Master Thesis

SUITABILITY OF BANKED FFPE BLOCKS OF NON-SMALL CELL LUNG CANCER FOR RESEARCH PROJECTS CONSIDERING DATA OF THEIR MORPHOLOGICAL ASSESSMENT (DIAGNOSIS, TUMOR CELL COUNT, NECROSIS)

Submitted by

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Statutory Declaration

I declare on my honor that I have written this dissertation independently and without assistance, that no sources other than those cited were used and that the sources used verbatim or in substance have been marked as such.

Graz, …… *Signature Halyna Chytaieva*

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List of abbreviations

AJCC	— American Joint Committee on Cancer
FFPE	— formalin fixed paraffin embedded
NGS	— next-generation sequencing
NSCLC	— non-small cell lung cancer
SCC	— squamous cell carcinoma
WHO	— World Health Organization

Abstract (English)

Cancer is the largest clinical, social and economic crisis of all human diseases, second only to cardiovascular pathology in mortality, but is projected to become the leading cause of death by 2060. Lung cancer is one of the deadliest cancers in the general population, with 85% of cases being non-small cell lung cancer (NSCLC), which includes adenocarcinoma, squamous cell carcinoma (SCC), and other, rarer histological types. Despite numerous studies and medical advances, the 5-year survival rate for NSCLC does not exceed 25%, the incidence in never-smokers is increasing, and many aspects of pathogenesis, progression, drug resistance, and targeted therapy require further study. Most studies of solid tumors are conducted on formalin-fixed paraffin embedded (FFPE) blocks, which have several advantages over other samples: low cost, ease of storage and transportation, suitability for molecular genetic studies of blocks aged 10 years or more, thanks to modern techniques. There are large pathological archives with huge collections of FFPE blocks, as well as biobanks with structured and certified systems for processing and storing material and relevant demographic and clinical data. There are a lot of requirements for blocks used in research projects, one of the most important is tumor and necrosis content. Although this threshold varies depending on the study, the most common is tumor content of ≥30% and necrosis of ≤30%. NSCLC is often characterized by multiple / extensive necrosis. Here, a collection of 592 NSCLC samples collected for the Audubon Bioscience biobank in Ukraine between 2020 and 2022 was analyzed to determine whether they met these requirements to find out which blocks could be offered for further research. 494 FFPE blocks were identified for further analysis; the remaining 98 (16.55%) were rejected due to discrepancies in clinical and pathological diagnosis. 403 (81.58%) blocks showed satisfactory tumor and necrosis percentage. According to the statistical analysis, a correlation was found between tumor size and tumor percentage, and tumor size and necrosis percentage. In further NSCLC material collection, the identified correlations should be considered and at least 20% more samples should be collected than required by the project to select blocks with ≥30% tumor and ≤30% necrosis for use in further studies.

Abstrakt (German)

Krebs ist die größte klinische, soziale und wirtschaftliche Krise aller menschlichen Krankheiten und steht hinsichtlich der Sterblichkeit an zweiter Stelle nach Herz-Kreislauf-Erkrankungen, wird aber voraussichtlich bis 2060 zur häufigsten Todesursache werden. Lungenkrebs ist eine der tödlichsten Krebsarten in der Allgemeinbevölkerung. 85 % der Fälle sind nicht-kleinzellige Lungenkarzinome (NSCLC), zu denen Adenokarzinome, Plattenepithelkarzinome (SCC) und andere, seltenere histologische Typen gehören. Trotz zahlreicher Studien und medizinischer Fortschritte liegt die 5-Jahres-Überlebensrate für NSCLC nicht über 25 %, die Inzidenz bei Nichtrauchern steigt, und viele Aspekte der Pathogenese, Progression, Arzneimittelresistenz und gezielten Therapie müssen noch weiter untersucht werden. Die meisten Studien zu soliden Tumoren werden an formalinfixierten, in Paraffin eingebetteten (FFPE) Blöcken durchgeführt, die gegenüber anderen Proben mehrere Vorteile haben: geringe Kosten, einfache Lagerung und Transport, Eignung für molekulargenetische Untersuchungen von Blöcken, die dank moderner Techniken 10 Jahre oder älter sind. Es gibt große pathologische Archive mit riesigen Sammlungen von FFPE-Blöcken sowie Biobanken mit strukturierten und zertifizierten Systemen zur Verarbeitung und Speicherung von Material und relevanten demografischen und klinischen Daten. An Blöcke, die in Forschungsprojekten verwendet werden, werden viele Anforderungen gestellt, eine der wichtigsten ist der Gehalt an Tumor- und Nekrosegewebe. Obwohl dieser Schwellenwert je nach Studie variiert, ist der häufigste Wert ein Tumorgehalt von ≥30 % und ein Nekrosegehalt von ≤30 %. NSCLC ist oft durch multiple/ausgedehnte Nekrosen gekennzeichnet. Hier wurde eine Sammlung von 592 NSCLC-Proben, die zwischen 2020 und 2022 für die Audubon Bioscience Biobank in der Ukraine gesammelt wurden, analysiert, um festzustellen, ob sie diese Anforderungen erfüllen, und um herauszufinden, welche Blöcke für weitere Forschungszwecke angeboten werden können. 494 FFPE-Blöcke wurden für die weitere Analyse identifiziert; die restlichen 98 (16,55 %) wurden aufgrund von Diskrepanzen in der klinischen und pathologischen Diagnose abgelehnt. 403 (81,58 %) Blöcke wiesen einen zufriedenstellenden Tumor- und Nekroseanteil auf. Die statistische Analyse ergab eine Korrelation zwischen Tumorgröße und Tumoranteil sowie zwischen Tumorgröße und Nekroseanteil. Bei der weiteren Sammlung von NSCLC-Material sollten die identifizierten Korrelationen berücksichtigt werden, und es sollten mindestens 20 % mehr Proben gesammelt werden als vom Projekt gefordert, um Blöcke mit ≥30 % Tumor und ≤30 % Nekrose für die Verwendung in weiteren Studien auszuwählen.

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1. Introduction

1.1. Lung cancer is one of the main oncological diseases of our time

Cancer is a major social, public health and economic problem of the 21st century, causing about 16.8% of all deaths and 22.8% of deaths from non-communicable diseases (Bray F, et al, 2024).

Lung cancer ranks first in both morbidity and mortality from cancer, with a tendency to increase in indicators with a poor prognosis (3-year survival in various types does not exceed 31%) (Deshpande R, et al., 2022). Lung cancer is most common in men in 37 countries, including China, most of Eastern Europe, the Middle East, and Southeast Asia. In women, lung cancer is most common in the United States, Northern Europe, and Western Europe. Lung cancer rates among men and women are lowest in West, Central and East Africa (Bray F, et al., 2024; Thandra KC, et al., 2021).

It is estimated that 1 in 5 people will develop cancer in their lifetime, and that 1 in 9 men and 1 in 12 women will die from lung cancer (Smolarz B, et al., 2025). Approximately 38.9% of men and women have a lifetime risk of being diagnosed with lung cancer (Zhou J, et al., 2024). In general, the 5-year survival rate for lung cancer does not exceed 27%, and in most countries of the world it is 20% (Bray F, et al., 2024; Jiajing Sun, et al., 2022).

1.1.1. Development of cancer in general and of lung cancer specifically

Cancer development is a complex, multi-stage process that is not fully understood. Numerous studies of solid tumors, from epidemiological to molecular, allow us to unravel the mysteries of the pathogenesis and progression of malignant tumors, including lung cancer.

As is known, tumor growth is caused by two main deviations from the norm: the induction of proliferation stimuli and the simultaneous compensatory suppression of cell death.

The molecular pathogenesis of lung cancer is quite complex and heterogeneous. Lung cancer can arise from a sequence of genetic factors and epigenetic changes (e.g., point mutations, insertions, deletions, translocations) (Cai JS, et al., 2022; Smolarz B, et al., 2025) (Fig. 1.1.1.1).

The cancer process begins when cells lose control of the mechanisms that determine their division and localization. In this case, the cell cycle is like normal, but cells do not obey regulatory mechanisms and become insensitive to signals from other cells. Alterations in genes that control the cell cycle play a key role in neoplastic transformation. Tumor initiation and progression involve the following steps:

- cell cycle regulation disorders;
- mutations of proto-oncogenes and tumor suppression genes;
- disruption of DNA repair processes;
- overexpression of growth factors and angiogenesis;
- avoidance of apoptosis (mutations of anti- and pro-apoptotic genes);
- increased telomerase activity;
- invasive growth and metastasis.

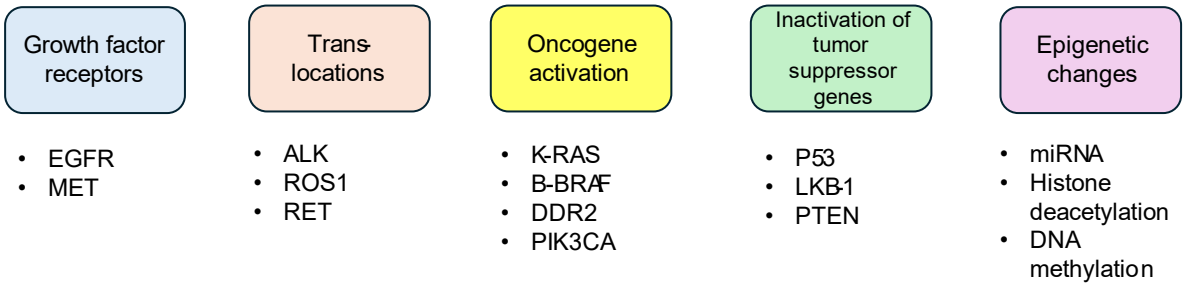


Fig. 1.1.1.1. Molecular alterations in lung cancer (according to Smolarz B, et al., 2025).

An important role is plays the the instability of the cellular genome, which occurs at the beginning of the carcinogenesis process, leading to the gradual accumulation of various genetic disorders, weakening of the DNA structure, and increasing the likelihood of new mutations (Smolarz B, et al., 2025).

Thus, mutations in the p53 gene are considered a key point in carcinogenesis. Against this background, mutations in other genes (MYC, RAS, EGFR) significantly increase the risk of neoplastic transformation. Abnormal changes in specific genes are very important for clinical practice as molecular predictive factors that can contribute to the search for more effective and targeted therapy and, consequently, better prognosis.

A very important stage of diagnosis, especially in the later stages of the disease, is the study of biomarkers, in particular, EGFR, ALK, KRAS, B-RAF, HER2, ROS1, PD-L1, RET and MET, which are detected with varying frequency. For example, EGFR mutations

are present in 15–62% of cases (more common in patients of Asian descent and those who have never smoked), ROS mutations in 1–2%, BRAF mutations in 1–7%, KRAS mutations in 19–30% of cases, ALK rearrangements are less common (4–22%, mainly in young patients and those who have never smoked). The identification of molecular targets has contributed to the emergence of new standards for the diagnosis and treatment of lung cancer, including in the presence of metastatic disease (Casal-Mouriño A, et al., 2021; Feng H, et al., 2015; Smolarz B, et al., 2025).

Over the past 10 years, numerous molecular abnormalities that cause the development of lung cancer have been identified and studied. Tyrosine kinase inhibitors that target EGFR mutations, ALK and ROS1 translocations have been developed for the treatment of lung adenocarcinoma and have been approved for use in late-stage disease. Continuous progress in diagnostic and treatment methods gives hope for improving the prognosis for patients with lung cancer.

1.1.2. Lung cancer frequency and incidence

Lung cancer was the most diagnosed cancer in 2022 (12.4% of all cancer cases worldwide). The second most common types were breast cancer in women (11.6% of cases), colorectal cancer (9.6%), prostate cancer (7.3%), and stomach cancer (4.9%) (National Cancer Institute. Cancer Statistics [Internet]. National Cancer Institute. 2025. Available from: https://www.cancer.gov/about-cancer/understanding/statistics) (Fig. 1.1.2.1). In 2022, 2,480,675 new cases of lung cancer were diagnosed, including 1,572,045 in men and 908,630 in women. The largest number of lung cancer cases in 2022 were reported in China, the United States, and Japan; Asia accounted for more than half of all new lung cancer cases (World Cancer Research Fund. Lung cancer statistics | World Cancer Research Fund [Internet. World Cancer Research Fund. 2024. Available from: https://www.wcrf.org/preventing-cancer/cancer-statistics/lung-cancer-statistics/; Zhou J, et al., 2024) (Fig. 1.1.2.1). Lung cancer also took the leading position in cancer mortality — 1.8 million cases (18.7%). In women, the leading oncological pathology detected in 2022 and causing patient death was breast cancer, in men — lung cancer, respectively (National Cancer Institute. Cancer Statistics [Internet]. National Cancer Institute. 2025. Available from: https://www.cancer.gov/about-cancer/understanding/statistics). The main risk factors for lung cancer are long-term active smoking (80–90% of cases) or long-term exposure to radon, asbestos, polycyclic aromatic compounds, arsenic, cadmium, silicone, vinyl chloride, etc. (Cai JS, et al., 2022; Smolarz B, et al., 2025; Yanqian Huang, et al., 2021). 15–20% of lung cancer cases occur in non-smokers (Jiajing Sun, et al., 2022).

Lung cancer incidence and mortality rates are significantly higher in men, due to many factors: smoking (cigarette smoke contains over 60 different carcinogens), genetic predisposition, and potential hormonal influences (Smolarz B, et al., 2025; Zhou J, et al., 2024). Thus, malignant neoplasm of the chest, namely lung cancer ranks first in cancer-related mortality, both among men and women, and is a serious global public health problem (Baiu I, et al., 2021; Jiajing Sun, et al., 2022; Tolwin Y., et al., 2020).

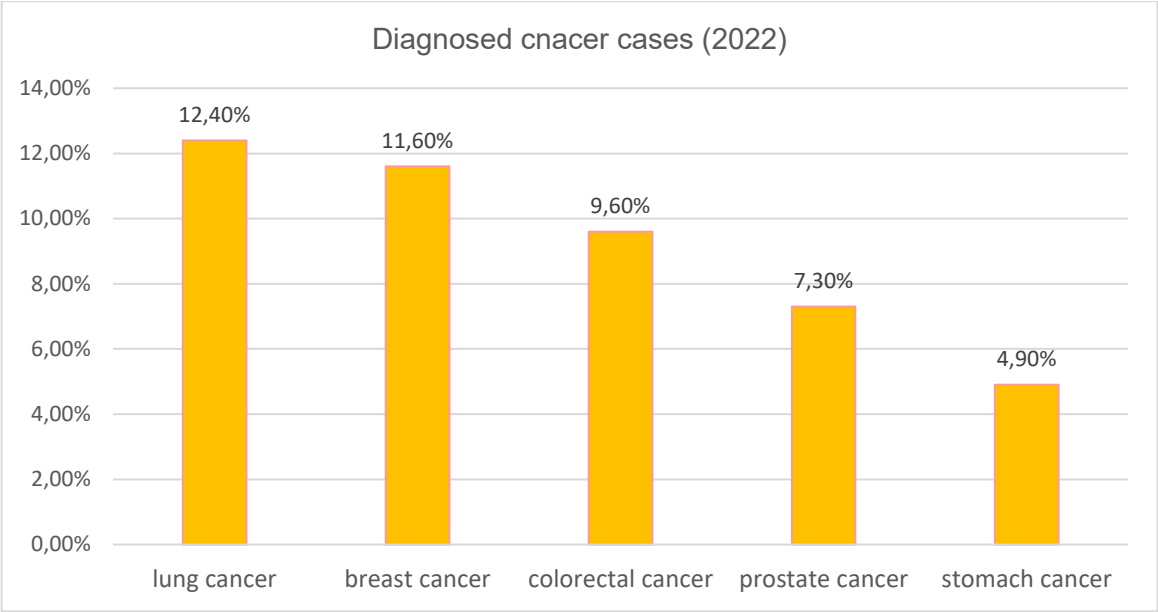
Over the past decade, despite significant progress in the prevention, screening, and treatment of lung cancer, huge racial, socioeconomic, and geographic disparities in incidence and mortality persist worldwide. Lung cancer incidence is increasing today, especially in low- and middle-income countries. By 2050, lung cancer incidence is projected to increase by 86.2% from 2022 to 4.62 million, and mortality is projected to increase by 95% from 2022 to 3.55 million (Zhou J, et al., 2024).

According to the National Cancer Registry of Ukraine, in 2020, the incidence of lung cancer was 26.8 per 100.000 population (male/female ratio 46.0/10.2 per 100.000); mortality was 22.6 cases per 100.000 population (male/female ratio 39.4/8.0 per 100.000). Of the newly identified cases, 31.8% had stage III disease, 42.2% had stage IV; 57.9% of patients identified in 2019 did not survive even 1 year. The rate of morphologically verified cases is far from optimistic — only 60.4% (National Cancer Registry of Ukraine. Cancer incidence and mortality in Ukraine, 2021–2022. Cancer in Ukraine, 2021–2022. Incidence, mortality, and indicators of oncology service activity. Available from: http://www.ncru.inf.ua/publications/BULL_24/index.htm.).

