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Immunohistochemical Approach to the Diagnosis of Adenocarcinoma of the Lung

Jelena Stojsic*

Department of Thoracopulmonary Pathology, Service of Pathohistology, Clinical Center of Serbia, Serbia

Abstract

Introduction: Differentiation between adenocarcinoma of the lung (LADC) from the other non-small cell lung carcinoma (NSCLC) is possible by immunohistochemistry. When diagnosis of LADC is established, the rest of lung cancer tissue could be used for molecular testings, particularly EGFR analysis

Aim: To evaluate 4 antibodies TTF-1, Napsin-A, Cytokeratin7 and Surfactant B in diagnosis of LADC.

Material and method: Fifty small-sized samples, obtained upon bronchoscopy or transthoracic fine-needle lung biopsy, were included in this study. Diagnosis of LADC was performed after routine hematoxylin-eosin examination and TTF-1, Napsin-A, Surfactant B and Cytokeratin7 immunohistochemistry. Descriptive statistical method (%) was used in the study.

Results: Cytokeratin7 was expressed in 90% (45/50), than TTF-1-86% (43/50) and Napsin-A - 82% (41/50) and Surfactant B in 6% (28/50) in LADCs.

Conclusion: TTF-1 is the most useful for diagnosis of LADC and Napsin-A in cases where TTF-1 is not expressed. Cytokeratin7 is useful in differentiation from digestive adenocarcinoma and Surfactant B in cases where TTF-1 and Napsin-A, individually or both are not expressed.

Introduction

Lung cancer is the first cause of mortality from malignancy worldwide. Adenocarcinoma of the lung (LADC) patients increased in number in the past few decades [1,2]. According to 2004, World Health Organization lung carcinoma classification, adenocarcinoma is defined as malignant epithelial tumor with glandular morphological pattern or mucus secretion. They show acinar, papillar, solid, bronchioloalveolar or mixture pattern. International Association for the Study of Lung Cancer excluded bronchiloalveolar carcinoma and mixture morphological pattern of adenocarcinoma in use. Invasive adenocarcinoma of the lung included: lepidic, acinar, papillary, micropapillary and solid subtypes [3,4].

Despite of growth pattern of LADC, its immunophenotype remained the same. In diagnostic algorithm of LADC is emphasized the significance of Thyreoid-Transcriptive-Factor-1 (TTF-1) for differentiation LADC from the other non-small cell lung carcinoma. Cytokeratin7 confirmed lung origin of adenocarcinoma, excluding digestive system origin. Terry et al. suggested TTF-1, Cytokeratin7 and Napsin-A for differentiation adenocarcinoma of the lung from squamous cell carcinoma. Napsin-A and Surfactant B with high specificity for adenocarcinoma of the lung confirm its origin [5].

It means that in cases of poorly differentiated LADC after routinely hematoxyllin-eosin (H&E) analysis and immunohistochemical staining and final diagnosis, the rest of lung cancer tissue could be used for molecular testings on small tissue samples. Oncological treatment depends of histopathological diagnosis on small tissue samples in majority of patients. Only 1/6 of our patients went under surgery after histopathological diagnosis on small tissue samples according to our previous investigations [6,8]. Molecular testings, particularly EGFR analysis could be performed on paraffin blocks after histopathological diagnosis.

In adenocarcinoma, the most common EGFR mutations are deletions in exon 19 and the L858R point mutation in exon 21. In adenocarcinoma, the prevalence of EGFR mutation is 45% in Pacific Asians and 24% in Caucasians, significantly more frequent in never smokers, women. EGFR mutations were significantly associated with adenocarcinoma in situ, minimally invasive adenocarcinoma, and lepidic- and papillary-predominant adenocarcinoma subtypes, whereas they were rarely detected in mucinous subtype tumors [8-11].

The ALK gene rearrangement is a recently identified, in non-small cell lung carcinoma. EML4-ALK rearrangement is present in about 4% of all non-small cell lung carcinoma. It is more frequent in younger patients, never smokers, and predominantly solid adenocarcinoma and signet ring cell subtypes. The guidelines recommend a stepwise-testing: first, analyses of the most common mutations, EGFR and KRAS, are performed and, if the results of these are negative testing for ALK rearrangement [9-11]. Personalized lung cancer therapy is based on the individual access to the patient and avoid the toxic effects of chemotherapy improving quality of life and prolongate survival rate [8,11].

Aim

To find out optimal immunohistochemical markers for diagnosis

***Corresponding author:** Jelena Stojsic, Service of Pathohistology, Clinical Center of Serbia, Serbia, Tel: 381 11 3663485; Fax: +381 11 2681591; E-mail: dr.jelenastoj@sezampro.rs

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of LADC on small tissue samples and save enough tumor tissue for the most common molecular testings.

Material and Method

Fifty biopsy samples were included in our retrospective single center study. Small-sized biopsy samples were obtained upon bronchoscopy or transthoracic fine-needle lung biopsy. Diagnosis of LADC was performed after routine H&E examination and immunohistochemistry. These biopsy samples were processed and diagnosed at the Department of thoracopulmonary pathology, Service of Pathology, Clinical Center of Serbia in Belgrade, in 2011.

