“CAROL DAVILA” UNIVERSITY OF MEDICINE AND PHARMACY, BUCHAREST

DOCTORAL SCHOOL

FIELD OF MEDICINE

MORPHOLOGICAL ASPECTS AND ANTIGENIC CONSTELLATION IN NON-SMALL CELL LUNG CANCER

DOCTORAL THESIS SUMMARY

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TABLE OF CONTENTS OF THE DOCTORAL THESIS

I. FUNDAMENTAL ISSUE

Lung cancer is a field of great interest for the international scientific community, as it ranks among the leading causes of morbidity and mortality worldwide. The origin of these neoplasms is most often epithelial (carcinomas). For no other cancer is the size of the neoplastic cells as important as it is in this disease, where diagnosis always begins by dividing the lesions into small cell bronchopulmonary carcinomas - 15%, and non-small cell lung carcinomas (NSCLC) - 85% [1, 2]. Given the disproportionate prevalence of these subtypes, the present study aims to evaluate NSCLC-type proliferations, as they place the greatest burden on the healthcare system.

This paper highlights the epidemiological situation (both globally and in Romania), as well as the morphological aspects of lung cancer, emphasizing the evolution of classifications according to the World Health Organization (WHO). *The Global Cancer Observatory* (GLOBOCAN) reported that in 2022, bronchopulmonary cancer ranked first among oncological diseases, both in terms of incidence (12.4%) and mortality (18.7%), with 2 480 675 newly diagnosed cases and 1 817 469 deaths, regardless of gender and age. In Romania, the incidence of bronchopulmonary cancer was 11 716 cases (11.2%), ranking third after colorectal and breast malignancies. In terms of mortality, it ranks first, with 10 530 deaths (18.7%) [2].

The morphology of pulmonary carcinomas is extremely complex, and their histopathological classification is a dynamic process, periodically revised. This reflects both the impact of environmental factors on human health and the reclassifications driven by the continuous acquisition of new scientific information regarding the genetics and histopathogenesis of malignant tumor processes [1].

One highly controversial aspect is the determination of the tumor's grade of differentiation (G). The 5th edition of the WHO classification introduces, as a new element, the establishment of clear criteria for grading pulmonary adenocarcinomas (ADK), based strictly on the architecture of neoplastic elements. In this context, the following have been classified as high-grade patterns: micropapillary, solid, and complex glandular (including the cribriform pattern) [1]. Well-differentiated ADK (G1) are considered those with a predominant lepidic pattern (in the absence or presence of less than 20% high-grade patterns). Moderately differentiated tumors (G2) are those with a predominantly acinar or papillary pattern (in the absence or presence of less than 20% high-grade pattern). Any

tumor that presents with at least 20% high-grade pattern is classified as poorly differentiated (G3) [1].

The situation is much more complicated in the case of squamous cell carcinomas (SCC), where there is no uniform, standardized grading system for either pulmonary tumors or those located in other areas (skin, esophagus, anus, vagina, etc.). A multitude of studies have been published in this regard, but the scientific community has not yet reached a consensus. In practice, most specialists consider nuclear pleomorphism and the presence of keratinization, but the assessment is extremely subjective and non-reproducible [1, 3-7].

A study conducted by M. Jesinghaus proposed an alternative grading method for SCC, called *the degree of cellular dissociation*. This approach integrates both quantitative parameters, such as the presence and extent of the tumor budding phenomenon (tumor cells arranged individually or in aggregates of up to five elements at the tumor invasion front), as well as qualitative parameters, such as the size of cellular islands within the tumor structure. By combining these elements, Jesinghaus offers a more precise, standardized method for evaluating tumor grade, which could improve prognostic stratification and guide therapeutic strategies [8].

Additionally, in the general section of the work, aspects related to the etiopathogenesis of lung cancer are highlighted, analyzing both the role of environmental factors and the molecular aspects. This condition has a multifactorial etiology and a complex pathogenesis [1].

Studies conducted on a group of miners from Eastern Europe who developed pulmonary malignancies have highlighted exposure to radon, which is considered the primary identified cause of lung cancer. Currently, smoking is the most frequently implicated factor in the development of this pathology, with 80-90% of cases diagnosed in smokers, closely correlated with the number of cigarettes consumed and the years of exposure. Passive smoking also plays an extremely important role. It is well known today that a smoker has a tenfold higher risk of developing lung cancer than a non-smoker, and this risk can increase up to 20 times in heavy smokers (≥ 25 cigarettes/day) [9-12].

Although the existence of genetic factors associated with the risk of lung cancer has been studied for a long time in the scientific community, validation required considerable technological progress to allow for thorough genetic analysis. The challenge was even greater given that NSCLC is characterized by a high genomic diversity. Although the identification of genetic mutations associated with NSCLC represents an important step,

developing targeted therapy is still a path full of challenges. Evidence of this is the fact that progress in the treatment of these cancers has been almost exclusively made for „non-squamous" neoplasms [10, 13].

This study analyzed the main genetic mutations involved in the etiopathogenesis of lung cancer: activating mutations of the *Epidermal Growth Factor Receptor* (EGFR), *anaplastic lymphoma kinase* (ALK) translocation, *Kirsten Rat Sarcoma* (KRAS) mutations, rearrangements of the receptor tyrosine kinase gene encoded by the ROS1 gene (ROS-1), *Rearranged during Transfection* (RET), or the neurotrophic tyrosine receptor kinase 1 (NTRK1), *mutations of v-Raf murine sarcoma viral oncogene homolog B* (BRAF), and the *Human Epidermal Growth Factor Receptor 2* (HER2) gene, *Mesenchymal-to-Epithelial Transition* (MET) mutations, *Phosphatase and Tensin Homolog* (PTEN) and *Cyclin-Dependent Kinase Inhibitor 2A* (CDKN2A) abnormalities [13-16].

