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CAROL DAVILA UNIVERSITY OF MEDICINE AND PHARMACY OF BUCHAREST
DOCTORAL SCHOOL
PATHOLOGY

The predictability of evaluating lung tumors using complex morphological methods for precision oncological treatment

PHD THESIS SUMMARY

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I. GENERAL PART - Current state of knowledge

1. EMBRYOLOGY OF THE RESPIRATORY SYSTEM

The development of the lungs involves two fundamental stages: an initial stage of forming the air ducts and a later stage of developing the respiratory interface. Pulmonary organogenesis is based on complex interactions between the endoderm and mesoderm, facilitated by growth factors, hormones, and intricate signaling mechanisms. It begins in the embryonic weeks 3 and 4 with the formation of primitive lung buds derived from the endoderm of the proximal intestine. This is followed by a complex process of three-dimensional development, where embryological stages are defined by cellular events and pulmonary morphology rather than a chronological timeline [1].

2. ANATOMY AND HISTOLOGY OF THE RESPIRATORY SYSTEM

The respiratory system consists of the two lungs and a series of ducts that conduct air to and from the lungs. Within the lungs, the airways branch into progressively smaller structures, culminating in the smallest airspace, the alveolus. This system fulfills three main functions: air conduction, filtration, and gas exchange at the alveolar level [2].

The trachea divides into the two main bronchi, right and left. At the pulmonary hilum, each bronchus divides into lobar bronchi (secondary bronchi). The left lung comprises two lobes, while the right lung has three, hence the right main bronchus divides into three lobar bronchi and the left into two. The left lung is further divided into eight bronchopulmonary segments, while the right lung has ten, each served by a tertiary or segmental bronchus [3].

Initially, the bronchi have the same histological structure as the trachea. After entering the lung parenchyma, cartilaginous rings are replaced by irregularly contoured cartilaginous plates, arranged linearly around the wall's circumference, giving the bronchi a cylindrical shape. As the bronchi branch and decrease in size, the cartilaginous content diminishes and disappears entirely when the diameter reaches 1 mm, becoming bronchioles. The second change in the intrapulmonary bronchial wall is the proportional increase in smooth muscle tissue as the cartilaginous content decreases, forming a continuous muscular layer that becomes interrupted in smaller bronchi [4].

Bronchopulmonary segments are further divided into pulmonary lobules, each with an afferent bronchiole. On the serous surface, lobules appear as polygonal areas delineated by discrete

connective tissue. Each lobule comprises several pulmonary acini. The smallest functional unit of the lung is the bronchiolar respiratory unit, consisting of a respiratory bronchiole and the alveoli it serves. Alveolar ducts are elongated airways without their own walls, delineated only by alveolar walls. Alveolar sacs are airspaces surrounded by clusters of alveoli opening into them, located at the ends of airways or along them [5].

Alveoli are the site of gas exchange between air and blood. Each alveolus is a polyhedral chamber with a diameter of 0.2 mm, bordered by a thin wall (alveolar septum), surrounded by a rich network of capillaries bringing blood close to the inspired air within the alveoli [2]. The alveolar epithelium consists of type I and type II alveolar cells and occasional brush cells. Constantly exposed to inhaled particles, pathogens, and toxins, the alveolar epithelium also contains specialized cells for defense [2,6].

3. LUNG CANCER

Globally, lung cancer continues to be the leading cause of cancer death in both men and women, with economic development playing a significant role, especially among female patients [7]. According to the 2018 Global Adult Tobacco Survey, 30.7% of Romanians over 15 years old regularly used tobacco in any form, with 30% smoking cigarettes, consuming an average of 16.5 cigarettes per day. The evolution of smoking in Romania reported by the Ministry of Health in 2019 shows that overall tobacco use increased from 26.8% in 2011 to 30.7% in 2018, a relative increase of 14.9% [8]. In Romania, according to the World Health Organization's 2020 report, lung cancer ranked second in total new cases for both sexes, representing 12.3% of the total cases. In 2020, 9030 new cases of lung cancer were diagnosed in men, ranking first, and 3092 cases in women, ranking fourth after breast, colorectal and cervical cancer [9].

At the time of diagnosis, over 90% of lung cancer patients present symptoms, most commonly cough [10]. Symptomatology is closely related to the tumor's location. Central tumors more frequently present symptoms associated with the injury of the main, lobar, or proximal segmental bronchi, including cough, hemoptysis, dyspnea, hoarseness, or stridor due to bronchial obstruction. Patients with peripheral tumors may experience cough, pleuritic pain, or dyspnea [10].

