

Diagnostic Value of Immunohistochemistry Panel of P63, CK5/6, TTF1, NapsinA in the Diagnosis of Adenocarcinoma and Squamous Cell Carcinoma of Lung

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Abstract

Background: Lung cancer is one of the most frequently diagnosed malignant neoplasms in the world. The pulmonary carcinomas are divided into two major categories, namely small cell lung carcinoma (SCLC) and non-small cell lung carcinoma (NSCLC). Traditionally, the treatment for NSCLC was based on tumor stage, irrespective of histologic subtypes; over the recent years, however, with the development of targeted therapies with different adverse or therapeutic effects on each subtype, it is crucial to correctly subcategorize NSCLC. Utilizing Immunohistochemical (IHC) markers may be conducive to obtain this objective, yet no single marker is sensitive or specific enough to differentiate SCC and adenocarcinoma. Therefore, in the present research, we want to use a panel consisting of P63, CK 5/6, TTF-1, and Napsin A.

Methods: 83 cases of NSCLC (36 adenocarcinoma, 37 squamous cell carcinoma and 10 poorly differentiated carcinoma) were selected. IHC examination for P63, TTF-1, Napsin A, and CK 5/6 were performed on tissue sections obtained from formalin-fixed, paraffin embedded blocks.

Results: TTF1 had 94% sensitivity and 69% specificity with PPV 73% and NPV 92%, and NapsinA had 91% sensitivity and 97% specificity with PPV 97% and NPV 92% as regards the diagnosis of adenocarcinoma. P63 had 100% sensitivity and 84% specificity with PPV 87% and NPV 100%, and CK5/6 had 100% sensitivity and 69% specificity with PPV 78% and NPV 100% in the diagnosis of squamous cell carcinoma.

Conclusion: Using an IHC panel of TTF-1, Napsin A, P63, and CK5/6 it is possible to reliably diagnose poorly differentiated NSCLC with no evident glandular or squamous differentiation.

Keywords: Lung, Adenocarcinoma, Squamous cell carcinoma, Napsin A, TTF1, P63, CK5/6

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Introduction

Lung cancer is among the most frequently diagnosed malignant

neoplasms and the most prevalent cause of cancer mortality in the world.¹ Pulmonary carcinomas

Table 1. List of antibodies used in this study

Monoclonal antibodies	Clone	Dilution	Cellular localization	Source
NapsinA	BC15	Ready to use	Cytoplasmic(granular)	BioCare, USA
TTF1	8G7G3/1	Ready to use	Nuclear	BioCare, USA
CK5/6	EP42&EP67	Ready to use	Cytoplasmic and cell surface	BioCare, USA
P63	4A4	Ready to use	Nuclear	BioCare, USA

are categorized into two major categories, namely small cell lung carcinoma (SCLC) and non-small cell lung carcinoma (NSCLC), further subdivided into squamous cell carcinoma (SCC), adenocarcinoma and large cell carcinoma.² Traditionally, the treatment for NSCLC was based on tumor stage, irrespective of histologic subtypes. However, over the recent years, given the development of targeted therapies with different adverse or therapeutic effects on each subtype, it has become crucial to correctly subcategorize NSCLC,^{3,4} which can be achieved by routine H&E examination in the majority of cases; nevertheless, in certain cancers, particularly poorly differentiated ones, and in specimens with few tumor cells, it may be necessary to conduct ancillary studies for the differentiation of adenocarcinoma and SCC.¹

To reach this objective, it might be helpful to use Immunohistochemistry (IHC) markers, yet no single marker is sensitive or specific enough to differentiate SCC and adenocarcinoma, hence the possible need for a panel of four to six IHC markers for the diagnosis of NSCLC.^{1,5-17} The proposed panel should contain the minimum number of IHC markers with acceptable accuracy because the number of tumor cells in small biopsy specimen may not be enough for the utilization of a wide range of IHC markers.