In recent decades, lung cancer therapy has made remarkable progress, primarily based on two major therapeutic approaches. The first is immunotherapy, which has revolutionized the treatment of advanced NSCLC through the use of immune checkpoint inhibitors, such as anti-*Programmed Death-1* (anti-PD-1) and anti-*Programmed Death-Ligand* 1 (anti-PD-L1), to stimulate the immune system to recognize and destroy tumor cells [13].

The second therapeutic approach is based on the identification of tumor-specific mutations, thus enabling the administration of targeted therapy aimed at blocking the molecular pathways involved in tumor growth and dissemination. This includes tyrosine kinase inhibitors, which are currently considered the standard of care for molecularly defined patient groups [13, 17-19].

The *Cluster of Differentiation* (CD)39 - CD73 - adenosine pathway represents an increasingly studied research area, considered a potential target for immunotherapy. Extracellular adenosine triphosphate (ATP) released by necrotic/degraded cells is one of the major biochemical components of the tumor microenvironment and has a pro-inflammatory effect. The ectonucleotidases CD39 and CD73 degrade extracellular ATP to adenosine, which has recently been recognized as one of the most potent immunosuppressive factors in the tumor microenvironment. Research has shown that CD39 has anti-inflammatory, antithrombotic effects and promotes the healing process, being involved in angiogenesis. Thus, CD39 inhibitors could have a broad range of potential therapeutic effects: stimulating the inflammatory response and blocking angiogenesis, thereby halting tumor growth [20-23].

The present research also includes a special section in which the prognostic value of the epidemiological, morphological, immunohistochemical, and molecular features of NSCLC were evaluated.

2. RESEARCH HYPOTHESIS AND GENERAL OBJECTIVES

The present study aims to conduct an extensive evaluation of NSCLC, based on the hypothesis that during the coronavirus pandemic causing severe acute respiratory syndrome type 2 (SARS-CoV2), lung tumors were diagnosed at advanced stages and in fewer numbers.

It has also been observed that there are atypical microscopic features of these tumors, presentations that deserve to be mentioned in order to improve diagnosis, especially since the prognosis of patients can vary.

Another hypothesis of the study is that there are a series of microscopic, immunohistochemical, or molecular arguments, other than the well-established ones (for example, micropapillary or solid patterns in ADK), which correlate with the disease prognosis. Morphological indicators are currently used in the grading of SCC, although there is no standardized model in this regard. Most specialists use nuclear pleomorphism and the degree of keratinization in practice for this assessment.

Additionally, the reports in the specialized literature regarding the prognostic value of PD-L1, EGFR, and ALK are contradictory and require extensive studies.

The specific objectives of this study are:

1. To evaluate the epidemiological and histopathological characteristics of NSCLC in the geographical context of our country and in relation to the SARS-CoV2 pandemic;

2. To assess the prognostic value of certain morphological features of SCC: the arrangement of neoplastic cells, tumor budding, Spread Through Airway Spaces (STAS), mitotic index, stromal desmoplasia, tumor necrosis, nuclear pleomorphism, nucleolar prominence, keratinization, degree of differentiation (G), pTNM staging, tumor size;

3. To evaluate the prognostic value of immunohistochemical or molecular features (such as PD-L1, EGFR, and ALK);

4. To assess the factors influencing PD-L1 expression;

5. To identify new prognostic indicators - *Cluster of Differentiation* (CD)39, CD73.

3. THE GENERAL RESEARCH METHODOLOGY

The doctoral research is retrospective, observational, and descriptive, and included a cohort of 1937 patients over 18 years old, diagnosed with NSCLC at the „Marius Nasta" Institute of Pneumoftiziology (IMN) between January 1, 2015, and December 31, 2021.

Subjects who withdrew or did not provide written informed consent, those with impaired mental status, those under judicial control, those with other histopathological types of primary lung cancer (typical/atypical carcinoids, non-epithelial tumors), or secondary tumors, those with benign lung tumors, exclusively in situ/dysplastic lesions, tumor recurrences, and those diagnosed exclusively by cytology, within a different time frame/at another institution than the ones mentioned, were excluded from the study.

The study was conducted in accordance with the obligations arising from the current legislation, with the approval of the Ethics Committee (number 8406/12.04.2022). The collected information (from the internal database, archived histopathological reports, discussions with patients/family members) was centralized using Excel (version 2401).

Tissue samples were sent to the Pathology Department from the Bronchology Laboratory/Thoracic Surgery, along with the *Accompanying Form for Biological Material*. This form contains mandatory information (patient identification data, medical record number, diagnosis and submitted specimen, referring physician and department, date of the medical intervention). After 24 hours of fixation in 10% formalin, the samples were macroscopically examined, recording the number and size of the specimens and lesions, their color and consistency, whether their borders were well-defined or ill-defined, the presence of necrotic/hemorrhagic areas, the appearance of the resection margins, and the distance between the margins and the lesion.