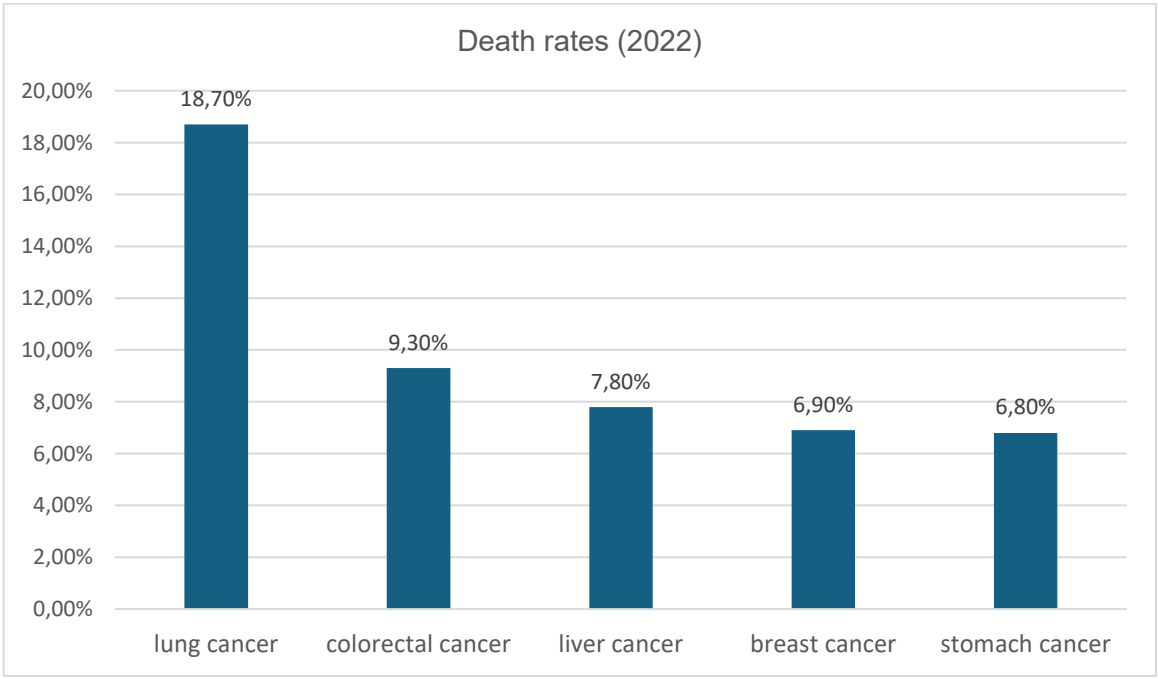
Even today, with the availability of screening and modern examination methods, most patients with lung cancer are diagnosed at a late stage, i.e. at stage III or IV. Thus, according to literature data, lung cancer at stages I–II is detected in 25% of cases, at stage III — in 35%, and at stage IV — in 40%, respectively (Cai JS, et al., 2022; Smolarz B, et al., 2025) (Fig. 1.1.2.1). Typically, clinical symptoms are nonspecific and fully manifest in the late stages, with nearly 75% of patients experiencing cough, chest pain, hemoptysis, dyspnea, and weight loss. Most common sites of lung cancer metastasis are lymph. nodes, bones, liver and brain (Artene SA, et al., 2013; Shimin Xie, et al., 2021). Metastases in the lymph. nodes are a critical event in lung cancer progression that influences clinical treatment and prognosis (Xiayao Diao, et al., 2022). Accurate and precise staging of lung cancer is critical for treatment and prognosis. Staging is based on guidelines of AJCC 8th ed, which was presented during the 16th World Congress of Lung Cancer and has been in effect since 2017 (AJCC Cancer Staging Manual. 2018; Casal-Mouriño A, et al., 2021).

The stage of lung cancer at the time of diagnosis plays a crucial role in choosing the treatment method and determining the prognosis of the disease (Smolarz B, et al., 2025).

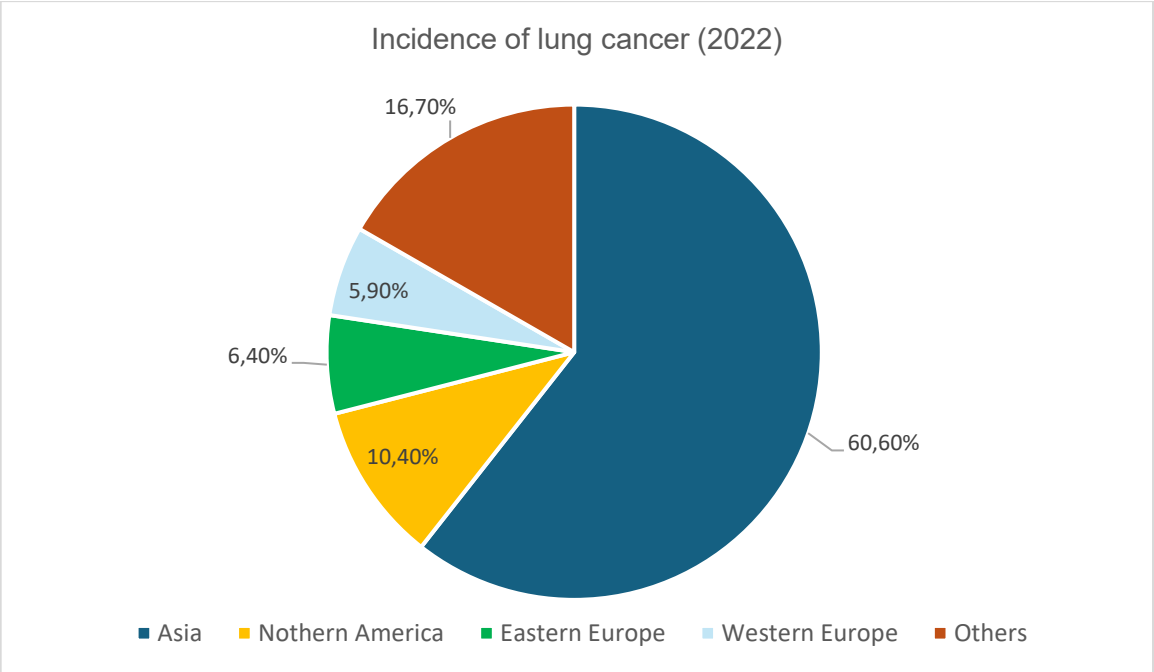
In the early stages, surgery is possible, with lobectomy being the method of choice; pneumonectomy is usually used in cases where lobectomy does not provide complete removal of the affected area. All types of resections should be accompanied by removal of hilar and mediastinal lymph. nodes. According to the standard, adjuvant treatment includes 3–4 cycles of chemotherapy and is used at stage II–III. When combined with surgery and chemotherapy, the risk of death from lung cancer is reduced by 13% (Deshpande R, et al., 2022). Postoperative radiotherapy is recommended in cases where microscopically the procedure was not radical. Palliative chemotherapy is used in stage IV, with a response rate of 20–30% (Smolarz B, et al., 2025). Targeted therapies for lung cancer can be personalized, allowing for specific molecules to be targeted and significantly improving prognosis. However, long-term disease control is not achieved in most patients, and 5-year survival rates remain far from optimistic (Deshpande R, et al., 2022).


Diagnosed cnacer cases (2022)
14,00%
12,00%
10,00%
8,00%
6,00%
4,00%
2,00%
0,00%
12,40%
11,60%
9,60%
7,30%
4,90%
lung cancer
breast cancer
colorectal cancer
prostate cancer
stomach cancer

A


Death rates (2022)
20,00%
18,00%
16,00%
14,00%
12,00%
10,00%
8,00%
6,00%
4,00%
2,00%
0,00%
18,70%
9,30%
7,80%
6,90%
6,80%
lung cancer
colorectal cancer
liver cancer
breast cancer
stomach cancer

B



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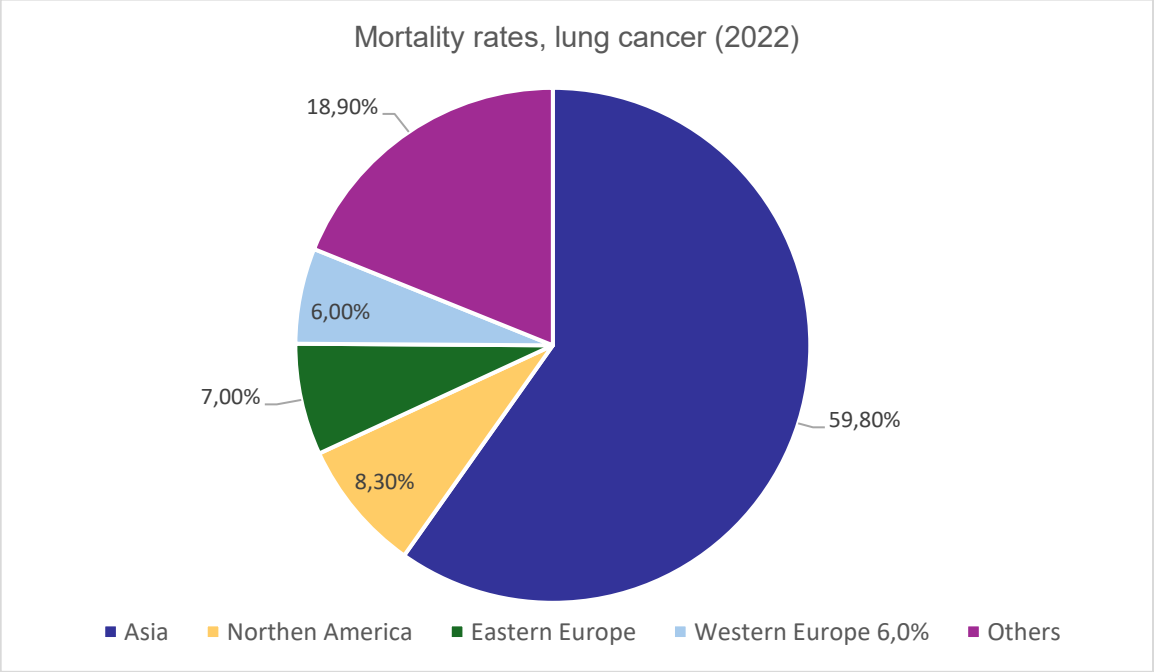

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Fig. 1.1.2.1. Incidence (A, C) and mortality (B, D) rates in lung cancer (2022) according to Zhou J, et al. (Zhou J, et al., 2024).

Lung cancer incidence and survival rates vary considerably between clinical trials. Between 2010 and 2017, lung cancer incidence decreased slightly, with an increase in the

number of cases detected at early stages and a decrease in stage IV cases mainly due to the mass introduction of screening methods into clinical practice.

According to 2018 data, thanks to the widespread implementation of screening studies, stage I lung cancer was detected in 40.25% of patients. The overall 5-year survival rate for stage I reached 87.68% (Chengdi Wang, et al., 2023). However, even at stage I, after complete resection, the frequency of postoperative recurrence or metastasis reaches 21.7% (Jiajing Sun, et al., 2022; Park SY, et al., 2011).

Stage III NSCLC encompasses a heterogeneous group of patients with varying location and extent of disease, and some aspects of its treatment are controversial. Stage III includes resectable and unresectable tumors. Treatment for stage III includes surgery, chemotherapy, radiotherapy and immunotherapy in various combinations. Despite advances in modern diagnostics and treatment, overall survival for stage III is from 9 to 34 months, with a 5-year survival rate of about 25.1% (Casal-Mouriño A, et al., 2021). Survival of patients (%) with lung cancer at different stages (according to Ganti AK, et al., 2021) is given on Fig. 1.1.2.2.

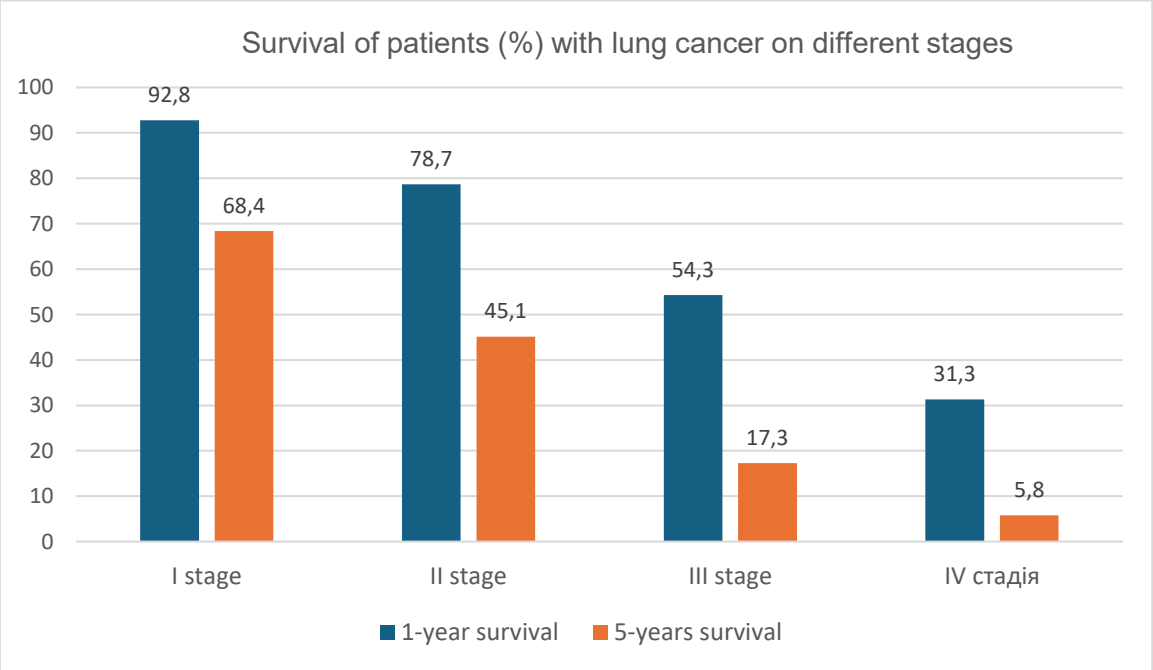


Fig. 1.1.2.2. Survival of patients (%) with lung cancer at different stages (according to Ganti AK, et al., 2021).

1.1.3. Lung cancer is complex and heterogenous disease

According to the WHO classification (2015), malignant lung neoplasms are divided into two large groups by histological type: NSCLC and neuroendocrine lung neoplasms (typical and atypical carcinoids, large and small cell neuroendocrine lung carcinomas) (Thandra KC, et al., 2021). Primary cancer (NSCLC) originates from epithelial cells and accounts for 80–85% of lung cancers (Smolarz B, et al., 2025). The main subtypes of NSCLC are adenocarcinoma (about 63.8%), SCC (27.7%), and large cell carcinoma (3.1%), which originate from different types of lung cells but are grouped together because their treatment and prognosis are often similar (Artene SA, et al., 2013; Wen Wang, et al., 2022). However, there are some differences. Adenocarcinoma and SCC originate from different epithelial cells, express different cellular markers, have various genomic profiles and molecular characteristics, and non-identical prognoses, but to date their different clinical characteristics and behavior are still largely unknown (Conde E, et al., 2013). SCC is more common in men, smokers, and usually at a later stage (larger tumor compared to adenocarcinoma). Wen Wang et al., believe that adenocarcinoma and SCC should be classified and treated as different cancers, with SCC considered a more aggressive type of NSCLC (Wen Wang, et al., 2022). According to Vinod SK et al., the survival of patients with different histological types of NSCLC at stage III differs, with lung adenocarcinoma showing the best results: median survival is 13 months, 3-year survival is 19.2%, in SCC — 10 months and 12.5 months, in large cell carcinoma — 9 months and 15.3 months, respectively (Vinod SK, et al., 2012).

1.1.4. Importance of tumor necrosis

The development of invasive cancer is associated with a switch from predominantly apoptotic cell death to necrosis. According to the literature, the presence of necrosis in a tissue sample may not only reflect the biology of the tumor but also be an additional reliable unfavorable prognostic factor (Caruso R, et al., 2012; Park SY, et al., 2011; Richards CH, et al., 2011). As a rule, when the tumor size is more than 3 mm, intensive development of its own angiogenesis is necessary, which is often imperfect (chaotic arrangement of vessels of different diameters, their walls are fragile and brittle), which contributes to the occurrence of hemorrhages and necrosis. Necrosis in a tumor occurs when there is insufficient blood supply and hypoxia, and indicates the aggressiveness of the neoplasm, which is usually associated with a poor clinical outcome, including in NSCLC. The presence of necrosis can also be one of the characteristic signs of the tumor itself; for example, foci of necrosis are most often detected in SCC and large cell undifferentiated carcinoma. Even moderately

differentiated SCC is characterized by multiple comedo-necroses, whereas necroses are rarely detected in primary lung adenocarcinomas except in cases of large or poorly differentiated tumors. Necrosis often correlates with the T-stage of NSCLC and, according to multivariate analysis, is a significant risk factor for recurrence and poor prognosis, even in the early stages (Caruso R, et al., 2012; Park SY, et al., 2011; Seok Whan Moon, et al., 2022). Thus, extensive necrosis reflects the aggressive phenotype of NSCLC and improves the predictive power of the TNM staging system.