Primary lung cancer manifests as a nodular lesion or tumor mass, sometimes taking complex forms. It is initially detected by chest X-ray and increasingly by computed tomography (CT), either incidentally or as part of a screening program [11].

Macroscopically, atypical adenomatous hyperplasia can be identified as millimetric, poorly defined, grayish-reddish nodular areas [11]. Lesions up to 3 cm, with a compact appearance or visible air spaces, are most frequently in situ adenocarcinomas [11]. Minimally invasive adenocarcinoma appears as a peripheral nodular lesion with a solid central area smaller than 5 mm, surrounded by collapsed lung tissue. It often associates anthracotic pigment and pleural retraction [12].

Invasive pulmonary adenocarcinoma can be non-mucinous and mucinous. It appears as a nodular lesion with poorly defined margins, grayish-white, with a central fibrous scar or low-consistency, gelatinous/mucoid appearance [11]. The same gelatinous, soft appearance is found in colloid adenocarcinomas.

Squamous dysplasia and carcinoma in situ are preinvasive lesions derived from bronchial epithelium. Macroscopically, they are most frequently seen near bifurcations as polypoid or nodular lesions of 1-2 mm. Larger, flat lesions over 1 cm are more common, presenting as focal thickened mucosa with an irregular appearance and increased vascularization [11][13]. Squamous cell lung carcinoma is a firm, poorly defined, whitish, gray, or brown lesion. Hemorrhage, necrosis, and cystic degeneration may occur, especially in large tumors. Central tumors appear as endobronchial lesions with an exophytic, papillary appearance [11].

Large cell carcinomas are typically large, circumscribed, solid tumors, often necrotic and rarely with cystic areas [11].

Carcinoid tumors are central lesions, often with endobronchial growth, appearing as round-oval lesions of varying sizes but over 5 mm, gray or yellowish, with low or firm consistency [14]. Large cell neuroendocrine carcinomas are often peripheral, large, well-defined, brownish-red lesions with necrotic areas [15]. Small cell neuroendocrine carcinomas are large, perihilar lesions, often unresectable and extensively invasive [11]. They appear as tumors that frequently mold to the bronchial contour, brown in color with extensive necrotic areas. Only 5% appear as circumscribed peripheral nodules [16].

Microscopically, atypical adenomatous hyperplasia is a precursor lesion to pulmonary adenocarcinoma in situ, consisting of a small proliferation (< 5 mm) of type II pneumocytes with moderate atypia and/or bronchiolar exocrine cells, also known as Clara cells (currently termed "club cells"). These cells line the alveolar walls and sometimes the respiratory bronchioles [11].

In situ pulmonary adenocarcinoma is a small lesion (< 30 mm) composed of neoplastic cells extending along pre-existing alveolar septa, creating a so-called lepidic growth pattern, without clear signs of stromal, vascular, airspace, pleural invasion or necrosis [17]. Tumor cells are arranged in a continuous row, single-layered, rarely pseudostratified, without forming papillary or micropapillary structures, and without tumor cells in the alveolar spaces. Depending on the tumor cells appearance, it can be non-mucinous or mucinous [11].

Minimally invasive adenocarcinoma is defined as a solitary lesion measuring less than 3 cm, predominantly exhibiting a lepidic growth pattern and an area of invasion smaller than 5 mm. It is most commonly non-mucinous, but rarely can also be mucinous and mixed [18]. The non-mucinous subtype consists of type II pneumocytes, with or without Clara cells. The mucinous type presents columnar cells with abundant apical mucin and basal nuclei, resembling colonic goblet cells. The invasion area is defined by the presence of other histological subtypes of adenocarcinoma besides lepidic: acinar, papillary, micropapillary, solid, colloid, enteric, fetal, or invasive mucinous, or by the presence of tumor cells infiltrating the myofibroblastic stroma [11].

Invasive non-mucinous pulmonary adenocarcinoma is a non-small cell lung carcinoma with glandular differentiation evident morphologically and immunohistochemically. Morphologically, it consists of a mix of architectural patterns (lepidic, acinar, papillary, micropapillary, and solid) and can be classified into subtypes based on the predominant pattern [17].