The processing of the biological material was performed automatically using a tissue processor, following standard techniques. Dehydration was carried out in ethanol solutions of increasing concentrations (from 70% to 100%), and clearing was done using toluene. After paraffin embedding, blocks containing the material to be analyzed were obtained, each with a unique identification number. These were sectioned using a microtome, resulting in thin, transparent sections of 3 microns, which were stained using the standard hematoxylin-eosin (HE) method. This process involved a series of successive steps, including deparaffinization (with benzene), hydration with alcohol, staining itself, rinsing with distilled water, and clearing (with toluene). After mounting the slides, the cases were

examined microscopically (BX46 Olympus). The images used in this study were captured using the attached digital camera, Olympus SC50.

4. SUMMARY OF CHAPTERS

This work is divided into two main sections: a general, theoretical part and a special section presenting the author's personal contributions.

The general section outlines the current state of scientific knowledge in the field of NSCLC and comprises two chapters (1–2): *The epidemiological context and morphological spectrum of bronchopulmonary cancer* (Chapter 1), and *The etiopathogenesis of bronchopulmonary cancer* (Chapter 2).

The special section comprises five chapters (3–7): *Research hypothesis and general objectives* (Chapter 3), *The general research methodology* (Chapter 4), *The epidemiological and morphological study of primary bronchopulmonary carcinomas. Aspects related to the impact of the SARS-CoV-2 pandemic* (Chapter 5), *The immunohistochemical and molecular study of primary bronchopulmonary carcinomas* (Chapter 6), and *The study of morphological aspects with prognostic implications in squamous cell bronchopulmonary carcinomas* (Chapter 7). Each of Chapters 5 to 7 represents a distinct research direction.

Chapter 5 is entitled *The epidemiological and morphological study of primary bronchopulmonary carcinomas. Aspects related to the impact of the SARS-CoV-2 pandemic.*

The research is retrospective, observational, and descriptive, and included 1815 patients diagnosed with NSCLC at the Department of Pathology of the IMN in Bucharest between January 1, 2019, and April 30, 2021.

Initially, a comparative analysis of the pre- and post-pandemic periods was conducted, evaluating the entire patient cohort. Subsequently, the research was divided into two directions (A and B), based on the diagnostic method, in order to capture the uneven impact of the pandemic on different areas of the medical sector. Direction A of the study represents an analysis of surgically diagnosed cases, while Direction B focuses on biopsy-diagnosed cases.

The majority of NSCLC cases were diagnosed in men (>70% of cases), most commonly between 60-69 years-old. The predominant histopathological type across the

entire cohort was adenocarcinoma (ADK).

Several diagnostic challenges were highlighted - some extrinsic, such as the impact of the SARS-CoV-2 pandemic, and others intrinsic, including the identification of rare morphological features: carcinomas with signet-ring cell or clear cell components, pseudoangiosarcomatous and acantholytic forms, and large-cell neuroendocrine carcinomas exhibiting carcinoid-like morphology (Fig. 1–6).

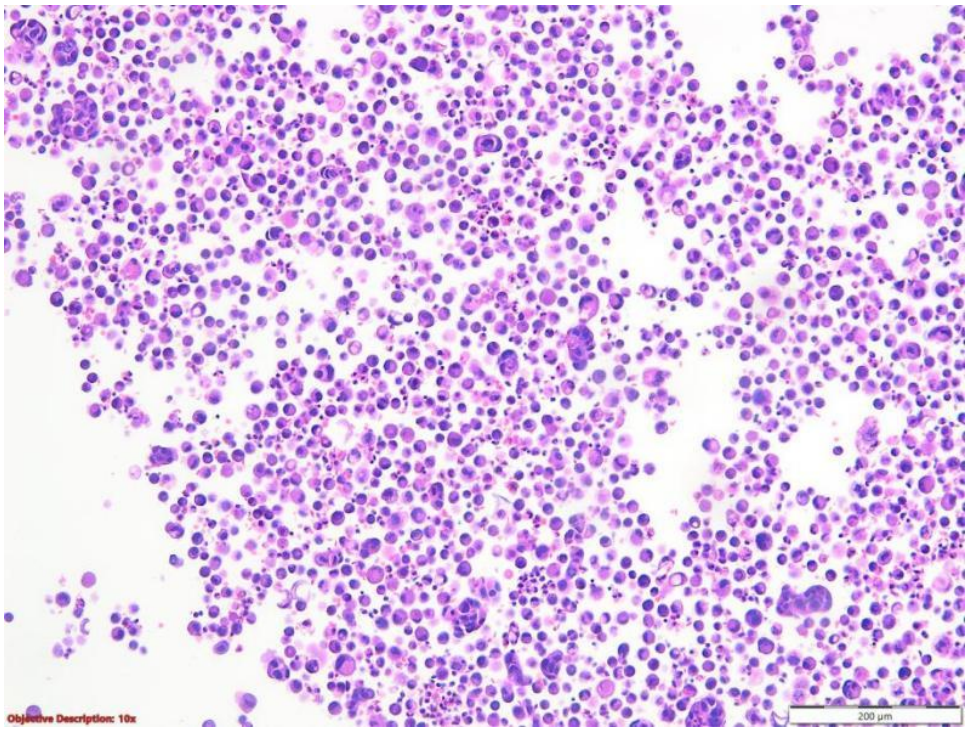

Figure 1. Cell block from pleural fluid – mesothelial cells, rare inflammatory cells, and neoplastic elements with signet-ring cell appearance; HE, 100x.