1.2. The role of biobanks in general and in cancer research

A biobank is a broad concept that is difficult to define unambiguously. A human biobank is a specialized legal repository that systematically collects, processes, stores, tests, analyzes and distributes various biological samples: blood and its derivatives, tissue, DNA and other biological materials. Relevant clinical, demographic and other data are stored with the samples, allowing not only the assessment of disease information, but also response to treatment and outcome (Annaratone L, et al., 2021; Yuanyuan Chen, et al., 2021; Mohanty A, 2024). Samples are collected and processed according to approved protocols and subsequently stored under strictly controlled conditions to ensure their integrity for future research and medical purposes. Biobanks play a key role in biomedical research as they provide a valuable resource in the form of well-annotated biospecimens for studying diseases, understanding their underlying mechanisms, and developing personalized medicine (Mendy M, et al., 2018).

The roots of biobanking can be traced back to early scientific attempts to understand human biology and the nature of disease. From ancient anatomical collections to modern tissue preservation techniques, humanity has for centuries practiced the systematic collection and storage of biological specimens, albeit in a rudimentary format. However, advances in genetics, molecular biology, and medical diagnostics in the mid-20th century emphasized the need for well-structured repositories of biological samples. Clinicians and scientists have realized that access to a variety of well-annotated samples will help them unlock the secrets of many diseases, including cancer. The implementation of this idea marked the emergence of the practice of biobanking (Mohanty A, 2024).

Since 1996, when Loft and Poulsen first coined the term “biobank” for the management of human biological materials, the biobanking field has experienced tremendous growth and played an important role in the development of medical research

(Parvizpour F, et al., 2025). Biobanking involves long-term storage and the possibility of using biological samples for future research projects.

In general, the decision to create and operate a biobank is influenced by many factors, but very often it all starts with the desire of medical doctors and scientists to create a resource useful for diagnostics, prognostics and research purposes. Collection, processing and storage of biological material must be carried out strictly in accordance with ethical, legal, clinical, scientific and technical requirements and guidelines. The quality of the stored material is one of the key aspects of successful biobanking.

Depending on the purpose and type of collected material, a biobank can be population-based or disease-oriented. There are case-control biobanks, tissue biobanks, cell-based biobanks (stem cell, cord blood), biobanking in the context of clinical trials, biomolecular resource centers, etc. (Artene SA, et al., 2013).

At the very beginning biobanks were created for the needs of a specific research project, now large biobanks are used for population projects and the study of various diseases; often disease-oriented biobanks are supported by academic institutions. According to a study conducted in 23 European countries, most biobanks were associated with academic institutions and only 3% were privately owned (collecting samples for commercial purposes) (Yuanyuan Chen, et al., 2021). Clinical biobanks are also developing rapidly. They are typically based in university hospitals, which have large resources of biological samples and patient health data.

A biobank provides valuable information about the methods of collection, processing, storage, and shipment of each sample. Other important aspects of the work of a biobank are the depersonalization / anonymization of samples, maintaining anonymity, the rights and interests of donors, state standards and principles of operation of a particular type of biobank; the priority is a focus on participation in long-term projects. Modern biobanks are complex structures that can be used not only as a collection of physical samples, but also as a source of epidemiological data in a specific area. Today, the biobank system has a huge number of archival samples linked to clinical and molecular data, which is very important for oncological research and diverse partnerships.

Due to the vast amount of information associated with samples, biobanks are an important reservoir for the development and validation of new diagnostic markers and therapeutic agents. In oncology, biobanks are a key resource for genomic, proteomic, metabolomic based research, molecular and epidemiological studies, biomarker studies, development of therapeutic targets and discovery of new drugs (Artene SA, et al., 2013; Izbicka E, et al., 2014).

Access to well-annotated banked samples allows researchers to draw correlations between genetic and molecular data and clinical outcomes, identify biomarkers, and study disease progression. It should be noted that each oncological patient / specimen is unique due to genetic variations, environmental factors and lifestyle / habits. The non-renewable nature of biological samples should be taken into account when considering scientific and research priorities. Biobanks play a key role in precision oncology by providing biological samples for molecular profiling and biomarker studies (Andry C, et al., 2017). By analyzing the genetic and molecular characteristics of tumors, mutations can be identified, and appropriate targeted therapy can be selected that is most likely to be effective in a particular patient. Targeted approach reduces the risk of treatment failure and potential side effects (Ballester PJ, et al., 2021).

The biomarker research process involves large scale analysis, collaboration of multidisciplinary teams and validation studies — these inconveniences can be eliminated with the help of biobanks. Biobanks often collaborate with research institutes and pharmaceutical companies, sharing data and samples for the research projects (Patil S, et al., 2018). Notably, underutilization of stored specimens and lack of sharing can negatively impact clinical and biological research.

In general, progress in medicine depends on innovation and the translation of laboratory findings into clinical practice. Access to human biospecimens and associated data is a prerequisite for such research and innovation. Moreover, large biobanking collections allow for large-scale research projects. Biobanks engaged in the collection, processing, storage and dissemination of biological samples can be represented as small biobanks working only with one specific project, with one research laboratory or university, or large academic or commercial biobanks, integrated into networks, working with big medical centers, several sizeable projects at a time, large laboratories and pharmaceutical companies.

To date, the exact number of biobanks in the world is unknown due to assessment methods and tracking limitations. Some studies evaluate that there are several thousand biobanks worldwide: according to other, more rigorous estimates, several hundred. Thus, according to the Biobank Resource Center locator, there are 413 registered or listed biobanks (Biobank Resource Centre: Biobanks\en [Internet]. biobanking.org. Available from: https://biobanking.org/locator). According to other sources, one major European directory alone listed 617 biobanks in 2023 and there are at least 650 biobanks in the United States alone. According to O'Donoghue S. et al., there are 11–30 medical research biobanks per 1 million population (2 large biobanks with >1000 samples and another 9–28

medium or small biobanks) (O'Donoghue S, et al., 2022). According to a study conducted in 23 European countries over the last decade, 77% of biobanks had <5000 samples, 25% of biobanks were defined as small biobanks (<1000 samples). Yuanyuan Chen et al. reported that although biobanks in China have most frequently collected and stored blood derivatives, including plasma and serum, over the past 10 years (like biobanks in the United States), FFPE accounted for only 17.14% of banked samples. However, the use of FFPE blocks in oncology and proteomics research has increased significantly recently, and biobanks are having to keep up with this trend (Yuanyuan Chen, et al., 2021). Continuous developments in the field of biobanking reflect the high demand for high-quality biological samples for scientific biomedical research.

According to Parvizpour F, et al, as of November 2022, there were 540 biobanks in 44 countries: 224 in North America, 259 in Europe, 35 in Asia, 13 in Australia, 7 in Africa and 2 in South America. The most common types of biobanks were tissue and cell-based biobanks (30.4%), tissue-based (27.93%) and cell-based biobanks (25.15%) (Parvizpour F, et al., 2025). The diagram is shown in fig. 1.2.1.

A tissue biobank is an organized repository of biomedical samples originally collected from biopsy / surgical material, processed and analyzed in a professional manner and stored under strictly controlled conditions. In addition to the samples themselves, relevant general, epidemiological and clinical data are available, which can later be used in the clinic or for research. In 2022, the largest number of tissue-based and cell-based biobanks was in the UK, Canada and the USA, gene-based biobanks — in the UK and the United States. Tissue and cell-based tissue-based biobanks predominated in the world, most of them were in Canada and the UK. While tissue and gene-based biobanks existed at the same time only in two countries — Spain and the UK. In the Middle East, tissue-based biobanks (Egypt, Saudi Arabia, Iran) and tissue and cell-based biobanks (Egypt and Saudi Arabia) also prevailed. Cell and gene-biobanks were available only in Israel (Parvizpour F, et al., 2025).

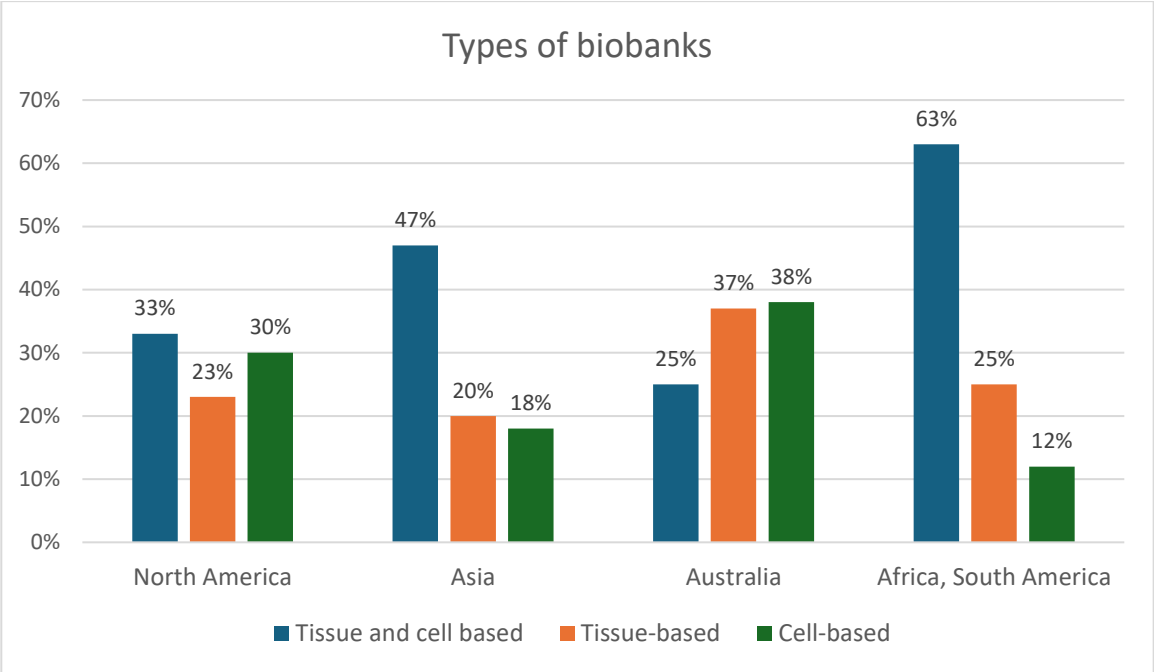


Fig. 1.2.1. Types of biobanks worldwide in 2022 (according to Parvizpour F, et al., 2025).

The development of personalized medicine has contributed to the fact that biobanking has become one of the top ten ideas of the century, thanks to which laboratory developments are introduced into clinical practice. Biobanks are a great help for basic research and progress in studying the effects of various drugs, and allow sharing knowledge, not only biomaterials and associated data among different countries.

Poor database organization and imperfect management can negatively impact a biobank's ability to collaborate with other biobanks and researchers. In addition, the infrastructure of different biobanks may differ significantly. To overcome these inconsistencies, BBMRI proposed a manageable network creation project. Many researchers see the future of biobanking in oncology in technical improvements, harmonization, data integration and international collaboration.

A biobank, as already mentioned, is a collection of human biological samples for biomedical research with associated phenotypic and epidemiological data. Often samples are collected specifically for a specific project, although frequently the collection also contains residual samples or leftovers (consented material taken during clinical care or diagnostic biopsy or surgery / tissue removal). Both material options are suitable for various studies (Giesbertz NAA, et al., 2012).

For decades, human biological samples have been widely used for research in oncology, to study the pathogenesis of diseases, test modern scientific hypotheses, and

evaluate biomarkers identified in experimental studies. The advent of new technologies has opened exciting opportunities to study the human genome, the complex relationships between multiple biomolecules, and the functional consequences and clinical manifestations of their disruption (Malhotra R, et al., 2023; Van Allen EM, et al., 2014). Thus, research using human biospecimens has become a critical and important resource for studying the causes of cancer, its progression, response or resistance to treatment, and predicting clinical outcomes.

Today, biobanks are a valuable source of samples for three rapidly developing areas of biomedical science:

- molecular and genetic epidemiology (with the aim of assessing the genetic basis and environmental influence on the occurrence of cancer both in the population and in individual families);
- molecular pathology (to create a molecular classification and methods for diagnosing oncological diseases);
- pharmacogenomics and pharmacoproteomics (to study correlations between a particular patient's genotype or phenotype and response to drug treatment).

According to rough estimates, about 400–500 million (or even 1 billion) archival FFPE blocks of cancer tissues with corresponding clinical and demographic data are stored in biobanks of the world, which represent a very valuable resource for elucidating new molecular mechanisms and biomarkers for solid tumors, as well as a potential tool for monitoring personalized treatment (Gassmann M, et al., 2012; Sah S, et al., 2013; Velasquez E, et al., 2021).

Generally, FFPE block storage is preferred for clinical specimens due to cost-effectiveness, ease of use, and superior preservation of morphological structures. Biobanks around the world house vast numbers of FFPE samples used in both large-scale longitudinal and genomic studies. There is a tendency to increase collections of FFPE blocks in biobanks with a view to their further use in precision medicine. Unfortunately, many biobanks fail to develop targets for their collections, which contain many samples that do not meet specific study criteria. However, samples that imperfect for certain research projects may be appropriate for others (Deshpande R, et al., 2022).

1.3. FFPE blocks are a valuable material for modern biomedical research in the field of oncology

Formalin fixation is one of the main methods in clinical diagnostics and basic biomedical research for decades (or more precisely since the 1890s). First, the tissue is excised from the donor. Then, no later than 30 minutes, the sample should be placed in a 10% neutral formalin solution to preserve the structure of tissues and proteins. The fixation process takes 18–24 hours. The fixation time is extremely important, as if it is not observed, the blocks become useless for diagnostic or molecular studies. The tissue is then dehydrated and infused with increasing concentrations of ethanol. After embedding, paraffin blocks are made, which are used in pathological anatomy in everyday workflow for diagnostic and research purposes. Directly in research, slides from paraffin blocks are used, which are stained with hematoxylin and eosin or (immuo-)histochemical dyes to enhance visualization of cells and structures of interest. Archival FFPE blocks are stored in large quantities in repositories for long-term diagnostic purposes and for biomedical research, including genetic and epigenetic analysis, which require the use of modern nucleic acid isolation methods (Oba U, et al., 2022; Vallicampa GE, et al., 2021). It should be noted that low-quality samples may cause anomalies in research data leading to misleading deductions.

Today, FFPE blocks are widely used for routine diagnostics and more in-depth studies of various diseases. One of the highest priority areas of use of FFPE blocks is oncological pathology.

Standard use of FFPE blocks:

1. Diagnostic and predictive studies: a) histological diagnostics of various conditions and diseases, including oncological pathology; b) evaluation of the primary microscopic structure of tissues and cells and detection of structural abnormalities, increased mitotic activity, etc.; c) assessment of tumor and necrosis content in cancer samples, which plays an important role in selecting samples for further scientific research; d) today FFPE samples play a crucial role in cancer staging and characterization, as well as in cancer diagnosis and treatment decisions.

2. Immunohistochemistry (IHC): a) IHC, which is performed on FFPE blocks, is often used in cancer diagnostics for the detection of various specific proteins in tissue, providing crucial diagnostic and differentiated diagnostic data for further specific requests; b) IHC allows to evaluate the expression of various diagnostic and predictive biomarkers, assess

the response to potential treatment, and study the role of specific proteins in different tissues.

3. Genomic and trancriptomic studies: a) FFPE samples are valuable material for genomic and trancriptomic studies; b) FFPE blocks are precious material for study of genetic changes, gene expression alterations, and other genetic disorders in tissues.

4. DNA and RNA suitable for research can be isolated from FFPE blocks, even after long term storage.

5. Different diseases: a) comparative research: to compare diseased and healthy tissues using FFPE blocks; b) immunology: analysis of FFPE samples from donors with an autoimmune disease can help to determine the cause of the pathological state and develop a therapy for such conditions. Also, there is analysis how effectively the immune system resists tumor development, as well as the interaction of immune cells and tumor cells; c) in infectious diseases, both the pathogens themselves and characteristic morphological changes can be detected in FFPE tissue; d) bone marrow studies in hematological disorders; also FFPE blocks can be used in genetics, toxicology, tissue regeneration and other fields; e) FFPE biopsy blocks are used for endocrine pathology diagnostics and treatment effectiveness monitoring; f) FFPE blocks are used for pathohistological diagnostics each time when the patient underwent a biopsy or surgery. But cancer research is one of the most important areas of FFPE material application [Prompt: FFPE use in modern research, Gemini 2.5 Pro, Google, Inc., 09-Sep-2025, https://gemini.google.com/].