Invasive mucinous adenocarcinoma is a primary pulmonary neoplasm characterized by a proliferation of cuboidal-columnar epithelial cells with abundant intracytoplasmic mucin and basally located bland nuclei. The arrangement of tumor cells can vary, with lepidic, acinar, papillary, micropapillary, solid, or cribriform growth patterns [19]. Tumors with a heterogeneous morphology, mucinous and non-mucinous, are called mixed adenocarcinomas if each component occupies more than 10% of the tumor surface [20].

Squamous cell carcinoma is a malignant epithelial tumor characterized by the presence of keratinization, intercellular bridges and an immunophenotype specific for squamous differentiation [21].

Based on microscopic appearance, it is divided into keratinizing, non-keratinizing, and basaloid carcinoma. The keratinizing carcinoma consists of a proliferation of polygonal epithelial cells arranged in nests and cellular strands, with intercellular bridges and, especially in well-differentiated tumors, extensive areas of keratinization, often forming keratin pearls. When keratin formation is not identified, immunohistochemical testing is recommended in order to establish the diagnosis of non-keratinizing squamous carcinoma [22].

The basaloid carcinoma is characterized by the presence of small to medium-sized cells with scant cytoplasm, organized in nests with a lobular arrangement and peripheral nuclear palisading. The mitotic rate is often high, as is the Ki-67 mitotic index [23].

Pulmonary neuroendocrine neoplasms are divided into neuroendocrine tumors (NET), which include low-grade tumors called typical carcinoids and intermediate-grade tumors called atypical carcinoids, and neuroendocrine carcinomas (NEC), which include large cell neuroendocrine carcinomas and small cell carcinomas [24].

Carcinoid tumors are well-differentiated cytologically and architecturally neuroendocrine malignant tumors. They are characterized by an organoid growth pattern, forming trabeculae, rosettes, islands or sheets of uniform cells with moderate to abundant eosinophilic cytoplasm and central round nuclei with finely dispersed chromatin, giving a "salt and pepper" appearance [24]. Typical carcinoid has reduced mitotic rate (< 2 mitoses/2 mm^2) and lacks tumor necrosis, unlike atypical carcinoid [11].

The most common type of neuroendocrine neoplasm found in the lung is small cell carcinoma. This is a high-grade malignant epithelial tumor composed of small round-oval cells with scant cytoplasm, poorly defined cell borders and nuclei with finely dispersed chromatin, lacking nucleoli, often with nuclear molding. The tumor presents densely cellular areas, numerous atypical mitoses, and extensive areas of tumor necrosis [25].

Large cell neuroendocrine carcinoma is a high-grade non-small cell carcinoma with neuroendocrine morphology and more than 10 mitoses per 2 mm^2 [11]. Microscopically, it presents an organoid growth pattern, with cellular nests and strands, with peripheral palisading and rosette

formation. Unlike other neuroendocrine tumors, the tumor cells have prominent medium-large nucleoli and abundant cytoplasm, with evident cell borders. The nuclei may have finely granular chromatin but also a vesicular appearance. Necrosis is present in almost all cases of large cell neuroendocrine carcinoma, as are numerous atypical mitoses (mean value 70 mitoses/2 mm^2) [26].

The diagnosis of lung carcinoma is based on histological criteria, but sometimes the differentiation of histological subtypes can be difficult, and in these conditions, immunohistochemistry can contribute [27].

Minimally invasive non-mucinous adenocarcinoma shows positivity for the pneumocyte markers TTF1 and Napsin A, while the mucinous type is negative for pneumocite markers and positive for CK20 and HNF4α [28].

Invasive non-mucinous pulmonary adenocarcinoma does not have markers that are positive in 100% of cases, but it is most frequently positive for the pneumocyte markers TTF1 and Napsin A. Dual positivity for TTF1 and p40 characterizes an adenocarcinoma or an adenosquamous carcinoma [29]. CK7 is not specific for pulmonary adenocarcinoma [29].

To establish the neuroendocrine nature and categorize the tumor into one of the followings: small cell lung carcinoma, large cell neuroendocrine carcinoma or carcinoid tumor, positivity for at least one of the markers Chromogranin A, Synaptophysin, CD56 (NCAM1), or INSM1 must be demonstrated [30].

Lung carcinoma is a disease with marked histological and molecular heterogeneity. Accumulating information about the molecular patterns of lung carcinoma formation has led to their classification into specific subtypes based on the targeted therapy they respond to. Thus, the use of immunohistochemistry can be an easy screening method [31].