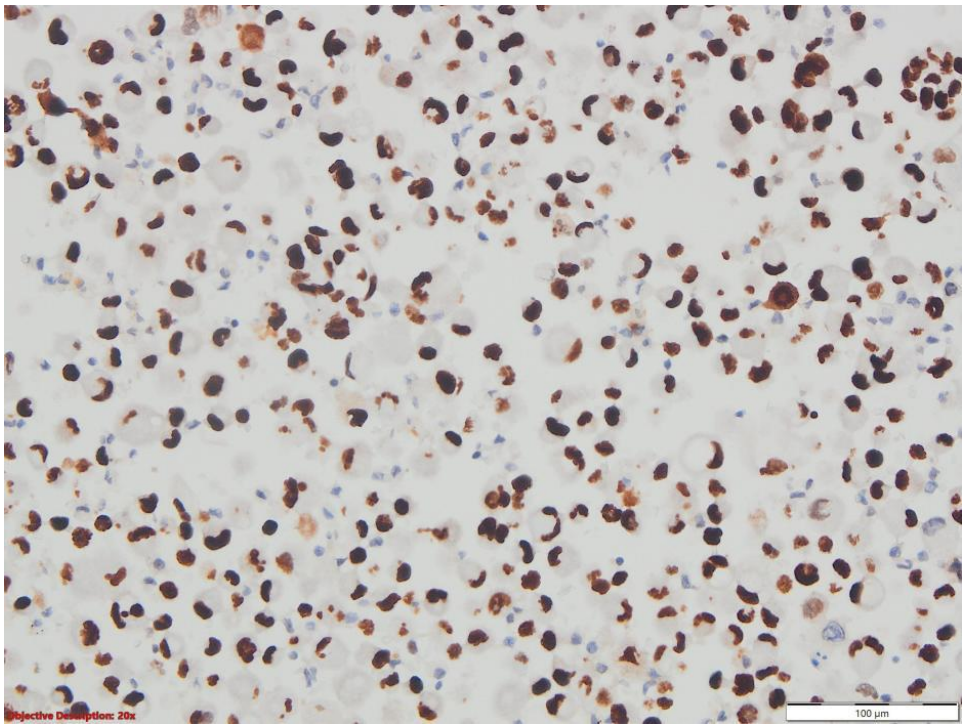

Figure 2. Cell block from pleural fluid – neoplastic cells with a signet-ring cell appearance, showing positive nuclear reaction for TTF; IHC (TTF1), 200x.

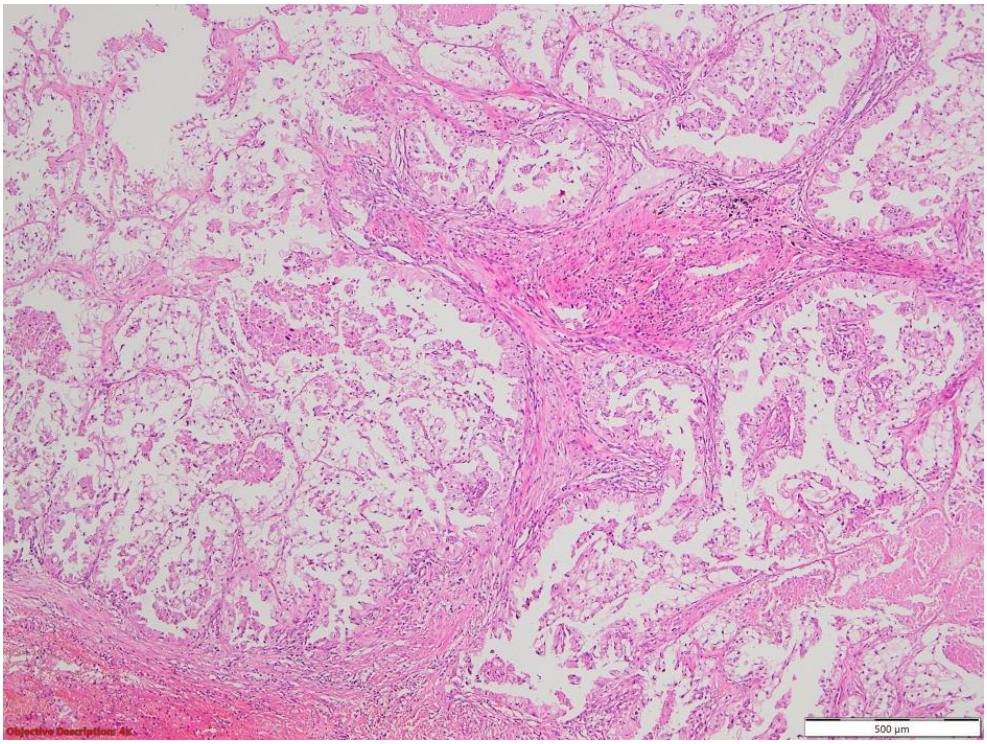

Figure 3. Pulmonary ADK with clear cells exhibiting a lepidic pattern; HE, 40x.