Thyroid transcription factor-1 (TTF-1) is a marker which stains pneumocyte epithelial cells. Adenocarcinomas are usually positive for TTF-1, while squamous and large cell carcinomas are

rarely positive. Napsin A is an aspartic proteinase expressed in lung and kidney. In lung, it is involved in the maturation of surfactant protein B and it is also expressed in type II pneumocytes and lung adenocarcinomas. Considered as a SCC marker, CK5/6 is a type of high molecular weight cytokeratin widely expressed in pulmonary bronchial epithelial basal cells. Tumor protein p63, a member of the p53 family of transcription factors, plays a key role in regulating epithelial proliferation and differentiation programs. Moreover, its staining in tumoral cells favors SCC, especially in conjunction with CK5/6.¹⁻⁵

Therefore, in the current research, we used a panel comprising P63, CK 5/6 (as markers for SCC) and TTF-1, Napsin A (as markers for adenocarcinoma) for a reliable differential diagnosis of SCC and adenocarcinoma in small biopsy and large specimen.

Materials and Methods

As a cross-sectional study done between 2010 and 2016, the Archive of Surgical Pathology Department of Faghihi and Nemazee Hospitals, affiliated to Shiraz University of Medical Sciences was searched for cases of NSCLC, diagnosed on small biopsy and large specimen (endobronchial, transbronchial, CT guided core biopsies or mass excision). 83 cases of NSCLC (36 adenocarcinoma, 37 SCC and 10 poorly differentiated carcinoma) were selected. The H&E slides were reviewed by a pathologist for confirmation of diagnosis and subsequent grading

Table 2. Frequencies of Napsin A, TTF1, CK5/6 and P63 in different subtypes of lung carcinoma (diagnosis by histologic examination)

	Napsin A	TTF-1	CK5/6	P63
Adenocarcinoma (36)	81% (29/36)	83% (30/36)	36% (13/36)	14% (5/36)
SCC (37)	3% (1/37)	30% (11/37)	95% (35/37)	97% (36/37)
Poorly differentiated carcinoma (10)	20% (2/10)	40% (4/10)	50% (5/10)	40% (4/10)

