

IMMUNOHISTOCHEMICAL APPROACH TO DIFFERENTIAL DIAGNOSIS OF NON-SMALL CELL LUNG CARCINOMA IN BRONCHOSCOPIC BIOPSY SPECIMENS

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Non-small cell lung carcinoma (NSCLC) includes squamous cell carcinoma, adenocarcinoma and large cell carcinoma. Subtyping of NSCLC is essential for therapy. Classification of NSCLC into more specific histological subtypes is carried out by morphologic examination or immunohistochemistry.

The aim of this study was immunohistochemical analysis of NSCLC (squamous cell carcinoma and adenocarcinoma) in bronchoscopic biopsies.

Formalin-fixed, paraffin-embedded bronchoscopic mucosal samples from 40 patients with NSCLC (20 patients with squamous cell carcinoma and 20 patients with adenocarcinoma) were retrieved from pulmonary pathology archives at Center for Pathology and Pathological Anatomy, Clinical Center Niš. Serial histological sections of 4 μm thickness were stained with hematoxylin and eosin, and immunohistochemical method DAKO LSAB for TTF-1, p63, and CK5/6 antibodies.

Positive immunoreactivity for p63 was found in 95% of squamous cell carcinomas (19/20), while for CK5/6 in 90% of squamous cell carcinomas (18/20), and in 5% of adenocarcinomas (1/20). In 80% of adenocarcinomas (16/20), a positive TTF-1 immunophenotype was found, while all squamous cell carcinomas were negative for this marker (0/20).

Immunohistochemical analysis (panel p63, CK5/6 and TTF-1) is a useful ancillary tool for distinguishing squamous cell lung carcinoma from adenocarcinoma in bronchoscopic biopsy specimens. *Acta Medica Medianae* 2016;55(2):31-34.

Key words: immunohistochemistry, non small-cell lung carcinoma, squamous cell lung carcinoma, adenocarcinoma

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Introduction

Lung cancer classifications by the World Health Organization (WHO) have traditionally been based on the histological characteristics of resected tumors with little guidance about diagnosis based on small biopsies. The focus has mainly been on the separation of small cell lung carcinoma (SCLC) and non-small cell lung carcinoma (NSCLC) (1). NSCLC comprises approximately 80 % of lung carcinoma. NSCLC includes several histological types, most commonly adenocarcinoma or squamo-

us cell carcinoma. Until recently, there have been no therapeutic implications for further classification of NSCLC, so little attention has been given to the distinction of adenocarcinoma and squamous cell carcinoma. The emergence of treatments with differential activity (e.g., pemetrexed) or limited indication (e.g., bevacizumab) in subtypes of NSCLC has placed a new emphasis on the importance of accurate subtyping (2).

Classification of NSCLC into more specific histological subtypes is carried out by morphologic examination or immunohistochemistry.

The aim of this study was immunohistochemical analysis of NSCLC (squamous cell carcinoma and adenocarcinoma) on bronchoscopic biopsies.

Material and methods

Formalin-fixed, paraffin-embedded bronchoscopic mucosal samples from 40 patients with NSCLC (20 patients with squamous cell carcinoma and 20 patients with adenocarcinoma) were retrieved from pulmonary pathology archives at Center for Pathology and Pathological Anatomy,

Clinical Center Niš. Serial histological sections of 4 μ m thick-ness were stained with hematoxylin and eosin, and with a panel of antibodies using the labeled streptavidin biotin-peroxidase complex method. The primary antibodies used were monoclonal antibodies for thyroid transcription factor-1 (TTF-1), P63 and cytokeratin 5/6. The chromagen was 3, 3'-diaminobenzidine (DAB), and the slides were lightly counterstained with Meyer's hematoxylin. All re-agents were acquired from the Dako Company (Glostrup, Denmark).

Results

Positive immunoreactivity for p63 was found in 95% of squamous cell carcinomas (19/20), while for CK5/6 in 90% of squamous cell carcinomas (18/20) and in 5% of adenocarcinomas (1/20). In 80% of adenocarcinomas (16/20), a positive TTF-1 immunophenotype was found, while all squamous cell carcinomas were negative for this marker (0/20) (Figures 1-3).

