Review / Derleme

2015 World Health Organization Classification of Pulmonary Tumors "A Valid Classification Until the New Classification"

2015 Dünya Sağlık Örgütü Pulmoner Tümör Sınıflandırması "Yeni Sınıflandırmaya Kadar Geçerli Bir Sınıflama"

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Abstract

In 2011, a research panel funded by the International Association for the Study of Lung Cancer (IASLC), American Thoracic Society and European Respiratory Society proposed a variety of significant improvements to all lung tumours prior to lung adenocarcinoma. Furthermore, the recommendations were adopted in this study panel with only minor changes and were identified as the 2015 World Health Organization (WHO) classification of lung tumours. Most of the studies conducted to establish this classification were realised by 'the IASLC Pathology Committee'. Leading experts in these fields have given their approval to certain subheadings for the organization and have accepted the WHO book. The most recent classification of lung, pleura, thymus and heart tumours was published by the WHO in 2015. Over the last 15 years, the classification of lung cancer has undergone major changes since the WHO classification in 2004 due to the effects of genetic research on lung cancer and important advances in target therapies. In this review article, these changes will be assessed from a pathologist's perspective.

Öz

2011 yılında Uluslararası Akciğer Kanseri Çalışmaları Derneği (IASLC), Amerikan Toraks Derneği, ve Avrupa Solunum Derneği'nin sponsor olduğu çalışma panelinde, öncelikle akciğer adenokarsinomu olmak üzere tüm akciğer tümörleri için birçok önemli değişiklik önerilmiştir. Bu çalışma panelinde önerilenler, yalnızca minor değişiklikler ile kabul edilerek 2015 Dünya Sağlık Örgütü (DSÖ) akciğer sınıflaması olarak tanımlanmıştır. Bu sınıflamayı oluşturmak için yapılan çalışmaların çoğu, "IASLC'nin Patoloji Komitesi" tarafından gerçekleştirilmiştir. DSÖ kitabının düzenlenmesi ve kabulü için, bu konularda rehber niteliğindeki uzmanlar belli alt başlıklar için onay vermişlerdir. Böylece, DSÖ 2015 yılında, akciğer, plevra, timus ve kalp tümörlerinin en son sınıflamasını yayınlamıştır. Son on beş yıl içinde akciğer kanser genetiği ile ilgili çalışmaların sonuçlarına ve hedef tedavilerinde ortaya çıkan önemli gelişmelere bağlı olarak, bir önceki 2004 DSÖ sınıflamasından sonra akciğer kanser sınıflamasında önemli değişiklikler olmuştur. Bu derleme yazısında, bu değişiklikler patolog gözüyle değerlendirilmeye çalışılacaktır.

Introduction

The World Health Organization (WHO) has published the most recent classification of lung, pleura, thymus, and heart tumors (1). Previously, in chronological order, WHO published classifications of lung tumors in 1967, and 1981, lung, and pleura tumors in 1999, and lastly lung, pleura, thymus, and heart tumors in 2004 (2-5). Within the last 15 years, related to the results of the genetic studies on lung cancer, and important developments in the target treatments, classification of lung cancer has undergone important changes after 2004 WHO classification. In this review article these changes will be evaluated from the perspective of a pathologist. In the year 2011, at a study panel sponsored by the International Association for the Study of Lung Cancer (IASLC), American Thoracic Society (ATS), and European Respiratory Society (ERS), many important changes for all lung tumors priorly for lung adenocarcinoma were recommended (6). At this study panel the recommendations were accepted with only minor changes, and defined as 2015 WHO classification of lung tumors. Most of the studies performed to create this classification were realized by "the Pathology Committee of the IASLC". This committee has supported organization of annual meetings, and participation in international multidisciplinary meetings for more than 10 years. Leading experts in these fields have provided their consent for certain subheadings for the organization, and acceptance of the WHO book. When compared with 2004 WHO classification of lung tumors, the most important changes in this classification will be considered one by one in the following sections.

1- We are Starting to Use Immunohistochemistry More Frequently

In previous WHO classifications, the diagnosis of lung cancer was made by microscopic examination of hematoxylin-eosin stained tumor tissue samples. Rarely mucin dyes were required for the diagnosis of adenocarcinoma. Immunohistochemical applications came into use firstly in 1999 WHO classification. In fact, more accurately it has begun to be used in 2004 WHO classification. It was then used only for large-cell neuroendocrine carcinomas (LCNEC), sarcomatoid carcinomas, and all of other carcinomas included in the differential diagnosis (2,5). However in 2015 WHO classification, use of immunohistochemical methods for small biopsies, cytologic specimens, and pulmonary resection materials has been recommended. For the analysis of resection materials containing some certain tumors as solid adenocarcinoma, non-keratinized squamous cell carcinoma (SCC), large-cell carcinoma (LCC), and neuroendocrine carcinoma, use of immunohistochemical methods is strongly advised. The rationale for this recommendation is application of target treatments for some subtypes of non-small cell lung cancers (NSCLC). For example for tumors with non-squamous histology type, chemotherapeutic agents as bevacizumab, and pemetrexed are used. In brief, more precise nomenculature for histopathological subtypes have become a necessity. However, during the application of immunohistochemical methods, supportive measures as use of good quality stains, in addition to ensuring quality control, and internal control have been strongly emphasized.