ALK rearranged lung adenocarcinomas account for 4-5% of lung adenocarcinomas and represent tumors found in young, non-smoking patients, that are positive for TTF-1 and show acinar architecture, mucinous secretion, or signet ring cells [32]. Immunohistochemical testing for ALK is a widely used, inexpensive, and rapid screening method for detecting ALK rearrangement, alongside in situ hybridization (FISH) [33].

ROS1 is an oncogenic driver found in approximately 1-2% of lung adenocarcinomas [34]. These tumors are more commonly found in young, non-smoking women and are characterized by a solid appearance, cribriform morphology, extracellular mucin, or signet ring cell morphology

[35]. Like ALK, immunohistochemical testing for ROS1 rearrangement is a good screening method. The immunohistochemical appearance depends on ROS1 fusion partners, so carcinomas with CD74-ROS1 fusion, the most common, show globular cytoplasmic immunoreactivity for ROS1, while those with EZR-ROS1 fusion show membranous positivity [35].

EGFR mutation is one of the most common driver mutations in lung adenocarcinoma, found more frequently in Asian, non-smoking female patients, histologically often presenting a "hobnail" appearance, micropapillary architecture, and positivity for TTF-1 [36]. Currently, immunohistochemical testing is not recommended as a screening method, as antibodies developed to detect exon 19 deletion (clone 6B6) and exon 21 L858R mutation (clone 43B2) have not proven specific [37].

PD-L1 (CD274) is an immune modulator that promotes immunosuppression by binding to PD1. PD-L1 on the surface of tumor cells prevents the cytotoxic action of T lymphocytes when binding to PD1 on their surface [38]. In this context, anti-PD1/PD-L1 therapy inhibits the binding of PD-L1 to PD1, allowing immune-mediated attack against tumor cells, and numerous studies have already shown the benefit of immune therapy in prolonging the survival of lung cancer patients [39].

II. SPECIAL PART - Personal contribution

4. PURPOSE OF THE WORK

The purpose of this work is to conduct a detailed analysis of one of the most aggressive types of tumors - lung carcinoma. By conducting an extensive evaluation of clinical, demographic, histological, and immunohistochemical characteristics, I aimed to identify possible prognostic factors for early detection and improved patient survival.

Analyzing the tumor microenvironment in lung cancer can provide numerous insights with direct clinical implications. This information can contribute to diagnosis, prognosis, and personalized treatment of patients.

Evaluating the type and density of immune cells, such as T lymphocytes and macrophages, can offer clues about the body's immune response to the tumor. A rich inflammatory infiltrate may

be associated with a better prognosis and longer survival, and I wanted to analyze this correlation in cases from Romania as well.

Identifying immunological markers, such as PD-L1 or LAG-3, can help predict response to immunotherapy, providing important information for selecting patients who would benefit from such treatments.

A detailed analysis of the tumor microenvironment can uncover new proteins and signaling pathways that play a role in the progression of lung cancer. These discoveries could become the basis for developing new targeted therapies.

By integrating this information into clinical practice, the management of lung cancer patients can be significantly improved, leading to more effective and personalized treatments, increased survival, and improved quality of life.

Additionally, this work aims to present a method for improving the current reporting system of lung cancer cases in Romania by developing a web application designed to ease the work of clinicians, standardize histopathological results, and enhance patient stratification and timely application of appropriate treatment.

The results of this study are expected to contribute to a better understanding of the pathological mechanisms of lung cancer and to provide a solid basis for developing more efficient and personalized treatment strategies. Understanding cellular interactions in the tumor microenvironment and identifying new biomarkers and therapeutic targets could lead to improved survival and quality of life for lung cancer patients.

5. STUDY OBJECTIVES

To identify and evaluate prognostic factors in 160 lung cancer patients to better understand their influence on disease progression and survival.

To investigate the relationships between demographic factors (age, sex, origin) and clinical factors (disease stage, histological type, comorbidities) and prognostic factors in order to identify relevant patterns and trends.

To assess the potential of demographic and clinical factors to contribute to early detection of lung cancer and improved survival through early interventions.

To analyze the predominant histological types, architectural structure, and differentiation degree of tumor cells in lung carcinoma cases.

To analyze the tumor microenvironment to discover cellular interactions as potential therapeutic targets that could be used in more efficient and personalized treatments for lung cancer patients.

