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ORIGINAL ARTICLE

Contribution of immunohistochemistry in the differential diagnosis of non-small cell lung carcinomas on small biopsy samples

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Summary

Purpose: Targeted therapy increases survival and the quality of life of non-small cell lung cancer (NSCLC) patients but it needs precise histological subtyping. The present study evaluated 6 monoclonal antibodies for the differential diagnosis of NSCLC on small-sized tissue samples.

Methods: 50 small-sized tissue samples were obtained by bronchoscopy or fine needle aspiration biopsy (FNAB). According to morphology before immunohistochemistry 2 squamous cell carcinomas (SCC), 6 adenocarcinomas (AC), 9 NSCLC-probably SCC, 11 NSCLC-probably AC and 22 unclassified NSCLCs were diagnosed. Thyroid transcription factor-1 (TTF-1), cytokeratin 5/6, cytokeratin 7, p63, and the neuroendocrine markers CD56 and synaptophysin were used in the differential diagnosis of NSCLC.

Results: After immunohistochemistry 13 (26.0%) SCC, 27 (54.0%) AC, 3 (6.0%) NSCLC with neuroendocrine differentiation (NSCLC-NE) and 7 (14.0%) NSCLC- unclassified were diagnosed. Twenty-two NSCLC- unclassified were further diagnosed as SCC (n=7), AC (n=7) NSCLC-NE (n=2) and 6 remained NSCLC- unclassified. Significant difference was found between definitely diagnosed 8 NSCLCs and 15 ACs (20.5 vs. 38.5%, p=0.008). TTF-1 and cytokeratin 7 were expressed in 85.2% (23/27) of AC, and cytokeratin 5/6 and p63 in 100% (13/13) of SCC. Positivity of CD56 and synaptophysin in 3 NSCLC determined NSCLC-NE.

Conclusion: No one monoclonal antibody is totally specified for one histological type of tumor and its origin. Combination of TTF-1, cytokeratin 7, p63, cytokeratin 5/6, CD56 and synaptophysin allows for differentiation of NSCLC but Napsin-A for AC differentiation and chromogranin A for NSCLC-NE differentiation should be added in an optimal panel.

Key words: diagnosis, immunohistochemistry, lung cancer, monoclonal antibodies

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Introduction

Lung carcinoma is a leading cause of death both in our country and worldwide. Current therapies of lung carcinoma are based on individual patient approach, hopefully leading to prolonged survival and improved quality of life, both during the oncological treatment and thereafter. Individual approach to treatment of NSCLC comprises precisely established histological subtype. In addition to its characteristic morphological findings, each histological subtype of NSCLC also displays a specific immunophenotype [1-5].

The vast majority of NSCLCs is diagnosed on small bioptic specimens obtained by bronchoscopy or transthoracic FNAB, while surgical biopsy is performed only in a small number of them. Over the last 20 years in our laboratory lung cancer is diagnosed approximately 6 times more frequently on small samples than on surgical bioptic specimens [6,7].

The previously used classification of NSCLCs is replaced by the current 2004 World Health Organization classification (Table 1) [5].

The purpose of this study was to examine the accuracy of diagnosis of NSCLCs on small bioptic samples based first on morphological findings, and then after applying immunohistochemical staining using specific monoclonal antibodies to discriminate the various histological subtypes of NSCLCs and to propose an optimal panel of the monoclonal antibodies to be used for precise diagnosis of NSCLCs.

Methods

The analysis included 50 small bioptic samples obtained by bronchoscopy or transthoracic FNAB of the lungs, based on which diagnosis of the most probable histological type of the carcinoma was established, while the definitive subtype was determined after immunohistochemistry. Bioptic samples were processed and studied at the Department of Thoracopulmonary Pathology, Service of Pathology, Clinical Center of Serbia in Belgrade, during 2010-2011.

The subtypes of NSCLCs included in this analysis were based first on the morphological appearance of the carcinoma after standard processing and hematoxylin-eosin staining as follows:

1. Squamous cell lung adenocarcinoma (SCC)
2. Adenocarcinoma (AC)
3. Large cell lung carcinoma with neuroendocrine differentiation (LCLC, NE).
4. Other NSCLCs with unclassified type or not otherwise specified (NSCLC, NOS).

Most probable types of NSCLCs were:

1. SCC – when some other histological type of carcinoma was previously diagnosed.
2. Probable SCC – with lower grade of differentiation.
3. AC – in patients with previously diagnosed AC in other sites.
4. Probable AC – with lower grade of differentiation.
5. Other NSCLCs with unclassified type or NOS.

Immunostaining

Immunostaining was performed by incubating tissue sections with appropriate sera for 30-60 min at room temperature in a hotplate humidity chamber using the streptavidin/biotin method (DAKO Cytomation, Denmark; NOVACASTRA™ HD Leika Biosystems, UK; and Thermo Scientific Lab Vision, USA). The cell nuclei were stained with Mayer's heamatoxylin. At the same time a tissue sample with appropriate positive immunostaining was used as indicator that the target retrieval procedure was carried out correctly.