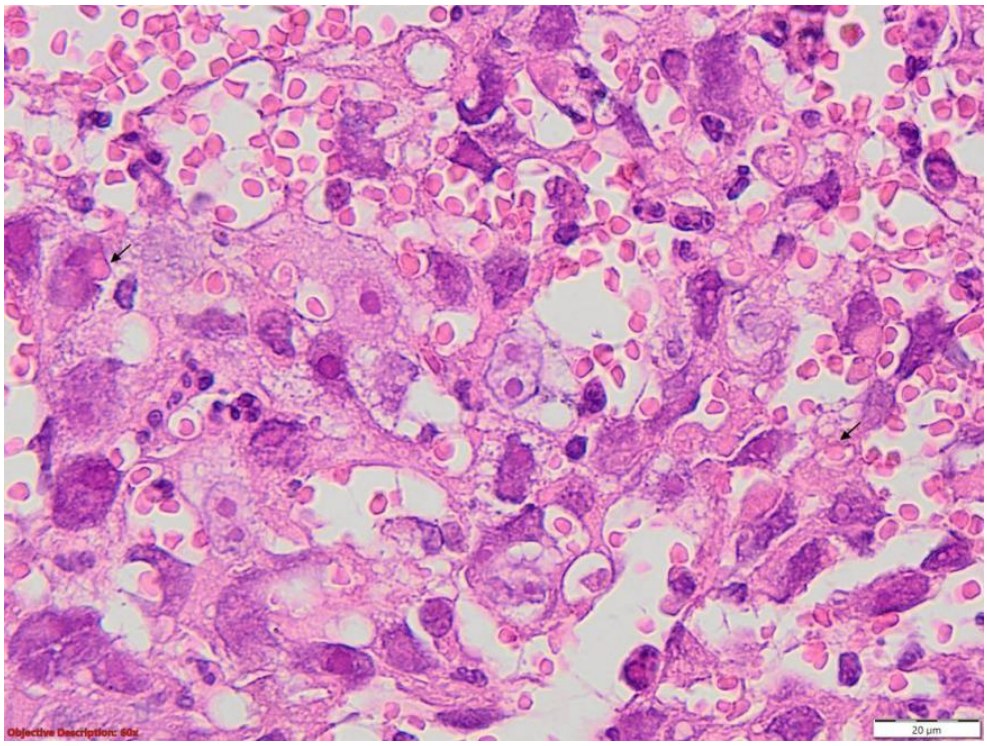

Figure 4. Pulmonary ADK with a pseudoangiosarcomatous phenotype, consisting of large neoplastic cells with moderate nuclear-cytoplasmic atypia and the presence of intracytoplasmic red blood cells (→); HE, 6000x.

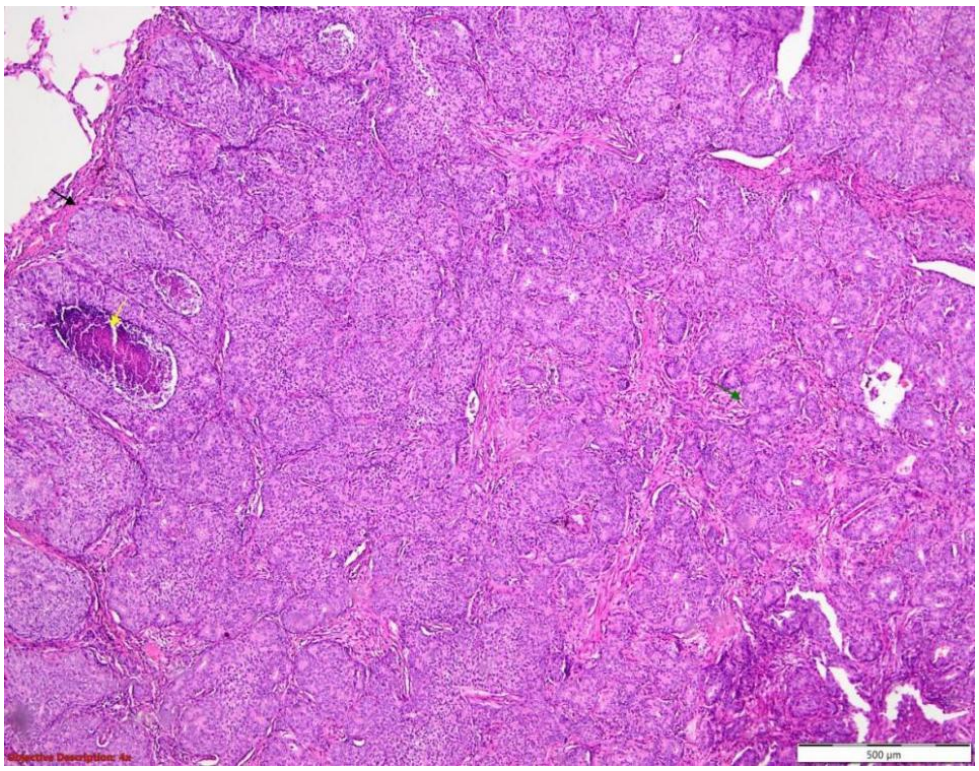

Figure 5. Lung (top left) remodeled by tumour proliferation (→) with focal necrosis (→), organoid pattern, and rosette formation (→); HE, 40x.

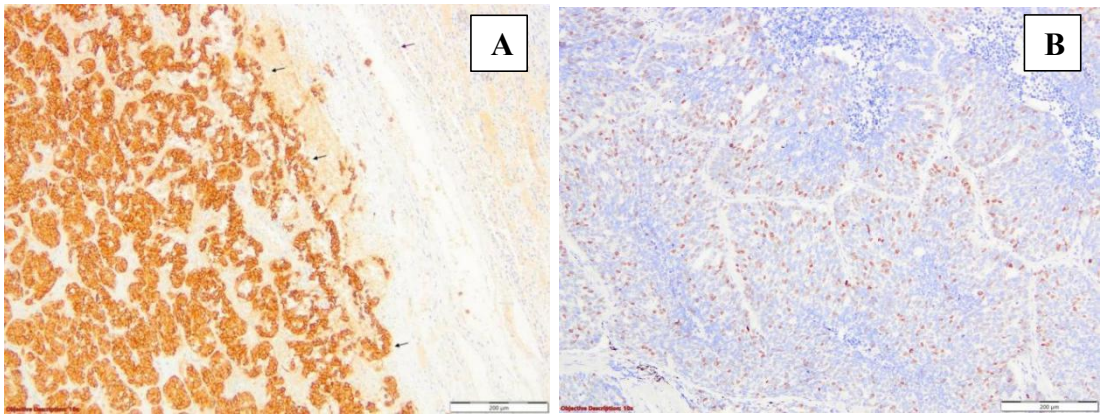

Figure 6. Lung (→) with lesions of large-cell neuroendocrine carcinoma exhibiting carcinoid-like morphology (→); (A) positive reaction for synaptophysin and (B) Ki67 35%; IHC (A - synaptophysin and B - Ki67), 100x.

