Chapter 1

Precise Diagnosis of Histological Type of Lung Carcinoma: The First Step in Personalized Therapy

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Additional information is available at the end of the chapter

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Abstract

This chapter is a combination of personal experience of a pulmonary pathologist and available references in the diagnosis of non-small cell lung cancer (NSCLC) types. The morphological appearance of poorly differentiated lung carcinoma is not characteristic, so immunohistochemical staining is used for further differentiation. In order to save tumor tissue from paraffin blocks, the most rational way is to use only two antibodies, p40 for squamous cell carcinoma and TTF-1 for adenocarcinoma of the lung, and if necessary or if cancer growth is organoid, also one of two neuroendocrine markers (CD56 or Synaptophysin) can be used. If there is enough tumor tissue in the paraffin block to confirm the diagnosis, NapsinA, p63, Cytokeratin5/6 or Cytokeratin5 can be used. It should be kept in mind that no antibody is highly specific for one histological type of carcinoma or its origin and if the immunohistochemical finding is unspecific, it should be concluded that this is "not otherwise specified" (NOS) carcinoma. The rest of tissue must be preserved for current and future molecular testing and predictive immunohistochemical staining for the purpose of personalized NSCLC therapy.

Keywords: non-small cell lung cancer, diagnosis, immunohistochemical staining, paraffin block, TTF-1, p40, p63, CD56, Synaptophysin, NapsinA, Cytokeratin5/6, Cytokeratin5

1. Introduction

1.1. Epidemiology as basis for developing a strategy in the treatment of advanced non-small cell lung cancer

Lung carcinoma is the most commonly diagnosed malignancy worldwide. There is a different lung cancer incidence and mortality statistics throughout the world. In male population, lung

cancer has the highest incidence rate, especially in developing countries, while in developed countries, it is immediately behind the prostate cancer. In female population, the incidence rate of lung cancer is rising, and it is higher than cervical carcinoma, but still lower than breast cancer. Lung carcinoma mortality rate is still alarmingly high, both in developing and developed countries [1–3].

This high mortality rate has led to research of drugs, which will, in the era of personalized therapy, prolong the survival of diseased patients for more than 5 years and also improve the quality of their lives during and after the treatment. Individual approach to the treatment of lung cancer is based on a precisely diagnosed pathohistological type of non-small cell lung cancer (NSCLC) [4, 5].

1.2. Types of biopsy specimens and pathohistological classification of lung cancer

Two most common histological types of NSCLC are adenocarcinoma and squamocellular carcinoma [6–8]. At the time of diagnosis, about 75% of lung cancer is in an inoperable, advanced stage and less than 15% of patients survive more than 5 years. As these patients cannot be surgically treated, diagnosis of NSCLC is based on bronchoscopic and fine-needle aspiration biopsy (*fnab*) or video-assisted thoracic surgery (*VATS*). That is why there is a huge responsibility on the shoulders of the pathologist to diagnose a histological type of NSCLC on a small biopsy specimen and preserve paraffin-embedded carcinoma tissue for further genetic and immunohistochemical testing in order to determine the effective personalized therapy. It aims at prolonging survival of patients and improving the quality of their lives during and after the therapy [9, 10].

In the final interpretation of the pathohistological findings, pathologists use the classification of the World Health Organization (WHO) from 2004 [6], that is, an improved version from 2015 [7].

According to WHO classification from 2004, histological subtypes of NSCLC are as follows:

- squamous cell carcinoma (**Figure 1**)
- adenocarcinoma (**Figure 2**)
- large-cell carcinoma
- adenosquamous carcinoma
- sarcomatoid carcinoma
- carcinoid tumors
- salivary gland tumors.

Each of these histological NSCLC subtypes has its own variants that are diagnosed based on their morphological picture and specific immunophenotype [6].

In the WHO classification of lung carcinoma from 2015, there have been some changes because adenocarcinoma took over the first place from squamous cell carcinoma, which previously had primacy. The greatest change in this classification compared to the previous one

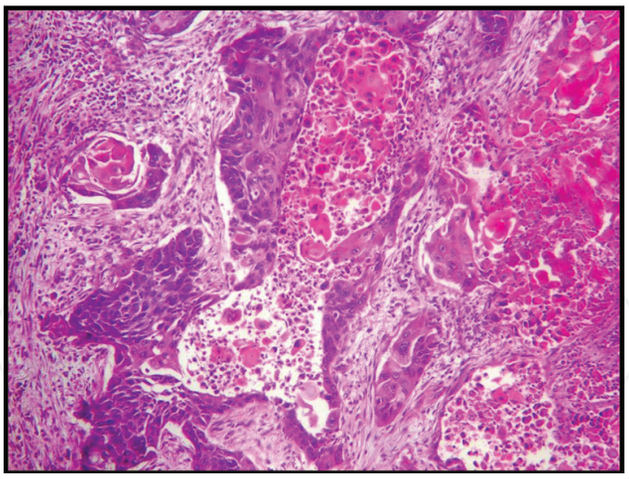

Figure 1. Keratinizing squamous cell carcinoma of the lung, H&E 100×.