Standard tissue histopathology examination begins with the preparation of slides from FFPE blocks and their staining with hematoxylin and eosin to study the morphology, tissue structure, immune cell infiltration, inflammation, identification of potentially altered cells, etc. (or other staining methods are used, depending on the objectives of the study).

Since 1991, with the publication of Shi et al., IHC staining, based on the detection of certain proteins, has gradually but firmly entered routine pathohistological practice and is used for clarifying and differential diagnostics (Criswell SL, et al., 2022; Kokkat TJ, et al., 2013). In IHC, tissue sections are placed in a solution containing antibodies that bind to specific proteins or structures. A dye is then used to visualize the antibodies, allowing the identification of specific proteins and their localization (Lexogen. Applications of FFPE samples in modern research | Lexogen [Internet]. Lexogen. 2024 [cited 2025 Oct 13]. Available from: https://www.lexogen.com/applications-of-ffpe-samples-in-modern-research/). IHC can identify cancer biomarkers — specific proteins that provide information about the tumor type, its aggressiveness, and potential therapeutic targets. Traditionally, different slides are used for histological diagnostics and IHC, which consumes tissue (which

is especially important for small biopsies), somewhat limits their capabilities and makes it impossible to directly compare individual cells; however, these days this problem can be overcome by modern technologies that allow simultaneous H&E and IHC staining on the same slide using specialized chromogens that do not interfere with each other.

Traditionally, FFPE blocks have been widely used for histological diagnostics and IHC analysis, but recently they have also become a valuable material for proteomic studies, which have great potential for understanding the mechanisms of many disorders, since most diseases manifest through changes in protein expression, and most treatments target proteins (Kokkat TJ, et al., 2013; O'Rourke MB, et al., 2016; Tüshaus J, et al., 2025).

FFPE blocks are widely used in a variety of research areas, including oncology, immunology, and drug development to study the molecular mechanisms underlying the origin of various diseases, drug resistance, and responses to treatment. One of the most extensive areas of FFPE blocks application is the study of oncological pathology to determine the cellular and molecular components and tumor microenvironment, as well as the causes of normal cells transformation into tumor cells (Gaffney EF, et al., 2018; Pabla S, et al., 2021). FFPE blocks are indispensable in retrospective studies to analyze effects of long-term storage on tissue (morphology and molecular changes over time).

Formalin is used to stop cellular processes and preserve tissue and cell structures. Proteins, RNA, and DNA are preserved in the tissue through formalin fixation during the production of FFPE blocks and can later be isolated for research. For example, in cancer research, analysis of nucleic acids extracted from FFPE samples sheds light on why a particular treatment is effective in some patients and not in others, why individual resistance to treatment develops, and what mutations and transcriptional changes are associated with these phenomena.

Although fresh-frozen tissue is considered better for nucleic acid isolation, these samples are not as common, and their storage and transportation require high costs. In this situation, FFPE samples are quite acceptable for modern research, if DNA is carefully extracted and bioinformatic analysis is used (Gao XH, et al., 2020; Okojie J, et al., 2024; Zhang P, et al., 2017). If high-quality FFPE blocks are used, sequencing results will be comparable to those using fresh-frozen samples. Several studies have shown that archival FFPE blocks can be used for molecular testing using polymerase chain reaction and next-generation sequencing (NGS), despite nucleic acid degradation over time (Gastman B, et al, 2020; Hedegaard J, et al., 2014; Sah S, et al., 2013; van Deventer BS, et al., 2022).

As mentioned, FFPE blocks preserve cellular proteins almost perfectly, which is important for IHC, but the situation with nucleic acids is somewhat different: formalin can

cause their fragmentation, destruction, or cross-linking with proteins (Zhang P, et al., 2017). Even though formalin fixation, paraffin embedding, and subsequent long-term storage lead to DNA damage, fragmentation, and even the appearance of artifacts resembling mutations, FFPE tissue is the most used method in practice for processing clinical biospecimens processing. DNA damage in FFPE tissues results in increased artifacts during sequencing, requiring greater effort to distinguish true mutations from "noise", which is achieved using specialized DNA repair kits and subsequent bioinformatics to mitigate these issues (Okojie J, et al., 2024; Watanabe M, et al., 2017). The negative impact of formalin fixation is most pronounced on the integrity of RNA, causing its chemical modification and degradation (Chung JY, et al., 2006). However, thanks to advances in molecular biology, attempts to overcome cross-linking in FFPE tissues have been successful, and it is now possible to extract DNA, RNA, and proteins, albeit in fragmented form, which can be used in the analysis of short fragments of macromolecules. Long-term storage of FFPE samples negatively affects both the qualitative and quantitative content of nucleic acids and proteins, which is most noticeable in the surface layers of the paraffin block (Grillo F, et al., 2017; Chen H, et al., 2020). But according to Kokkat TJ, et al. (2013), there is no significant difference between macromolecules extracted from FFPE blocks stored over 11–12 years, 5–7 years and 1–2 years in the comparison to the current year material. The miRNA and DNA extracted from FFPE samples have currently reached levels which allow their application as a first-line approach in the search for biomarkers (Kokkat TJ, et al., 2013). In the era of personalized medicine, the analysis of large cohorts of samples is necessary to study various biomarkers used for prognosis and targeted therapy, so archival FFPE biobank samples, even stored for many years, are an easily accessible and valuable resource to meet such needs.

FFPE tissue samples, especially native tumor tissue (collected before radio-, chemo- or immune therapy), are a valuable resource for retrospective and prospective biomedical research, offering significant potential for modern molecular diagnostics using analytical omics tools. Typically, FFPE specimens are routinely prepared for pathological)examination and archived with detailed clinical and demographic data, allowing for the analysis of large, unique datasets during the study.

Advantages of FFPE blocks: they are relatively easy and quick to produce, the technique is not very expensive, the samples themselves are stable and can be stored for a long time and shipped in ambient conditions. As already mentioned, FFPE specimens are more durable than fresh frozen samples. Moreover, a single FFPE block can be used multiple times for various studies. A single 10 μm tissue section provides sufficient RNA for RNA-sequencing with gene-sets which is more effective than single-gene analysis in

distinguishing cancer subtypes. In clinical settings cut slides are often used instead of original blocks for molecular studies, including biomarker analysis. And although under the influence of formalin degradation and chemical modification of macromolecules occurs, advanced NGS enables the analysis of genomes, epigenomes and transcriptomes using limited and fragmented nucleic acids from FFPE samples at a relatively reasonable cost. Genomic samples obtained by FFPE samples are of great value in oncology because they allow the identification of mutations, gene copy variations, and epigenetic changes that are important for understanding the genetic abnormalities underlying cancer. Undoubtedly, reliable NGS methods for FFPE specimens would unlock pathology archives for high-throughput profiling, facilitating large-scale retrospective and prospective clinical studies (Lewis F, et al., 2001).

Making a histological diagnosis and assessing morphological changes is of great importance. Assessment in morphological diagnostics the content of tumor tissue and the percentage of necrosis in the histological specimen also plays an important role, which determines the value of the FFPE block itself and its suitability for subsequent examination.

A quality FFPE sample must contain sufficient material for analysis. Tumor cells content is critical for biomarkers testing (Capello F., at al., 2022; Dufraing K., et al., 2018). For FFPE blocks used in different research, including molecular testing, the tumor content must be at least 30% for reliable results, however, specific requirements may vary depending on the type of study. For example, according to various sources, to detect specific proteins in FFPE tissue using IHC, the preferred tumor content ranges between 20% and 60% (Chen Xu, et al., 2017; Gastman B, et al., 2020; Masashi Mikubo, et al., 2020; Smolarz B, et al., 2025; Javey M, et al., 2021). Some IHC assays are more sensitive and can tolerate lower tumor percentages, while others require higher cellularity for accurate interpretation and to minimize the impact of non-tumor cells on the results. It is believed that the more tissue in the sample, the higher the percentage of tumor cells and the more accurate the results will be obtained (Sone M, at el., 2022; Steiert TA, 2023).

The threshold necrosis percentage can also vary significantly depending on the clinical and research purpose of FFPE blocks' use and the methods of analysis. IHC staining can be affected by necrotic tissue which causes false-positive or false-negative results interpretation. A false-positive signal occurs because antibodies bind to nonspecific components in necrotic tissue. A false-negative signal is due to decreased antigen presentation, since some antigens are destroyed during necrosis, while others remain intact, are masked, and become difficult for antibodies to access. Misinterpretation of IHC results is also possible due to cross-reaction of proteins from destroyed cells in necrotic

tissue that are not target antigens. False-positive, false-negative and misinterpreted IHC results can lead to inaccurate tumor characteristics and prognosis assessment, incorrect staging and prognosis. It is desirable that FFPE samples used for IHC contain up to 30% necrosis. In case of extensive necrosis, alternative approaches should be used to optimize diagnostic accuracy. For some research methods, a high percentage of necrosis is allowed, while for others there are significant limitations. High percentage of necrosis can cause RNA degradation and decrease of RNA Integrity Number (Masuda N, et al., 1999). Excess necrotic tissue can trigger DNA fragmentation, so the necrosis percentage for NGS, where DNA integrity is critical, and should not exceed 50% (Conroy JM, et al., 2018).

As mentioned, minimal tumor content required for research is 20% (Javey M, et al., 2021), but in fact in most cases FFPE blocks with tumor content 30% and higher are requested. Other limitations are related to the percentage of necrosis in the sample (Hernandes S, 2022). Usually, samples with maximally 30% necrosis are required for research projects. But III–IV stages of lung cancer (aggressive, fast-growing tumors) are often associated with vast necrosis and / or hemorrhages (Li L, eta l., 2021; Moosn SW, 2022). Even at stage II and with moderately differentiated SCC, multiple comedo-necroses are characteristic. For this reason, considering tumors' nature and characteristics, in some cases even 40% of necrosis is acceptable. It is necessary to realize what tolerance to tumor cell count and necrosis meets clients' needs and what samples are appropriate to collect for biobanking and to be further proposed for research projects. This will also help estimate the number of specimens that need to be collected to be enough for the project, considering rejections after morphological examination.

1.4. Summarizing

In terms of morbidity and mortality, oncological pathology occupies a leading position worldwide, despite the progress of modern technology, medicine, and early diagnostics. According to statistics, in 2020, lung cancer ranked third in the world in terms of incidence among all cancer cases (22.4 per 100.000 population), but mortality ranked first (18.0 per 100.000 population), significantly exceeding the rates of malignant neoplasms of other localizations. Nearly 85% of lung cancer cases are NSCLC, typically classified as adenocarcinoma or SCC. Most NSCLC cases are diagnosed at late stages (stages III–IV), resulting in a short life expectancy and poor prognosis.

NSCLC specimens, including archival ones, are in high demand in the modern world, especially native tumor samples (materials collected before chemotherapy, radiation

therapy, or any other treatment). In Ukraine, it was possible to collect native lung cancer samples (resections) in large quantities until 2023, before new treatment protocols were widely introduced into clinical practice. It's important to keep in mind that many samples stored in hospital archives or biobanks may be collected for different projects. However, if the same approved methods and protocols were used for collection, processing, and storage of the material, and the samples are consented, this makes them universally applicable to virtually any project.

Thanks to high quality standards, organized sample collection, and accompanying data sets, tissue biobanks are today one of the most valuable sources of material for comprehensive research with reliable and accurate results. However, despite the constant development of biobanks, many aspects of their work require improvement. For example, even minor flaws in database organization and management, insufficient public communication, and sample distribution can negatively impact a biobank's ability to provide researchers even with rare and high-quality specimens. Adherence to strict standards for processing and storage of the material is also important. The inconveniences arising in this area may complicate collaboration between biobanks and researchers from different countries. According to many experts, the future of biobanking lies in technical improvement, harmonization, data integration, and the development of close international collaboration.

One of the main methods for long-term storage of solid tumor tissue is FFPE blocks. Worldwide, clinical archives and biobanks contain at least 500 million (according to other sources, at least 1 billion) well-annotated samples ready for modern research, including in the field of oncological pathology. It is a valuable resource for histological, IHC, molecular, and other studies because scientists have access to extensive archival collections of blocks with preserved tissue morphology; these samples are cost-effective and are easy to store and transport in ambient conditions. FFPE samples are a vital resource for many retrospective studies. FFPE block archives can be used to study disease progression, identify and evaluate biomarkers, and assess the correlation of molecular analysis results with clinical data and patient survival. FFPE blocks, even decades-old, are increasingly used for genomic, proteomic, and biomarker studies, providing critical insights into cancer mechanisms, progression, and therapeutic targets. FFPE blocks also play an important role in personalized medicine by targeting the analysis of genetic mutations, biomarkers, and tumor profiles from archived tissue samples, which allow clinicians to make more precise therapeutic decisions based on individual characteristics, improving specific patient outcomes.

However, before offering blocks for research projects, their quality and compliance must be re-evaluated. One key requirement is adherence to standardized procedures for collecting, recording, processing, and storing the material. But no less important is the percentage of tumor and necrosis in each FFPE block (according to pathohistological examination data). According to various literary sources, blocks with a minimum tumor tissue content of 20–60% are suitable for IHC, genomic and other studies; necrosis, depending on the type of study, should not exceed 30–50%, since extensive necrosis is a significant obstacle to conducting various studies and can lead to false-positive or false-negative results, making it difficult to correctly evaluate the material. Based on the requests our biobank has received and continues to receive, the preferred / acceptable threshold for most clients is a minimum of 30% tumor and a maximum of 30% necrosis.

In our further work, we focused specifically on these indicators. We analyzed lung cancer specimens collected for the Audubon Bioscience Biobank between 2020 and 2022 to determine what portion of this collection could be further offered for research and to identify possible influences on tumor and necrosis content in the specimens, to use this knowledge in the future when collecting new samples.

Core theory of the Thesis: Some collected FFPE samples of NSCLC don’t meet the needed morphological criteria. We’ll estimate what proportion of specimens meet the requirements (minimum tumor content ≥30%, maximum necrosis ≤30%) and can be proposed for further research. In the future this may help in planning the collection rates of NSCLC samples for research.

2. Materials and methods

The study has been coordinated with the company management and Legal Department of Audubon Bioscience Company. The researcher has access to the biobank database within the scope of her professional competence and job responsibilities (pathologist, biobank manager).

All cases are consented, material have been collected for different projects before chemotherapy, radio-, or immunotherapy prescription in patients over 18 years old with lung cancer, treated in different hospitals of Ukraine, excluding temporarily occupied territories in 2020–2022 years (before the war in Ukraine began). The cold ischemia time did not exceed 30 minutes.

Standard histological techniques were used to prepare FFPE blocks. Tissue samples were placed in 10% formalin solution (from 18 to 24 hours). Dehydration was performed in a series of alcohols of increasing concentration followed by fabrication of FFPE blocks. Sections 5 μm thick were prepared from each block, deparaffinized, and stained with hematoxylin and eosin. At clinical sites, histological preparations were examined under a microscope in the histopathological laboratory to establish a histological diagnosis and assess tumor content and necrosis.

Clinical sites provided our company with a histopathological report for each case, indicating the histological diagnosis, tumor size, stage, degree of tumor differentiation (grade), percentage of tumor and necrosis, and the presence of secondary lesions. Each pathology report was assigned to the same ID as the donor. The diagnosis of NSCLC was established based on morphological examination; if necessary, IHC was used for differential diagnosis. Data from case repost form, material processing log (tissue), and pathology report were entered into the Audubon Bioscience Biobank specimen database; FFPE blocks transferred from clinical sites were sent for storage.

The study analyzed information from a database of 243 unique treatment-naive donors (negative for Hep A, Hep B, C and HIV) with a clinical diagnosis of lung cancer (592 FFPE tumor blocks) stored in the Audubon Bioscience biobank, including data from corresponding archival pathology reports. The blocks were collected between 2020 and 2022 (before the start of the war) in various hospitals in Ukraine (except Crimea and temporarily occupied territories) with which the company cooperated. All patients were of Caucasian race (Ukrainian), all cases provided informed consent; data are de-identified. Access to archived data is strictly regulated within the competence and position of the company's employees. Pathological anatomical reports (paper version of documents) are

organized, sorted and stored in cardboard boxes in a separate archive room with limited access. Scanned documents are anonymized, organized, and stored on the company's Google Drive, access to which is strictly restricted. The study did not make additional histological preparations or examine them under a microscope, nor did it calculate percentages of tumor and necrosis; instead, existing sample data were used and analyzed.

All FFPE samples were resectioned. We have 1–6 samples from the same donor in the biobank, however, some of the specimens can meet the needed morphological criteria, while others do not.