The SARS-CoV-2 pandemic had a significant impact on the diagnosis of NSCLC, leading to a 17% reduction in the total number of newly diagnosed cases at the IMN. The greatest impact was on the surgical domain, where, compared to the pre-pandemic period, cases were halved (66.9% pre-pandemic versus 33.1% pandemic), with limited, atypical interventions predominating (46.26% pre-pandemic versus 59.43% pandemic). These changes can be interpreted in the context of the epidemiological and sanitary restrictions imposed, which led to a reduction in large-scale interventions (in terms of duration, complexity, and the need for pre- and post-operative in patient monitoring).

The influence was also felt in the biopsy diagnosis of lung cancer, with a reduction (by 17%), although this was less significant.

The minimum number of NSCLC diagnosed cases was recorded in April 2020, at the onset of the pandemic. The numerical variation in cases reflected the pandemic waves, the restrictive measures imposed, as well as the panic generated among the population by the emergence of a virus with an incomplete understanding of its pathogenesis at that time.

A decrease in the diagnosis of lung cancer was observed among elderly patients, although the peak incidence remained between 60-69 years-old in both analyzed periods, with no variations dependent on the diagnostic method or gender.

The SARS-CoV-2 pandemic was associated with an increase in the proportion of diagnoses in women and individuals exposed to smoking or other respiratory toxicants. This can be interpreted in the context that bronchopulmonary conditions, which are common in smokers (chronic bronchitis ± emphysema/chronic obstructive pulmonary

disease, bronchiectasis, etc.), led to greater healthcare seeking, with respiratory symptoms exacerbated by the co-existence of cancer. Additionally, the symptoms of NSCLC can mimic a SARS-CoV-2 infection, thereby delaying diagnosis.

In contrast, the diagnosis of pulmonary malignancies decreased among individuals from rural areas (34.31% pre-pandemic versus 29.58% pandemic; $\chi 2=4.510$, $p=0.034$), most likely due to more difficult access to healthcare services.

In both analyzed periods, tumor sizes were statistically similar, being larger in men. However, during the pandemic, more patients presented with very large tumors (125–150 mm), while in the pre-pandemic period, the largest tumor measured 110 mm.

In both analyzed periods, the majority of lesions were diagnosed in stage III (almost half of the cases), followed by stage II and, less frequently, by stage I. Although during the pandemic, a slight decrease in stage III NSCLC and an increase in stage II was observed, the differences were not statistically significant.

In both analyzed periods, ADK predominated in surgical specimens (with most being non-mucinous), while SCC predominated in biopsy. The method of approach reflects the localization (peripheral versus central) of these tumors, in accordance with international publications, as it was described long time ago. Although the proportion of the two tumor types has changed, with ADK now being more common, their localization has not been affected.

Analyzing the entire study cohort, only a small gap was observed between the proportion of ADK (40%) and SCC (38%), which can be explained in the context of the disruptions caused by the SARS-CoV-2 pandemic (increased diagnosis of centrally located tumors due to greater availability of bronchoscopic methods).

Chapter 6 is entitled *The immunohistochemical and molecular study of primary bronchopulmonary carcinomas.*

The analysis is retrospective, observational, and descriptive, and was conducted on a cohort of 245 adult patients who underwent surgery at IMN between January 1, 2019, and December 31, 2021, and were diagnosed with common types of NSCLC in the Pathology Department.

Immunohistochemical testing was performed using PD-L1 (clone 22C3, Dako and Ventana®), ALK (clone D5F3, Roche). Molecular analysis for the detection of EGFR mutations involved DNA extraction using the cobas® DNA Sample Preparation Kit (Roche Diagnostics). The samples were analyzed for the detection of genetic mutations

using the cobas® EGFR Mutation Test v2 (Roche Diagnostics). Results were interpreted using the cobas® 4800 software (Roche Diagnostics).

Additionally, CD39 (clone EPR20627, Abcam) and CD73 (clone RM431, Bio SB) were tested on a subset of 32 patients.

Immunohistochemical and molecular analysis of the cases highlighted a positive prognostic impact of PD-L1, EGFR mutations, and ALK rearrangements, with all of these variables being associated with higher survival (the median survival was almost 13 months higher in cases with PD-L1 positivity or EGFR mutations, and nearly 33 months higher in patients with ALK rearrangements).

In the studied cohort, 38.77% of tumors were PD-L1 positive, which was associated with a more than twofold decrease in the hazard of death. Analyzing ADK, of the 61 PD-L1 positive cases, most (n=27) exhibited a solid pattern, followed by micropapillary (n=17). All lepidic ADKs were PD-L1 negative, while papillary and acinar types were predominantly negative.