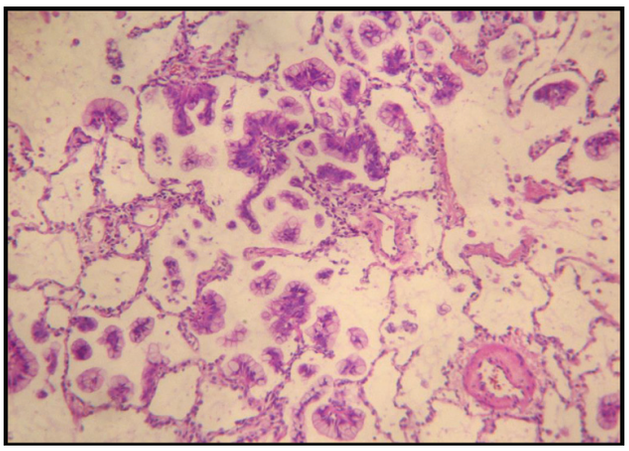

Figure 2. Adenocarcinoma of the lung with lepidic growth pattern, H&E 100×.

is a grouping of all carcinoma with neuroendocrine differentiation: carcinoid tumors, typical carcinomas (TC) and atypical carcinomas (AC), large-cell neuroendocrine carcinomas (LCC-NEC) and small-cell neuroendocrine carcinomas (SCLC) into one group of carcinomas due to specific biological behavior and special therapy, regardless of the different morphological picture. Remaining classification is identical to that of 2004 [7].

1.3. Processing of lung carcinoma tissue samples taken during bronchoscopy, FNA biopsy or VATS method

Bioptized lung samples obtained during bronchoscopy are fixed in 10% buffered formalin and brought to the laboratory. It is considered that sampling of tissue is representative if at least five biopsy samples have been delivered in diameter larger than 2 mm. Tissue samples taken during FNA biopsy should be delivered in 10% buffered formalin in the form of punctuate cylinder in order to use the whole tissue material for which is believed to contain lung cancer for morphological analysis and for immunohistochemical staining, while the rest of the tissue would be preserved for molecular testing (EGFR, optional KRAS), predictive

immunohistochemical staining (ALK, ROS1 and PDL-1), as well as for fluorescent *in situ* hybridization (FISH) if this method is accepted by consensus [11].

Basic information about the patient which is to be submitted in the biopsy referral for the pathohistological laboratory is: name and surname, gender, age, place of residence, occupation and smoking status. It is necessary to deliver clear and concise clinical picture, for example, whether there is a suspect tumor shadow in the lung, mediastinal lymphadenopathy, superior vena cava syndrome (SVCS), and so on. Also, it is necessary to note that there is a previously diagnosed pulmonary or some other kind of malignancy in patient, which histological type of tumor is diagnosed on that occasion and how long before the current examination. This data help to set a new pathohistological diagnosis by applying a smaller number of immunohistochemical staining to prove metastatic malignancy or a new primary carcinoma of the lung. An endoscopic finding of the mode of tumor growth and its localization must also be given. In this way, we save tumor tissue for methods which would be used in personalized therapy for advanced lung cancer. If these data are not available, the pathologist should consider that this is a biopsy of primary lung cancer. Incomplete data because of sloppiness and lack of interest of the doctor who performed the biopsy can lead to vagueness and difficulty in diagnosing and thus, to disrespect of the patient and pathologist. Correct communication at the patient-clinician level and clinician-pathologist level is the basis for setting a precise diagnosis.

According to NSCLC morphology, many pathologists would not agree on a definitive diagnosis, but after immunohistochemical stainings, the same diagnosis should be made in a high percentage of them. Also, crush phenomenon that can appear on obtained tissue samples during bronchoscopy and inadequate fixation of them can put pathologist on misdiagnosis.

1.4. Routine treatment of biopsy samples when there is a suspicion of NSCLC

Biopsy samples are routinely fixed in a 10% buffered formalin and then dehydrated in xylol and rising alcohol concentrations, embedded into paraffin block, cut at thickness of 2 μm, using hematoxylin-eosin stained, covered with medium (Canada balsam) and analyzed under the microscope. There should be only one, two at most cross sections, on one object plate in order to determine whether there is cancer in the first two cross sections by routine hematoxylin-eosin (H&E) staining and also to assume a histological type of cancer, based on the morphological characteristics of malignant cells. The following sections are cut separately on two respective plates for immunohistochemical staining in order to determine the precise histological type of NSCLC. The pathologist concludes that there are no malignant cells on both cross sections and to report this in his definitive pathohistological finding. If NSCLC is found, two immunohistochemical stainings are applied, TTF-1 and p40, which determine the histological subtype of two most histological subtypes of NSCLC, adenocarcinoma and squamous cell carcinoma. In the end, it is desirable for the pathologist to indicate in his report whether there is enough and how much tissue material is left for the next molecular testings. The number of plates is 4: for EGFR molecular testing and ALK, ROS1 and PDL-1 immunohistochemical evaluation (**Figure 2**). In the era of personalized therapy, it is desirable to cut eight tissue sections at the same time from paraffin block: two for morphological analysis based on H&E stained preparations, two for basic immunohistochemical staining (TTF-1 and p40) and the last four for molecular and immunohistochemical predictive staining only in the case that NSCLC is found on H&E stained cross sections [12, 13].

2. Immunohistochemistry

2.1. Immunohistochemical staining method

The labeled streptavidin-biotin staining method uses a highly "refined" avidin-biotin complex (ABC) three-stage technique in which a biotinylated secondary antibody reacts with several streptavidin molecules conjugated by peroxidase or alkaline phosphatase [12].