The program IBM SPSS “Statistics 28” (license No. Z125-3 301-14) was used to process the research results.

During the study, nonparametric statistical methods were used.

The median test was used in the data analysis because the data were nonparametric and, in addition, medians, unlike the arithmetic mean, are less sensitive to outliers. We also used the Pearson χ^2 test was used to identify the relationship between two categorical variables, and the Kruskal-Wallis test to check the equality of medians in several samples (3 or more). Spearman rank order correlation and Kendall Tau correlation were used to assess the strength and direction of the relationship between two variables (for example, the presence of a relationship between tumor % / necrosis % and the size of NSCLC of different histological types and at different stages).

This study aimed the dependence of the tumor and necrosis content in the specimens on various factors (stage, histological type, etc.) to use this data in the future when collecting new samples, and to reduce the number of rejections due to insufficient content of tumor and / or necrosis in FFPE blocks.

3. Results

In total, the biobank collection for 2020–2022 contained 592 FFPE blocks characterized as NSCLC from 243 donors. According to the preliminary analysis, discrepancies between clinical and pathological diagnoses were found in 9 (3.70%) cases (32 (5.41%) FFPE blocks). Morphological examination revealed inflammatory lesions in 3 (1.23%) patients with a clinical diagnosis of lung cancer: pneumonia, chronic nonspecific granulomatous inflammation, and abscess. In 6 (2.47%) cases (14 (2.36%) blocks), the detected lung tumors did not belong to lung cancer itself: hamartoma (1), pneumocytoma (1), B-cell lymphoma (1), malignant epithelioid tumor (1), inflammatory myofibroblast tumor (1), and neurofibroma (1). Another 15 (6.17%) patients (44 (7.43%) blocks) were diagnosed with neuroendocrine lung neoplasms of varying degrees of malignancy. In 2 (0.82%) cases (8 (1.35%) blocks), the diagnosis of primary lung cancer was not confirmed. Instead, metastatic lung involvement from colon and ovarian cancer was observed, which was established using IHC. Thus, after comparing clinical and pathological diagnosis, 208 (85.6%) cases (respectively 494 (83.45%) blocks) were selected for further study (Fig. 3.1.1).

According to the preliminary calculations made during the selection and justification of the topic of the Master Thesis, the minimum sample size required to evaluate the results with an error of 5% is 323 elements (FFPE blocks). Therefore, the sample satisfies these criteria.

The study included the following data: donor age and gender, clinical diagnosis, pathological diagnosis, TNM and stage (evaluated according to AJCC 8th edition) (AJCC Cancer Staging Manual. 2018), grade of malignancy, percentage of tumor and necrosis, and information on smoking. However, not in all 100% of cases were these data available in the database and primary documentation (as the samples were collected at different times, for different projects, using different CRF templates). In 8 (3.85%) observations, tumor's grade was not specified, in 11 (5.29%) tumor size was not indicated, and in 36 (17.31%) there was no information on smoking.

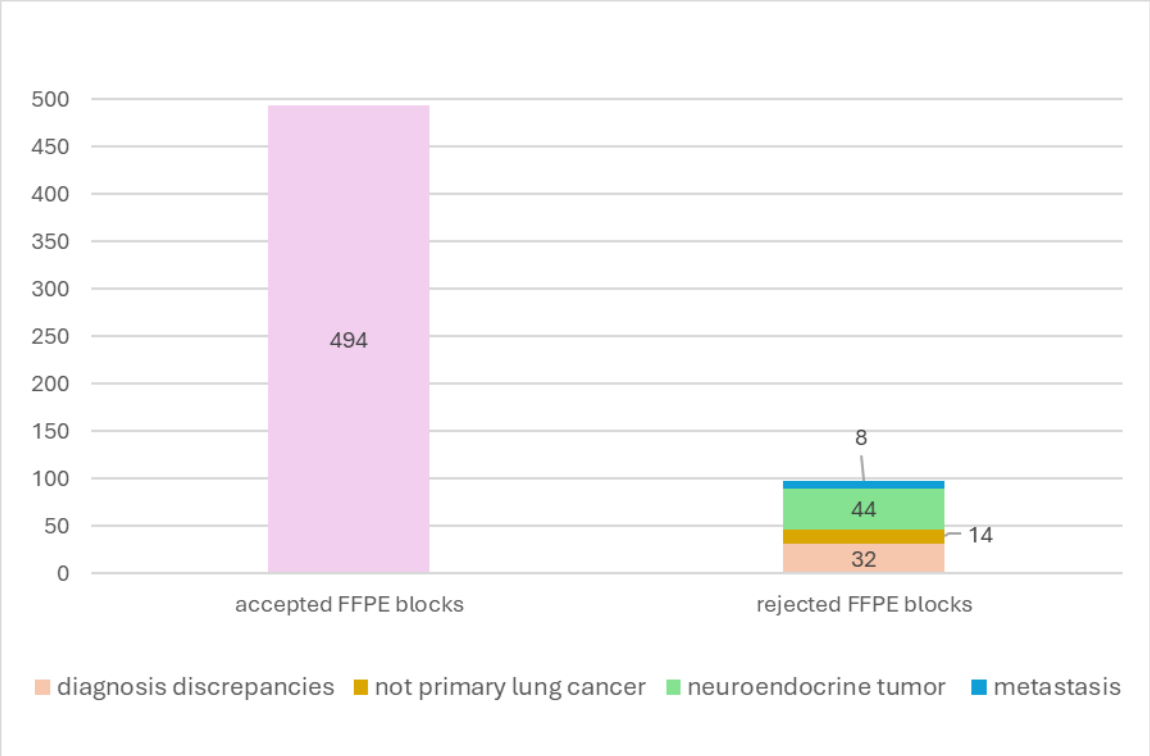


Fig. 3.1.1. Rejected and accepted samples from the general sample depending on the diagnosis compliance.

Among the patients included in the study, men predominated by 4.78 times with 172 (82.69%) men and 36 (17.31%) women. The age of donors ranged from 35 to 83 years, with an average of (61.06±7.43) years. All observations were divided by age according to the WHO classification: young age (25–44 years), middle age (45–60 years), elderly patients (61–75 years), and senile age (76–90 years). The largest group in our study was the elderly patient group: 113 (53.1%) individuals (91 men and 22 women), respectively, the number of FFPE blocks was the largest — 256 (51.82%). The least numerous was the group of the young age: just 1 (0.20%) male patient aged 35 years (2 (0.40%) FFPE blocks). There were 90 (43.63%) middle aged donors (77 male and 13 female patients), the number of blocks in this group was 229 (46.36%). In the senile aged group, there were 4 (1.92%) donors (3 male and 1 female) and, respectively, 7 (1.42%) FFPE blocks.

The size of the detected tumors ranged from 1.2 to 15 cm, with an average of (5.06±2.41) cm. For the convenience of further analysis, all cases were divided into stages I, II, III, and IV, without additional detail.

Tumor content ranged from 3 to 100%, on average (70.63±22.37%). Necrosis in the studied samples ranged from 0 to 100%, on average (17.22±17.76%). For further use in

scientific research, blocks with tumor content ≥30% and necrosis ≤30% were considered suitable, since these parameters are most often the inclusion criteria in the requests we receive; in addition, they are necessary conditions for IHC, comprehensive genetic testing, for accurate molecular analysis, and interpretation.

Acceptable tumor percentage was noted in 479 (96.96%) samples, necrosis — in 417 (84.41%) blocks. Thus, 403 (81.58%) FFPE samples met the specified criteria, and 91 (18.42%) were “rejected”.

3.1. Histological subtypes characteristics

NSCLC is represented by adenocarcinoma, SCC, and other, less common histological variants (adenosquamous carcinoma, large cell carcinoma, carcinosarcoma, etc.), which were combined into one group for the convenience of further analysis. NSCLC samples for further analysis were divided into groups according to histological subtype: group 1 — adenocarcinoma, group 2 — SCC, group 3 — other types.

Among NSCLC samples, SCC predominated — 108 (50.92%) cases, adenocarcinoma was diagnosed in 75 (36.06%), other types of NSCLC — in 25 (12.02%). At the same time, SCC was detected in 101 (59.41%) men and 7 (19.44%) women, adenocarcinoma in 48 (27.91%) men and 27 (75.0%) women, other types of NSCLC — in 23 (13.37%) men and 2 (5.56%) women. The study included 268 (54.25%) SCC blocks, 170 (34.41%) adenocarcinomas and 56 (11.34%) other histological types.

The age of patients with adenocarcinoma (group 1 by histological type) ranged from 35 to 76 years, with an average of (60.65±7.48) years. The size of the detected neoplasm ranged from 1.2 to 12 cm, with an average of (4.11±1.92) cm. Tumor content ranged from 5 to 100%, on average (70.56±24.24%), necrosis — from 0 to 70%, on average (13.19±10.24%). Tumor content was too low (<30%) in 5 (2.40%) observations (7 (1.42%) blocks), necrosis content was too high in 12 (5.77%) cases (21 (4.25%) blocks). A total of 28 (5.66%) adenocarcinoma blocks were deemed unsuitable for further use (representing 16.47% of all adenocarcinoma specimens).

The age of donors with SCC (group 2 by histological type) ranged from 45 to 83 years, with an average of (61.57±7.34) years. The tumor size ranged from 1.7 to 1 cm, on average (5.60±2.53) cm. The tumor content in the preparations ranged from 3 to 100%, on average (71.89±21.43%), necrosis — from 0 to 100%, on average (18.58±17.97%). In 8 (1.62%) samples, the tumor content was less than 30%, in 45 (9.11%) cases the necrosis

content exceeded 30%; in total, 44 (8.91%) blocks were considered unsuitable for further use (i.e. 16.42% of all ICC samples).

In group 3 (other, less common types of NSCLC), the age of patients ranged from 45 to 73 years, with an average of (59.86±7.61) years. Tumor size ranged from 3 to 10.8 cm at the time of diagnosis, with an average of (5.34±2.35) cm. The tumor content in the samples was estimated from 30 to 100%, on average (82.23±18.51)%, necrosis — from 0 to 100%, on average (22.89±19.94)%. Tumor content in all samples was satisfactory (≥30%), 11 (2.23%) blocks were rejected due to high percentage of necrosis (19.64% of all samples of other types of NSCLC).

3.2. Grading subtype characteristics

Most NSCLC specimens in our study were grade 2 or grade 3 — 443 (86.68%) blocks, respectively. NSCLC grade 1 (well-differentiated) was detected in 35 (7.09%) samples, grade 2 (moderately differentiated) — in 290 (58.7%), grade 3 (poorly differentiated) — in 153 (30.97%) blocks; in 16 (3.24%) specimens the grade was not determined. Adenocarcinoma grade 1 was detected in 13 (2.63%) samples, grade 2 — in 78 (15.79%), grade 3 — in 76 (15.38%), grade was not specified in 3 (0.61%) blocks. SCC grade 1 was detected in 12 (2.43%) blocks, grade 2 — in 209 (42.31%), grade 3 — in 45 (9.11%), grade was not indicated in 2 (0.40%) specimens. Grade 1 of other types of NSCLC was detected in 10 (2.02%) FFPE blocks, grade 2 — in 3 (0.61%), grade 3 — in 32 (6.48%), grade was not mentioned for 11 (2.23%) samples.

NSCLC stage I was diagnosed in 53 (25.48%) cases (118 (23.88%) samples); stage II — in 73 (34.09%) observations (171 (34.62%) blocks); stage III — in 70 (33.65%) patients (172 (34.82%) blocks); stage IV — in 12 (5.77%) cases (33 (6.68%) blocks). In 143 (68.75%) cases, NSCLC was diagnosed at stages II and III of the disease. In late stages of the disease (III and IV), NCSLC was first detected in 182 (39.42%) cases. In our study, the number of women in stages I and II was approximately the same (14 and 13 people, respectively), while the number of men differed slightly in stages II and III of the disease (60 and 66 respectively). In terms of the number of observations, stages I and IV were the least numerous.

NSCLC was diagnosed in 53 (25.48%) donors (39 men and 14 women), which amounted to 118 (23.89%) blocks. The age of the patients ranged from 45 to 73 years, with an average of (59.86±7.61) years. The size of the neoplasm ranged from 1.2 to 4 cm, with an average of (3.05±0.74) cm. Adenocarcinoma was detected in 65 (13.16%) blocks, SCC

in 35 (7.09%), other histological types of NSCLC in 18 (3.64%) samples. The distribution of lung cancer blocks at stage I was as follows: grade 1 (well-differentiated tumors) — 17 (3.44%) samples, grade 2 (moderately differentiated tumors) — 64 (12.96%) and grade 3 (poorly differentiated tumors) — 37 (7.49%). The tumor content in the samples ranged from 5 to 100%, on average (66.57±23.58%), and necrosis ranged from 0 to 80% (12.81±16.63%). 5 (4.24%) blocks were rejected due to low tumor content, 12 (10.17%) due to high necrosis content (in total, 17 (14.41) blocks at stage I were found to be unsuitable).

In 73 (35.10%) cases (60 men and 13 women), which amounted to 171 (34.62%) blocks, NSCLC was diagnosed at stage II. The age ranged from 45 to 78 years, with an average of (61.01±8.084) years. The tumor size ranged from 2 to 7 cm, with an average of (4.66±1.23) cm. Adenocarcinoma was detected in 51 (10.32%) blocks, SCC in 105 (21.26%), other histological types of NSCLC in 15 (3.04%) samples. The distribution of NSCLC blocks at stage II was as follows: grade 1 — 13 (2.63%) samples, grade 2 — 113 (22.87%) and grade 3 — 45 (9.11%). The tumor content in the blocks ranged from 3 to 100%, on average (70.88±22.17%), necrosis — from 0 to 70% (16.29±16.20%). 4 (2.34%) blocks were not eligible due to low tumor content, and 27 (15.79%) blocks — due to high necrosis. In general, considering the above criteria, 30 (17.54%) blocks from donors at stage II of the disease cannot be further offered for scientific research.

Stage III NSCLC was first detected in 70 (14.17%) donors (66 men and 4 women), which amounted to 172 (34.41%) blocks. The age of patients in whom the disease was detected at stage III ranged from 35 to 75 years, with an average of (61.15±6.6) years. The tumor size ranged from 2 to 15 cm in the largest dimension, with an average of (6.92±2.80) cm. Adenocarcinoma was detected in 40 (8.10%) blocks, SCC in 114 (23.08%), other histological types of NSCLC in 18 (3.64%) samples. The distribution of blocks of stage III NSCLC was as follows: grade 1 — 5 (1.01%) samples, grade 2 — 100 (20.24%) and grade 3 — 59 (11.94%), for 8 (1.62%) blocks the grade was not specified. The area occupied by the tumor in the samples ranged from 5 to 100%, on average (76.63±21.44%), the area of necrosis ranged from 0 to 100%, on average (20.22±19.11%). Tumor content was <30% in 6 (3.49%) specimens, necrosis was greater than 30% in 32 (11.63%) samples. A total of 38 (22.09%) blocks were considered ineligible for further use in stage III NSCLC.

Stage IV NSCLC was first detected in 12 (14.17%) donors (7 men and 5 women), which amounted to 33 (6.68%) blocks. The age of patients in whom NSCLC was first detected at stage IV, with distant metastases in the liver, bones, brain, etc., ranged from 45 to 70 years, with an average of (61.27±6.96) years. The size of the primary tumor ranged from 2 to 6.7 cm, with an average of (4.63±1.73) cm. Adenocarcinoma was detected in 14

(2.83%) blocks, SCC in 14 (2.83%), and other histological types of NSCLC in 5 (1.01%) samples. The distribution of stage IV lung cancer blocks was as follows: grade 1 — no samples, grade 2 — 13 (2.63%) and grade 3 — 12 (2.43%); for 8 (1.62%) blocks, the grade was not specified. The tumor area in the preparations ranged from 35 to 100%, on average (82.12±16.77%), necrosis — from 0 to 80%, on average (22.12±18.67%). The tumor percentage in all cases satisfied the conditions of the study. Due to an unacceptable rate of necrosis, 6 (18.18%) samples at stage IV were rejected.

Thus, 14.41% of blocks at stage I NSCLC, 17.54% at stage II, 22.09% at stage III, and 18.18% at stage IV were unsuitable for further use due to low tumor content or high percentage of necrosis (Fig. 3.2.1, 3.2.2).

General characteristics of the research material are presented in table 3.2.1.