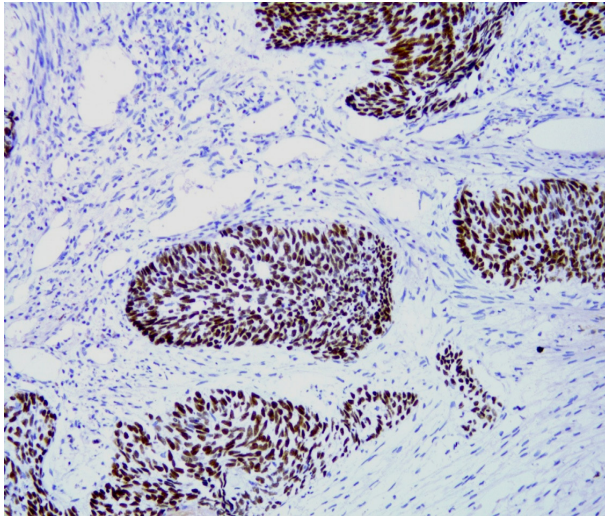


Figure 1. Squamous cell carcinoma of the lung. p63 expression (original magnification, x200).

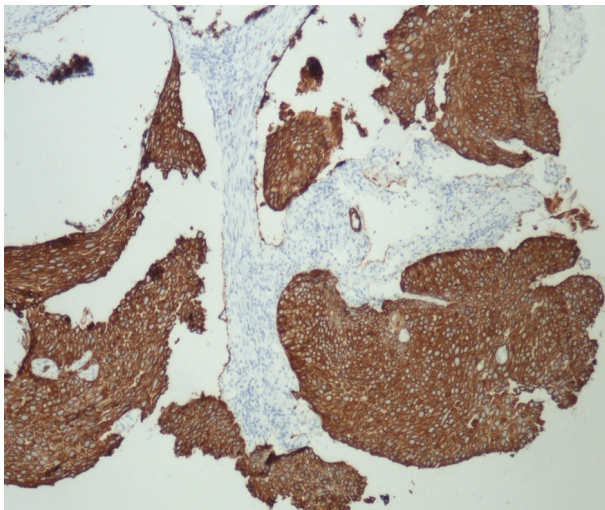


Figure 2. Squamous cell carcinoma of the lung. CK5/6 expression (original magnification, x100).

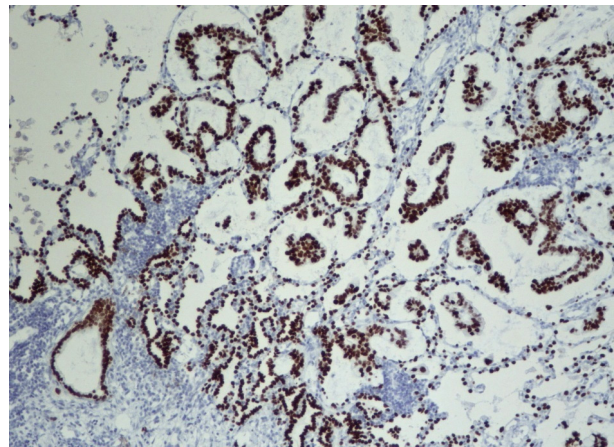


Figure 3. Adenocarcinoma of the lung. TTF1 expression (original magnification, x100).

Discussion

World Health Organization (WHO) (2004) (3) identifies multiple forms of NSCLC, but the major forms are squamous cell carcinoma, adenocarcinoma and large cell carcinoma. The distinction of squamous cell carcinoma and adenocarcinoma is based on morphologic criteria, with keratinization and intercellular bridges (hallmarks of squamous cell carcinoma), and glandular architecture or cytoplasmic mucin (hallmarks of adenocarcinoma). However, distinction can be difficult in some poorly differentiated tumors, where glandular or squamous features are unremarkable. On the other hand, 70% of NSCLC presents at an unresectable stage when the only diagnostic material guiding systemic therapy in the majority of such patients are small specimens (4).

Unlike previous WHO classifications where the primary diagnostic criteria were based on hematoxylin and eosin examination of resected tumors, the International multidisciplinary classification of lung adenocarcinoma (2011) emphasizes the use and integration of immunohistochemical, histochemical, and molecular studies, as specific therapies are driven histological subtyping. This International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society (IASLC/ATS/ERS) lung adenocarcinoma classification addresses an approach to small biopsies and cytology in lung cancer diagnosis (5, 6). An algorithm for adaptation of the classification of small biopsies and cytology using immunohistochemical markers has been proposed (adenocarcinoma marker *i.e.*, thyroid transcription factor -TTF-1 and mucin stain, squamous marker p 63 and/or CK5/6 staining (7), as well as recommendations on molecular testing (*e.g.*, epidermal growth factor receptor mutations). NSCLC may be further classified into a more specific type, such as adenocarcinoma or squamous cell carcinoma, whenever possible. The term large cell carcinoma should not be used for the diagnosis in small biopsy and should be restricted to resection specimens

where the tumor is thoroughly sampled to exclude a differentiated component (5). The term NSCLC-not otherwise specified (NSCLC-NOS) should be used as little as possible, and it should be applied only when a more specific diagnosis is not possible by morphology and/or special stains. Neuroendocrine (NE) immunohistochemical markers (CD56, chromogranin, and/or synaptophysin) should only be performed in cases where there is suspected NE morphology.