2.2. Immunohistochemical staining procedure

Tissue samples for immunohistochemical staining are deparaffined according to the prescribed procedure of the manufacturer and then incubated with a specific serum at room temperature in a damp chamber for a prescribed duration. It is used the labeled streptavidin-biotin (LSAB) technique. The antigen-antibody complex is visualized by 3-amino-9-ethylcarbazole or diaminobenzidine hydrochloride solution. Mayer's hematoxylin is used as a counterstain. A "positive control" is used to evaluate the effectiveness of a method or reaction.

As already stated, "internal positive control" is used, since there are normal tissue structures on the preparation itself, in this case, lung, which express the administered antibodies. In the part of the chapter in which we analyze individual monoclonal antibodies, we will also indicate which lung structures are expressing them regularly [12, 14, 15].

3. Monoclonal antibodies in the diagnosis of non-small cell lung cancer

There is a question, which two monoclonal antibodies should be rationally applied in order to establish the exact diagnosis of the histological subtype of NSCLC. Currently, these are thyreoid-transcription-factor-1 (TTF-1) and p40. These two antibodies have a role in distinguishing two most common histological subtypes, adenocarcinoma and squamous cell carcinoma. TTF-1 (clone 8G7G3/1, DAKO Cytomation, Denmark) is a diagnostic marker for adenocarcinoma of the lung and p40 (BC28, Ventana, USA) for squamous cell carcinoma [16, 17].

However, in the past, other less specific antibodies were used in the differentiation of histological subtypes of NSCLC. They can also be used now as an additional confirmation about histological subtype of NSCLC and differentiation of primary from secondary lung cancer. The following antibodies are also useful for differentiation: NapsinA (clone IP64, Novocastra™ HD, Leica Biosystems, UK), p63 (clone 7JUL, Novocastra™ HD, Leica Biosystems, UK), Cytokeratin5/6 (cloneD5/16B4 DAKO Cytomation, Denmark) or Cytokeratin5 (clone EP1601Y, Cell Marque RUO, USA) and Cytokeratin7 (clone OV-TL 12/30 DAKO Cytomation, Denmark) [18, 19]. If the morphological picture of NSCLC has organoid appearance, there is a suspicion that this is large cell lung carcinoma with neuroendocrine differentiation (LCC-NEC). To confirm this suspicion, it is necessary to use at least one of two neuroendocrine markers, CD56 (clone NCAM-1 Ab-2, Thermo Scientific LabVision, USA) or Synaptophysin (clone 27G1 Novocastra ™ HD, Leica Biosystems, UK) [18, 20]. If TTF-1, p40 and optionally CD56 were not expressed, for the purpose of storing malignant tissue for molecular testing, it should

be concluded that this is non-small-cell lung carcinoma, or "not otherwise specified" (NOS) carcinoma [7, 16]. It should be noted that no antibody is not highly specific for one organ or one histological type of cancer, that is, each antibody is specific for more than one histological types of cancer or more organs [21].

For precise pathohistological diagnosis of histological subtype of NSCLC, it is necessary to use two antibodies, TTF-1 and p40. If it is estimated that there is enough tumor tissue in a paraffin mold, it is possible to use additional antibodies to confirm the diagnosis and to preserve it for molecular testing [22, 23].

The advantages and disadvantages of each of these antibodies are discussed in the next section.

3.1. Thyroid transcription factor-1 (TTF-1)

Thyroid Transcription factor-1 (TTF-1) is a nucleic specific protein transcriptional factor expressed by thyroid gland and thyroid tumors as well as adenocarcinoma of the lung (**Figures 3** and **4**). This marker is expressed in the majority of lung adenocarcinoma (75%), but also in about 10% of squamous cell carcinoma. TTF-1 is significant in the differential diagnosis between primary and metastatic adenocarcinoma. At a time, when less antibodies were known, if TTF-1 with Cytokeratin7 was positive and Cytokeratin20 negative, diagnosis of adenocarcinoma of the lung was established [5, 24–28]. Diagnostic algorithms in the diagnosis of adenocarcinoma and differentiation of adenocarcinoma from squamous cell carcinoma are shown in papers of IASLC/ATS/ERS and Terry et al. [29, 30]. In various studies, TTF-1 was specific from 88 to 97% [6, 30, 31]. This marker is also expressed in lung cancer with neuroendocrine differentiation, in typical and atypical carcinoids, as well as in more than 90% of small cell lung carcinomas with neuroendocrine differentiation and in 50% of large cell lung carcinomas with neuroendocrine differentiation [30]. Literature reveals that TTF-1 can be expressed in breast and ovarian carcinoma [31, 32].

In one of our studies, we found that TTF-1 was expressed in 85.2% and in the other study, in 86% of lung adenocarcinomas [12, 33]. We also found that TTF-1 was also expressed in benign lung tumors [34, 35].