Table 3.2.1. General characteristics of the research material

Stage	Cases		FFPE blocks							
	Male	Female	Total number	NSCLC histological type			Grade			
				adeno-carcinoma	SCC	other types	G1	G2	G3	N/A
I	39	14	118	65	35	18	17	64	37	—
II	60	13	171	51	105	15	13	113	45	—
III	66	4	172	40	114	18	5	100	59	8
IV	7	5	33	14	14	5	—	13	12	8
Total	172	36	494	170	268	56	35	290	153	16

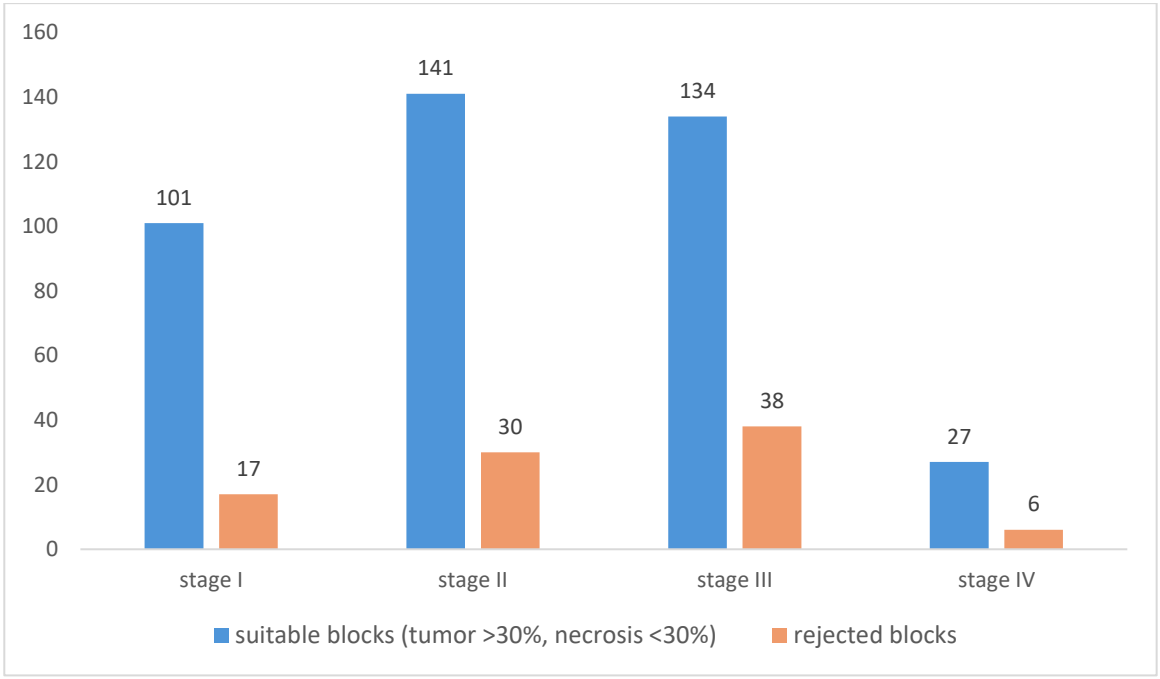


Fig. 3.2.1. FFPE blocks at different stages of NSCLC, suitable and unsuitable for further use.

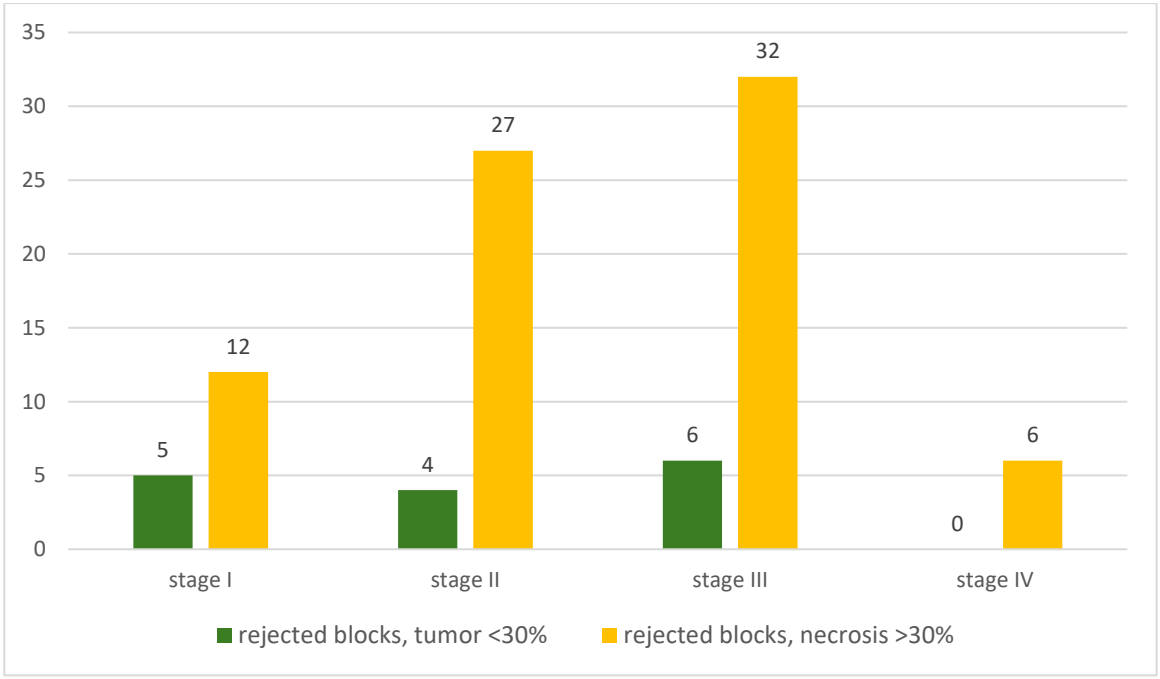


Fig. 3.2.2. Causes of FFPE blocks rejection at different stages of NSCLC.

3.3. Statistical results

As mentioned, we wanted to test the dependence of the tumor and necrosis content in the specimens on various factors (stage, histological type, etc.) to use this data in the future when collecting new samples, and to reduce the number of rejections due to insufficient content of tumor and / or necrosis in FFPE blocks.

The overall median tumor percentage is = 0.800000 across different stages of NSCLC; χ^2 = 15.76012 (p=0.0013), Kruskal-Wallis rank test = 22.68011 (p=0.0000) (Fig. 3.3.1 A, B). The indicator was identical in stages I and II and then increased towards stages III and IV. An outlier occurred at stage III.

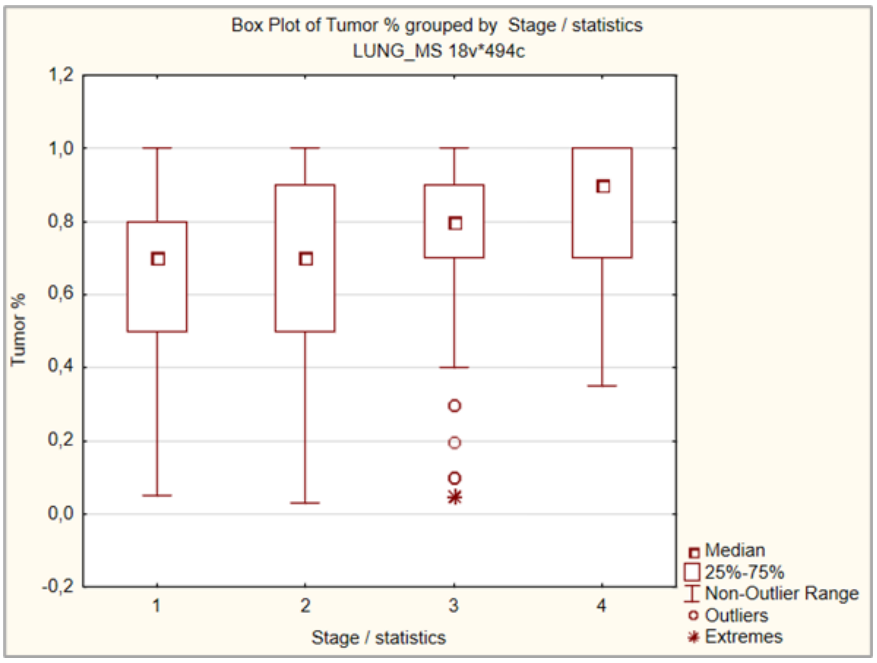
The median tumor percentage values depending on the histological type of NSCLC are shown in Fig. 3.3.2 A, B. The rate for group 1 (adenocarcinoma) and group 2 (SCC) was identical and increased in group 3 (other, rarer histological types of NSCLC). There were outliers for SCC and other histologic types of NSCLC (Fig. 3.3.2 A, B).

The mean values of tumor percentage (% ± 95% confidence interval) at different stages of lung cancer are presented in Fig. 3.3.3.

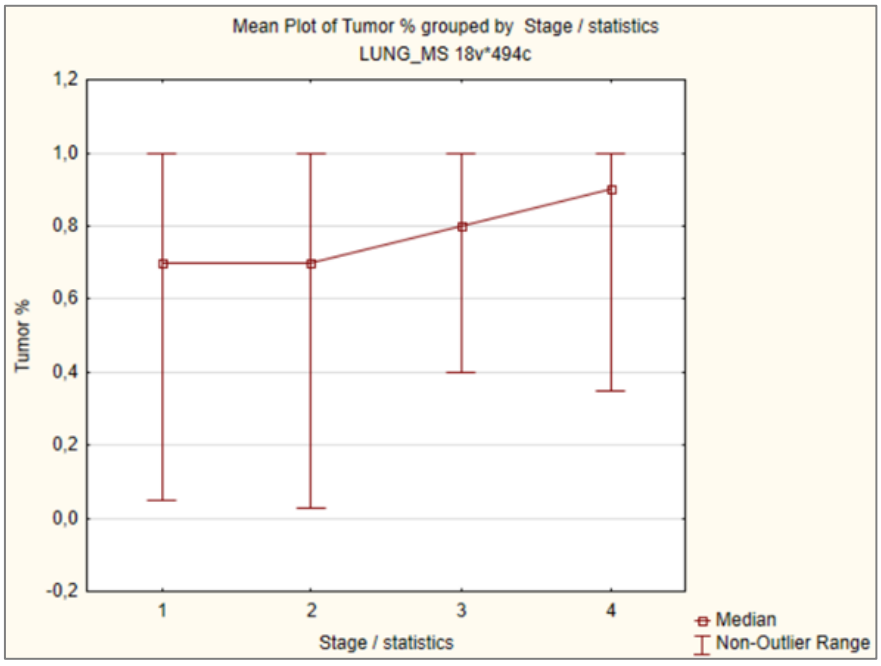
Overall median necrosis percentage = 0.100000 across NSCLC stages; χ^2 = 16.83767 (p=0.0008), Kruskal-Wallis rank test = 21.19448 (p=0.0001) (Fig. 3.3.4 A, B). The indicator gradually increased from stage I to stage IV, with outliers observed at each stage.

The median necrosis percentage values depending on the histological type of NSCLC are shown in Fig. 3.3.5 A, B. The rate for group 1 (adenocarcinoma) was the lowest, for group 3 (other, rarer histological types of NSCLC) it was the highest, and for group 2 (SCC) it was intermediate. There were outliers for each histological type.

Mean values of necrosis percentage (% ± 95% confidence interval) at different stages of NSCLC are presented in Fig. 3.3.6.

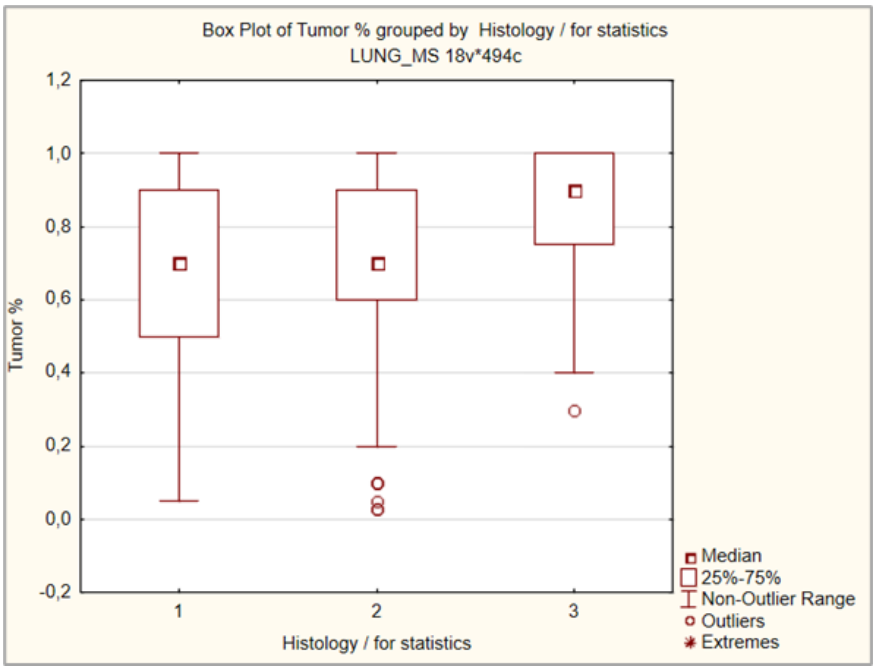


A

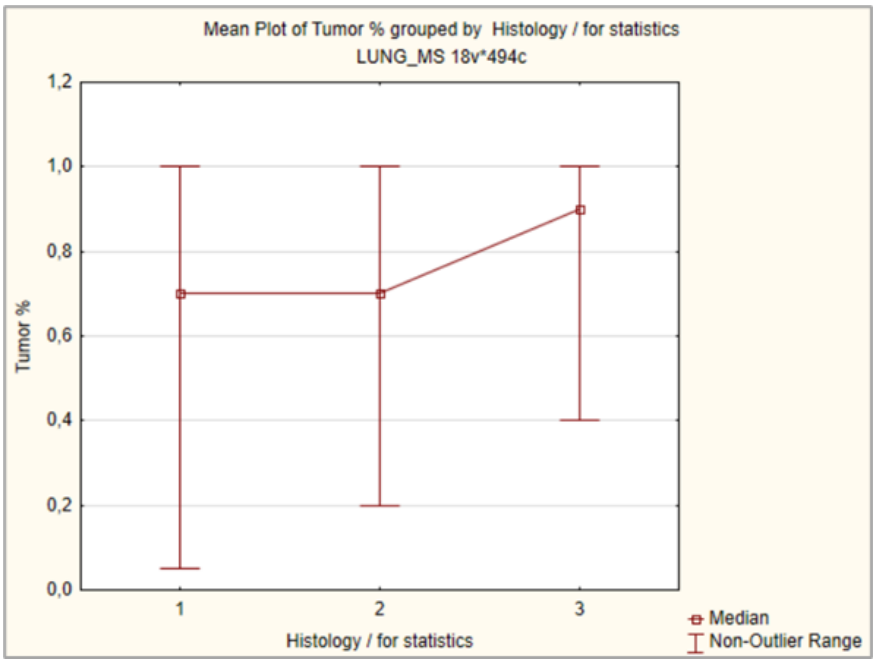


B

Fig. 3.3.1. Box-plot of tumor percentage (A) and mean plot of tumor percentage (B) grouped by stage of NSCLC.



A



B

Fig. 3.3.2. Box-plot of tumor percentage (A) and mean plot of tumor percentage (B) grouped by histological type of NSCLC.

