Content

I. General Part (Current state of knowledge)

1. Introduction to the Definition of Soft Tissue Sarcomas

Soft tissue sarcomas are a rare and heterogeneous group of tumours of mesenchymal origin. The term comes from the Greek words “sarcos” and “oma,” meaning fleshy tumour. These can develop from any connective tissue such as smooth or striated muscles, tendons, blood vessels, adipose tissue, or nerve tissue.

Estimates from the American Cancer Society for soft tissue sarcomas in the United States for 2024 are: approximately 13,590 new soft tissue sarcomas will be diagnosed (7,700 in men and 5,890 in women). About 5,200 people (2,760 men and 2,440 women) will die from soft tissue sarcomas. These statistics include both adults and children, thus necessitating the optimization (personalization) of treatment regimens from the early stages of the disease.

Ideally, quantifying tumour characteristics would help select the most effective therapeutic approach for each patient diagnosed with soft tissue neoplasm (surgical excision, chemotherapy/radiotherapy, neoadjuvant/adjuvant/palliative). However, the main challenge in this step is the heterogeneity of these types of sarcomas.

To stimulate optimal (personalized) treatment, it is important to distinguish the tumour type based on clinical-imaging characteristics, histopathological and immunohistochemical diagnosis, and molecular diagnosis. Sarcomas are defined as a distinct group of malignant tumours originating from mesenchymal cells, representing almost 21% of all paediatric solid malignant cancers and less than 1% of solid neoplasms in adults.

Histopathological classification has significantly evolved over the last few decades with the help of new diagnostic techniques such as immunohistochemistry, electron microscopy, and cytogenetic analysis. These advances have led to the reclassification of several tumours. For example, rhabdomyosarcoma and fibrosarcoma, common histological subtypes 30 years ago, are now considered rare. In their place, malignant fibrous histiocytoma is now recognized as the most common histological subtype.

The incidence of sarcomas varies by country and study date, making it difficult to specify accurately. Similarly, the incidence of each individual histological subtype is unclear, and sarcomas are misdiagnosed in up to 30% of cases, leading to patients being improperly treated according to clinical guidelines, which recommend that the management of patients diagnosed with soft tissue neoplasm should be carried out by a dedicated multidisciplinary team. The incidence of soft tissue sarcomas has been challenging to estimate due to the heterogeneous nature of these types of tumours, and controversies regarding the classification of its various subtypes have contributed to this difficulty.

Prognostic Factors

Clinical-Pathological Factors: Understanding the clinicopathological factors affecting prognosis is essential in formulating a treatment plan for patients diagnosed with soft tissue sarcoma. The three major clinicopathological factors that establish the risk profile for a specific patient are tumor size, histological grade, and disease extent (nodal or metastatic involvement), as reflected in the 8th edition of the AJCC staging system. Tumour size is of particular interest, with tumors grouped as 1-5 cm, greater than 5 cm, greater than 10 cm, and greater than 15 cm. Besides the aforementioned factors, histological subtype and resection margins are also significant, but these criteria are not included in the current staging system. Unlike other solid tumors, factors predisposing to local recurrence differ from those predicting distant metastasis and tumor-associated mortality. In other words, patients with a constellation of prognostic factors predisposing to local recurrence do not necessarily have an increased risk of distant metastasis or tumor-associated death, and vice versa.

Classification and Prognostic Significance of Surgical Resection Margins: Surgeons should use the UICC classification system for resection margins to integrate surgical outcomes and surgical resection margins. In this system, an R0 resection is defined as a macroscopically complete resection of the sarcoma with negative microscopic surgical margins. An R1 resection is a macroscopically complete resection of the sarcoma with positive microscopic surgical margins, and an R2 resection is a macroscopically incomplete resection (macroscopic residual lesion) of the sarcoma with positive microscopic surgical margins. The type of positive microscopic margins is also important. For example, an R1 resection for a low-grade liposarcoma or an R1 resection after preoperative radiotherapy, where a positive microscopic excision margin is anticipated (and accepted) to conserve essential structures, has a relatively low risk (less than 10%) of local recurrence. Additionally, an anticipated positive margin on a critical structure does not increase the local recurrence rate as supported by the TMCC classification. Using this classification system, positive margins were divided into positive margins near noble structures, positive margins after an unplanned excision or re-excision, and inadvertent positive margins. Positive margins near essential structures had similar local recurrence rates to those where noble structures were also excised. Patients undergoing unplanned excision followed by re-excision with positive margins (i.e., R1 resection) or patients with unexpected positive margins after primary resection have an increased risk of local recurrence, with rates approaching 30%. Therefore, the specific clinical context must be considered when interpreting the relative risk of local recurrence after an R1 resection.

II. Original Contribution (Personal Contributions)

II.1 Working Hypothesis and General Objectives

Soft tissue sarcomas represent a group of rare malignant tumors with an incidence of approximately 1% of all cancers in adults. These tumors exhibit considerable genetic and phenotypic heterogeneity, making accurate diagnosis and effective treatment challenging. The purpose of this research study stems from the lack of standardized diagnostic methods and the identification of prognostic factors that impact treatment response rates, overall survival, and mortality. The research aims to use precision medicine and personalized treatment approaches based on the genetic and molecular characteristics of soft tissue sarcomas, significantly improving patient prognosis and quality of life. It is anticipated that by identifying genetic mutations and specific biomarkers, targeted therapies can be developed that are more effective and have fewer adverse effects compared to standard treatments.