To evaluate the distribution of the inflammatory infiltrate in the tumor microenvironment and correlate it with patient prognosis to understand the impact of the immune response on lung cancer evolution and identify potential therapeutic targets.

To create a web application that improves the current reporting system of lung cancer cases in Romania. The application will facilitate the work of clinicians, standardize reported results, and contribute to more precise patient stratification, allowing for the timely application of appropriate treatment.

6. MATERIALS AND METHODS

The study cohorts were selected from the histopathological records of the Pathology Department of the University Emergency Hospital of Bucharest. The histological samples examined were derived from both bronchopulmonary biopsies and surgical resection specimens.

Macroscopic examination was conducted, and fragments were collected for microscopic examination, using ORDER NO. 1217/2010 on the approval of Medical Practice Guidelines for Pathology, Annexes 1 and 3, as well as the examination protocol for specimens from patients with primary non-small cell lung carcinoma, small cell lung carcinoma, or pulmonary carcinoid tumors, from the College of American Pathologists (CAP).

Following the macroscopic examination, representative fragments were collected, processed to obtain histological sections, and stained using routine or immunohistochemical staining methods. Microscopic evaluation was then performed, and a histopathological result was recorded in the electronic database of the University Emergency Hospital of Bucharest.

The data for this study were collected from the database of the University Emergency Hospital of Bucharest, clinical observation sheets of patients admitted to the Thoracic Surgery and Oncology Departments of the University Emergency Hospital of Bucharest, and histopathological result records of the Pathology Department of the University Emergency Hospital of Bucharest.

Image acquisition was done using the Leica DM750 microscope equipped with a Leica ICC50 HD camera from the University Emergency Hospital of Bucharest and the Olympus BX43 microscope equipped with a high-definition Olympus XC50 camera from the Center of Excellence in Translational Medicine, Fundeni, Bucharest.

Digital images of histological slides were created using the scanner from the Clinical County Emergency Hospital in Constanta, within the Clinical Pathology Service.

Clinical-morphological, histopathological, and immunohistochemical data were recorded in an electronic database using Microsoft Excel, part of the data being interpreted within the same platform. Statistical evaluation and interpretation of the data were performed using the GraphPad Prism V.10.2.0 software (GraphPad Software, Boston, Massachusetts, USA, www.graphpad.com).

7. RESULTS

The analyzed cohort included 160 patients treated in the Thoracic Surgery Department of the University Emergency Hospital of Bucharest and diagnosed within the Pathology Department of the University Emergency Hospital of Bucharest between April 2016 and October 2020.

Descriptive analysis of the studied cohort revealed that most patients were males, with the age range of 61-70 years having the highest incidence for both sexes, and most patients came from urban areas, particularly Bucharest and surrounding counties.

The surgical procedures conducted to obtain tissue fragments primarily consisted of lobectomies, excisional biopsies (atypical subsegmental resections), pneumonectomies, segmentectomies, and needle biopsies.

Tumor lesions were most frequently identified in the right lung, with tumor diameters ranging from 0.8 cm to 16.1 cm, with an average of 4.31 cm.

Most cases exhibited locoregional, lymph node, pleural, mediastinal, costal, parietal, pericardial, and diaphragmatic invasion, with distant metastases most frequently found in the central nervous system (6% brain or cerebellar metastases), followed by hepatic metastases (3%).

Most lung cancers were diagnosed at advanced stages, with stage IV being the most frequently established at diagnosis, followed by stage I, and the histological types, in descending order of frequency, were adenocarcinoma, squamous cell carcinoma, neuroendocrine tumors, with

the rarest being undifferentiated carcinomas, adenosquamous carcinomas and mucoepidermoid carcinomas.

Adenocarcinomas were predominantly acinar (54%), followed by NOS (not otherwise specified - 22%) and solid (10%). Mucinous adenocarcinomas constituted 7% of total adenocarcinomas, with another 3% presenting a mucinous component greater than 10% of the tumor surface. 59% of adenocarcinoma cases were confirmed by immunohistochemical testing at the time of diagnosis.

Squamous cell carcinomas were non-keratinizing in two-thirds of cases, with only three cases having immunohistochemical confirmation.

The main subtypes of neuroendocrine neoplasms found in the studied cohort were small cell lung carcinomas in two-thirds of cases, followed by large cell neuroendocrine carcinomas and finally typical and atypical carcinoid tumors.