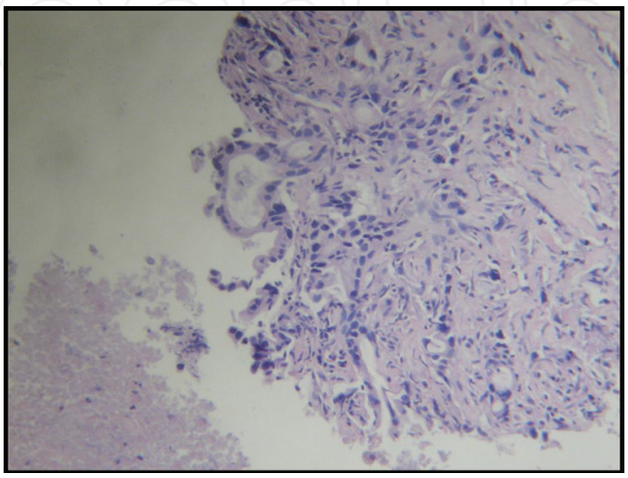

Figure 3. Adenocarcinoma of the lung with acinar growth, fnab, H&E 100×.

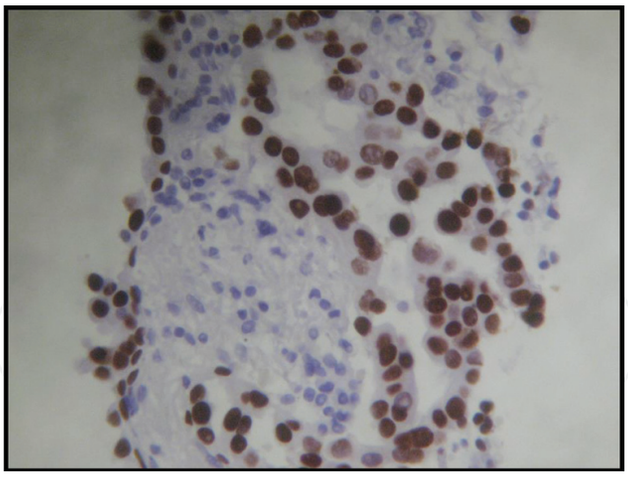

Figure 4. Adenocarcinoma of the lung, nucleic expression, TTF-1 200×.

3.2. p40

Role of p40 is to distinguish adenocarcinomas from squamous cell carcinomas in small samples (**Figure 5**) as well as on cytological smears. Squamous cell carcinoma is confirming with p40 antibody which is also known as DNp63. p40 is expressed in the nucleus of malignant cells of squamous cell carcinoma (**Figure 6**). This antibody is more specific for squamous cell lung carcinoma from p63. p40 is expressed in a smaller number of lung adenocarcinoma cells than p63. That is why it is recommended to use p40 instead of p63 for diagnostics of squamous cell carcinoma [36, 37]. If in malignant cells of carcinoma are not expressed TTF-1 and p40, diagnosis of non-small cell lung carcinoma -not otherwise specified (NSCLC-NOS) on a small biopsy sample will be established [16].

However, p40 is not a highly specific marker for only squamous cell lung carcinoma. It is also highly specific marker for urothelial carcinoma. This means that metastatic urothelial carcinoma in the lung is difficult to distinguish from primary squamous cell lung carcinoma both for morphological similarity these two carcinomas and similar immunophenotype [38]

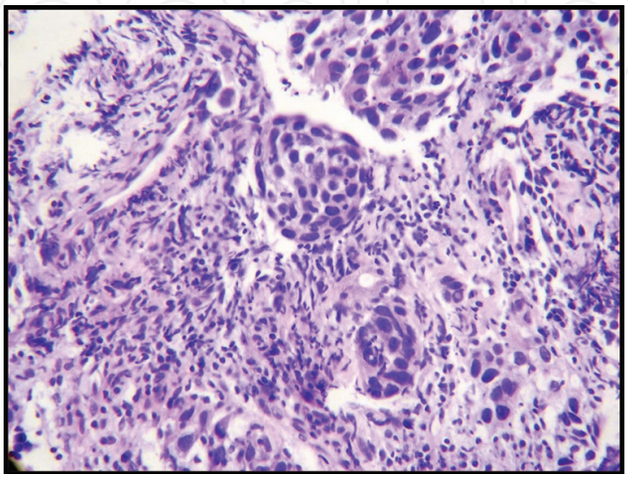

Figure 5. Moderately differentiated squamous cell carcinoma of the lung on bronchoscopic biopsy, H&E 20×.

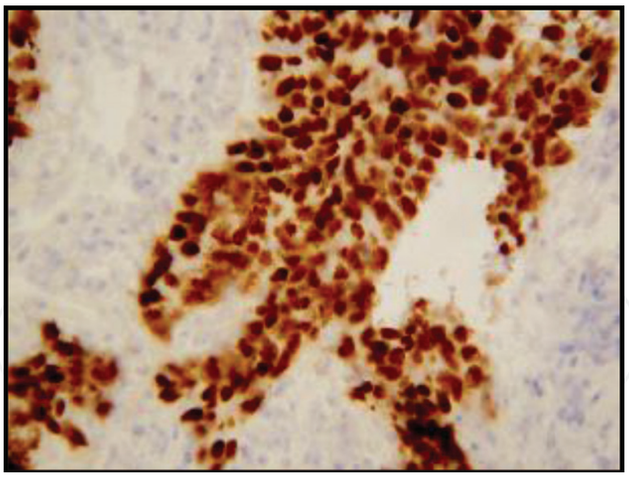

Figure 6. p40 expression in the squamous cell lung nuclei, 400×.