TTF-1: Thyroid transcription factor-1; SCC: Squamous cell carcinoma

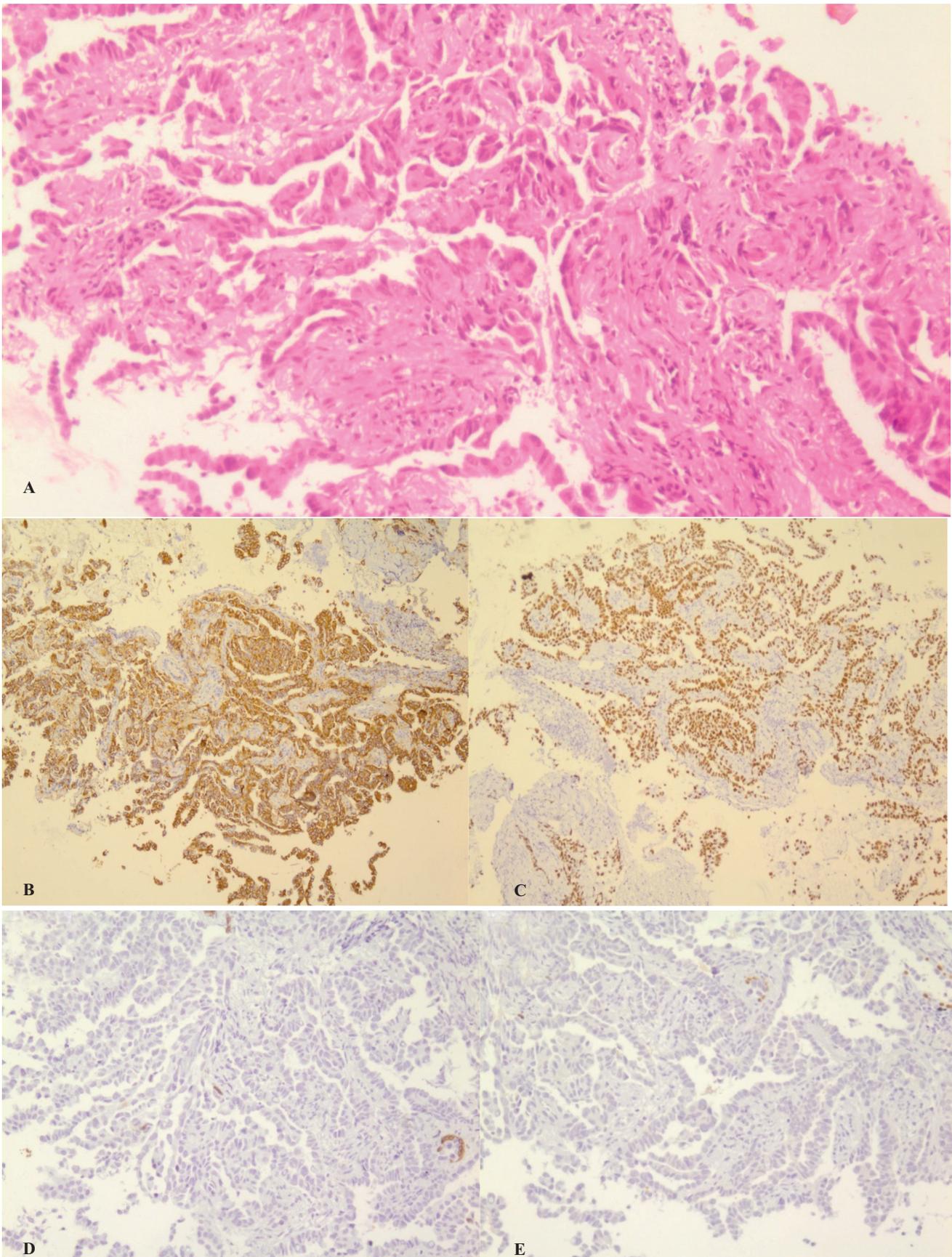


Figure 1 (A-E). Adenocarcinoma, A: routine H&E stain, B: positive NapsinA staining, C: positive TTF-1 staining, D: negative CK5/6 staining, E: negative P63 staining.

Table 3. Subtyping lung carcinoma (diagnosis after IHC examination)

Diagnosis by histologic examination	Subtype after IHC examination
Adenocarcinoma(36)	Adenocarcinoma (27/36) SCC (2/36) Adenosquamous cell carcinoma (3/36) Metastatic (4/36)
SCC(37)	SCC (34/37) Adenosquamous cell carcinoma (1/37) Metastatic (2/37)
Poorly differentiated carcinoma(10)	SCC (6/10) adenocarcinoma (3/10) Metastatic (1/10)

IHC: Immunohistochemistry, SCC: Squamous cell carcinoma

of the tumor. The lesion was regarded as SCC if showing keratinization or intercellular bridges, adenocarcinoma if showing glandular or mucin formation and poorly differentiated carcinoma if showing neither squamous nor glandular differentiation. Ethics Committee of Shiraz University of Medical Sciences approved the study (IR.SUMS.MED.REC.1394.s72).

IHC techniques of staining

IHC examinations of P63, TTF-1, Napsin A, and CK 5/6 were performed on the tissue sections obtained from formalin-fixed (neutral buffered 10% formalin) and paraffin embedded blocks. Table 1 shows the IHC markers which were used in the study.

Immunostained slides were evaluated by a pathologist for immunoreactivity in tumor cells. If more than 10% of the cells were positive for each marker, the reaction was considered as positive. P63 and TTF-1 are nuclear markers, while Napsin A and CK 5/6 are cytoplasmic / membranous.

In each slide, the immunostaining of pneumocyte and basal epithelial layer of bronchi, next to the tumor, were considered as internal positive control for TTF-1 and NapsinA, CK5/6

and P63, respectively

Statistical analysis

Data analysis was done by SPSS15. The diagnosis value (sensitivity, specificity, positive predictive value, and negative predictive value) of immunoreactivity pertaining to each marker and combination of both markers (P63 and CK5/6, Napsin A and TTF-1) were evaluated in comparison to the gold standard represented by the morphologic diagnosis. Percentages were rounded to an integer and may not add up to 100.

Results

In this study, 83 lung carcinoma cases were included and categorized into three groups by histologic examination with different grades of differentiation: 36 adenocarcinoma, 37 SCC, and 10 poorly differentiated carcinoma.

Each case was stained with Napsin A, TTF-1, P63, and CK5/6. The results of IHC expression in each histologic subtype of carcinoma are summarized in table 2.