11.83% of cases presented EGFR gene mutations, most commonly located in exons 19 and 20. The detection of these genetic abnormalities was associated with the absence of PD-L1 expression, except in five cases (all affected by mutations in exon 19). EGFR mutations were associated with a 2.31-fold decrease in the hazard of death.

3.67% of cases had ALK rearrangements, which were associated with a 6.61-fold decrease in the hazard of death.

Attempts were made to identify new prognostic markers, CD39 and CD73, but no statistically significant association was found between their immunohistochemical expression and the overall survival of the patients. However, it should be noted that this study has several limitations, including budget constraints (which restricted the evaluation of these markers on a larger cohort), as well as the retrospective and observational nature of the study.

Chapter 7 is entitled *The study of morphological aspects with prognostic implications in squamous cell bronchopulmonary carcinomas*.

The study is retrospective, observational, longitudinal with a cross-sectional analysis on July 1, 2024, non-randomized, conducted on a cohort of 130 adult patients who underwent surgery for invasive primary bronchopulmonary squamous cell carcinoma (SCC), diagnosed in the Pathology Department of IMN between January 1, 2015, and December 31, 2021.

The arrangement of tumor cells, mitotic index, STAS, tumor budding, staging (pTNM), G, and tumor size were associated with the overall survival of patients diagnosed with this pathology.

The hazard of death was twice as high for tumors exhibiting STAS and 40% higher for those with a high mitotic index (≥15 mitoses/10 HPF) or with tumor budding.

Analyzing the arrangement of neoplastic cells both at the center and at the periphery of the tumor, it was significantly correlated with survival.

The identification of isolated neoplastic cells in the central portion of the tumor was associated with a death hazard almost 3 times higher compared to the arrangement in small cellular islands (2-4 neoplastic cells) and 6 times higher compared to the arrangement in large cellular islands (≥ 5 neoplastic cells). Similarly, when analyzing the tumor periphery, the presence of isolated neoplastic elements was associated with a death hazard twice as high compared to the arrangement in small cellular islands and nearly 3 times higher compared to the arrangement in large islands.

Another statistically significant variable was pTNM, with stage III tumors having a 3.07 times higher death hazard compared to those in stage I, and stage II tumors having a 2.04 higher death hazard. Lymph node metastases were associated with an increased death hazard as follows: 1.13 times for pN1 tumors, 1.56 times for pN2 tumors, and 4.61 times for pN3 tumors.

Compared to well-differentiated tumors, moderately differentiated tumors had a 1.44 times higher death hazard, while poorly differentiated tumors had a 2.64 times higher death hazard.

An objectively measurable macroscopic factor that influenced survival was tumor size, with a 1 cm increase in tumor proliferation being correlated with a 16% higher death hazard.

In contrast, patient gender and age, stromal desmoplasia, keratinization, tumor necrosis, prominent nucleoli, and nuclear pleomorphism did not prove to have prognostic value in the studied cohort.

5. CONCLUSIONS AND PERSONAL CONTRIBUTIONS

The first objective of the doctoral research consisted of the epidemiological and morphological analysis of NSCLC, placing it within a clearly defined spatial and temporal context.

A thorough evaluation of pulmonary malignancies diagnosed in a national reference center for this pathology in Romania revealed that, within the studied cohort, the majority of lesions were ADK, occurring predominantly in male, smokers, between 60-69 years-old. All assessed characteristics were consistent with international reports.

A novel aspect of the study was the integration of this evaluation within the context of the SARS-CoV-2 pandemic, particularly given the highly heterogeneous results reported in international literature. The pandemic was associated with a decrease in the number of newly diagnosed cases at IMN, with the lowest point recorded in April 2020, at the onset of the crisis. The surgical sector was the most severely affected, with the number of cases reduced by half and a predominance of limited, atypical procedures.

A decrease in the diagnosis of lung cancer was observed among elderly patients, although the highest incidence remained in the interval 60-69 years-old across both analyzed periods. Patients from rural areas were also more significantly affected. In contrast, there was an increase in the proportion of diagnosed cases among women and individuals exposed to smoking or other respiratory toxicants.

Contrary to expectations, the cases were not more advanced at the time of diagnosis. The pandemic led to a slight decrease in stage III NSCLC and a corresponding increase in stage II cases; however, these differences were not statistically significant.

Across the entire cohort, the majority of tumors were ADK, more frequently diagnosed in women and identified on surgical specimens, while SCC predominated in men and were more commonly diagnosed through biopsies, regardless of the period.

The study highlighted a series of rare morphologies of the most common types of NSCLC, associated with a high degree of diagnostic complexity: clear cell or signet ring cell morphology, pseudoangiosarcomatous or acantholytic appearance, and carcinoid-like morphology in large cell neuroendocrine carcinomas. According to the literature research, the case presented in this work is the first pseudoangiosarcomatous ADK described in the pulmonary context.

The second direction of the doctoral study focused on the immunohistochemical and molecular analysis of NSCLC. The research highlighted an association between patient survival and the following factors: PD-L1 positivity, the presence of EGFR mutations, and ALK rearrangements (listed in ascending order of impact).

PD-L1 positivity was associated with more than a twofold decrease in the hazard of death and an average survival increase of 12.81 months. An important feature of the study was the correlation between the ADK pattern and PD-L1 expression, with the highest frequencies of positivity found in high-grade phenotypes (solid and micropapillary).

In the case of tumors with EGFR mutations, exons 19 and 21 were the most frequently involved (consistent with international reports). These were associated with a 2.31-fold reduction in the hazard of death and an average survival increase of nearly 13 months. All tumors with EGFR mutations were PD-L1 negative, except for five cases, all of which had exon 19 affected.

