small cell lung carcinoma-not otherwise specified

a diagnostically challenging subgroup remains, particularly among poorly differentiated tumors and mucinous adenocarcinomas showing negativity for both markers. In these cases, subclassification may be difficult because classical morphologic features such as gland formation or keratinization are frequently absent in limited tissue samples [2-4,7].

In the present study, histochemical mucin positivity was identified in 96.7% of TTF-1- and p40-negative NSCLC cases. Only four cases remained mucin-negative and consequently retained the diagnosis of NSCLC-NOS. Furthermore, a statistically significant association was observed between mucin positivity and adenocarcinoma-oriented subclassification. These findings suggest that mucicarmine staining provides meaningful diagnostic support for a subgroup of tumors that often prove problematic when diagnosed on the basis of morphology and immunohistochemistry alone.

Our findings are consistent with previous studies evaluating the contribution of histochemical mucin stains to NSCLC subclassification. Loo et al. [10] demonstrated that the combined use of TTF-1 and mucin staining provided one of the most effective approaches for predicting adenocarcinoma differentiation and substantially reduced the frequency of NSCLC-NOS diagnoses in small biopsy specimens. These observations are also consistent with recent studies showing that the judicious use of ancillary stains in small biopsy specimens improves diagnostic confidence while preserving tissue for molecular profiling [5].

Similarly, Nicholson et al. [9] reported that incorporation of mucin stains into limited diagnostic panels improved the refinement of NSCLC subclassification and increased diagnostic concordance among pathologists.

More recently, WHO 5th edition-based studies have confirmed that a substantial proportion of poorly differentiated NSCLCs can be accurately subclassified using limited ancillary testing, with mucin positivity serving as supportive evidence of glandular differentiation in selected cases [3,4].

Although the increasing availability of immunohistochemical and molecular techniques has transformed thoracic pathology, recent reviews continue to highlight that ancillary histochemical stains retain an important role in selected diagnostically challenging small biopsy specimens, particularly when tissue preservation for molecular testing is a priority [12]. Mucicarmine staining is inexpensive, technically simple, widely available, and requires considerably less tissue than extended immunohistochemical panels. Therefore, it remains an attractive adjunctive method in resource-limited settings and in cases where tissue preservation is a priority for molecular testing [5,9]. Accordingly, contemporary diagnostic recommendations continue to recognize histochemical mucin stains as valuable ancillary tools in selected diagnostically challenging cases.

The biological plausibility of our findings is further supported by the distinctive characteristics of invasive mucinous adenocarcinoma. Previous studies have demonstrated that these tumors frequently exhibit reduced or absent expression of TTF-1 and Napsin A, likely reflecting alterations in lineage-specific differentiation pathways [7,8].

Consequently, reliance solely on immunohistochemical markers may result in diagnostic uncertainty. In such settings, histochemical demonstration of intracellular mucin may provide valuable supportive evidence favoring adenocarcinoma differentiation.

Another important consideration is the growing recognition that TTF-1-negative NSCLCs may represent a biologically distinct subgroup. Recent studies have suggested that loss of TTF-1 expression is associated with less favorable clinical outcomes in advanced non-squamous NSCLC, further emphasizing the importance of accurate characterization of these tumors beyond conventional immunophenotypic classification [13].

Another notable finding in our study was the absence of a significant association between biopsy modality and mucin positivity. The diagnostic contribution of mucicarmine staining appeared comparable between transthoracic needle biopsies and bronchoscopic biopsy specimens, suggesting that the utility of mucin evaluation is not dependent on sampling technique. Similar observations have been reported in cytology-based studies, where mucicarmine staining improved subclassification concordance and reduced NSCLC-NOS diagnoses in bronchial brushing specimens [11].

Study Limitations

The present study has several limitations. First, its retrospective, single-center design may introduce selection bias. Second, molecular data were not available for all cases, precluding assessment of the correlation between mucin positivity and specific genomic alterations. Third, the absence of a control group composed of definitively classified cases of squamous cell carcinoma precluded formal assessment of diagnostic sensitivity and specificity. Finally, mucin positivity alone cannot establish a primary pulmonary origin and should always be interpreted in conjunction with morphologic, immunophenotypic, clinical, and radiologic findings.

Conclusion

Our findings demonstrate that histochemical mucicarmine positivity provides substantial diagnostic support for adenocarcinoma-oriented subclassification in TTF-1- and p40-negative NSCLC cases diagnosed on small biopsy specimens. As a practical, inexpensive, and tissue-preserving ancillary technique, mucicarmine staining may help reduce the frequency of NSCLC-NOS diagnoses while improving diagnostic confidence in diagnostically challenging small biopsy specimens.

Ethics

Ethics Committee Approval: This study was approved by the Scientific Studies Ethics Committee of University of Health Sciences Türkiye, Ankara Atatürk Sanatorium Training and Research Hospital (approval no: 2024-BÇEK/559, date: 06.05.2026).

Informed Consent: This retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: N.G., F.D., M.B.K.Y., Ç.Y.M., H.A., P.A.K., B.B.İ., Concept: N.G., Design: N.G., Data Collection or Processing: N.G., F.D., M.B.K.Y., Ç.Y.M., H.A., P.A.K., B.B.İ., Analysis or Interpretation: N.G., F.D., M.B.K.Y., Ç.Y.M., Literature Search: N.G., Writing: N.G.

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