Adenocarcinoma was mostly immunoreactive for Napsin A (81%) and TTF-1(83%), and the reactivity of these markers was homogeneously strong and diffuse in the majority of cases (Figure

Table 4. Frequencies of Napsin A, TTF-1, CK5/6, and P63 in different subtypes of lung carcinoma (diagnosis following IHC examination) Biomarkers, Number of positive/total (%)

	Napsin A	TTF-1	CK5/6	P63
Adenocarcinoma(27)	96% (26/27)	100% (27/27)	22% (6/27)	0% (0/27)
SCC(36)	0% (0/36)	30% (10/36)	100% (36/36)	100% (36/36)

IHC: Immunohistochemistry; SCC: Squamous cell carcinoma; TTF-1: Thyroid transcription factor-1

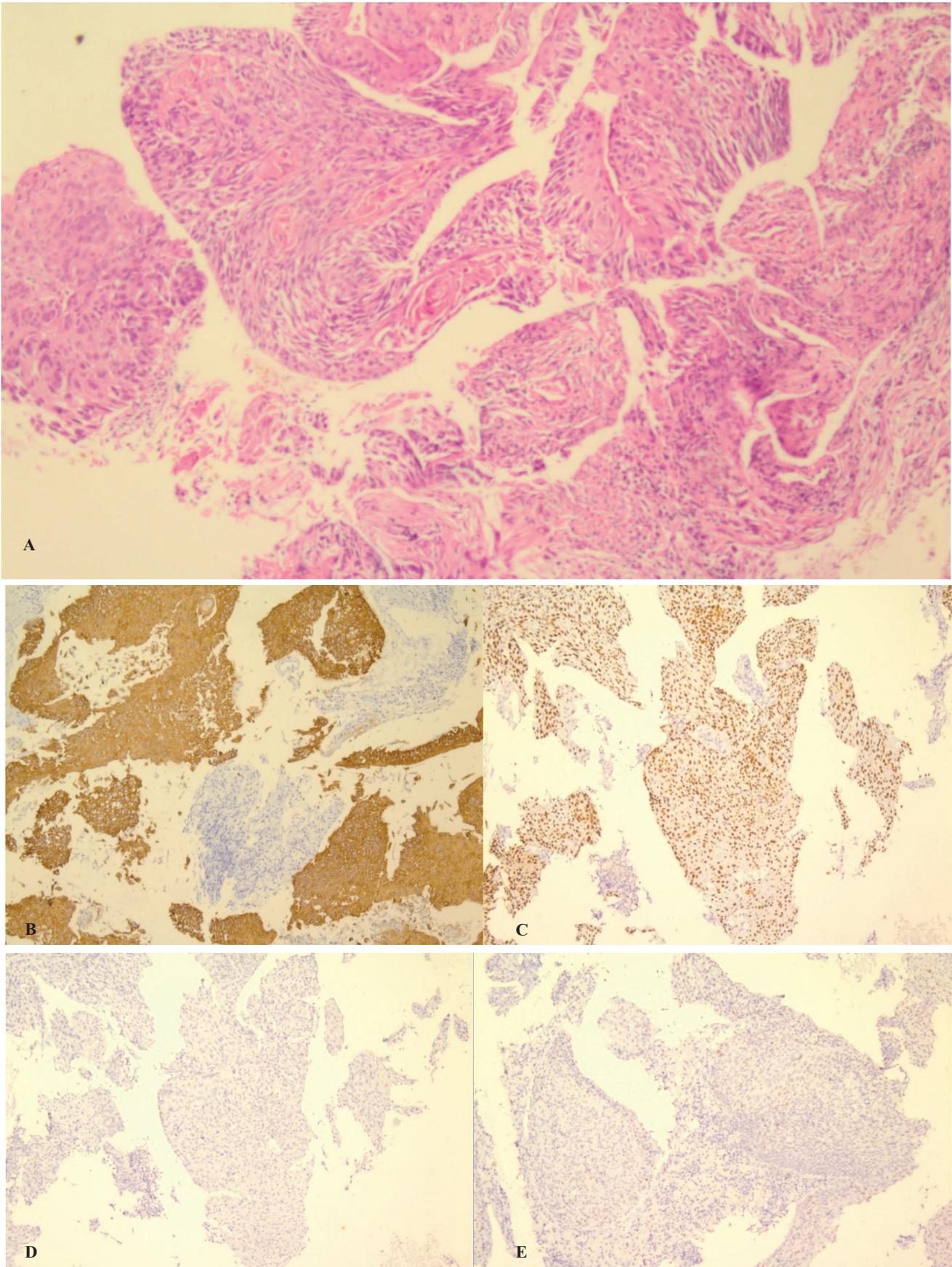


Figure 2 (A-E). Squamous cell carcinoma, A: routine H&E stain, B: positive CK5/6 staining, C: positive P63 staining, D: negative TTF-1 staining, E: negative NapsinA staining.