Table 1. Current 2004 World Health Organization classification of non-small cell lung carcinomas

Histological type of NSCLCs
Squamous cell carcinoma
Adenocarcinoma
Large cell carcinoma
Adenosquamous carcinoma
Sarcomatoid carcinoma
Carcinoid tumors
Salivary gland tumors

Table 2. Characteristics of monoclonal antibodies used in this study

Antibody and clone	*Manufacturer*	*Solution*	*Reaction*
CD56/ NCAM-1 Ab-2	LabVision	1:100	Cell membrane
Cytokeratin5/6, CloneD5/16B4	DAKO	1:30	Cytoplasm
Cytokeratin 7, Clone OV-TL 12/30	DAKO	1:100	Cytoplasm
p63 Protein, Clone 7JUL	Novocastra	1:50	Nucleus
Synaptophysin, Clone 27G1	Novocastra	1:100	Cytoplasm
TTF-1,Clone 8G7G3/1	DAKO	1:100	Nucleus

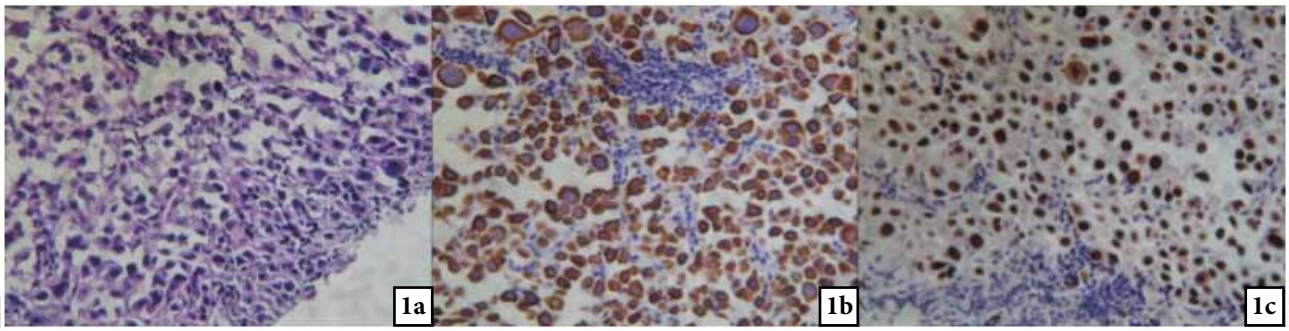

Figure 1. Poorly differentiated squamous cell lung carcinoma; morphology and immunophenotype, transbronchial biopsy: a) H&E x40; b) Cytokeratin5/6 x40; c) p63 x40.

The following monoclonal antibodies were used in this study:

1. Cytokeratin 5/6 and p63 – for verification of SCC.
2. TTF-1 and cytokeratin 7 – for verification of AC.
3. CD56 or NCAM and synaptophysin, for verification of NSCLC-NE.

Monoclonal antibodies and their clones used in this study are presented in Table 1.

The definite diagnosis of the histological type of NSCLCs after immunohistochemical staining was: (1) SCC, (2) AC, (3) NSCLC-NE, (4) Other NSCLCs unclassified type or NOS.

Presence and absence of immunoreactivity in the tumor cells were marked as (1) and (0), respectively.

Diagnostic dilemmas were resolved by a second or, if necessary, by a third pathological opinion.

Statistics

Fisher's exact probability test was used in the analysis of contingency tables with significance level at $p<0.05$. This test was employed because of small sample sizes. Descriptive statistics were also used.

Results

Changes in diagnosis of all biopsy samples from probable to final diagnosis of histological subtypes of NSCLC after immunohistochemistry are detailed in Table 2.

Histopathological diagnosis of suspected SCC was established in 9 biopsy samples, to be confirmed immunohistochemically in 4 (44.4%) of them, changing the diagnosis in 5 (55.6%) specimens. Definite diagnosis of 2 SCCs was confirmed by immunohistochemistry.

Out of 11 biopsy samples with suspected lung

Table 3. Detailed review of all biopsy specimens, applied immunohistochemistry, changes diagnosis from probable to final diagnosis of histological types of non-small cell lung carcinoma