Only 27% of the analyzed patients were alive in May 2024, with survival ranging between 7 years and 6 months and 3 years and 7 months. Higher survival was observed among women, who were most frequently diagnosed at an early tumor stage compared to men.

Analysis of the Tumor Microenvironment in Pulmonary Adenocarcinoma

I analyzed 50 cases of pulmonary adenocarcinoma from the initial cohort and using the Tissue Array method, I obtained three recipient blocks composed of fragments collected from all 50 cases.

The collection was conducted at the Center of Excellence in Translational Medicine, using the Tissue Arrayer MiniCore® 3 and its associated software. The resulting paraffin blocks were sectioned for routine and immunohistochemical staining.

Sections with a thickness of four microns obtained from the recipient blocks were stained for immunohistochemical markers CD3 (clone EP41), CD4 (clone EP204), CD8 (clone SP16), CD68 (clone Kp-1), CD163 (clone EP324), PD-L1 (clone Cal10), PD1 (clone NAT105), and LAG-3 (clone 17B4).

In the studied cohort, a statistically significant correlation was observed between the density of CD4+ and CD8+ cells, indicating a possible reciprocal dependence and supporting their interaction in the tumor microenvironment in regulating the immune response against tumor cells.

An increased CD4/CD8 ratio, used as a measure of the tumor's capacity to evade the immune system's destructive action, was also observed in the evaluated pulmonary adenocarcinomas.

The analysis of intratumoral histiocytes showed a linear distribution of the densities of cells positive for CD68 and CD163, with a statistically significant correlation between the density of CD68+ cells and tumor diameter, indicating a role of inflammation and macrophages in tumor growth.

Additionally, the identified correlation between the density of CD68+ cells and the proliferation index Ki-67 provides important information about the relationship between macrophages and tumor proliferation. The positive relationship between CD68 and Ki-67 suggests that CD68+ macrophages may contribute to the growth and aggressiveness of pulmonary adenocarcinomas by promoting cell proliferation, and they may be preferentially recruited in more aggressive tumors (with a high Ki-67 index), where more frequent tumor cell lysis and more pronounced antigen release occur.

Regarding the evaluation of immune cell markers, significant correlations were identified between the densities of CD4+ cells and those of CD68+ and CD163+, as well as between CD8 and CD163. Thus, a significant correlation between the density of T helper cells (CD4+) and macrophages (CD68+) suggests an interaction between these immune cells. CD68+ macrophages may be involved in recruiting and activating T helper cells, which can influence the immune response in the tumor environment. The significant correlation between CD4+ and CD163+ suggests an interaction between T helper cells and immunosuppressive macrophages, indicating a possible role of T cells in mediating the activity of M2 macrophages in tumors.

CD8+ cells are cytotoxic T cells that play a crucial role in the antitumor immune response. A significant and strong correlation between CD8+ and CD163+ suggests that M2 macrophages may influence or interact with cytotoxic T cells, possibly suppressing their activity and contributing to tumor immune evasion.

These correlations indicate important interactions between different immune components in the tumor environment. Specifically, significant relationships between T lymphocytes (CD4+ and CD8+) and macrophages (CD68+ and CD163+) suggest that macrophages can significantly influence the immune response in the tumor, either by recruiting and activating T lymphocytes or by suppressing their activity.

The evaluation of immune evasion mechanisms through the expression of PD-L1, PD1, and LAG-3 proteins identified a significant correlation between PD-L1 and LAG-3 expression, which may suggest that these two proteins are co-regulated or have a synergistic role in the tumor environment, with functional interaction between them in the context of suppressing the antitumor immune response.

A highly significant correlation was also identified between LAG-3 and CD163, suggesting that in the tumor microenvironment, the presence of LAG-3 is associated with the presence of M2 macrophages (CD163+). This may indicate that both components contribute to an immunosuppressive tumor environment. Thus, CD163+ macrophages and LAG-3-expressing cells could work synergistically to suppress the antitumor immune response, favoring tumor progression.

Additionally, the results showed statistically significant correlations between PD1 expression and the populations of CD4+ and CD68+ cells. CD4+ T helper lymphocytes are essential in mediating and regulating the immune response. The correlation between PD1 and CD4+ suggests a significant interaction between these cells and PD1 expression in the tumor microenvironment, which could contribute to tumor-induced immunosuppression. The correlation between PD1 and CD68+ suggests that macrophages, which play a crucial role in innate and adaptive immunity, may have a significant influence on PD1 expression.