Tissue samples for immunohistochemical staining are deparaffinized according to the proposed procedure, incubated with the specific serum at room temperature in moist chamber for 30 to 60 minutes. The cell nuclei were contra stained with Mayer's haematoxylin. A positive control was used.

Monoclonal antibodies analyzed in the study:

1. TTF-1 - DAKO Cytomation, Denmark;
2. Cytokeratin7 - DAKO Cytomation, Denmark;
3. Napsin-A - NOVACASTRA™ HD Leica Biosystems, UK and
4. Surfactant B - NOVACASTRA™ HD Leica Biosystems, UK.

Monoclonal antibodies used in this study, their clone, solution and reaction are given in Table 1.

Presence and absence of immunoreactivity in the tumor cells were marked as (1) and (0), respectively. Diagnostic dilemma was resolved by the second or, if necessary, by the third pathological opinion. Descriptive statistical method (%) was used in the study.

Results

Nº	Monoclonal antibody	Clone	Solution	Reaction
1.	Thyroid Transcription Factor-1	8G7G3/1	1:100	Nuclear
2.	Napsin-A	IP64	1:400	Cytoplasmatic
3.	Cytokeratin7	OV-TL 12/30	1:100	Cytoplasmatic
4.	Surfactant B	19H	1:50	Cytoplasmatic

Table 1: Used antibodies for diagnosis of LADC.

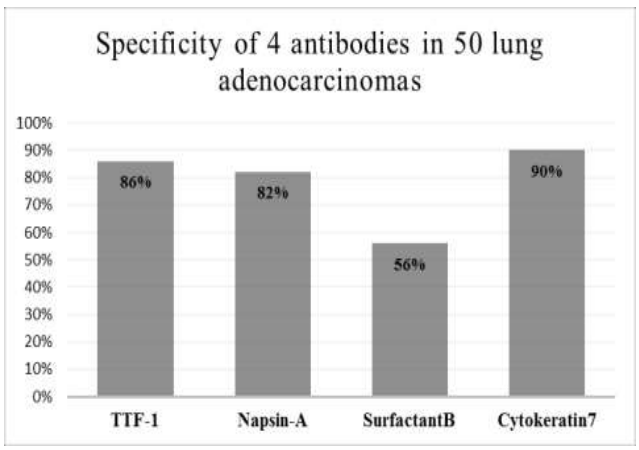

Chart 1: Specificity of 4 antibodies in 50 adenocarcinoma of the lungs.

No.	Monoclonal antibody				Diagnosis
	TTF-1	Napsin-A	SurfactantB	Cytokeratin7	
1.	1	1	0	1	ADC
2.	0	1	0	1	ADC
3.	1	1	1	1	ADC
4.	1	1	0	1	ADC
5.	1	1	1	1	ADC
6.	0	1	1	0	ADC
7.	1	1	0	1	ADC
8.	1	1	1	0	ADC
9.	1	1	1	1	ADC
10.	1	1	0	1	ADC
11.	1	0	0	1	ADC
12.	1	1	0	0	ADC
13.	1	1	1	1	ADC
14.	1	1	0	0	ADC
15.	0	1	0	1	ADC
16.	1	1	1	1	ADC
17.	1	1	1	0	ADC
18.	1	1	1	1	ADC
19.	1	1	1	1	ADC
20.	1	1	1	1	ADC
21.	1	1	0	1	ADC
22.	1	1	0	1	ADC
23.	1	1	1	1	ADC
24.	1	0	0	1	ADC
25.	1	1	1	1	ADC
26.	1	1	1	1	ADC
27.	1	1	0	1	ADC
28.	1	1	1	1	ADC
29.	1	1	1	1	ADC
30.	1	0	1	1	ADC
31.	1	0	0	1	ADC
32.	1	1	1	1	ADC
33.	1	1	1	1	ADC
34.	1	1	0	1	ADC
35.	1	0	0	1	ADC
36.	1	1	0	1	ADC
37.	1	0	0	1	ADC
38.	1	1	1	1	ADC
39.	1	1	1	1	ADC
40.	0	1	1	1	ADC
41.	1	1	0	1	ADC
42.	0	1	1	1	ADC
43.	1	0	1	1	ADC
44.	1	1	0	1	ADC
45.	0	1	1	1	ADC
46.	1	0	0	1	ADC
47.	0	1	1	1	ADC
48.	1	1	0	1	ADC
49.	1	1	1	1	ADC
50.	1	0	1	1	ADC
Total	43	41	28	45	50

Abbreviations: No: Number of Sample; TTF-1: Thyreoid-Transcriptve-Factor-1; ADC: Adenocarcinoma; 1: Positive; 0: Negative

Table 2: Immunophenotype of LADC diagnosed on 50 small-sized lung samples.

TTF-1 specificity was 86% (43/50), Napsin-A - 82% (41/50), Surfactant B - 56% (28/50) and Cytokeratin7- 90% (45/50) in ADCs (Chart 1). Two monoclonal antibodies were positive in 24% (12/50) ADCs, one of them was necessary TTF-1 or Napsin-A. Three

monoclonal antibodies were positive in 40% (20/50) and all 4 in 36% (18/50) ADCs, respectively.