Sample number	*Probable diagnosis*	*Monoclonal antibody*						*Final diagnosis*
		TTF-1	*CK7*	*CK5/6*	*p63*	*CD56*	*Syn*	
1.	pb.SCC	1	1	0	0	0	0	AC
2.	pb.SCC	1	0	0	0	0	0	AC
3.	NSCLC	0	1	1	0	0	0	NSCLC, NOS
4.	pb.AC	1	0	0	0	1	1	LCLC,NE
5.	AC	1	1	0	0	0	0	AC
6.	NSCLC	1	1	0	0	1	1	NSCLC, NOS
7.	NSCLC	0	1	0	0	0	0	NSCLC, NOS
8.	pb.AC	1	1	0	0	0	0	AC
9.	NSCLC	0	0	1	1	0	0	SCC
10.	NSCLC	1	1	0	0	0	0	AC
11.	NSCLC	0	0	0	0	1	1	LCLC, NE
12.	pb.AC	1	1	0	0	0	0	AC
13.	AC	1	1	0	0	0	0	AC
14.	pb.SCC	1	1	0	0	0	0	AC
15.	NSCLC	1	1	0	0	0	0	AC
16.	SCC	0	1	1	1	0	0	SCC
17.	NSCLC	1	1	0	0	0	0	AC
18.	pb.AC	1	1	0	0	0	0	AC
19.	NSCLC	0	1	1	0	0	0	NSCLC,NOS
20.	NSCLC	0	0	0	0	1	1	LCLC,NE
21.	pb.AC	1	1	0	0	0	0	AC
22.	AC	1	1	0	0	0	0	AC
23.	pb.AC	0	1	0	0	0	0	NSCLC,NOS
24.	AC	1	1	0	0	0	0	AC
25.	NSCLC	0	0	1	1	0	0	SCC
26.	pb.AC	1	1	0	0	0	0	AC
27.	NSCLC	0	0	1	1	0	0	SCC
28.	pb.SCC	0	0	1	1	0	0	SCC
29.	pb.AC	1	0	0	0	0	0	AC
30.	AC	1	1	0	0	0	0	AC
31.	pb.SCC	1	1	0	0	0	0	AC
32.	pb.AC	1	1	0	0	0	0	AC
33.	NSCLC	0	0	1	1	0	0	SCC
34.	pb.AC	1	1	0	0	0	0	AC
35.	NSCLC	1	1	0	0	0	0	AC
36.	NSCLC	1	0	0	0	0	0	NSCLC,NOS
37.	NSCLC	0	1	0	0	0	0	AC
38.	NSCLC	0	1	1	1	0	0	SCC
39.	pb.AC	1	1	0	0	0	0	AC
40.	NSCLC	1	1	0	0	0	0	AC
41.	NSCLC	0	0	1	1	0	0	SCC
42.	pb.SCC	1	1	1	0	0	0	AC
43.	LCC	0	0	0	0	0	0	NSCLC,NOS

Continued on next page

44.	LCC	1	1	0	0	0	0	AC
45.	AC	1	1	0	0	0	0	AC
46.	pb.SCC	0	0	1	1	0	0	SCC
47.	NSCLC	0	0	1	1	0	0	SCC
48.	pb.SCC	0	0	1	1	0	0	SCC
49.	NSCLC	0	0	1	1	0	0	SCC
50.	pb.SCC	0	0	1	1	0	0	SCC

pb: probable, SCC: squamous cell carcinoma, AC: adenocarcinoma, LCLC,NE: large cell lung carcinoma with neuroendocrine differentiation, NSCLC: non small cell lung carcinoma, NOS: not otherwise specified, TTF-1: thyroid transcription factor-1, CK5: cytokeratin5, CK7: cytokeratin7, Syn: synaptophysin

Table 4. Sensitivity of the method - changes in diagnosis after immunohistochemistry

		Final diagnosis				
		SCC	*AC*	*NSCLC, NOS*	*LCLC, NE*	*Total*
SCC	N	2	0	0	0	2
	Preliminary diagnosis %	100	0	0	0	100
	Final diagnosis %	15.4	0	0	0	4.0
pb. SCC	N	4	5	0	0	9
	Preliminary diagnosis %	44.4	55.6	0	0	100
	Final diagnosis %	30.8	18.5	0	0	18.0
AC	N	0	6	0	0	6
	Preliminary diagnosis %	0	100	0	0	100
	Final diagnosis %	0	22.2	0	0	12.0
pb.AC	N	0	9	1	1	11
	Preliminary diagnosis %	0	81.8	9.1	9.1	100
	Final diagnosis %	0	33.3	14.3	33.3	22.0
NSCLC	N	7	7	6	2	22
	Preliminary diagnosis %	31.8	31.8	27.3	9.1	100
	Final diagnosis %	53.8	25.9	85.7	66.7	44.0
Total	N	13	27	7	3	50
	Preliminary diagnosis %	26.0	54.0	14.0		100
	Final diagnosis %	100	100	100	100	100

For abbreviations see footnote of Table 3

Table 5. TTF-1 specificity in various non-small cell histological subtypes

		TTF-1		
Final diagnosis		*Negative*	*Positive*	*Total*
SCC	*N (%)*	13 (100)	0 (0)	13 (100)
AC	*N (%)*	4 (14.8)	23 (85.2)	27 (100)
NSCLC, NOS	*N (%)*	6 (85.7)	1 (14.3)	7 (100)
LCLC, NE	*N (%)*	1 (33.3)	2 (66.7)	3 (100)
Total	*N (%)*	24 (48.0)	26 (52.0)	50 (100)