(**Figures 7** and **8**). Therefore, for definitive differentiation lung squamous cell carcinomafrom urotothelial carcinoma is also required clinical data on current local status in previously operated patients with urothelial carcinomas (degree of cancer invasion at the time of surgery, presence of angioinvasion, state of resection margins, the presence of metastases in local and remote lymph nodes).

p40 is also expressed in squamous cell carcinoma of other localizations (head and neck, larings, trachea, cervix, skin). That is why it is not possible to differentiate primary squamous cell lung carcinoma from metastatic lung carcinoma of the same histological type by using p40. Differentiation of primary from secondary squamous cell carcinoma in the lung is possible only in clinicopathological correlation [39].

3.3. NapsinA

NapsinA is expressed in the cytoplasm of preserved lung parenchyma in the form of functional aspartic proteinase, homologous to the polypeptide Tao2 and included in maturation of the

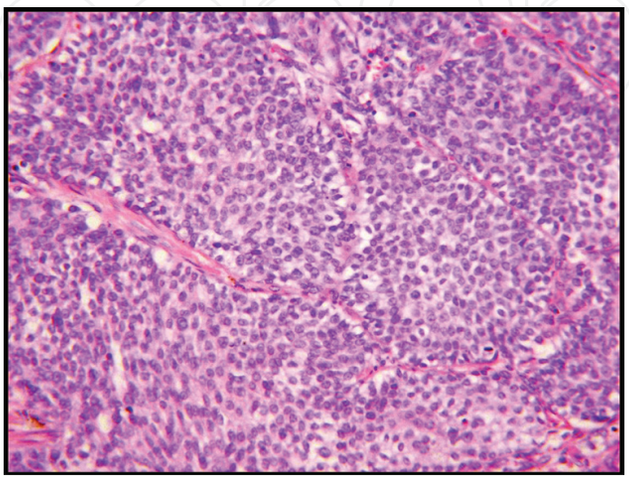

Figure 7. Urothelial carcinoma metastases in the lung, 400×.

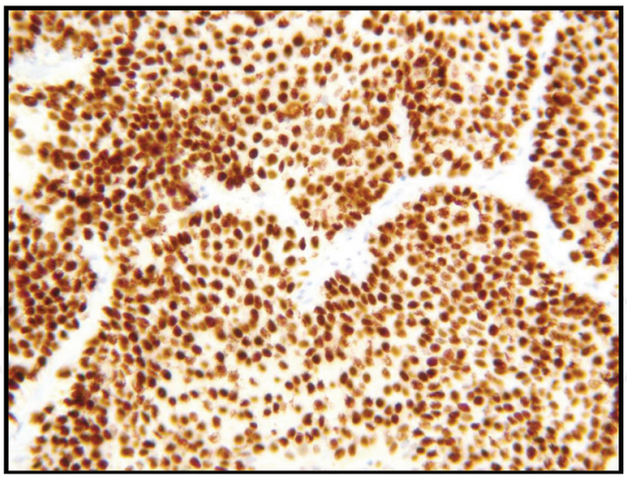

Figure 8. p40 expression in urothelial carcinoma metastases in the lung, 400×.

biologically active surfactant protein B. It also consists of 38-kDa protein, protein chain which is expressed in type II pneumocytes, alveolar macrophages and renal tubules, secretion channels of exocrine glands and pancreas. NapsinA is staining as coarse-grained intracytoplasmic marker [40].

There are papers that favor the use of NapsinA in regard to TTF-1 in differentiation of lung adenocarcinoma from other histological subtypes of NSCLC. Papers were done on a large number of lung adenocarcinomas wherein NapsinA showed higher specificity compared to the TTF-1. It is even recommended double immunohistochemical staining, so-called cocktail, which would beside NapsinA for cytoplasmic staining, contain and p40 for nuclear staining for squamous cell lung carcinoma [17, 36, 40, 41]. A simple diagnostic algorithm from 2010 recommends that NapsinA should be applied in order to diagnose adenocarcinoma, if TTF-1 is not exposed in NSCLC and if squamous cell carcinoma is not proved [30]. Our study on 50 adenocarcinomas showed a greater specificity of TTF-1 as compared to NapsinA [34]. If NapsinA is not expressed and the presence of mucin has not been proven, it should be concluded that it is about NSCLC-NOS. The definitive conclusion is that NapsinA is supplemental to TTF-1 which serves as the main marker for proving of lung adenocarcinoma (**Figure 9**).

3.4. p63

This antibody is known in the two isoforms, DNp63 and TAp63. It is expressed in the nucleus of the malignant cells. According to previous diagnostic algorithms, before occurrence of p40, p63 was the main marker in the differentiation of NSCLC on small biopsy samples beside TTF-1 [29]. Beside TTF-1, NapsinA and Cytokeratin5/6 are considered to be useful markers for the diagnosis of NSCLC on small biopsy samples [28]. The degree of p63 expression increases with the decrease in the degree of keratinization, that is, differentiation of squamous cell carcinoma [25] (**Figure 10** 200×; **Figure 11** 400×).

However, p63 as well as p40 is not a highly specific marker only for squamous cell carcinoma. It is also a marker for urothelial carcinoma. That is why it is hard to differentiate metastatic carcinoma of urothelial type from primary squamous cell lung carcinoma [39] (**Figure 12** 200×; **Figure 13** 400×).