Immunoprofile of each of investigated 50 LADCs is given in Table 2. Positivity of two antibodies was enough for diagnosis of LADC on 22.0% (11/50), three was enough on 36.0% (18/50) and all four on 42% (21/50) biopsies, respectively. Positive immunohistochemical staining of each of 4 antibodies is present in Figure 1. Useful immunohistochemical algorithm for diagnosis of LADC is shown in Figure 2.

Discussion

Thyroid Transcription factor-1 (TTF-1) is a transcription factor protein expressed in the nucleus. This protein is expressed in thyroid, pneumocytes, thyroid tumors and LADC. According to the WHO Classification of the Lung Tumours Blue Book (Travis 2004), TTF-1 is expressed in 75% of adenocarcinoma. TTF-1 expression varied in different studies, from 80% LADC [12] and 88% LADC [5] to 100% [13]. TTF-1 was positive in 82.4% LADC [14].In our previous study TTF-1 expression was evidenced in 85.2% of LADC [15], with similar result in this one (86%).

Cytokeratin7 is a basic 54-kD type II keratin presents in a single-layer pseudostratified epithelium as well as in ductal epithelium and mesothelial cells. This is an intensive and diffuse cytoplasmic staining. Cytokeratin7 is widely (100%) expressed in LADC (Mukhopadhyay & Katzenstein and Tan & Zander studies). In combination with TTF-1, Cytokeratin7 is useful for diagnosis of LADC and differential diagnosis of adenocarcinoma of digestive origin [12,16].

Napsin is an aspartic protease and belongs to pepsin family. Using *in situ hybridization* napsin mRNA was detected in pneumocytes type II, proximal renal tubules and B lymphocytes. Human genes for napsin have two isoforms, A which is expressed in kidney and lung, and B expressed in the spleen. Its expression is revealed in alveolar macrophages but could be expressed in renal, thyroid, breast, biliary tract and colon and endometrial carcinoma exocrine part of pancreas. Napsin-A expression appears to be granular [17]. Kadivar and Boozari considered that Napsin-A is useful marker for differentiation primary lung from metastatic adenocarcinoma because it was positive in all 18 (100%) lung adenocarcinoma [18]. Napsin-A was expressed in 58% (11/19) of LADC [12] and is useful for diagnosis of LADC in cases where TTF-1 was not expressed [14]. Combination TTF-1 and Napsin-A increased sensitivity for diagnosis of LADC [19]. In our study Napsin-A was expressed in 5 LADCs where TTF-1 was not.

Pulmonary surfactants contain a number of proteins included surfactant proteins. But, surfactant protein A and B are not specific only for LADC. They are expressed in 63% LADC and 46% metastatic adenocarcinoma. *Surfactant protein B* is a 79-amino acid, hydrophobic peptide associated with surfactant phospholipids. It is synthesized as a preproprotein by alveolar type II epithelial cells and non-ciliated bronchiolar (Clara) cells. This antibody was suggested in textbooks of pulmonary pathology, as this may assist in the identification of LADC [20,21].

Limitation of this study is a small number of lung tissue samples but in the future investigations we will continue to use antibodies related to diagnosis of LADC. Beside only 50 investigated LADC, our results are similar to those in respectable literature.

In conclusion, TTF-1 is the most useful antibody for diagnosis and differential diagnosis LADC because of its highest specificity. Napsin-A is useful antibody because of its high specificity and positivity in cases

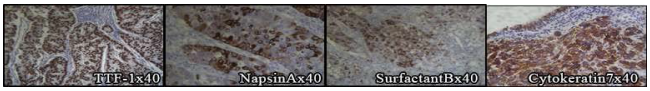

Figure 1: Positive immunostaining of 4 antibodies in adenocarcinoma of the lung.

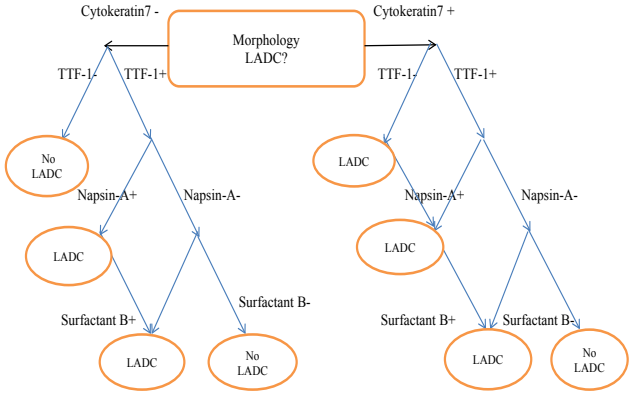

Figure 2: Diagnostic algorithm of lung adenocarcinoma based on immunohistochemistry on 50 small-sized biopsies.

where TTF-1 is negative. Cytokeratin7 is high specific antibody, widely useful in differentiation from digestive adenocarcinoma and in cases where TTF-1 and Napsin-A are not expressed in LADC. Surfactant B is less specific antibody and useful where one or two of the rest three investigated antibodies are not expressed in LADC.

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