2- We Should Include Molecular Tests in Our Routine, We are Approaching to Genetic Tests One Step Closer

Application of molecular tests has been recommended for the achievement of individualized treatment modalities in advanced lung cancer patients. In other words, the trend is to combine histological, and genetic methods, and towards implementation of a molecular classification. Indeed as is the case with many cancer types, important developments have been achieved in the treatment decisions based on histological, and genetic characteristics of the tumor, in other words in individualized medical practices. Nowadays, discrimination between lung cancers as small-cell or non-small cell is not sufficient. Differentiation of non-small-cell carcinomas into specific pathological subtypes as adenocarcinomas or SCCs has become a necessity for us, the pathologists. Indeed this discrimination enables implementation of molecular tests, and treatment strategies.

In patients with advanced pulmonary adenocarcinoma, mutations of epidermal growth factor receptors (EGFR), and rearrangements of anaplasic lymphoma kinase (ALK) have been discovered to be effective targets for EGFR thyrosine kinase or ALK inhibitors. This condition has laid the responsibility of evaluating tumor histology, together with tumor biology, in other words, the construction of the basis of translational approach for the implementation of treatment strategies

on pathologists (6). Pathologist is compelled to discriminate histologically between SCC, and adenocarcinoma. The reason for this condition is the correlation between histological type of tumor, and its biology, and related treatment alternatives which can be summarized as follows; a) EGFR mutations, ALK and ROS1 rearrangements were priorly detected in adenocarcinomas, b) determination of higher effectiveness of pemetrexed in advanced pulmonary adenocarcinoma versus SCC, c) contraindication of bevacizumab in SCC, d) effectiveness of nivolumab in advanced SCC (6). In conclusion experienced clinicians, and pathologists recommend application of molecular tests in tumors classified as adenocarcinoma for EGFR mutation, and rearrangement of ALK. Even, this recommendation is valid for all tumors with a suspicion of having a component of adenocarcinoma (7,8).

3- We Should Use Our New Criteria for Small Biopsies and Cytologic Specimens

2015 WHO classification firstly advised standardized criteria, and terminology to be used for the evaluation of small biopsies (bronchoscopic, needle or core biopsies), and cytologic specimens so as to diagnose lung cancer. In previous classifications of WHO (1967, 1981, 1999, and 2004) resected samples were predominantly considered for the classification of lung cancer. However 2015 WHO classification recommend use of the identical criteria not only for resected tumors, but also for the diagnosis of small biopsy, and cytologic samples. The approach to the small biopsies is summarized in Table 1. This recommendation is also important, and valuable for some reasons: a) At the time of diagnosis most of the patients with lung cancer are at an advanced stage of their disease, b) generally diagnosis is made based on only small biopsy/cytology material, c) in recent years, treatment alternatives based on histological subtypes have demonstrated differences, therefore as the term of non-small cell lung carcinoma- not otherwise specified (NOS) should be used as little as possible. These small biopsy samples, and cytologic materials are extremely valuable for both establishment of accurate diagnosis, and their use in molecular tests. Therefore these materials should be used rationally, too many tissue samples should not be used for immunohistochemical analysis. In addition, demand for the most accurate histopathological diagnosis are compelling issues for the pathologists (6,9). During routine practice, we find it hard to obtain tissue sections for immunohistochemical tests to be used in the differential diagnosis between adenocarcinoma, and SCC. Which is more correct approach? Making the fewest number of sections to preserve the integrity of tissue for further molecular tests or preparing blank sections to be used for additional immunohistochemical staining in case of need? If a tumor has a recognizable morphology of SCC as

Table 1. The histopathological and immunohistochemical approach in the small biopsies

In small biopsies
If morphologic adenocarcinoma patterns clearly present
Adenocarcinoma with lepidic pattern
Invasive mucinous adenocarcinoma
Adenocarcinoma with colloid features
Adenocarcinoma with fetal features
Adenocarcinoma with enteric features
If morphologic adenocarcinoma patterns not present but supported by special stains (i.e., TTF-1 or napsin-A)
Non-small cell carcinoma, favor adenocarcinoma
If morphologic squamous cell patterns
Squamous cell carcinoma
If morphologic squamous cell patterns not present but supported by stains (i.e. p40, p63 or CK5/6)
Non-small cell carcinoma, favor squamous cell carcinoma
If no clear adenocarcinoma, squamous or neuroendocrine morphology or staining pattern
Non-small cell carcinoma, not otherwise specified

keratinization, and intercellular bridges or carries morphological patterns of adenocarcinoma as acinar, lepidic, papillary, and micropapillary formations, then respective diagnoses of SCC and adenocarcinoma can be made without immunohistochemical examination. In poorly differentiated tumors which can not be diagnosed without immunohistochemical analysis or those can not be discriminated under light microscopic examination limited immunohistochemical procedures are recommended taking care to spare as much tissue as possible. Usually with only one marker of squamous cell (p63 or p40), and adenocarcinoma (TTF-1 or mucin) classification can be made. If differential diagnosis can not be established between SCC and adenocarcinoma in non-small cell carcinomas using these methods, then definition of these tumors in the group of NOS has been recommended. Besides refraining from use of the letter ''L'' to signify ''lung'' is advised, accordingly WHO guidline indicates that using the abbreviation non-small cell carcinoma instead of NSCLC is more appropriate. Indeed in small biopsy or cytologic samples, absence of marker of pneumocyte expression does not rule out the possibility of metastatic carcinoma. Therefore before making a diagnosis of primary lung tumor, another primary focus should be clinically ruled out. TTF-1, and Napsin-A have been accepted as markers of adenocarcinoma in the differential diagnosis of adenocarcinoma (10,11). Since TTF-1 is a nuclear marker, it is easy to determine its value. Though cytokeratine 5/6, and p63 are firstly recommended markers for squamous differentiation, currently p40 has been reported as the most specific, and sensitive marker for SCC (12,13). In case of need, for immunohistochemical analysis, generally in the determination of squamous, and glandular differentiation use of at least one or at most two markers has been recommended. Generally firstly panels of TTF-1, and p40 are said to be suitable, each pathology laboratory is routinely using its own marker for evaluation. If one group of tumor cells has TTF-1 reactivity, and the other group has positivity for squamous cell marker, then the possibility of adenosquamous carcinoma should be thought of. However it should not be forgotten that it will be more appropriate to make such a diagnosis "adenosquamous carcinoma'' based on histopathological examination of a resection specimen.