Table 5. Sensitivity and specificity of Napsin A and TTF-1 in lung adenocarcinoma

	Napsin A	TTF-1
Sensitivity	91%	94%
Specificity	97%	69%
Positive predictive value	97%	73%
Negative predictive value	92%	92%

TTF-1: Thyroid transcription factor-1

1A-E); however, few cases of adenocarcinoma were positive for CK5/6 (36%) and p63 (14%), but the stain intensity were weak in all of these adenocarcinoma cases. On the contrary, SCC cases were immunoreactive for CK5/6 (95%) and P63 (97%) with strong and diffuse pattern (Figure 2A-E); certain cases were positive for TTF-1(30%) while rare cases were positive for Napsin A (1/35, 3%).

After IHC examination, the cases were reclassified. Tumors in which none of the markers were positive were regarded as probably metastatic, hence excluded (Table 3). Once again, the frequency, sensitivity, and specificity, of IHC markers were calculated and summarized in tables 4-8.

Discussion

In this study, we employed a panel comprised of P63, CK 5/6 (as markers for SCC) and TTF-1, Napsin A (as markers for adenocarcinoma) for a reliable differential diagnosis of SCC and adenocarcinoma in small biopsy and large specimen. Several recent studies have evaluated IHC markers to diagnose non-small cell carcinoma; however, given the recent advances in targeted therapies, the subclassification of NSCLC category into adenocarcinoma and SCC is becoming increasingly important.³ In fact, the subclassification can be done via routine H&E examination without IHC in the majority of cases; however, in poorly differentiated cancers and specimens

with few tumor cells, it is difficult and may be necessary to use IHC studies.¹

TTF-1 is highly sensitive (94%) but not specific for adenocarcinoma (69%) due to its weak positivity in some cases of lung SCC (11/37). This marker was highly specific but not very sensitive regarding the diagnosis of lung adenocarcinoma in the study of Zhao et al.¹⁰ and Argon et al.; however, demonstrated 100% sensitivity and specificity with regards to TTF-1.¹²

Napsin A is also a marker for the diagnosis of pulmonary adenocarcinoma.² In the present study, Napsin A was more specific (97%) but slightly less sensitive (91%) than TTF-1 as regards adenocarcinoma; Zhao et al. reported the same result,¹⁰ but Turner et al. observed a higher sensitivity in Napsin A compared with TTF-1 as far as primary lung adenocarcinoma is concerned; moreover, a higher specificity was documented for Napsin A in comparison with TTF-1 concerning primary lung adenocarcinoma versus all tumors, except for kidney which was independent of tumor type.⁹

Combination of adenocarcinoma related markers (of NapsinA and TTF-1 and Napsin A or TTF-1) in our study is sensitive in 90% and less specific for diagnosis of adenocarcinoma. Only one sample in our study was positive for TTF-1 but negative for Napsin A that fulfilled the WHO morphologic criteria for adenocarcinoma.

In our study, although P63 was highly sensitive

Table 6. Sensitivity and specificity of CK5/6 and P63 in lung SCC

	CK5/6	P63
Sensitivity	100%	100%
Specificity	69%	84%
Positive predictive value	78%	87%
Negative predictive value	100%	100%

Sensitivity, specificity, PPV and NPV were calculated in 32 cases of adenocarcinoma and 35 cases of SCC; SCC: Squamous cell carcinoma

Table 7. Sensitivity and specificity of a combination of markers (Napsin A and/or TTF-1) in lung adenocarcinoma

	Napsin A and TTF-1	Napsin A or TTF-1
Sensitivity	91%	94%
Specificity	69%	97%
Positive predictive value	72%	97%
Negative predictive value	89%	94%

TTF-1: Thyroid transcription factor-1

(100%) in terms of SCC, it was not entirely specific (84%) as it was also expressed in adenocarcinoma (14%). Similar results were observed in other studies.¹⁵⁻¹⁷