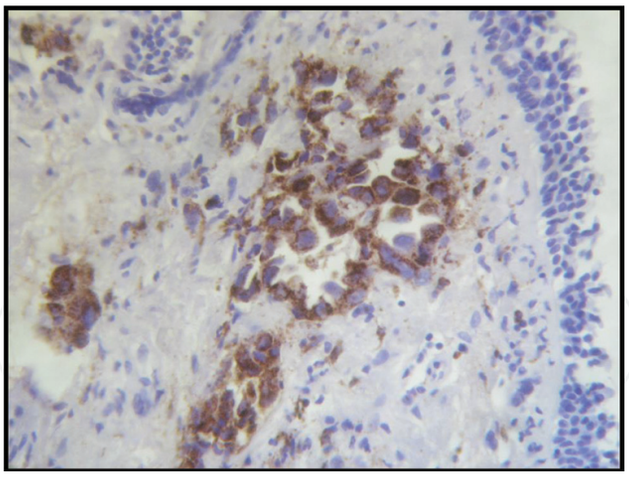

Figure 9. Bronchoscopic biopsy, infiltrate of the acinar adenocarcinoma of the lung in the mucosallayer of the bronchi, proven by using NapsinA 200×.

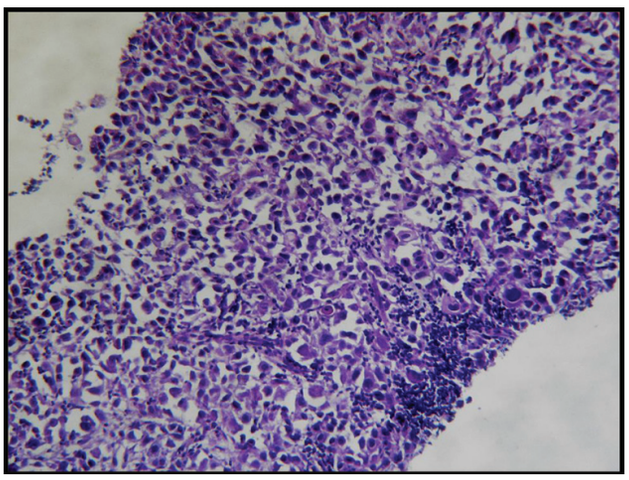

Figure 10. NSCLC deposit in the lymphatic tissue, where squamous cell carcinoma was immunohistochemically proven, H&E 200×.

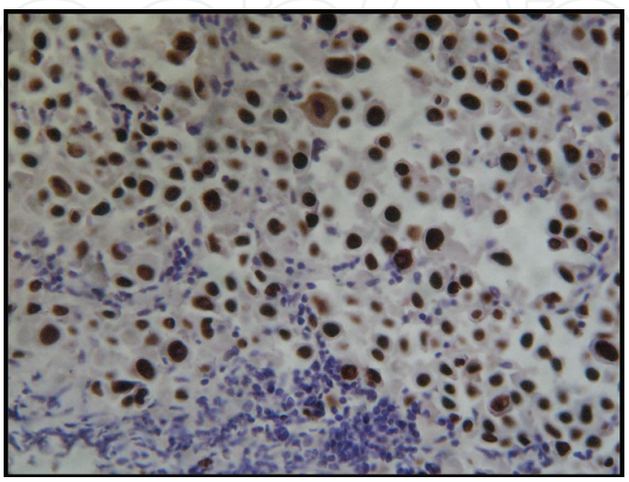

Figure 11. Strong nuclear expression of p63 in the cells of poorly differentiated squamous cell lung carcinoma, 400×.

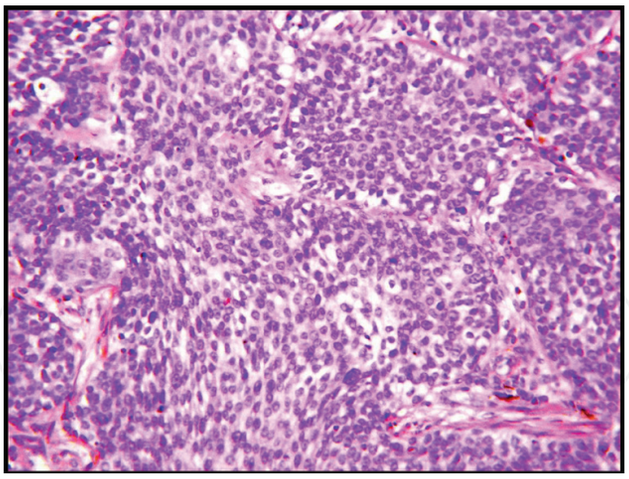

Figure 12. Urothelial carcinoma with lung metastases, H&E 400×.

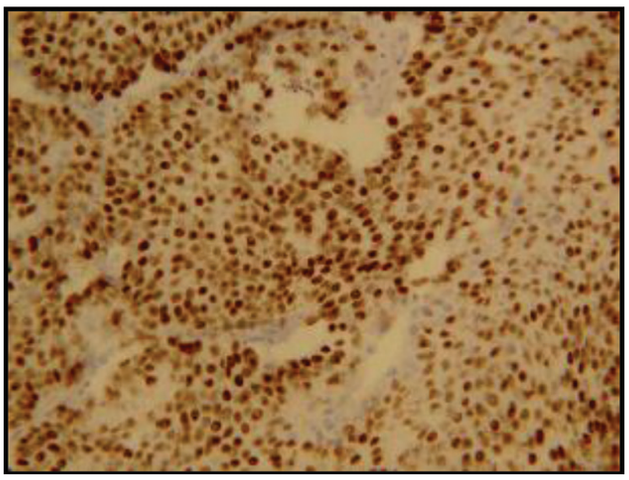

Figure 13. p63 expression in urothelial carcinoma with lung metastases, 400×.