Application of new diagnostic criteria, and recommendations for small biopsy, and cytologic specimens will aid in the collection of important data for further studies to be performed in the future. Besides, introduction, and use of new criteria for both small biopsies, and resection materials in routine practice so as to provide data for large-scale genetic studies to be conducted in the future convey utmost importance. For example for classification of a poorly differentiated tumor as solid adenocarcinoma or non-keratinized SCC immunohistochemical methods should be used.

Even absolute use of 2015 WHO classification for small biopsies, and cytologic specimens has utmost importance, interestingly for most lung tumors a histological grading system is not available. For neuroendocrine tumors we can indicate presence of a grading system [typical carcinoid (TC) -low-grade, atypical carcinoid (AC)- intermediate grade, high-grade LCNEC, and small-cel carcinoma]. Some tumors are entirely of high grade (large cell, and pleomorphic carcinoma etc). As recommended, grading of adenocarcinomas in resection samples, should not only be based on structural, but also on nuclear characteristics (14). However, the grading system based on predominant pattern is being currently used, because it is a simple, and satisfactory ranking system. For example, tumors with lepidic, acinar- papillary, and solid- micropapillary predominant patterns were classified in low-, moderate, and poorly differentiated groups, respectively. Though limited information is available for SCCs, size of nuclei has been suggested as a parametre of worse prognosis (15). Besides tumor ''budding'' in both adenocarcinoma, and SCC has been reported to be an indicator of poor prognosis (16).

4- Mostly Classification of "Adenocarcinoma" Has Changed, Nowadays We are Using a Different Approach (Bronchioloalveolar Carcinoma?)

In the year 2011 a new classification was proposed by IASLC/ATS/ERS for lung adenocarcinomas. This classification contained important changes from 2004 WHO classification. They can be summarized as follows: a) Terms of bronchioloalveolar carcinoma, and mixed type adenocarcinoma are not used anymore. b) "Atypical adenomatous hyperplasia" was included in the class of preinvasive lesions in addition to adenocarcinoma *in situ* (AIS). c)

Minimally invasive adenocarcinoma (MIA) was included in the classification, d) classification of all subtypes of invasive adenocarcinomas is based on predominant histological pattern, e) use of the term "lepidic" for non-invasive component of an invasive adenocarcinoma (old term: bronchoalveolar carcinoma). f) use of ''invasive mucinous carcinoma'' instead of the old term ''mucinous bronchoalveolar carcinoma.'' (excluding tumors meeting criteria of AIS or MIA), g) Terms of clear-cell, and signet cell are only indicated as a characteristic feature in 2015 WHO classification not as a subtype, h) term of mucinous cystadenocarcinoma is excluded from the list, and included in the category of colloid adenocarcinomas.

As an important issue even lack of mucin secretion, in 2015 WHO classification for the tumors expressing pneumocyte markers as TTF-1 or napsin A the term ''solid adenocarcinoma'' has been recommended. In previous classification this group of tumors were termed as "large cell carcinoma" (1). Besides in solid adenocarcinomas five or more than five cells should contain intracytoplasmic mucin at least two high magnification microscopic fields of vision.

Diagnostic criteria for AIS, and MIA are summarized in Graphic 1, 2. If the predominant pattern in resection material of invasive adenocarcinoma is lepidic pattern, then it should be called as lepidic adenocarcinoma or adenocarcinoma lepidic subtype. Estimated percentages of the lepidic components, and all components of all other patterns should be provided. In tumors with lepidic component extent of invasion may be falsely measured. If the tumor is small, and only one focus is invaded then measurements can be made directly using microscope. If the tumor is not limited to a single microscopic field of view or multiple number of foci of invasion exist, then it is difficult to make measurements. In these cases, a measurement method is recommended based on the calculations of the percentage of the invasive component, size of the tumor, the extent of invasion (17). If invasive foci are smaller than 5 mm, then it is classified as MIA, if it is larger, then as lepidic carcinoma. In some cases even if tumor containing tissues are reexamined, size of the tumor can not be precisely determined. In such cases high-resolution computed tomography may aid in diagnosis. Usually areas resembling ground glass correspond to a lepidic, and solid areas to an invasive component.

As will be guessed, diagnoses of AIS, and MIA can be made only for resection materials. Sometimes, both macroscopic, and microscopic evaluation of all resection materials can be required. If small biopsy material contains only non-mucinous lepidic pattern then it should be termed as "adenocarcinoma with lepidic pattern". However as pathologists we should indicate some definitions in our reports. While indicating the diagnosis of adenocarcinoma with lepidic pattern in a small biopsy specimen, we should also write in the report that this histologic appearance

	Diagnostic Criteria for Adenocarcinoma *in Situ*
!	A small tumor ≤3 cm and a solitary adenocarcinoma ⇓ Growth pattern must be pure lepidic type ⇓ No invasion Stromal, vascular or pleural ⇓ No spread through air spaces ⇓ No pattern of invasive adenocarcinoma ⇓ Cell type mostly non-mucinous, less mucinous type ⇓ Nuclear atypia is absent or inconspicuous

Graphic 1. Diagnostic criteria for adenocarcinoma *in situ*

	Minimally Invasive Adenocarcinoma
!	A small tumor ≤3 cm and a solitary adenocarcinoma ⇓ Growth pattern predominantly lepidic type ⇓ Invasion ≤0.5 cm (greatest dimension in any one focus) ⇓ Invasive component - Any histologic subtype other than a lepidic pattern - Tumor cells infiltrating myofibroblastic stroma ⇓ No invasion: Stromal, vascular or pleural ⇓ No spread through air spaces ⇓ No tumor necrosis ⇓ Cell type mostly non-mucinous, less mucinous

Graphic 2. Diagnostic criteria for minimally invasive adenocarcinoma

may represent a lesion with AIS, MIA or invasive adenocarcinoma with a lepidic component. Rarely, also metastatic tumors may demonstrate lepidic pattern. In this case presence of TTF-1 negativity, and characteristic signs detected by radiological means differentiate between similar entities.