For abbreviations see footnote of Table 3

Table 6. Cytokeratin 7 specificity in various non-small cell histological subtypes

		Cytokeratin7		
Final diagnosis		*Negative*	*Positive*	*Total*
SCC	*N (%)*	11 (84.6)	2 (15.4)	13 (100)
AC	*N (%)*	4 (14.8)	23 (85.2)	27 (100)
NSCLC, NOS	*N (%)*	3 (42.9)	4 (57.1)	7 (100)
LCLC, NE	*N (%)*	2 (66.7)	1 (33.3)	3 (100)
Total	*N (%)*	20 (40.0)	30 (60.0)	50 (100)

For abbreviations see footnote of Table 3

Table 7. Cytokeratin 5/6 specificity in various non-small cell histological subtypes

		Cytokeratin 5/6		
Final diagnosis		*Negative*	*Positive*	*Total*
SCC	*N (%)*	0 (0)	13 (100)	13 (100)
AC	*N (%)*	25 (92.6)	2 (7.4)	27 (100)
NSCLC, NOS	*N (%)*	5 (71.4)	2 (28.6)	7 (100)
LCLC, NE	*N (%)*	3 (100)	0 (0)	3 (100)
Total	*N (%)*	33 (66.0)	17 (34.0)	50 (100)

For abbreviations see footnote of Table 3

Table 8. p63 specificity in various non-small cell histological subtypes

		p63		
Final diagnosis		*Negative*	*Positive*	*Total*
SCC	*N (%)*	0 (0)	13 (100)	13 (100)
AC	*N (%)*	26 (96.3)	1 (3.7)	27 (100)
NSCLC, NOS	*N (%)*	7 (100)	0 (0)	7 (100)
LCLC, NE	*N (%)*	3 (100)	0 (0)	3 (100)
Total	*N (%)*	36 (72.0)	14 (28.0)	50 (100)

For abbreviations see footnote of Table 3

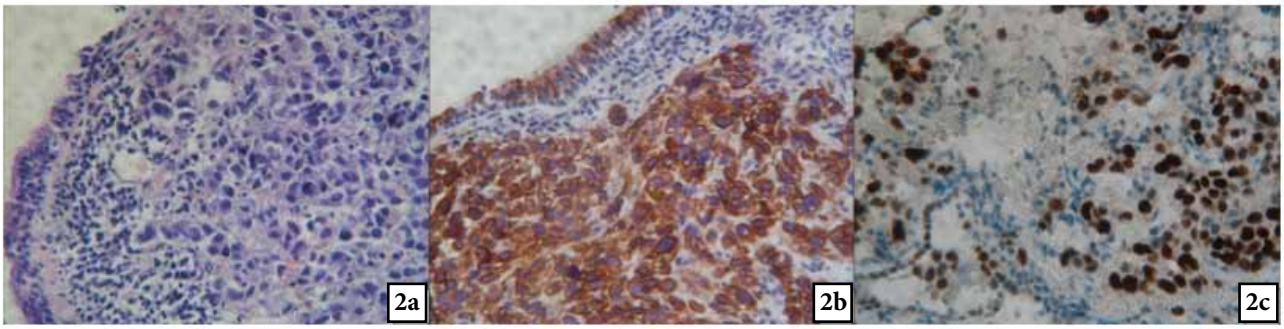

Figure 2. Poorly differentiated lung adenocarcinoma; morphology and immunophenotype; transbronchial needle biopsy: a) H&E x40; b) Cytokeratin7 x40; c) TTF-1 x40.

AC, the diagnosis was confirmed in 9 (81.8%), while NSCLC with/without neuroendocrine differentiation was diagnosed in 1 (9.1%) sample each. Diagnosis of *de novo* lung AC was confirmed by immunohistochemistry in all 6 biopsies.

Out of 22 NSCLCs 6 (27.3%) remained unclassified even after immunohistochemical study, 2 (9.1%) expressed neuroendocrine markers and were classified as NSCLCs-NE, while 7 (31.8%) were equally diagnosed as SCC and AC.

Table 3 shows the sensitivity of the immunohistochemical method. After immunohistochemistry only 7 lung carcinomas remained unclassified as NSCLC, NOS.

Out of 11 NSCLCs suspected to be SCCs, immunohistochemistry confirmed the histological type in 6 (54.5%) cases. A total of 7 (31.8%) NSCLCs were diagnosed as SCC after application of immunohistochemistry (Figure 1). Out of 17 ACs, the diagnosis was confirmed in 15 (88.2%) cases (Figure 2), while NSCLCs with or without neuroendocrine differentiation were evidenced in 1 (5.9%) case each. After immunohistochemistry, 7 (31.8%) NSCLCs were classified as SCC and AC, neuroendocrine differentiation was diagnosed in 2 (9.1%; Figure 3), while 6 (27.3%) remained as NSCLCs NOS (Figure 4).