Statistically significant correlations were identified between patient age and the expression of PD-L1 and LAG-3. It was observed that PD-L1 and LAG-3 expressions decrease with age, which may indicate that tumors in older patients are less capable of evading the immune response through these specific pathways. This could influence the response to therapies targeting immune checkpoint inhibitors.

Among lymphocyte and histiocyte markers, only the density of CD4+ cells showed a significant negative correlation with patient age. The decrease in CD4+ cell density with aging may indicate an age-related alteration in immune system function. Changes in CD4+ cell density with age could affect the body's ability to respond to tumors and control their progression.

Analysis of the spatial distribution of inflammatory infiltrate in the tumor microenvironment

The analysis was conducted on the cohort of 50 patients with pulmonary adenocarcinoma described earlier.

In this stage of the study, I analyzed the spatial distribution of inflammatory infiltrate in the tumor microenvironment in two regions of the tumor – the tumor center and the tumor margin. To limit the number of variables, I chose to analyze the correlations between different inflammatory cells, their densities in the tumor center and periphery, in two categories of tumors: acinar and non-acinar adenocarcinomas.

For acinar adenocarcinomas, moderate and significant positive correlations were identified between the expressions of CD8+, CD3+, CD68+, and CD163+ in the tumor center and margin, suggesting that the distribution of these cells is relatively uniform between the center and margin, with a coordinated and efficient immune response in these tumors.

Differences in the correlation between inflammatory cells in the tumor center and margin between acinar and non-acinar adenocarcinomas suggest that the histological type of the tumor can influence the distribution and immune response. Thus, the lack of significant correlation in non-acinar adenocarcinomas may indicate greater variability in the distribution of inflammatory cells or a difference in how these cells infiltrate the tumor.

These results underscore the importance of precise characterization of immune infiltrate in different histological types of pulmonary adenocarcinomas to better understand the mechanisms of the antitumor immune response and to develop personalized therapeutic strategies.

Regarding the evaluation of marker expressions in the tumor center and margin and their correlation with patient age, the results indicate a significant correlation between patient age and the expression of inflammatory markers (CD4+, CD68, CD163) in the tumor center but not in the tumor periphery. This suggests that aging affects the tumor compartments differently. The more adverse microenvironment in the tumor center, characterized by hypoxia and necrosis, may accentuate the effects of immunosenescence, leading to a more pronounced decrease in immune cell density in elderly patients. In contrast, more favorable conditions in the tumor periphery may maintain a relatively constant immune cell density regardless of patient age.

Digital pathology analysis of malignant pulmonary tumors

In this study, I analyzed the histological characteristics and prognostic factors of pulmonary carcinomas in 40 patients from the studied cohort using digital pathology.

A decrease in inflammatory infiltrate was noted with increasing tumor diameter, with large tumors more frequently showing a densely cellular fibroblastic stroma. This stroma can provide better structural and nutritional support for tumor cells, promoting tumor growth and expansion. In contrast, collagenous stroma is dominated by densely organized collagen fibers, which can create a physical barrier to tumor cell proliferation and migration. Lower cellular density and decreased remodeling activity can limit tumor expansion in this type of stroma.

The prognostic factors analyzed included spread through air spaces (STAS), necrosis and vascular invasion. STAS refers to the dissemination of tumor cells through alveolar air spaces adjacent to the primary tumor, without being connected via the tumor stroma or blood/lymphatic vessels. This morphological characteristic has been associated with poorer prognosis and higher recurrence rates after surgical resection of the tumor. In this study, this type of invasion was noted only in adenocarcinomas, most frequently in the mucinous subtype and in acinar adenocarcinomas with a significant micropapillary component.

The presence of tumor necrosis is associated with larger tumor sizes. Necrosis often occurs in large tumors, where the blood supply is insufficient to nourish the entire tumor mass, leading to cell death in the tumor center. Large tumors can create a hypoxic microenvironment, promoting necrosis, which may be an indicator of tumor aggressiveness.

Lymphovascular invasion, indicated by the presence of tumor cells in lymphatic or blood vessels, is a negative prognostic factor associated with a higher risk of metastasis and recurrence. Significant differences in tumor size variances suggest that tumors with lymphovascular invasion have greater size variability, which may reflect increased tumor heterogeneity and variable biological behavior.