5- Why Do We Report Subtypes of Lung Adenocarcinoma in Detail?

Since lung adenocarcinomas generally consist of a mixture of histological subtypes, in 2015 WHO classification, the term ''predominant'' has been started to be used in the diagnosis of adenocarcinoma as lepidic or acinar predominant adenocarcinoma (Figure 1). This terminology has replaced 2004 WHO classification term of "adenocarcinoma, mixed subtype". Reporting each adenocarcinoma pattern semiquantitatively in 5% sections has enabled pathologists to evaluate all histologic subtypes instead of focusing on only one predominant type. In most of the previous studies for analyses of patterns, and proportional values a cut-off value of 10% was used. Nowadays use of a cut-off value of 5% is helpful in the determination of predominant type especially in tumors having two subtypes in nearly similar percentages. Besides this approach will prevent overlooking subtypes with prognostic value even though they are present in very small percentages as micropapillary pattern.

In pulmonary adenocarcinomas two predominant pattern may present in the same proportions (as percentages). However in reports only one predominant pattern should be chosen. Another important issue for pathologists is reporting adenocarcinomas of respiratory tract demonstrating acinar or lepidic pattern containing tumor cells with micropapillary pattern (Figure 2). It has been recommended that these types of tumors should be classified as micropapillary tumors rather than acinar or lepidic predominant tumors.

Following recommendations of 2011 IASLC/ATS/ERS for the classification of pulmonary adenocarcinomas, spread through air spaces (STAS) has become more understandable among pathologists. STAS can be defined as progression or invasion of the tumor into surrounding lung tissue as single cells, solid nests or micropapillary clusters. It won't be a false assumption to predict that this tumoral invasion will induce risk of recurrence in early stage tumors which were not completely resected. Some researchers also indicated this condition as a worse prognostic factor (18,19). STAS is not taken into consideration during histological subtyping or measurement of the extent of invasion. Though STAS is not considered in the measurement of invaded area, if STAS is seen, diagnosis should not be given as AIS/MIA, and STAS should be reported separately as visceral pleural invasion or vascular invasion.

In the presence of multiple pulmonary adenocarcinomas, it is difficult to discriminate multiple primary tumors from intrapulmonary metastasis of a single tumor. In the pathology report, in addition to predominant pattern, specification of the percentages of each component in detail will

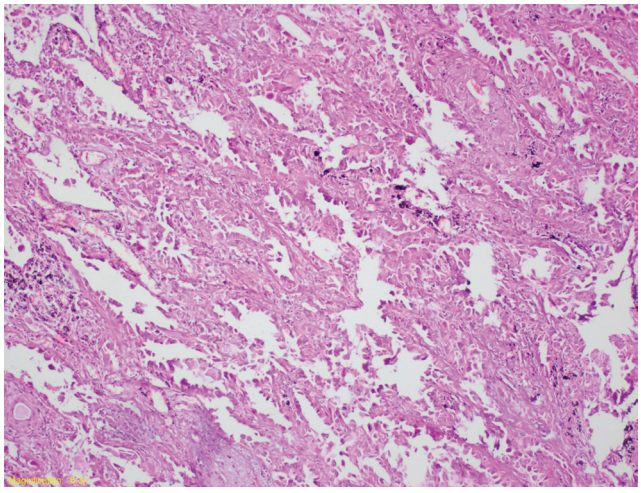


Figure 1. Resection material shows an "adenocarcinoma with a lepidic pattern"

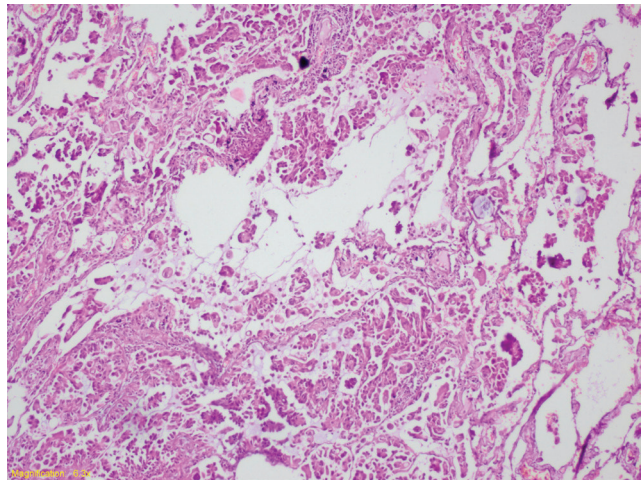


Figure 2. Tumor cells consists of micropapillary clusters within airspaces in the lung parenchyma

facilitate this differentiation process. This method of reporting will provide information of utmost importance, in conditions where re-assessments of tumor preparations of the same patient are required and/or archival materials can not be accessed. Besides, these data will be needed in retrospective studies, and in novel classifications to be recommended in future years. In addition, reporting the presence of tumor cells containing clear cell or signet cell type, and stromal characteristics as desmoplasia or reactive immune response against tumor carries crucial importance. Since in metastatic tumor foci, instead of most frequently seen predominant component, poorly differentiated components as solid or micropapillary patterns can be observed in the primary tumor, preparation of a detailed report has an utmost importance. Besides, this approach will enable conduction of various genetic studies. However centers where genetic studies will be performed, the methods to be used, and criteria of interpretation are not clearly established yet. Besides, during these procedures, tumor tissue should be carefully utilized, and it should not be wasted for unnecessary tests.