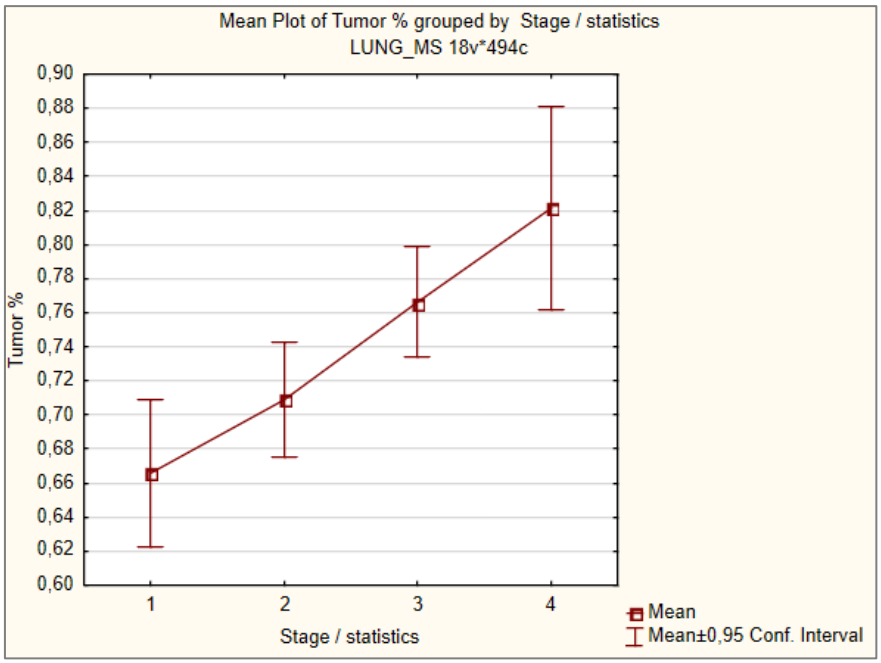
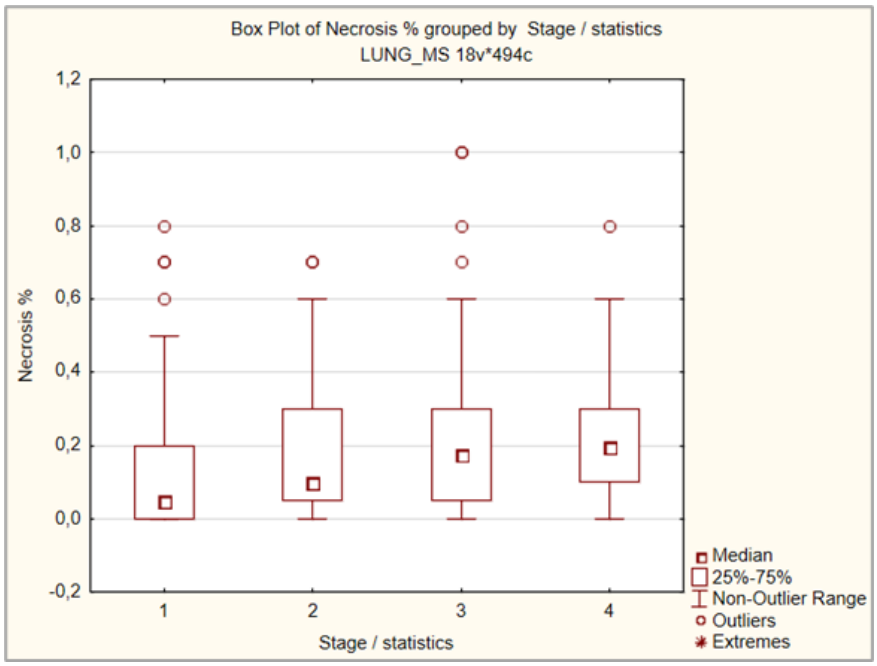
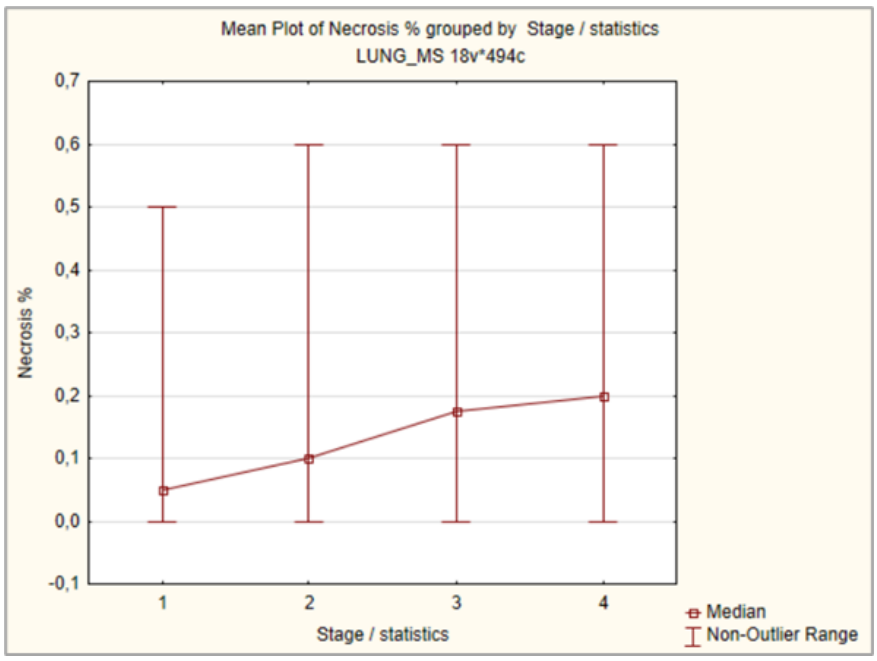


Fig. 3.3.3. Mean values of tumor percentage (% ± 95% confidence interval) at different stages of NSCLC.

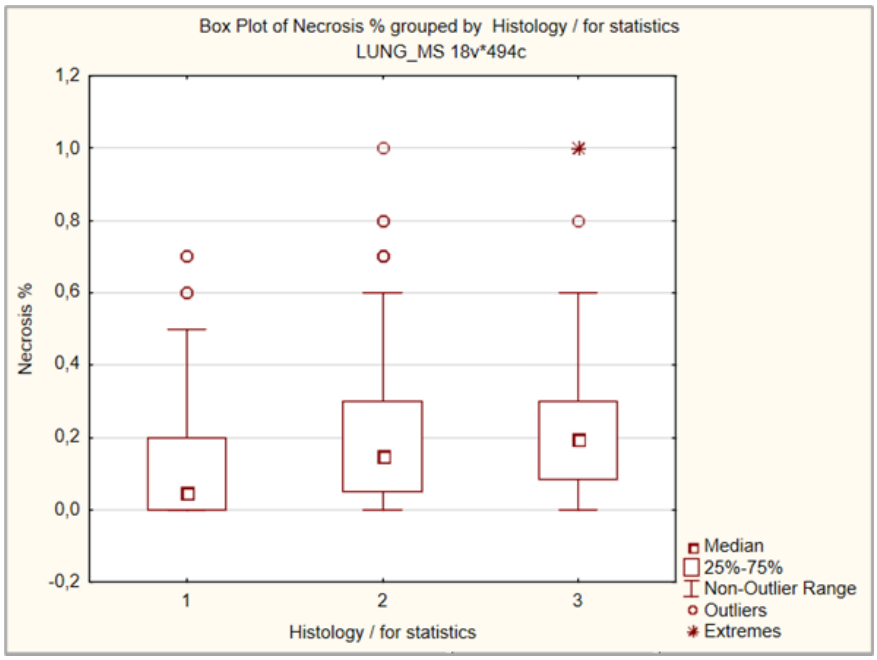


A

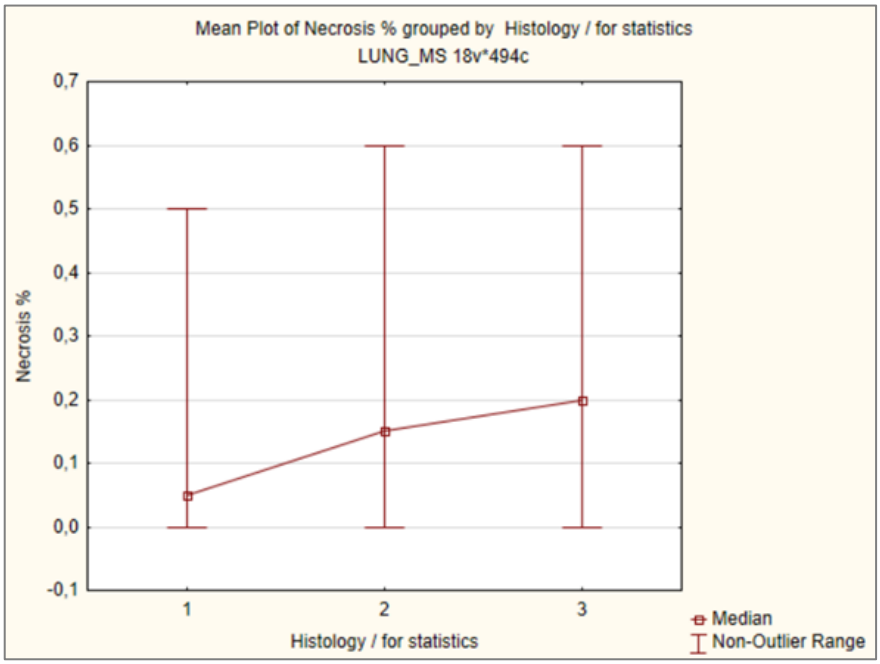


B

Fig. 3.3.4. Box-plot of necrosis percentage (A) and mean plot of necrosis percentage (B) grouped by stage of NSCLC.



A



B

Fig. 3.3.5. Box-plot of necrosis percentage (A) and mean plot of necrosis percentage (B) grouped by histological type of NSCLC.

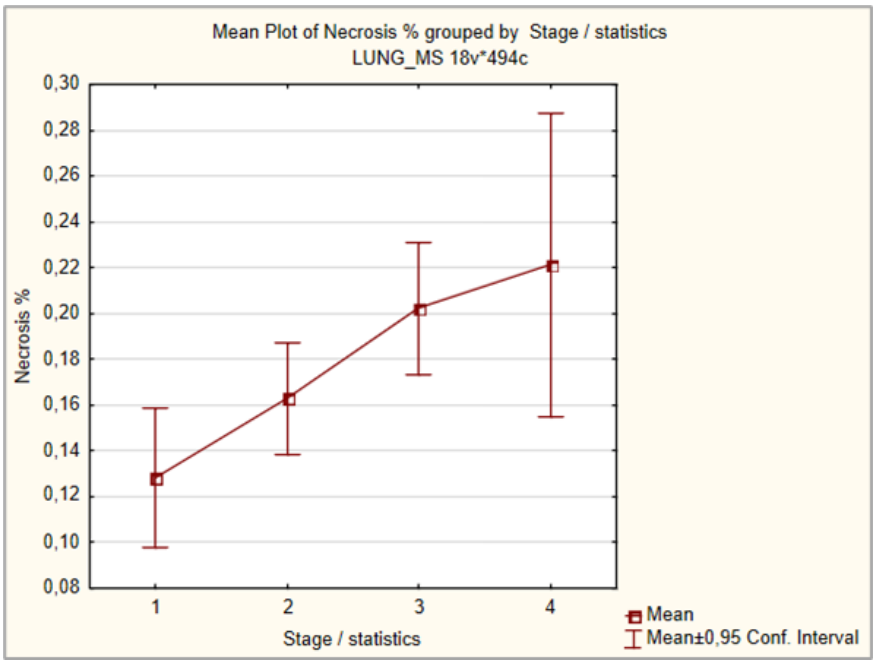


Fig. 3.3.6. Mean values of necrosis percentage (% ± 95% confidence interval) at different stages of NSCLC.

No statistically significant differences were found between blocks that met the selection criteria and rejected samples by stages (χ^2 = 2.38583, p=0.496279) and histological type (χ^2 = 0.793256, p=0.672584) and grade (χ^2 =4.42995, p=0.109156).

According to our data, a weak positive correlation (Spearman correlation) was detected between tumor size and tumor percentage at stage II (ρ = 0.217422), and moderate positive correlation — at stage III (ρ = 0.395305) (p<0.05). For stages I and IV, the relationship between the indicators was not statistically significant. The Kendall Tau correlation was τ = 0.162710 for stage II and τ = 0.293431 for stage III (p<0.05).

A moderate positive correlation (Spearman correlation) was detected between tumor size and tumor percentage for group 1 (adenocarcinoma) and group 2 (SCC) ρ = 0.317285 and ρ = 0.367729 respectively) (p<0.05). For group 3 (other histological types of NSCLC), the association between the parameters was not statistically significant. Kendall Tau correlation was τ = 0.226209 for adenocarcinoma и τ = 0.266357 for SCC (p<0.05).

Similar calculations were performed for the necrosis percentage. A weak positive association (Spearman correlation) was detected between tumor size and necrosis percentage at stages I and III of NSCLC (ρ = 0.204942 and ρ = 0.163619), a moderate positive association was found at stage II (ρ = 0.337260) (p<0.05). For stage IV, the

association was not statistically significant. Kendall Tau correlation was $\tau = 0.161176$ at stage I, $\tau = 0.247157$ at stage II, and $\tau = 0.121445$ at stage III ($p<0.05$).

A weak positive correlation (Spearman correlation) was detected between tumor size and necrosis percentage for group 1 (adenocarcinoma) and group 2 (SCC) $\rho = 0.299382$ and $\rho = 0.155483$ respectively) ($p<0.05$). For group 3 (other histological types of NSCLC), the association was moderately positive ($\rho = 0.364319$) ($p<0.05$). Kendall Tau correlation was $\tau = 0.161176$ for adenocarcinomas, $\tau = 0.117014$ for SCC, and $\tau = 0.287216$ for other histological types of NSCLC ($p<0.05$).

Also, we hypothesized that the tumor necrosis rate might be related to smoking, but this was not confirmed. No statistically significant association was found between smoking and necrosis percentage.

4. Discussion

Today, oncological pathologies ranks second among the causes of death of the population, second only to cardiovascular diseases.

The term “lung cancer” was introduced into clinical practice in the 19th century and today this type of cancer is the most common type of tumor oncology in most countries. This is especially true in low- and middle-income countries due to high levels of smoking, air pollution, hazardous production often without adequate protective equipment, and limited access to healthcare facilities, which negatively impacts treatment options and prognosis.

Despite constant progress in medicine, lung cancer is often detected at late stages, in the presence of metastatic lesions, and the outcome is far from satisfactory; the mortality rate for lung cancer is increasing year after year (Smolarz B, et al., 2025). Approximately 2.5 million new cases are diagnosed worldwide each year. The lung cancer incidence is projected to increase by 86.2% by 2050 compared to 2022 levels (to 4.62 million), and mortality by 95% compared to 2022 levels (to 3.55 million) (Zhou J, et al., 2024). Many countries have national registries, and in recent decades, numerous national studies have been conducted in both Asian and European countries to analyze the causes, progression, and treatment of lung cancer. In most cases of lung cancer, a link can be traced to long-term heavy smoking, air pollution, or exposure to other unfavorable factors, but in approximately 11–15% of patients such a direct link is absent, which requires additional studies, including genetic and molecular ones.

Approximately 85% of lung cancer cases are NSCLC. According to the literature, the proportion of different histological types of NSCLC varies by country, but in general, 40 to 70% are adenocarcinoma, approximately 25–30% are SCC, and the remainder are rarer types of lung cancer (adenosquamous carcinoma, large cell lung cancer, carcinosarcoma, etc.) [Prompt: SCC % of NSCLC, Gemini 2.5 Pro, Google, Inc., 29-Sep-2025, https://gemini.google.com/]. (Smolarz B, et al., 2025). Although these different histological variants are combined into one group of NSCLC due to the origin of tumors from epithelial cells and the commonality of treatment approaches, some authors believe that adenocarcinoma and SCC of the lung are fundamentally different neoplasms, with different pathogenesis, aggressiveness and prognosis, and therefore require different approaches (Smolarz B, et al., 2025; Wen Wang, et al., 2022).

According to the literature, 30–40% (or more) of NSCLC cases manifest metastases at the time of diagnosis. Distant metastases are the main cause of death for most cancer patients. Most often, distant metastases in NSCLC are detected in the bones, lungs, brain, liver and adrenal glands, in the chest cavity, and distant lymph nodes, which significantly reduces patient survival. Metastasis appears to be a random process that has not been properly characterized. Some researchers suggest that oncogenic drivers such as EGFR, ALK, ROS1 may induce the occurrence of metastases (Yu Chen, et al., 2021; Tamura T, et al., 2014).

Many aspects of the pathogenesis of lung cancer and its progression remain unresolved, and the search for effective treatment methods continues, requiring new, large-scale research.

Despite advances in diagnostics and treatment, lung cancer outcomes, unfortunately, remain far from optimistic. It should be considered that up to 40% of NSCLC cases are still detected at stages III–IV (due to the non-specificity of symptoms, the unavailability of screening in some cases, etc.). According to rough estimates, 5-year survival at stage I NSCLC ranges from 65–84%, at stage II — 40%, at stage III — 15%, and at stage IV does not exceed 5%. However, even at stage I, after complete resection of the part of the lung containing the tumor, the risk of recurrence or metastasis exceeds 20% (Jiajing Sun, et al., 2022; Park SY, et al., 2011). The overall 5-year survival rate for NSCLC in most countries worldwide does not exceed 20%.

Research into the comprehensive study of lung cancer is relevant — from retrospective and epidemiological to molecular and genetic studies, the development and testing of new drugs, and the identification of new targets for their action — anything that will contribute to a better understanding of the nature, mechanisms of occurrence and development, and resistance of lung cancer, as well as increasing the life expectancy of patients.

Most of these biomedical studies are conducted using solid lung tumor tissue, namely FFPE blocks.

Fixation of tissues in formalin followed by paraffin embedding has been used as a routine procedure in histopathology for over a century. The blocks are used to assess the morphological features of tumors, make a diagnosis, compare changes in tissue before and after treatment, etc. IHC is also an integral part of diagnostics, especially differential diagnostics or detection of primary tumors in the presence of distant or anonymous metastases. Both standard histologic evaluation and IHC are widely used in histopathology. The blocks are stored in large quantities in hospital archives or in specialized biobanks, the

number of which is increasing every year due to the high demand for such samples for biomedical research. FFPE blocks are a valuable and practical material for most studies due to their low cost, ease of storage and transportation, and their ability to withstand long-term storage. Fixation of tissue in formalin and subsequent long-term storage are known to negatively affect the quantity and quality of nucleic acids; however, thanks to modern methods of their extraction, FFPE blocks can be used for DNA and RNA research, even on samples stored for 10–12 years or more. Although each patient's samples are unique due to genetic characteristics, environmental influences, lifestyle, habits, etc., as a rule, enough sections can be obtained from each FFPE sample for various studies. In addition, archives and biobanks can share not only samples themselves with other repositories and researchers, but also the results of tests already conducted. In this regard, the use of standardized methods for collecting, processing, and storing material, protecting personal data and patient confidentiality, and adhering to local and international legal standards are of great importance. Collaboration with biobanks is currently at the peak of relevance, as such legally structured repositories offer well-annotated, high-quality samples for various studies, from population-based to molecular. Entire networks of biobanks and online repositories are being created to improve sample distribution and data exchange.