Regarding the tumor growth pattern, the solid pattern is more than twice as frequent in deceased patients compared to those alive, suggesting a strong correlation between this growth pattern and poor prognosis. Tumors with a solid pattern tend to be more aggressive, with higher rates of proliferation and invasion, which may explain the increased mortality among these patients.

PathAdapt – A reporting tool of lung cancer for pathologists in Romania

We aim to create a standardized histopathological reporting method based on the latest European and American guidelines, to assist pathologists and their colleagues in related specialties.

The proposed application will be intuitive, easy to use, and accessible from any mobile or web browser, ensuring that the obtained results are standardized and comprehensive, thereby eliminating reporting variability. The major advantage will be for the pathologist, who will select from the available options and enter some numerical values, with the application then generating a comprehensible and thorough result, saving time needed for searching the guidelines and the usual formulations. This application will significantly aid patients, who will be diagnosed according to current standards, in a standardized and detailed manner, hoping to contribute to improved treatment and survival outcomes.

8. CONCLUSIONS AND PERSONAL CONTRIBUTIONS

This study provides a detailed description of the clinical and morphological aspects of different types of malignant pulmonary tumors, contributing to a better understanding of the characteristics of these lesions. The demographic analysis of the patients, including sex distribution, age at diagnosis and patient origin, offers valuable information about the epidemiology of lung cancer in Romania.

Identifying the most common symptoms and surgical interventions (lobectomies and excisional biopsies) in patients with lung cancer can help optimize diagnostic and treatment strategies. A better understanding of disease progression can be achieved by detailing the distribution of clinical stages of tumors and the types of metastases encountered.

The study has made significant contributions to understanding the tumor microenvironment in lung cancer through the immunohistochemical analysis of lymphocyte and histiocyte markers, as well as the mechanisms of immune evasion.

A significant correlation was found between CD4+ and CD8+ cells, suggesting a close interaction between these subpopulations of T lymphocytes in the tumor context and a frequently supraunitary CD4/CD8 ratio.

The significant correlation between CD68 and CD163 expression indicates an association between M1 and M2 macrophages in pulmonary tumors, with CD68+ correlating with tumor diameter and Ki-67 index, suggesting a link between the presence of macrophages and the tumor proliferation rate.

Mechanisms of immune evasion were evaluated through immunohistochemical expression of PD1, PD-L1, and LAG-3, with significant correlations identified between PD-L1 and LAG-3 and between PD1 and CD4 and CD68, highlighting the complexity of interactions between immune cells and tumor cells through immune evasion mechanisms.

Regarding the spatial distribution of inflammatory cells in the tumor microenvironment, CD8+ lymphocytes were less frequent in the center of tumors compared to CD4+ and CD163+ cells, which could be a consequence of functional exhaustion of the immune process.

Significant correlations in acinar adenocarcinomas between CD8+, CD3+, CD68+, and CD163+ cells in the tumor center and margin suggest that in acinar adenocarcinomas, the presence of inflammatory cells in one region of the tumor is relatively well reflected in the other region. This could indicate a coordinated and uniformly distributed immune response throughout the tumor.

The lack of significant correlation in non-acinar adenocarcinomas suggests that the immune response in these tumors may be more heterogeneous or localized. This could indicate less efficient penetration of immune cells in certain parts of the tumor, which could negatively influence the efficiency of the antitumor immune response.

Digital analysis showed that large tumors were associated with reduced inflammatory infiltrate and densely cellular fibroblastic stroma, while necrosis and lymphovascular invasion were more frequent in tumors with moderate and high cytonuclear atypia. Tumors with a micropapillary morphology most frequently showed invasion through air spaces, while those with a solid growth pattern and negative prognostic factors, such as necrosis and lymphovascular invasion, were more common in patients who had died compared to those alive in May 2024.

These findings contribute to a better understanding of cellular interactions and immune evasion mechanisms in lung cancer, offering valuable insights for developing more effective therapeutic strategies.

Evaluating patient survival based on various variables (age, tumor diameter, immunohistochemical markers) helps identify relevant prognostic factors for lung cancer.

The introduction of a prototype application for standardized histopathological reporting represents an innovation that can improve the quality and efficiency of histopathological diagnosis.

These contributions provide new and relevant information in the field of lung cancer, enhancing existing knowledge and providing a basis for future research.

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