In conclusion, we, the pathologists should arrive at a diagnosis by writing a detailed report which evaluate tumor tissue with the support of clinical, and radiological information. In addition, we should support appropriate molecular studies in order to be able to perform target treatments based on tumor biology. We should improve ourselves, and perform updated molecular tests. If we can not perform these tests, then we should evaluate samples containing tumor tissue very attentively.

6- We are Beginning to Classify SCCs as in the Case with Classification for Nasophayngeal Cancers

With new classification, SCC is divided into subgroups of ''keratinized'', ''non-keratiinzed'', and ''basaloid''. As is known, 2004 WHO classification contained papillary, clear cell, small cell, and basaloid subtypes. In new classification, the reasons for giving up using these terms for subtypes are indicated as follows; a) since papillary, clear cell, and small-cell subtypes are very rare, it is not meaningful to subtype them separately, b) small-cell subtype can be confused with small-cell carcinoma, c) clear cell modification is not a subtyype but a cytologic characteristics which can be seen in miscellaneous tumor types, d) some adenocarcinomas can appear like SCC (especially in non-keratinizied SCC's confirmation with p63, and p40 is a must), e) basaloid carcinomas included in the LCC group express squamous cell markers as detected with immunohistochemical analysis, and demonstrate similar genetic modifications (their inclusion in SCC subgroup is more appropriate).

Based on these reasons, lung SCCs have been divided into three groups just like WHO head and neck classification of nasopharyngeal carcinomas (20);

- Keratinized (presence of keratinization is important rather than its extent),
- Non-keratinized,
- Basaloid (keratinization present/absent, if basaloid areas are >50%).

A clear-cut prognostic difference has not been defined among these three types, and so diverse outcomes have been reported (1,15,21).

Therefore subtyping in SCC is not currently necessary. However, as is the case with classification of adenocarcinomas, detailed pathology report should be written. We should not forget that these subtypes can be targets of new treatment modalities in the future.

7- How Do We Define Large-cell Carcinoma?

With 2015 WHO classification definition of LCC can only be provided for only resection materials (for small biopsy/cytologic samples such a definition is not valid!!!!). With this classification tumors previously included in ''large-cell group'' were distributed into different groups. Thus the term ''LCC'' has been started to be used only for tumors which do not demonstrate any morphological and immunohistochemical differentiation.

In 2004 WHO classification, the following types of carcinoma were included in the group of LCC (2):

- Large-cell neuroendocrine carcinoma,
- Basaloid carcinoma,
- Lymphoepithelioma-like carcinoma,
- Clear cell carcinoma,
- LCC demonstrating rhabdoid.

As is known, according to 2004 WHO classification, in the diagnosis of LCC with solid pattern, immunohistochemical markers which will discriminate between adenocarcinoma, and SCC were not given due importance. However 2015 WHO classification, use of these markers has enabled differentiation of tumors with solid pattern as solid adenocarcinoma or non-keratinized SCC. Use of these markers has

gained increasingly widespread use in line with the results of some genetic, and immunohistochemical data. Indeed, tumors classified as LCC have been actually reported to represent a heterogenous group of tumors with adenocarcinoma, and squamous cell differentiation (22). As a matter of fact, this group also contains tumors which do not stain with pneumocyte, squamous, or an neuroendocrine marker, in other words they are termed as tumors with null immunological structure (22).

Conclusion; with 2015 WHO classification LCCs have been started to be evaluated immunohistochemically based on TTF-1, p40, and neuroendocrine markers. Currently tumors demonstrating solid pattern are accepted as solid adenocarcinoma if pneumocyte marker TTF-1 positivity is detected, and p40 (a marker of squamous differentiation) positivity indicates the presence of non-keratinized SCC.

(2015 WHO-LCC: Mucin-negative, lack of protein expressions of squamous cell or adenocarcinoma markers).

Other subtypes categorized in the LCC group in 2004 WHO classification were included in other groups in 2015 WHO classification as follows: a) LCC in 2004 WHO classification, was transferred into group of neuroendocrine tumors in 2015 WHO classification. b) Basaloid carcinoma in 2004 WHO classification was considered as a SCC subtype in 2015 WHO classification. c) Lymphoepithelioma-like carcinoma in 2004 WHO classification was placed in the group of other/unclassified tumors in 2015 WHO classification. The categories of clear cell carcinoma, and rhabdoid phenotype were removed from the classification, and considered as a cytologic characteristics of other histological types.

8- Neuroendocrine Tumors are Gathered Under a Single Roof

For the first time in 2015 WHO classification, all neuroendocrine tumors of the lung were gathered in a separate group. Besides diffuse idiopathic pulmonary neuroendocrine cell hyperplasia (DIPNECH) which is a preinvasive lesion was included in this group but under a different subheading (1).

• Small-cell carcinoma
- Combined small-cell carcinoma
• Large-cell neuroendocrine carcinoma
- Combined large-cell neuroendocrine carcinoma
• Carcinoid tumors
- TC
- AC
• Preinvasive lesion
- DIPNECH (it consists of distinct neuroendocrine cell hyperplasia, tumorlets localized along bronchial wall).