3.5. Cytokeratin5/6 or Cytokeratin5

Cytokeratin 5/6 or Cytokeratin5 is a cytoplasmic marker. Its expression is present at squamous cell carcinoma (**Figure 14**). Because of that, this marker can be used as a confirmation for this histological type, together with p40 and p63, especially if there are no technical conditions for using one of these antibodies. Except in the regular epithelium of bronchial airways, Cytokeratin 5/6 or Cytokeratin5 is also expressed in regular and reactive mesothelioma cells, but also in the epithelium cells of the malignant pleural mesothelioma. Ovarian carcinomas, especially those of serous type, are cytoplasmically expressed antibody [21, 28, 42].

3.6. Neuroendocrine markers

According to WHO recommendations about the classification of lung carcinoma from 2004, in order to establish the diagnosis of large cell lung carcinoma with neuroendocrine differentiation, it is necessary for the cells to be large and round in an organoidal arrangement, with a

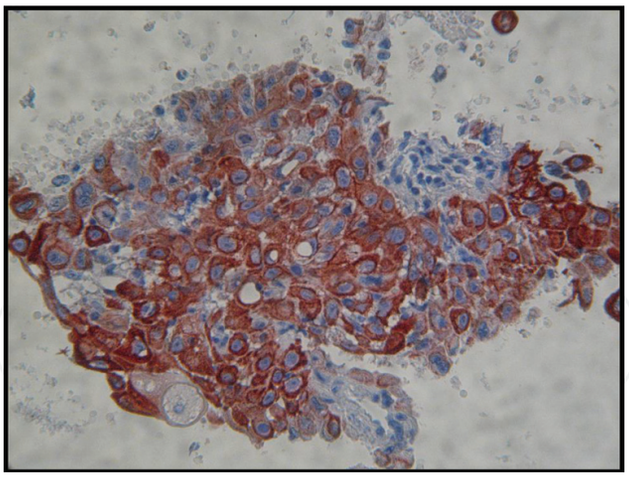

Figure 14. Expression of Cytokeratina5/6 in squamous cell carcinoma, fnab, 200×.

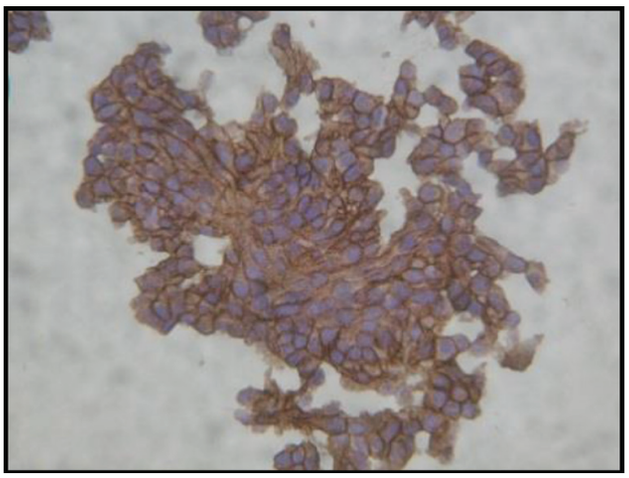

Figure 15. Membrane expression of CD56 into LCLC-NEC cells, 200×.

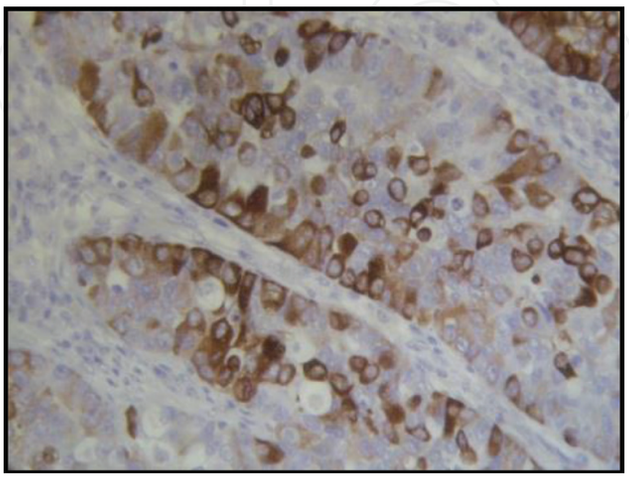

Figure 16. Cytoplasmic expression of Synaptophysin into NSCLC with partly adenoid growth pattern 200×.

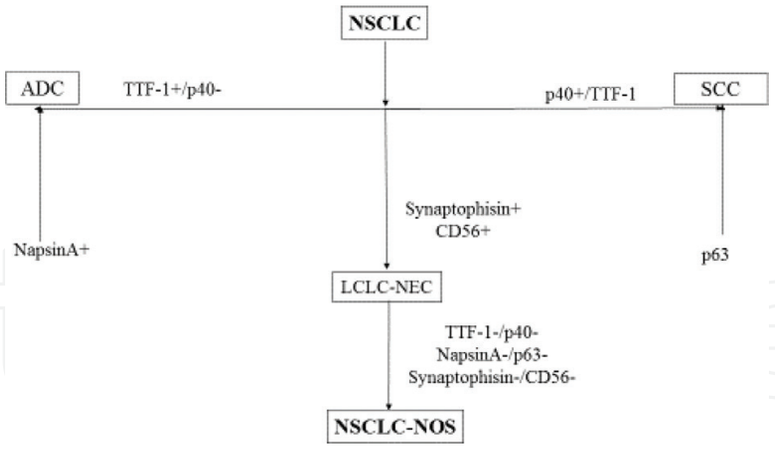

Figure 17. The proposed algorithm for the differential diagnosis of non-small cell lung carcinoma by using immunohistochemical stainings.