Of 11 biopsy samples suspected as SCCs, the diagnosis was confirmed in 6 (21.4%). Suspected AC was diagnosed in 17 biopsy samples, to be immunohistochemically confirmed in 15 (88.2%) of them. No statistically significant difference was found in immunohistochemical verification of definitive diagnosis between SCC and AC (21.4 vs. 53.6%, p=0.076).

NOS was suspected in 22 biopsy samples, however after immunohistochemical studies the histopathological diagnosis was confirmed in only 8 (24.2%) biopsies. No statistically significant difference was found in the definitive diagnosis between SCC and NSCLC after immunohistochemistry (18.2 vs. 24.2%, p=0.459).

Statistically significant difference was found in the verification of definitive diagnosis of NOS (8; 20.5%) and definitive diagnosis of AC (15; 38.5%) (38.5 vs. 20.5%, p=0.001).

TTF-1 expression was evidenced in 85.2% of the verified ACs, in 14.3% of NSCLCs without neuroendocrine differentiation and in 2 (66.7%) with neuroendocrine differentiation. Expression of the antibody was not evidenced in SCC (Table 4).

Presence of cytokeratin 7 was evidenced in 23 (85.2%) of ACs and in 2 (15.4%) of SCCs. Cytokeratin 7 was expressed in 4 (57.1%) NSCLCs without neuroendocrine differentiation and in 1 (33.3%) with neuroendocrine differentiation (Table 5).

Cytokeratin 5/6 was expressed in all (100%) SCC as well as in 2 (7.4%) ACs and in 2 (28.6%) NSCLCs without neuroendocrine differentiation (Table 6).

p63 was expressed in all (100%) SCCs and in 1 (3.7%) AC. p63 was not expressed in NSCLC, NOS (Table 7).

Three NSCLCs-NE were diagnosed based on the expression of the neuroendocrine markers CD56 and synaptophysin. Both markers were diagnosed in both NSCLCs (Tables 8 and 9).

Discussion

TTF-1

In addition to lung ACs, TTF-1 is also present in 50% of NSCLCs, with or without neuroendocrine differentiation. According to the World Health Organization data and data reported in some papers in the available journals, it may be also expressed in SCC [12,15,16].

TTF-1 is expressed in more than 90% of small-cell lung carcinomas. One third of typical and atypical lung carcinoids also express TTF-1. TTF-1 is present in normal pneumocytes and bronchial epithelia and is sensitive in 62% and specific in 92% of ACs according to one study [6], in comparison to 97% and 88%, respectively according to another study [9]. In one study [15], TTF-1 was positive in 80% (16/20) of ACs, none of 15 SCCs and in 50% (2/4) of NSCLCs-NE and NOS together. Some authors reported a clear correlation between the degree of expression of TTF1 and survival of patients with lung ACs [18]. TTF-1 is an optimal marker for differentiation of NSCLCs [6].

In our series, TTF-1 was positive in 85.2% (23/27) of ACs, in 14.3% (1/7) of NSCLC– unclassified type and in 66.7% (2/3) of NSCLC– NE.

Cytokeratin 7

Cytokeratin 7 is widely applied in the differential diagnosis of the different histological subtypes of NSCLCs (typical and atypical carcinoid and NSCLC with and without neuroendocrine differentiation). Tan and Zander [14] reported in their

Table 9. CD56 specificity in various non-small cell histological subtypes

		CD56		
Final diagnosis		*Negative*	*Positive*	*Total*
SCC	*N (%)*	13 (100)	0 (0)	13 (100)
AC	*N (%)*	27 (100)	0 (0)	27 (100)
NSCLC, NOS	*N (%)*	7 (100)	1 (0)	7 (100)
LCC, NE	*N (%)*	0 (0)	3 (100)	3 (100)
Total	*N (%)*	46 (92.0)	4 (8.0)	50 (100)

For abbreviations see footnote of Table 3

Table 10. Synaptophysin specificity in various non-small cell histological subtypes

		Synaptophysin		
Final diagnosis		*Negative*	*Positive*	*Total*
SCC	*N (%)*	13 (100)	0 (0)	13 (100)
AC	*N (%)*	27 (100)	0 (0)	27 (100)
LCC, NOS	*N (%)*	7 (100)	0 (0)	7 (100)
LCC, NE	*N (%)*	0 (0)	3 (100)	3 (100)
Total	*N (%)*	47 (90.0)	3 (100)	50 (100)