Carcinoid tumors differ from high-grade small-cell carcinomas, and large- cell neuroendocrine carcinomas from clinical, epidemiological, histological, and genetic perspectives. Patients with carcinoid tumors are younger, have a better prognosis, and non-smokers which discriminate them from large- or small-cell neuroendocrine carcinoma patients. Morphologically much greater number of mitosis, and diffuse necrosis are observed in small cell carcinoma, and LCNEC. Besides in genetic analyses greater number of genetic disorders have been detected in small- cell carcinoma, and LCNEC when compared with carcinoid tumors (23). Most of the time, examination of well-fixated cytologic samples and/or hematoxylin-eosin stained tumor tissue slides of good quality prepared using conventional methods under light microscope is sufficient for the diagnosis of small-cell carcinoma (Figure 3). Only in case of need, neuroendocrine markers aid in diagnosis.

Another important feature of 2015 WHO classification, is that for the first time Ki-67 proliferation index is included in differential diagnosis (Figure 4). Ki-67 especially plays a role in the differentiation especially between carcinoid tumors, high-grade small cell carcinoma, and LCNEC in crushed or necrotic tissue samples (24).

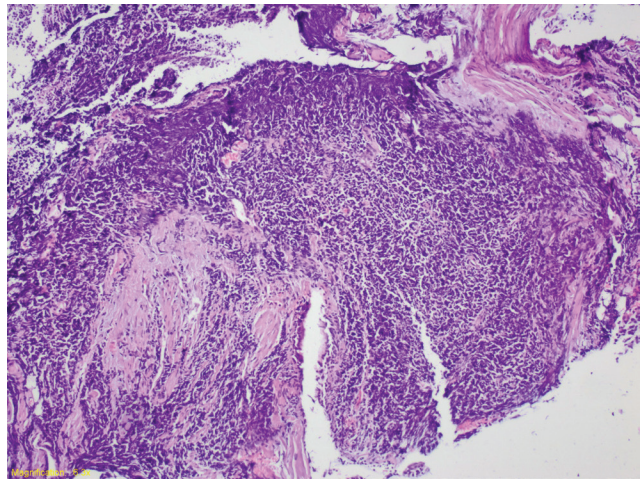


Figure 3. Small cell carcinoma

Acceptable ranges of Ki-67;
- for small cell carcinoma, 50-100%,
- for LCNEC, 40-80%,
- for AC, 20%,
- for TC, 5%.

In the 2015 WHO classification, number of mitoses are described in more detail. Number of mitoses should be counted on areas with the highest mitotic activity. Number of mitoses on an area of 2 mm^2 are evaluated. Since areas of high magnification can differ among different models of microscopes, evaluation of an area of 2 mm^2 instead of an area of high magnification is recommended. Average number of mitoses on at least three different areas of magnification are taken into consideration. Mitotic ratio, and state of necrosis should be always indicated in cases with typical, and AC tumors.

9- Classification of Sarcomatoid Carcinoma Has not Changed, but Please Specify

From 2004 on any change in the terminology, and diagnostic criteria of sarcomatoid carcinoma has not been made. Pleomorphic carcinoma is a general term used for carcinosarcoma, and pulmonary blastoma. However it is appropriate to specify rather than using a general term so as to avoid confusion with real sarcoma. In this type of tumors molecular analysis is recommended to evaluate genetic abnormalities based on the existing histological components. Indeed prognosis of these types of tumors is very bad, and based on the results of these genetic tests possibilities of target treatment which will be able to prolong survival times exist.

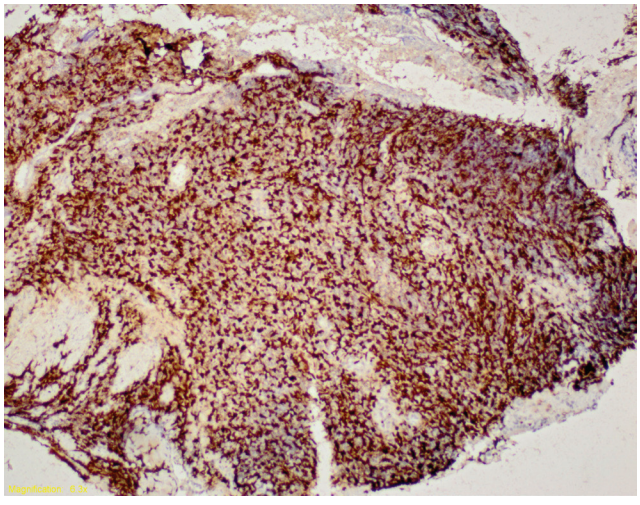


Figure 4. Ki-67 prolfieration index is very high (nearly 100%)

10- A New Type of Tumor- "NUT" Midline Carcinoma" Derived from *NUT* Gene

NUT midline carcinoma is defined as chromosomal reaarrengament in the *NUT* gene from which it originates, and this type of carcinomas are poorly differentiated tumors. It has been firstly included in 2015 WHO lung cancer classification (25). This tumor was defined as a thymic tumor in 2004 WHO classification. Histopathologically it consists of layers of small-intermediate sized undifferentiated monomorphic cells, and their nests (26). Using immunohistochemical method, it is detected nuclear positivity with granular characteristics in more than 50% of tumoral cells. Fortunately, it is a rarely seen aggressive malignancy.