luminous and large nucleus and noticeable nucleolus and to express at least one neuroendocrine marker: CD56 (**Figure 15**), Synaptophysin (**Figure 16**) or ChromograninA [5, 6]. It means that if on a small biopsy sample, non-small cell lung cancer is showing organoid, trabecular or acinar growth pattern, and two neuroendocrine markers, preferably CD56 and Synaptohysin [43], should be used to confirm this type of cancer (LCLC-NEC) (**Figure 17**).

4. Discussion

Finally, someone may ask why it is necessary to know of which histological type of non-small cell lung cancer is about and to save enough malignant tissue in paraffin block for molecular testings at the same time.

The answer is that it is necessary to know how to apply certain tests for the application of personalized oncology therapy. If the evaluation of the tests showed that a positive response on therapy is expected, the same will be applied.

Globally, adenocarcinoma of the lungs is the most common histologic type of lung cancer. EGFR molecular testing is used to assess the response to tyrosine kinase inhibitors (TKI) therapy in patients with adenocarcinoma. Positive response to the TKI therapy is more commonly expected in young nonsmoking woman. It is also recommended that this testing is done in young nonsmoking woman with squamous cell lung cancer because it showed positive results. The result of this molecular testing depends on the sensitivity of the method and number of cells remaining after morphological and immunohistochemical diagnostics, more than 5% of malignant cells in relation to the total number of cells on the cross section. EGFR mutations are diagnosed in 10–15% of Caucasians patients with NSCLC [22, 44].

ALK testing (clone D5F3, Ventana, USA) is done in patients with adenocarcinoma of the lungs in which EGFR mutations have not been detected. There is no consensus on whether this test should be performed only in lung adenocarcinoma or it can be performed in all

pathohistological subtypes of NSCLC. Namely, there are several diagnostic algorithms which include all histological types of NSCLC or only adenocarcinoma of the lungs. There are data indicating that this genetic rearrangement was detected only in about 4% of patients with adenocarcinoma of the lungs or in about 2% of patients with NSCLC. In order for this test to be valid, it is necessary that on the tissue cross section at least 50 malignant cells have to be present after primary diagnostics and EGFR testing. Then immunochemical testing would be valid and where there is this type of consensus, to confirm the presence of rearrangements, FISH test should also be done [13, 45, 46].

It is necessary to know the fact that about 70% of LCC-NEC is ALK positive, but that its expression is not in correlation with personalized ALK inhibitors. If NSCLC have an organoid morphological picture, it is necessary to apply CD56 and Synaptophysin on small biopsy samples in order to exclude LCC-NEC [13, 45, 46].

ROS1 testing (clone D4D6, Ventana, USA) is also done in adenocarcinoma of the lungs where EGFR and ALK tests did not show positive results. This fusion is present only in 1–2%, predominantly younger patients, nonsmokers, with adenocarcinoma of the lungs. The presence of fusion is immunohistochemically determined, and it is necessarily confirmed by the FISH method, so it is necessary to preserve tissue for these methods after diagnosis of adenocarcinoma [13, 46].

If any of these tests fail to give results, for the application of immunotherapy, it is applied PDL-1 testing (clone 22C3, DAKO Cytomation, Denmark), mainly in squamous cell lung cancer, but also in adenocarcinomas in which previous test did not show positive results. For this immunohistochemical testing, it is recommended that tissue cross section contains at least 100 malignant cells [47, 48].

The future of personalized lung cancer treatment is next-generation sequencing (NGS), polymerase chain reaction (PCR) method, in which, from the remaining of paraffin block in which NSCLS was diagnosed, at the same time is detecting all druggable mutations [23].

Liquid biopsy is a method that detects DNA tumor cells in whole or in its parts. This method is useful in early detection of lung cancer, but it is false negative if circulating malignant cells do not possess a mutation [49].

5. Conclusions

Precise diagnosis of NSCLC histological type is based only on morphological characteristics of the tumor, cell appearance and their growth pattern on H&E stained preparation is rarely possible. Based on morphological findings, keratinizing squamous cell carcinoma can be diagnosed with great certainty, without immunohistochemistry. The main disadvantage of all used antibodies is not highly specificity only for one histological type of cancer or originates from more than one organ. In order to preserve tumor tissue for molecular testing, one must use only one, the most specific antibody for one histological type of carcinoma. For adenocarcinoma of the lung, highly specific is TTF-1, and for squamous cell carcinoma, it is p40. Other

additional antibodies can be applied only if there is a greater amount of bioptic tumor material (*fnab* biopsy or *VATS* biopsy). NapsinA is an additional antibody, which can be applied in proving adenocarcinoma of the lung and p63 for squamous cell carcinoma. When LCLC-NEC is suspected, it is recommended to use only one neuroendocrine marker, and the most reliable is CD56. For saving, in order to apply all the appropriate antibodies and to preserve tissue for molecular testing, it is necessary to cut only one cross section on one plate.

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