For abbreviations see footnote of Table 3

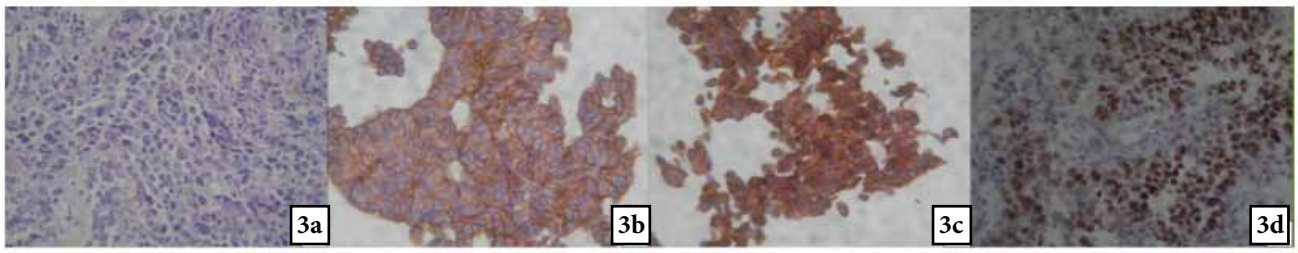

Figure 3. Large cell lung carcinoma with neuroendocrine differentiation; transthoracic needle biopsy: a) H&E x20; b) CD56 x40; c) Synaptophysin x40; d) TTF-1 x40.

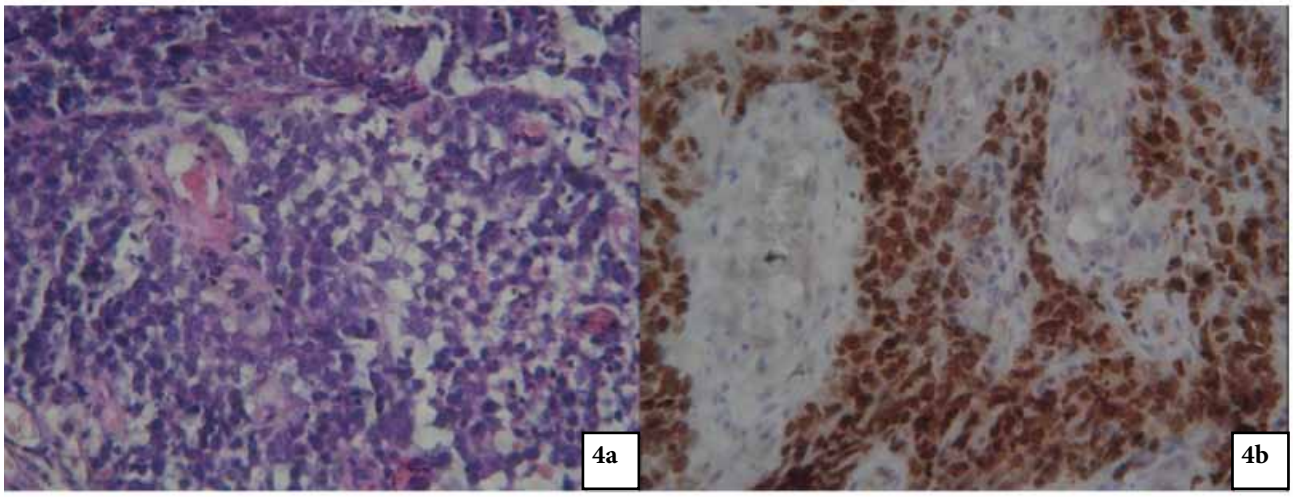

Figure 4. Large cell lung carcinoma; morphology and immunophenotype, unclassified type- NOS; transthoracic needle biopsy: a) H&E x20; b) TTF-1 x20.

review that cytokeratin 7 is widely present in ACs, and also in SCCs and unclassified NSCL In lung AC, sensitivity and specificity of cytokeratin 7 is 93% (range 90-97) and 63% (range 57-70), respectively [14]. Mukhopadhyay and Katzenstein [15] reported that cytokeratin 7 was present in 100% (19/19) of ACs, in 60% (9/15) of SCCs and in 75% (3/4) of unclassified NSCLCs. In the present study, cytokeratin 7 was positive in 15.4% (2/13) of SCCs, in 85.2% (23/27) of ACs, in 57.1% (4/7) of NSCLCs – unclassified type and in 33.3% (1/3) of NSCLCs – NE.

Cytokeratin 5/6

In normal tissues cytokeratin 5/6 is expressed in the keratinizing epithelium and non-keratinizing epithelium covering mucosal tissues. Cytokeratins are found in the stratified squamous epithelium, myoepithelial layer of the prostate, breast and salivary glands as well as in benign and malignant epidermal tumors, and carcinomas originating from stratified squamous epithelium and myoepithelium. Optimal panel used for differentiation between malignant mesothelioma and adenocarcinoma also contains cytokeratin 5/6. According to a WHO study group [6], cytokeratin 5/6 was positive in 93% (13/15) of the studied lung SCCs. Co-expression of cytokeratin 5/6 and p63 in more than 10% of tumor cells was highly specific (96%) and sensitive (77%) [9,18-20]. A study performed by Mukhopadhyay and Katzenstein [15] evidenced that cytokeratin 5/6 was expressed only by 73% (11/15) of the lung SCCs, while it was not expressed by ACs. It is not expressed by unclassified NSCLCs. Cytokeratin 5/6 is used as one of two essential monoclonal antibodies in the differential diagnosis between SCCs and ACs of the lung. Sensitivity (84%) and specificity (79%) of cytokeratin

5/6 are lower compared with p63. Sensitivity (92%) and specificity (74%) of cytokeratin 5/6 and p63 co-expression are the same as individual sensitivity and specificity of p63 [10,14,19]. Based on World Health Organization recommendations, cytokeratin 5/6 is included in the optimal panel of the monoclonal antibodies used in the differential diagnosis of SCC of the lung, however it is also included in the optimal panel for diagnosis on the NSCLC smears [6,9,14].