***Following 2004 WHO classification, as important changes pulmonary tumors apart from lung cancers have been defined which include sclerosing pneumocytoma, pulmonary hamartoma, PEComatous tumor group, epithelioid hemangioendothelioma, pulmonary myxoid sarcoma (EWSR1-CREB1 translocation), myoepithelial tumors, and Erdheim-Chester disease. Let's briefly describe these types of tumors:

•Sclerosing Pneumocytoma (Instead of Sclerosing Hemangioma)

The term sclerosing hemangioma was included in the group of "Miscellaneous tumors" in "1999, and 2004 WHO classification. In 2015 WHO classification instead of the term ''sclerosing hemangioma", it was named as ''sclerosing pneumocytoma'' in 2015 WHO classification, and included in the adenoma group. Indeed it is not a vascular tumor. It arises from primitive respiratory epithelial cells (TTF-1 positive) (27). Therefore ''adenoma'' is a more accurate term. This lesion contains two types of (dual) neoplasic cell population (28). They generally demonstrate a benign course, and rarely metastatize.

•Pulmonary Hamartoma (A True Neoplasia!)

Hamartomas seen in non-pulmonary locations are not considered to be neoplasic formations. However genetic studies on pulmonary hamartomas have demonstrated that pulmonary hamartomas are true neoplasias. Therefore this entity is included in "International Classification of Diseases for Oncology Code". Correct terminology is not hamartoma but "pulmonary hamartoma". Translocation t (3;12) (q27-28;q14-15) is frequently seen in pulmonary

hamartomas. At least two mesencymal components (cartilage tissue, connective tissue, adipose tissue, and smooth muscle etc), and surrounding respiratory epithelium are combined in various rates (29). Mesenchymal component is generally rich in chondroid or chondromyxoid tissue. For the diagnosis of pulmonary hamartoma immunohistochemical methods are not required. In most of the cases only morphology establishes the diagnosis.

•PEComatous Tumor Group

PEComatous tumors acceptedly arise from perivascular epithelioid cells. They are seen in three different forms in lungs;

-Diffuse multicystic proliferation [Lymphangioleiomyomatosis (LAM)],

-Clear cell tumor/PEComa benign localized mass (very rare),

-Diffuse proliferation where characteristics of both groups overlap (extremely rare).

In 1999 WHO classification LAM was included in the tumor-like lesions, in 2004 WHO classification it was shifted into the group of mesenchymal tumors (2,5). Clear cell tumors were included in the group of "Miscellaneous tumors" in both 1999, and 2004 classifications. In 2015 WHO classification all of these tumors have been included under one heading. "PEComatous tumors". PEComatous tumors consist of three subgroups;

- LAM,
- Benign PEComa,
- Malign PEComa.

•Epithelioid Hemangioendothelioma

It is a low-intermediate grade malign vascular tumor. Morphologically, it consists of solid nests, and short chords of endothelial cells resembling epitheloid appearance within the myxohyalin stroma (30). In this tumor translocation in *WWTR1*, and *CAMTA1* genes are detected. It is considered as an intermediate grade type in the presence of necrosis, increased mitosis (2/2 mm²), and dictinct nuclear atypia (30). Hemorrhagic pleural effusion in addition to diffuse intrapulmonary, and pleural invasion are associated with poor prognosis.

In the lasted classification of pulmonary tumors (4th edition WHO 2015) the authors have achived very important improvements in comparison with previous editions. Most importantly, it is a multidisciplinary for diagnostic and therapeutic decisions in the patient management. Up to the validated randomized studies including new established diagnostic and therapeutic algorithms, and molecular biomarkers, 2015 WHO classification seems to continue to be used.

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References

1. Travis WD, Brambilla E, Burke AP, Marx A, Nicholson AG. WHO classification of tumours of the lung, pleura, thymus and heart. Lyon: International Agency for Research on Cancer; 2015.
2. Travis WD, Brambilla E, Müller-Hermelink HK, Harris CC. Pathology and genetics: tumours of the lung, pleura, thymus and heart. Lyon: International Agency for Research on Cancer; 2004.
3. World Health Organization. Histological typing of lung tumours. Geneva: World Health Organization; 1967.
4. World Health Organization. Histological typing of lung tumors. Geneva: World Health Organization; 1981.
5. Travis WD, Colby TV, Corrin B, Shimosato Y, Brambilla E, in collaboration with LHS, Countries pf. Histological typing of lung and pleural tumors. Berlin: Springer; 1999.
6. Travis WD, Brambilla E, Noguchi M, Nicholson AG, Geisinger KR, Yatabe, et al. The new IASLC/ATS/ERS international multidisciplinary lung adenocarcinoma classification. J Thoracic Oncol 2011; 6: 244-85.
7. Lindeman NI, Cagle PT, Beasley MB, Chitale DA, Dacic S, Giaccone G, et al. Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology. J Thorac Oncol 2013; 8: 823-59.
8. Leighl NB, Rekhtman N, Biermann WA, Huang J, Mino-Kenudson M, Ramalingam SS, et al. Molecular testing for selection of patients with lung cancer for epidermal growth factor receptor and anaplastic lymphoma kinase tyrosine kinase inhibitors: American Society of Clinical Oncology endorsement of the College of American Pathologists/International Association for the study of lung cancer/association for molecular pathology guideline. J Clin Oncol 2014; 32: 3673-79.
9. Travis WD, Brambilla E, Noguchi M, Nicholson AG, Geisinger K, Yatabe Y, et al. Diagnosis of lung cancer in small biopsies and cytology: implications of the 2011 International Association for the Study of Lung Cancer/American Thoracic Society/European

Respiratory Society classification. Arch Pathol Lab Med 2013; 137: 668-84.