In accordance with these recommendations, we used a panel of immunohistochemical markers p63, CK5/6 and TTF-1 and analyzed 20 squamous cell carcinomas and 20 adenocarcinomas of the lung and compared our results with literature data. Similarly to our findings, Rekhtman et al. (2011) (4) showed that squamous cell carcinoma had a highly consistent immunoprofile (TTF-1-negative and p63/CK5/6-diffuse positive) with only rare variation. In contrast, adenocarcinoma showed significant immune heterogeneity for all "squamous markers" (p63 (32%), CK5/6 (18%)), and TTF-1 (89%). As a single marker, only diffuse TTF-1 was specific for adenocarcinoma whereas none of the "squamous markers", even if diffuse, were entirely specific for squamous cell carcinoma. In contrast, coexpression profiles of TTF-1/p63 had only minimal overlap between adenocarcinoma and squamous cell carcinoma, and there was no overlap if CK5/6 was added as a third marker. Kargi et al. (2007) (8) also used the same panel of antibodies: TTF-1, p63 and CK5/6 for the identifi-

cation of the glandular and squamous differentiation in bronchoscopic samples. In their study, 32 of 39 squamous cell carcinomas had a negative TTF-1/p63 positive immunoprofile. Adenocarcinomas were negative for p63 (10/10), and most (8/10) of them for CK5/6. In our study, all 20 squamous cell carcinomas were negative for TTF-1 and only 1 of 20 adenocarcinomas was positive for CK5/6. Sensitivity of CK5/6 for squamous cell carcinoma in different studies ranges from 75-100% (8-10). This marker has high specificity for squamous cell carcinoma, although the positivity was also found in a small percentage (2-8%) of adenocarcinomas (9).

TTF-1 has been used as an immunohistochemical marker for primary lung adenocarcinoma (11-15). Many studies have demonstrated TTF1 expression in 70% to 85% of resected lung adenocarcinomas. In the context of small biopsy or cytology samples, TTF-1 stains are positive between 60% and 92%. In the present study, we found TTF-1 positivity in 80% of adenocarcinoma cases in bronchoscopic biopsy specimens.

Conclusion

The proposed panel of immunohistochemical markers might help classification of NSCLC for identifying cell differentiation lineages (glandular or squamous). p63, CK5/6 and TTF-1 are useful ancillary tools in distinguishing squamous cell lung carcinoma from adenocarcinoma in bronchoscopic biopsy specimens.

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IMUNOHISTOHEMIJSKI PRISTUP U DIFERENCIJALNOJ DIJAGNOSTICI NESITNOĆELIJSKOG KARCINOMA PLUĆA U BRONHOSKOPSKOJ BIOPSIJI

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"Non-small cell" karcinom pluća (NSCLC) obuhvata planocelularni karcinom, adenokarcinom i makrocelularni karcinom. Subtipizacija NSCLC je od presudnog značaja za terapiju. Klasifikacija NSCLC u specifične podtipove vrši se morfološkom analizom ili imunohistohemijom.

Cilj ovog rada bio je imunohistohemijska analiza NSCLC (planocelularnog karcinoma i adenokarcinoma) u bronhoskopskim biopsijama.

Bronhoskopske biopsije, fiksirane u formalinu i kalupljene u parafinu, 40 bolesnika sa NSCLC (20 sa planocelularnim karcinomom i 20 sa adenokarcinomom pluća) izdvojene su iz arhiva za patologiju pluća Centra za patologiju i patološku anatomiju Kliničkog centra u Nišu. Serijski histološki isečci debljine 4 µm bojeni su hematoksilin-eozinom i imuno-histo-hemijskom metodom DAKO LSAB na TTF-1, p 63 i CK5/6 antitelo.

Pozitivna imunoreaktivnost na p 63 nađena je kod 95 % planocelularnih karcinoma (19/20), dok je na CK5/6 nađena kod 90% planocelularnih karcinoma (18/20) i 5% adenokarcinoma (1/20). Kod 80% adenokarcinoma (16/20) nađen je pozitivan TTF-1 imunofenotip, dok su svi planocelularni karcinomi bili negativni na ovaj marker (0/20).

Imunohistohemijska analiza (panel antitela p63, CK5/6 i TTF-1) je korisna dopunska metoda u diferenciranju planocelularnog od adenokarcinoma pluća na bronhoskopskim biopsijskim uzorcima. *Acta Medica Medianae* 2016;55(2):31-34.

Ključne reči: *imunohistohemija, "non-small cell" karcinom pluća, planocelularni karcinom, adenokarcinom*

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