As for our study, cytokeratin 5/6 was expressed in 100% of SCCs, in 7.4% of all diagnosed lung ACs and in 2 (28.6%) of 7 diagnosed NSCLCs – unclassified type.

p63

p63 is recommended as an optimal monoclonal antibody for differentiation between SCC and AC of the lung due to its high specificity and sensitivity.

In the World Health Organization classification from 2004, application of p63 is recommended for the differential diagnosis of SCC of the lung. According to some studies, p63 is expressed in 80% of the SCCs. Co-expression of p63 and cytokeratin 5/6 is recorded in 73% of SCCs in a study carried out by Kaufmann et al. [13].

Over the last years, the role of p63 has been repeatedly studied. Mukhopadhyay and Katzenstein [15] reported their diagnostic algorithm that 100 % of the poorly differentiated SCCs express p63. Tan and Zander [14] also reported that p63 is essential in the differential diagnosis of small-cell lung carcinoma and small-cell type of SCC of the lung.

Two studies established the presence of statistically significant difference ($p<0.001$) in p63 expression between SCCs and ACs of the lung. The number of SCCs with p63 expression and the number of ACs without expression was 22 (76%) and 10 (26%), respectively, in the first study, and 49 (54%) and 2 (7%), respectively, in the second one [11,15].

Owing to its high specificity (92%) and sensitivity (74%) p63 application is recommended exclusively for small biopsy samples for differentiating SCC from AC [9,10,15].

In our study, p63 was expressed in all 13 diagnosed SCCs, as well as in 1 (3.7%) of 27 diagnosed ACs.

p63 is also recommended for use in the cytological smears of NSCLCs [9,10,16,22]. German authors reported that the level of expression of p63 is inversely related to the degree of keratinization of lung SCC [23].

CD56/NCAM and *synaptophysin*

In addition to the presence of organoid distribution of tumor cells, the diagnostic criteria of NSCLCs-NE also include the presence of at least one neuroendocrine marker. CD56/NCAM, synaptophysin and chromogranin A are neuroendocrine markers used in the diagnosis of this histological type of lung cancer [9,15]. Our study indicated that two markers, CD56/NCAM and synaptophysin, are sufficient to differentiate this type of lung carcinoma. TTF-1 was expressed in 2 (66.7%) of 3 NSCLCs-NE.

Failure to obtain *kappa* index of agreement between the diagnoses before and after immunohistochemical analysis is a shortcoming of our study. This was impossible due to the lack of the same number of probable and definitive diagnoses to compare. Five probable diagnoses were available: SCC, AC, probable SCC, probable AC and NSCLC-unclassified type, while definitive diagnoses included SCC, NSCLC– NE and NSCLC– unclassified type.

Another shortcoming of the present study was the fact that definitive diagnosis of the histological subtype of lung carcinoma was not verified on larger tissue samples obtained from surgical material. Based on our experience, we know that only one sixth of the patients with diagnosed lung carcinoma undergo surgery, meaning that 8 out of 50 studied patients could be subjected to surgery. This small number of patients would lack statistical significance for drawing relevant conclusions.

Failure to investigate Napsin-A is another weakness of the study. This monoclonal antibody was not used in all 50 studied samples of the NSCLCs and thus its diagnostic role has not been studied. Napsin-A is sensitive in 59% and specific in 94% of

lung ACs while TTF1 is sensitive in 62% and specific in 92% of lung ACs [9]. According to another study [15], TTF-1 was expressed by 80% (16/20) of lung ACs while Napsin-A was expressed by 58% (11/19) of lung ACs.

According to our experience, MOC-31 is expressed by both AC and SCC of the lung. Therefore, due to its low specificity it was not analyzed. Our investigation did not include high molecular cytokeratin (34βE12) for the same reason.

Conclusion

Expression of cytokeratin 5/6 and p63 is specific for diagnosis of SCC of the lung. TTF-1 and cytokeratin 7 expression indicates diagnosis of lung AC. Introduction of Napsin-A and any of surfactants leads to increase in the number of diagnosed lung ACs at the expense of undifferentiated NSCLCs. If the malignant cells express only cytokeratin 7 or only TTF-1 this could only suggest NSCLC.

Expression of the neuroendocrine markers CD56/NCAM and synaptophysin is indicative for neuroendocrine differentiation of NSCLCs.