There are several hundred (or even thousands) of public and commercial biobanks worldwide (it all depends on the approach to assessing the criteria for a repository as a biobank), most of them collaborate with academic institutions and university clinics. Most biobanks offer blood and its derivatives, but the demand for FFPE blocks among researchers, especially in oncology, is only growing, and modern biobanks and biological sample archives must keep up with this trend.

Biobanks hold both archival collections and prospective samples collected for a specific purpose or project, but both are in high demand, especially if the samples are consented and well-annotated. In addition, biobanks provide information on the timing and methods of material collection, its fixation, storage conditions, and transportation. The samples themselves are accompanied by demographic and clinical data, sometimes the results of previous studies, which serve as a great help in new biomedical projects.

The most in-demand samples in oncopathology include resections of NSCLC, especially primary tumors (samples collected before patients are given chemotherapy, radiation therapy, or immunotherapy). In Ukraine, it was possible to collect such samples in large quantities until 2023 before the introduction of new, improved treatment protocols into clinical practice. This study included consented FFPE samples of NSCLC, collected for the

Audubon Bioscience biobank in 2020–2022 (before the war) from clinics with which the company legally cooperated, excluding temporarily occupied territories.

In addition to the quality criteria for sample preparation and storage, the content (%) of tumor and necrosis in the blocks is of great importance. This criterion varies for different studies, but the most common restrictions are minimum 30% tumor, maximum 30% necrosis. Insufficient tumor tissue (most often this applies to small biopsies) can lead to incorrect diagnosis, the same applies to excessive necrosis, especially with IHC, where the results can be false-positive or false-negative, which also affects the accuracy of the diagnosis. Also, the percentage of necrosis is of great importance for NGS, but the requirements there are less strict (up to 50% necrosis, according to literature) (Conroy JM, et al., 2018). In this study, blocks with a tumor percentage of 30% or more and necrosis of 30% or less were considered acceptable for further use; the remaining NSCLC blocks were rejected.

It should be noted that multiple and extensive necrosis is not uncommon in NSCLC, especially when it comes to SCC and large cell carcinoma. Necrosis is more often detected in SCC even in small sizes and moderately differentiated ones. On the contrary, necrosis is much less common in adenocarcinomas; as a rule, it is detected in poorly differentiated or large tumors (i.e., more often in late stages). This nuance should also be considered, since sometimes lung cancer blocks with a necrosis content of up to 40% are acceptable for research. It should also be noted that for many solid tumors, including NSCLC, the presence of necrosis is considered a criterion for tumor aggressiveness and a negative prognostic sign.

Yere, the Audubon Bioscience's FFPE collection of NSCLC samples was evaluated for diagnostic relevance and acceptable tumor and necrosis percentages to determine what proportion of specimens could be submitted for further research. I also attempted to analyze the influence of various factors on the percentage of tumor and necrosis in the sample (patient age, stage, histological type of tumor, size, stage, smoking) with the aim of using these data in the future when collecting new samples to minimize the number of possible rejections.

The study used information from the Audubon Bioscience biobank database, with some data taken from the pathology report (tumor size, TNM stage, histological type, grade, tumor percentage and necrosis). No new sections were prepared during the study, and no microscopic diagnostics were performed. Only existing data were analyzed.

The initial collection included 592 FFPE tumor blocks characterized as NSCLC from 243 donors.

During the selection process, 32 (5.41%) FFPE blocks from the collection were excluded due to discrepancies between clinical and pathological diagnosis (abscess, pneumonia, etc.). 14 (2.36%) blocks were rejected because the identified tumor was not primary lung cancer itself (hamartoma, B-cell lymphoma, etc.). 44 (7.43%) blocks were rejected because patients were diagnosed with neuroendocrine tumors of the lung. Another 8 (1.35%) blocks were excluded because primary lung cancer was not confirmed by pathological examination; instead, patients were found to have metastases of ovarian and colorectal cancer in the lungs.

The final study included 494 NSCLC blocks from 208 donors. The number of samples was deemed sufficient to evaluate the results with a 5% margin of error (according to preliminary calculations, the minimum required size was 323 samples).

According to statistics, lung cancer is more often diagnosed in men (55–85% in different countries). According to our data, NSCLC was detected in 82.69% of men and 17.31% of women (sex ratio 4.78), which is consistent with data from international studies.

Regarding gender differences, according to the literature, the development of lung cancer in men is often associated with long-term smoking and hormonal changes (Smolarz B, et al., 2025). In general, women smoke less often, although with the same intensity and duration of smoking, they have a higher risk of NSCLC development. SCC predominates in men, while adenocarcinoma predominates in women. However, mutations, such as those in the EGFR gene, are more common in women, and overall, they have a better prognosis for NSCLC.

The average age of patients with NCSLC was (61.06±7.43) years, which is consistent with the data of other authors (Lemaire M, et al., 2024; Tao Chen, et al., 2019). In each age group from young (25–44 years) to senile age (76–90 years), men predominated; the elderly patients' group (61–75 years) was the largest group in our study, while the smallest group was the young patients group. The age of patients with NSCLC of different histological types did not differ significantly. Thus, the average age of patients with adenocarcinoma was (60.65±7.48) years, with SCC — (61.57±7.34) years, and with other, rarer types of lung cancer — (59.86±7.61) years.

In this study, SCC was the predominant type of NSCLC (50.92%), which is typical for Eastern Europe [Prompt: SCC lung cancer in what countries prevails, Gemini 2.5 Pro, Google, Inc., 29-Sep-2025, https://gemini.google.com/]. Although according to the literature, adenocarcinoma predominates among lung cancer cases, this may depend on the region (for example, SCC predominates, as already mentioned, in Eastern Europe, as well as in Northern Europe and East Asia) (World Cancer Research Fund. Lung cancer

statistics | World Cancer Research Fund [Internet]. World Cancer Research Fund. 2024. Available from: https://www.wcrf.org/preventing-cancer/cancer-statistics/lung-cancer-statistics/). In this study, adenocarcinoma was diagnosed in 36.06% of samples, and other, rarer types of NSCLC were diagnosed in 12.02%.

The distribution of observations by stages in this study was as follows: stage I — 23.88% of samples, stage II — 34.62%, stage III — 34.82%, and stage IV — 6.68%. Thus, most NSCLC cases were detected at stages II and III. However, the number of samples at late stages is also high — 41.5%, which is consistent with literature data that, despite advances in early diagnosis, about 40% of lung cancer cases are detected at stages III and IV (Jiajing Sun, et al., 2022; Park SY, et al., 2011). This situation in Ukraine may be due to the non-specific symptoms of lung cancer, which most often develops against the background of existing chronic lung diseases (usually chronic bronchitis), limited availability of CT and the banal reluctance of most patients to see a doctor until their condition has critically worsened (something like a national trait).

In this study, moderately differentiated (G2) and poorly differentiated (G3) NSCLC samples predominated — 58.7% and 30.97%, respectively. Well-differentiated tumors (grade 1) accounted for 7.06%, grade was not specified for 3.24% of samples.

According to pathological reports, the tumor percentage in the samples ranged from 3 to 100%, with an average of (70.63±22.37%), and the necrosis percentage ranged from 0 to 100%, with an average of (17.22±17.76%). As mentioned, blocks with tumor content ≥30% and necrosis ≤30% were considered suitable for further use. Acceptable tumor percentage was noted in 479 (96.96%) samples, necrosis in 417 (84.41%). 403 (81.58%) FFPE samples met the required criteria, 91 (18.42%) were rejected. Thus, nearly a fifth of the NSCLC specimen collection failed to meet the requirements for tumor or necrosis content.

The percentage of samples rejected was 2.23% of samples grade I, 8.10% grade II, 5.06% grade III, and 0.81% of samples which grade was not indicated. Most unaccepted samples were rejected because of high necrosis, most of such samples were SCC and rare types of NSCLC (namely 72.73% of rejected samples). This is consistent with literature data, according to which necrosis is more often detected in the tissue of SCC and large cell cancer, and these can be moderately differentiated and not very large tumors (Caruso R, et al., 2012; Park SY, et al., 2011; Seok Whan Moon, et al., 2022). There were also 45.46% SCC and rare types of NSCLC among rejected grade I tumors, and another 6 (54.55%) tumors were adenocarcinomas. However, it is difficult to draw reliable conclusions here due

to the small number of observations. All samples with a high percentage of necrosis and an indeterminate grade were rare types of NSCLC.

At stage I, tumor content ranged from 5 to 100%, necrosis — from 0 to 80%. 5 (1.01%) FFPE blocks were rejected because of low tumor content, 12 (2.43%) due to high necrosis (in total 17 (3.44%) samples were found to be unsuitable). At stage II, tumor ranged from 3 to 100%, necrosis — from 0 to 70%. 4 (0.81%) samples were not eligible due to low tumor content, and 27 (5.46%) due to high necrosis. In general, considering the above mentioned criteria, 30 (6.07%) blocks at stage II cannot be further offered for medicobiological research. At stage III tumor area in the FFPE samples ranged from 5 to 100%, necrosis area — from 0 to 100%. 6 (1.21%) blocks had an unacceptably low tumor percentage, while 32 (6.48%) had a high necrosis percentage. 38 (7.69%) stage III blocks were deemed unsuitable for further use in research projects. At stage IV, the tumor content in the samples ranged from 35 to 100%, necrosis — from 0 to 80%. Tumor content in all blocks met the study criteria. All 6 (1.21%) stage IV samples were rejected due to high necrosis.

In the statistical analysis of the sample, median values were used, which are not as sensitive to outliers as mean values in the case of nonparametric data. The Pearson χ^2 test was used to identify the relationship between two categorical variables, and the Kruscal-Wallis test to check the equality of medians in 3 and more samples. Spearman rank order correlation and Kendall Tau correlation were used to assess the strength and direction of the relationship between two variables.

The percentage of tumor burden and percentage of necrosis was analyzed based on tumor stage, histological type, and other parameters. No statistically significant relationship was found between the tumor and necrosis percentage and the age of patients, as well as smoking. Weak positive Spearman correlation was detected between tumor size and tumor percentage at stage II ($\rho = 0.217422$, $p<0.05$) and moderate positive correlation at stage III ($\rho = 0.395305$, $p<0.05$). The Kendall Tau correlation was $\tau = 0.162710$ for stage II and $\tau = 0.293431$ for stage III ($p<0.05$). This may be due to the intense cell division in larger tumors and their higher cell density. The results for stages I and IV were not statistically significant, which is likely due to the small number of such observations.

A moderate positive Spearman correlation was detected between tumor size and tumor percentage for adenocarcinomas and SCC ($\rho = 0.317185$ and $\rho = 0.367729$ relatively) ($p<0.05$), Kendall Tau correlation was $\tau = 0.226209$ for adenocarcinomas и $\tau = 0.226357$ for SCC) ($p<0.05$). For rarer histological types of NSCLC, the results were not statistically significant, possibly due to the small number of such observations.

A weak positive association (Spearman correlation) was found between tumor size and necrosis percentage at stages I and III ($\rho = 0.204942$ and $\rho = 0.163619$), a moderate positive association was detected at stage II ($\rho = 0.337260$) ($p<0.05$). For stage IV the correlation was not statistically significant. This dependence may be due to the fact that as the tumor size increases (in NSCLC, the stage of the disease is determined by the tumor size), the likelihood of necrosis in the tumor also increases. Firstly, as the malignant tumor progresses, its aggressiveness increases. Secondly, angiogenesis is enhanced, and in malignant neoplasms it is imperfect; the vessels are located chaotically, are fragile and brittle, which provokes the formation of necrosis and hemorrhages. For stage IV, the statistical relationship was insignificant, possibly due to the small number of observations. Although stage IV is not limited to the primary tumor, it is defined by the presence of distant metastases.

A weak positive Spearman correlation was detected between size of the tumor and necrosis percentage for adenocarcinoma and SCC ($\rho = 0.299382$ and $\rho = 0.155483$ respectively) ($p<0.05$). The correlation was moderately positive for rare types of NSCLC ($\rho = 0.364319$) ($p<0.05$). Kendall Tau correlation was $\tau = 0.161176$ for adenocarcinomas, $\tau = 0.117014$ for SCC and $\tau = 0.87216$ for other histological types of NSCLC ($p<0.05$). As the tumor size increases (as it progresses), the risk of necrosis increases in all histological types of NSCLC, which is associated with TNM staging and a more serious prognosis.

Regarding tumor percentage in FFPE samples, the tissue sampling itself and the selection of areas that visually represent tumor tissue as much as possible are of great importance. As the tumor progresses and increases in size, the rate of cell division appears to increase and the cell density also increases, which may be related to the higher tumor content in stages II and III samples. An increase in tumor percentage with increasing tumor size was observed for the main histological types of NSCLC — adenocarcinoma (group 1) and SCC (group 2). For rare histological types of NSCLC (group 3), this relationship was not statistically significant, likely due to the small number of observations.

According to our study, tumor size (and, consequently, TNM stage) correlated with percentage of necrosis, and this indicator increased from stage I to stage III. The increase in tumor size is associated not only with increased proliferation of its cells, but also with rapid death. In addition, due to imperfect angiogenesis and the fragility of the tumor's own vessels, insufficient blood supply, and hypoxia, which is more pronounced in rapidly growing tumors and large neoplasms, large / multiple foci of necrosis are formed. For stage IV, the identified correlations were not statistically significant, possibly due to the small number of observations.

The significance of necrosis in NSCLC requires further study. Thus, according to the literature, necrosis in solid tumors is associated with a reduction in the overall life expectancy of patients (according to univariate statistical analysis), while according to multivariate analysis, only damage to the lymph nodes, blood vessel invasion and T-stage were identified as statistically significant prognostic factors. Central cavitation and foci of necrosis are often found in SCC of the lung, possibly due to rapid tumor growth and insufficient blood supply, which leads to necrosis (Nismat Javed, et al., 2023). Furthermore, the survival time of patients with necrosis >20% or more in SCC tissues was shorter than in patients without necrosis. These findings were confirmed by the results of univariate and multivariate statistical analysis, while similar correlations were not identified for adenocarcinoma. Extensive necrosis in lung tumor tissue was an independent prognostic factor useful in improving the predictive power of the TNM staging system at NSCLC stages I and II. However, the prognostic value of this histological parameter was reduced in patients with clinical stage III, and no association was found between tumor necrosis and angiogenesis (Gkogkou C, et al., 2014).

A collection of 592 blocks characterized as NSCLC was initially taken for analysis, but 98 (16.55%) blocks were rejected because of clinical and pathological diagnosis discrepancies. A total of 494 samples were included in the study, of which 91 (18.42%) blocks did not meet the minimum requirements for tumor and necrosis content. Thus, according to this study, 31.93% of blocks from the initial collection and 18.42% of samples with a suitable diagnosis cannot be offered for future research. This is, unfortunately, a rather high figure.

4.1. Conclusions

In the future, it will be necessary to pay closer attention to the correspondence of the pathological diagnosis to the nosology being collected and to rely specifically on the data of the pathohistological report when sending samples for storage. In my opinion, the low tumor content in the samples can be overcome by more careful sampling of the material, avoiding areas of fibrosis. The highest number of potential rejections is possible in stages II and III NSCLC due to the high percentage of necrosis, especially in the case of SCC, which must also be taken into account when collecting samples. At the stage of material cutting, it is necessary to take areas without visible cavitation, softening and hemorrhage. Also, if possible, it is worth increasing the number of samples from one patient (at least to 3), which will allow in the future to select specimens with the lowest, acceptable content of necrosis. My experience suggests that, if possible, 20% more NSCLC samples should be

collected, especially in stages II and III, to obtain the required number of samples with an acceptable percentage of tumor and necrosis. Blocks with 40% necrosis content can be retained in the biobank and offered to projects with less stringent selection criteria (40.66% of all rejections due to mismatch between tumor and necrosis content). Since there is a signed cooperation agreement between the National Medical University and Audubon Bioscience, we can offer some samples containing 20% of tumor and / or 50% necrosis for the production of educational slides, including digital ones, for medical students. Blocks with very low tumor content (3–15%) and high necrosis percentage (60–100%) can be discarded.

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