In previous reports, the sensitivity of p63 ranges from 75% to more than 95%; whereas, specificity is between 70% and 90%.¹³⁻¹⁵

Xiao-yan Xu et al. investigated CK5/6, CK34βE12, p63, TTF-1, and CK7 for differentiation between adenocarcinoma and SSC on biopsy specimens. In their study, P63 displayed the highest sensitivity (97%) and a better specificity (91%).³

In the present study, the immunoreactivity of CK5/6 in SCC was observed to have a similar sensitivity (100%) and a lower specificity compared to P63. Ck5/6 was positive in certain cases of lung adenocarcinoma (13/36). CK5/6 positivity has also been reported in lung adenocarcinoma with focal and weak staining in some other studies.^{5,18} Zhao and Turner et al. showed that CK5/6 was highly specific for the SCC of lung.^{9,10} CK5/6 was established as the most sensitive, though not entirely specific, in squamous differentiation. In addition to SCC, most mesothelioma were reported to be positive for CK5/6, while negative in most lung adenocarcinoma; therefore, it might be a useful marker for the differentiation of pulmonary adenocarcinoma from mesothelioma.⁵

Combination of SCC-related markers (P63 and CK5/6 and P63 or CK5/6) in our study was very sensitive (100%) for SCC diagnosis with

high negative predictive values (100%).

In Xiao-yan Xu et al., combination of CK5/6, CK34βE12, p63 showed 100% sensitivity and 98% specificity in diagnosing SCC, and in case of limited biopsy specimens, a combination of CK5/6 and P63 was recommended.³

In the current research, the panel of IHC stains, including Napsin A, TTF-1, P63, and CK5/6 allowed for a correct subclassification in more than 90% of SCC (34/35) and more than 80% (27/32) of adenocarcinoma.

Zhao et al. showed that the use of a panel of IHC stains, including (P63, CK5/6, TTF-1, and Napsin A) resulted in correct subclassification in more than 80% of the cases.¹⁰

In small specimens, while it is necessary to conserve the tissue for potential predictive testing, the optimal diagnostic algorithm, particularly the minimal marker combination, is useful for a definitive diagnosis.⁴

In our study, Napsin A was more specific than TTF-1 regarding the diagnosis of lung adenocarcinoma and P63, having more specificity than CK5/6, showed significant immunoreactivity with regards to lung SCC.

It is proposed that a two-marker panel of Napsin A/P63 is sufficient for subtyping a majority of tumors as adenocarcinoma versus SCC and addition of CK5/6 and TTF-1 is required in a small subset of cases. Whithaus et al. also documented a panel consisting of Napsin A/P63 with a specificity of 94% and a sensitivity of 96% for differentiating adenocarcinoma from

Table 8. Sensitivity and specificity of a combination of markers (CK5/6 and/or P63) in lung squamous cell carcinoma

	CK5/6 and P63	CK5/6 or P63
Sensitivity	100%	100%
Specificity	66%	87%
Positive predictive value	76%	90%
Negative predictive value	100%	100%

SCC.⁸

Natasha et al. recommended a two-marker panel of TTF-1/P63 for subtyping a majority of tumors as adenocarcinoma versus SCC.⁴

Terry et al. examined the expression of nine markers (P63, TTF-1, CK5/6, CK7, 34βE12, napsinA, mucicarmine, NTRK1, and NTRK2) on 200 cases of adenocarcinoma and 225 SCC cases of small tissue specimen. They recommended P63, TTF-1, CK5/6, CK7, Napsin A, and mucicarmine as the optimal panel to separate adenocarcinoma from SCC. They stated that the reduction in the panel to P63, TTF-1, CK5/6, and CK7 was less effective but possibly the best alternative when the tissue is limited.⁷

Conclusion

Based on the results of our study, it is suggested that IHC studies are necessary in all cases of NSCLC, in which no glandular morphologic feature or squamous morphologic features are identified after the examination of H&E stained slides.

Our recommended IHC profile is composed of Napsin A, TTF-1, CK5/6, and P63. Moreover, the most cost-effective tissue-preserving panel for small biopsy specimens regarding the differential diagnosis of lung adenocarcinoma versus SCC is a combination of P63 and Napsin A.

Conflict of Interest

None declared.

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