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References

1. Ries LAG, Melbert D, Krapcho M et al (Eds): SEER Cancer Statistics Review, 1975–2005. Bethesda, MD: National Cancer Institute; 2008. http://seer.cancer.gov/csr/1975_2005/. based on November 2007 SEER data submission, posted to the SEER web site.
2. Janssen-Heijnen M, Coebergh JW. The changing epidemiology in Europe. Lung Cancer 2003; 41:245–258.
3. Vukicevic A, Miljus D, Zivkovic S, Mickovski N, Rakocevic I, Plavsic P (Eds): Cancer incidence and mortality in central Serbia. Cancer Registry of central Serbia. Reports 1, 2 and 3. Belgrade: Institute of Public Health of Serbia, 2002, 2004 and 2005.
4. Mollberg N, Surati M, Demchuk C et al. Mind-mapping for lung cancer: Towards a personalized therapeutics approach. Adv Ther 2011; 28: 173–194.
5. Kerr KM. Personalized medicine for lung cancer: new challenges for pathology. Histopathology DOI: 10.1111/j.1365-2559.2011.03854.x
6. Travis WD, Brambilla E, Müller-Hermelink K, Harris CC (Eds): World Health Organisation Classification of Tumours. Pathology and Genetics of Tumours of the Lung, Pleura, Thymus and Heart. IARC Press, International Agency for Research on Cancer: Lyon, 2004.
7. Stojsic J, Radojicic J, Markovic J et al. Gender and age trends of histological types of lung cancer in a 20-year period – pathological perspective. J BUON 2010; 15: 136-140.
8. Stojsic J, Adzic T, Maric D et al. Histological types and age distribution of lung cancer operated patients in a period of twenty years – a pathohistological based study. Srp Arh Celok Lek 2011; 139: 619-624.
9. Terry J, Leung S, Laskin J, Leslie KO, Gown AM, Ionescu DN. Optimal immunohistochemical markers for distinguishing lung adenocarcinomas from squamous cell lung carcinomas in small tumor samples. Am J Surg Pathol 2010; 34:1805-1811.
10. Loo PS, Thomas SC, Nicolson MC, Fyfe MN, Kerr KM. Subtyping of undifferentiated non-small cell carcinomas in bronchial biopsy specimens. J Thorac Oncol 2010; 5:442-447.
11. Conde E, Angulo B, Redondo P et al. The use of P63 immunohistochemistry for the identification of squamous cell lung carcinoma of the lung. PLoS One 2010; 5: e12209.
12. Travis WD, Brambilla E, Noguchi M et al. International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society International Multidisciplinary Classification of Lung Adenocarcinoma. J Thorac Oncol 2011; 6: 244–285.
13. Kaufmann O, Fietze E, Mengs J, Dietel M. Value of p63 and cytokeratin 5/6 as immunohistochemical markers for the differential diagnosis of poorly differentiated and undifferentiated carcinomas. Am J Clin Pathol 2001;116:823-830.
14. Tan D, Zander DS. Immunohistochemistry for assessment of pulmonary and pleural neoplasms: a review and update. Int J Clin Exp Pathol 2008; 1:19-31.
15. Mukhopadhyay S, Katzenstein A-L.A. Subclassification of non-small cell lung carcinomas lacking morphologic differentiation on biopsy specimens: Utility of an immunohistochemical panel containing TTF-1, Napsin A, p63, and CK5/6. Am J Surg Pathol 2011; 35:15–25.

16. Jagirdar J. Application of immunohistochemistry to the diagnosis of primary and metastatic carcinoma to the lung. Arch Pathol Lab Med 2008; 132: 384-396.
17. Al-Zahrani IH. The value of immunohistochemical expression of TTF-1, CK7 and CK20 in the diagnosis of primary and secondary lung carcinomas. Saudi Med J 2008; 29: 957–961.
18. Barletta JA, Perner S, Lafrate JA et al. Clinical significance of TTF-1 protein expression and TTF-1 gene amplification in lung adenocarcinoma. J Cell Mol Med 2009; 13: 1977–1986.
19. Chu PG, Weiss LM. Expression of cytokeratin5/6 in epithelial neoplasms: an immunohistochemical study of 509 Cases. Mod Pathol 2002;15:6–10.
20. Ordonez NG. The immunohistochemical diagnosis of mesothelioma: a comparative study of epithelioid mesothelioma and lung adenocarcinoma. Am J Surg Pathol 2003; 27:1031-1051.
21. Ordonez NG. Value of cytokeratin5/6 immunostaining in distinguishing epithelial mesothelioma of the pleura from lung adenocarcinoma. Am J Surg Pathol 1998; 22:1215-1221.
22. Hasanovic A, Rekhtman N, Sigel SC, Moreira AL. Advances in fine needle aspiration cytology for the diagnosis of pulmonary carcinoma. Pathol Res Int 2011; doi:10.4061/2011/897292
23. Vollmer E, Schultz H, Stellamacher Fl et al. Tumors in the lung – morphologic features and the challenge of integrating biomarker signatures into diagnostics. Rom J Morphol Embryo 2010; 51:607–614.