10. Travis WD, Rekhtman N, Riley GJ, Geisinger KR, Asamura H, Brambilla E, et al. Pathologic diagnosis of advanced lung cancer based on small biopsies and cytology: a paradigm shift. J Thorac Oncol 2010; 5: 411-4.
11. Turner BM, Cagle PT, Sainz IM, Fukuoka J, Shen SS, Jagirdar J. Napsin A, a new marker for lung adenocarcinoma, is complementary and more sensitive and specific than thyroid transcription factor 1 in the differential diagnosis of primary pulmonary carcinoma: evaluation of 1674 cases by tissue microarray. Arch Pathol Lab Med 2012; 136: 163-71.
12. Nicholson AG, Gonzalez D, Shah P, Pynegar MJ, Deshmukh M, Rice A, et al. Refining the diagnosis and EGFR status of non-small cell lung carcinoma in biopsy and cytologic material, using a panel of mucin staining, TTF-1, cytokeratin 5/6, and P63, and EGFR mutation analysis. J Thorac Oncol 2010; 5: 436-41.
13. Bishop JA, Teruya-Feldstein J, Westra WH, Pelosi G, Travis WD, Rekhtman N. p40 (ΔNp63) is superior to p63 for the diagnosis of pulmonary squamous cell carcinoma. Mod Pathol 2012; 25: 405-15.
14. Kadota K, Suzuki K, Kachala SS, Zabor EC, Sima CS, Moreira AL, et al. A grading system combining architectural features and mitotic count predicts recurrence in stage I lung adenocarcinoma. Mod Pathol 2012; 25: 1117-27.
15. Kadota K, Nitadori J, Woo KM, Sima CS, Finley DJ, Rusch VW, et al. Comprehensive pathological analyses in lung squamous cell carcinoma: single cell invasion, nuclear diameter, and tumor budding are independent prognostic factors for worse outcomes. J Thorac Oncol 2014; 9: 1126-39.
16. Kadota K, Yeh YC, Villena-Vargas J, Cherkassky L, Drill EN, Sima CS, et al. Tumor budding correlates with protumor immune microenvironment and is an independent prognostic factor for recurrence of stage I lung adenocarcinoma. Chest 2015; 148: 711-21.
17. Kadota K, Villena-Vargas J, Yoshizawa A, Motoi N, Sima CS, Riely GJ, et al. Prognostic significance of adenocarcinoma in situ, minimally invasive adenocarcinoma, and nonmucinous lepidic predominant invasive adenocarcinoma of the lung in patients with stage I disease. Am J Surg Pathol 2014; 38: 448-60.
18. Kadota K, Nitadori J, Sima CS, Ujiie H, Rizk NP, Jones DR, et al. Tumor spread through air spaces is an important pattern of invasion and impacts the frequency and location of recurrences after limited resection for small stage I lung adenocarcinomas. J Thorac Oncol 2015; 10: 806-14.
19. Warth A, Muley T, Kossakowski CA, Goeppert B, Schirmacher P, Dienemann H, et al. Prognostic impact of intraalveolar tumor spread in pulmonary adenocarcinoma. Am J Surg Pathol 2015; 39: 793-801.
20. Barnes L, Eveson JW, Reichart P, Sidransky D. Pathology and genetics of head and neck tumours. Lyon: International Agency for Research on Cancer; 2005.
21. Wang LC, Wang L, Kwauk S, Woo JA, Wu LQ, Zhu H, et al. Analysis on the clinical features of 22 basaloid squamous cell carcinoma of the lung. J Cardiothorac Surg 2011; 6: 10.
22. Rossi G, Mengoli MC, Cavazza A, Nicoli D, Barbareschi M, Cantaloni C, et al. Large cell carcinoma of the lung: clinically oriented classification integrating immunohistochemistry and molecular biology. Virchows Arch 2014; 464: 61-8.
23. Clinical Lung Cancer Genome Project (CLCGP), Network Genomic Medicine (NGM). A genomics-based classification of human lung tumors. Sci Transl Med 2013; 5: 209ra153. doi: 10.1126/scitranslmed.3006802.
24. Pelosi G, Rindi G, Travis WD, Papotti M. Ki-67 antigen in lung neuroendocrine tumors: unraveling a role in clinical practice. J Thorac Oncol 2014; 9: 273-84.
25. Bauer DE, Mitchell CM, Strait KM, Lathan CS, Stelow EB, Lüer SC, et al. Clinicopathologic features and long-term outcomes of NUT midline carcinoma. Clin Cancer Res 2012; 18: 5773-9.
26. French CA, Kutok JL, Faquin WC, Toretsky JA, Antonescu CR, Griffin CA, et al. Midline carcinoma of children and young adults with NUT rearrangement. J Clin Oncol 2004; 22: 4135-9.
27. Devouassoux-Shisheboran M, Hayashi T, Linnoila RI, Koss MN, Travis WD. A clinicopathologic study of 100 cases of pulmonary sclerosing hemangioma with immunohistochemical studies: TTF-1 is expressed in both round and surface cells, suggesting an origin from primitive respiratory epithelium. Am J Surg Pathol 2000; 24: 906-16.
28. Niho S, Suzuki K, Yokose T, Kodama T, Nishiwaki Y, Esumi H. Monoclonality of both pale cells and cuboidal cells of sclerosing hemangioma of the lung. Am J Pathol 1998; 152: 1065-9.
29. Xiao S, Lux ML, Reeves R, Hudson TJ, Fletcher JA. HMGI(Y) activation by chromosome 6p21 rearrangements in multilineage mesenchymal cells from pulmonary hamartoma. Am J Pathol 1997; 150: 901-10.
30. Anderson T, Zhang L, Hameed M, Rusch V, Travis WD, Antonescu CR. Thoracic epithelioid malignant vascular tumors: a clinicopathologic study of 52 cases with emphasis on pathologic grading and molecular studies of WWTR1-CAMTA1 fusions. Am J Surg Pathol 2015; 39: 132-9.