ALK rearrangements were associated with a 6.61-fold reduction in the hazard of death and an average survival increase of nearly 33 months.

Another current focus of the study was the attempt to identify new prognostic markers in NSCLC by analyzing CD39 and CD73 at the tumor level. Although there were variations in expression, no statistically significant correlations were found with survival or other morphological or molecular aspects.

The third direction of the doctoral study focused on the analysis of morphological factors with prognostic implications in SCC.

The arrangement of neoplastic cells, mitotic index, STAS, tumor budding, pTNM, grade, and tumor size were all found to influence overall survival.

The hazard of death was twice as high in tumors with STAS present and 40% higher in those with a high mitotic index or tumor budding. The identification of isolated neoplastic cells in the central area of the tumor was associated with a death hazard almost three times higher compared to the arrangement in small cellular islands and six times higher compared to the arrangement in large cellular islands. Similarly, when analyzing the tumor's peripheral area, the presence of isolated neoplastic elements was associated with a death hazard twice as high compared to small cellular islands and almost three times higher compared to large cellular islands.

Tumors in stage III had a death hazard 2.37 times higher than those in stage I. Lymph node metastases were associated with an increased death hazard: 1.13 times for pN1 tumors, 1.56 times for pN2, and 4.61 times for pN3.

Compared to G1 tumors, G2 tumors had a death hazard 1.44 times higher, and G3 tumors had a death hazard 2.64 times higher. Tumor size also influenced survival, with a 1 cm increase in tumor growth being correlated with a 16% higher death hazard.

The innovative aspect of the research lay in the analysis of SCC morphology, focusing on their prognostic implications. In the present study, no prognostic role was identified for the following variables: gender, age, stromal desmoplasia, keratinization, tumor necrosis, prominent nucleoli, or nuclear pleomorphism.

The limitations of this research were both financial and methodological, arising from the study type (retrospective, observational, descriptive). The limited budget restricted the immunohistochemical testing of CD39 and CD73 to a small number of cases, preventing an extensive evaluation from which meaningful conclusions could be drawn. The study identified statistically significant associations between certain parameters but was unable to establish causal relationships (due to its observational nature). Additionally, the retrospective nature of the research imposed limitations regarding the accessibility of available data.

An unresolved issue remains the evaluation of the prognostic value of CD39 and CD73, as this study did not demonstrate a correlation between their immunohistochemical expression and patient survival. This represents one direction in which the research could be continued, by analyzing larger patient cohorts and potentially correlating with other variables (such as peri- and intratumoral inflammatory infiltrates or the serum levels of C-reactive protein).

Additionally, identifying other predictive and prognostic markers would represent an important step in the management of patients with lung cancer.

Other potential research directions could include the development of a standardized, objective grading system for SCC, as well as the integration of artificial intelligence in the diagnosis of these malignancies. Additionally, pulmonary co-infections associated with NSCLC represent an area that requires further exploration, as they may lead to atypical tumor phenotypes and influence the timing of oncological treatment (for example, tuberculosis).

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LIST OF PUBLISHED SCIENTIFIC PAPERS

1. **Marghescu AȘ**, Leonte DG, Radu AD, Măgheran ED, Tudor AV, Teleagă C, Țigău M, Georgescu L, Costache M., „Atypical Histopathological Aspects of Common Types of Lung Cancer-Our Experience and Literature Review". Medicina (Kaunas). 2024 Jan 7;60(1):112. doi: 10.3390/medicina60010112. PMID: 38256374; PMCID: PMC10818882, FI - 2,6/2024, https://www.mdpi.com/1648-9144/60/1/112 (Chapter 5, pages 35-84).

2. **Marghescu AȘ**, Vlăsceanu S, Mahler B, Bădărău IA, Țigău M, Dumitrache-Rujinski S, Leonte D, Măgheran E, Tudor A, Georgescu L, Negoescu IA, Bobocea AC, Costache M, Savu C, „The Impact Of the SARS-COV-2 Pandemic On the Diagnosis of Lung Cancer Patients", Internal Medicine vol. 21, no. 1, Sciendo, 2024, pp. 7-23, https://doi.org/10.2478/inmed-2024-0274, (Chapter 5, pages 35-84).

3. **Marghescu AȘ**, Vlăsceanu S, Preda M, Țigău M, Dumitrache-Rujinski Ș, Leonte DG, Măgheran ED, Tudor A, Bădărău IA, Georgescu L, Costache M., „Navigating the Maze: Exploring Non-Oncological Complexities in Non-Small-Cell Lung Cancer". Cancers (Basel). 2024 May 16;16(10):1903. doi: 10.3390/cancers16101903. PMID: 38791982; PMCID: PMC11120337, FI - 5,2/2024, https://www.mdpi.com/2072-6694/16/10/1903 (Chapter 5, pages 35-84).

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5. Vlăsceanu S, Mahler B, **Marghescu AȘ***, Bădărău IA, Moldovan H, Gheorghiță D, Costache M, Savu C. „The Nine-Year Survival of Patients Operated for Non-Small-Cell Lung Carcinoma in a Tertiary Centre: The Impact of the Tumour Stage and Other Patient-Related Parameters". Medicina (Kaunas). 2024 Feb 28;60(3):415. doi: 10.3390/medicina60030415. PMID: 38541141; PMCID: PMC10972444, FI - 2,6/2024, https://www.mdpi.com/1648-9144/60/3/415 (Chapter 7, pages 100-128).

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