II.2 Materials and Methods

The analytical observational cohort study was conducted over seven years (2016-2023) within the Orthopaedics – Traumatology department of the Bucharest University Emergency Hospital, in accordance with the Declaration of Helsinki and approved by the ethics committee (no. 2305/2016). Each participant received an informed consent form and adhered to international ethics and scientific research deontology norms.

The main objective of this analysis was to identify and monitor patients diagnosed with primary/recurrent soft tissue sarcomas localized at the extremities. Patient data such as age, sex, body mass index, origin, personal medical history, and hereditary-collateral antecedents were collected. Imaging techniques used (MRI, CT, Angiography, PET-CT, Specht CT) provided data on the extent of the tumor, vascular or bone invasion, nodal involvement, and the presence/absence of secondary determinations.

The definitive diagnosis was based on the analysis of histopathological and immunohistochemical reports. Additional details were obtained from molecular genetic analysis, collecting data provided by next-generation genomic sequencing. All patients included in the study underwent a clinical, imaging, and histopathological diagnostic algorithm (including cases of recurrent soft tissue sarcomas).

I. Study on the Association of Immunohistochemical and Imaging Markers with Prognosis

I.1. Immunohistochemistry combines histological and biochemical methods with immunological ones to identify cellular and tissue elements as precisely as possible. In histopathological diagnosis, immunohistochemistry uses molecular criteria that allow phenotypic analysis of histogenesis differentiation, classification, and tumor proliferation. Additionally, the

prognostic and predictive role of certain markers is identified and demonstrated through IHC techniques. IHC techniques have improved the elaboration of a comprehensive anatomopathological diagnosis, especially for low-differentiation tumors, rare tumors, tumors with uncertain malignancy, or tumor formations that cannot determine the origin of primary cellular proliferation through usual staining.

Immunohistochemical Lymphoid Markers:

CD4 – a common marker for helper T cells, is used to classify and differentiate lymphomas from inflammatory conditions.

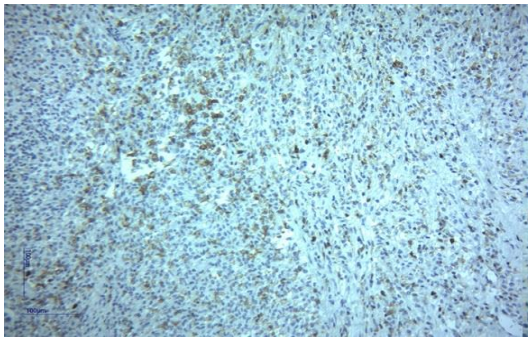


Fig. I.9 CD4 – MPNST – 20x

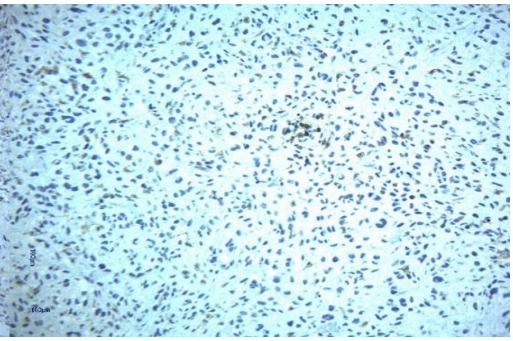


Fig. I.10 CD4 – mixofibrosarcoma 100x

CD8 – a marker for cytotoxic T cell populations, is recommended for detecting specific antigens in normal and neoplastic tissues as an adjunct to conventional histopathological examination using non-immunological histochemical stains.

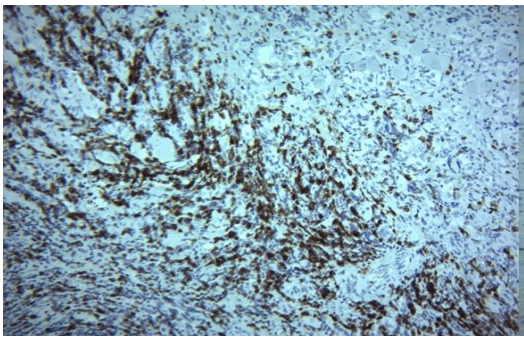


Fig. I.11 CD8 – angiosarcoma 200x

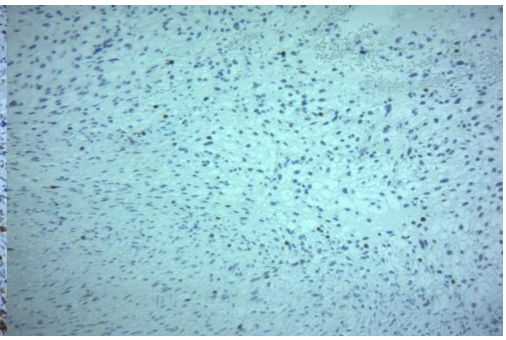


Fig. I.12 CD8 – pleomorphic sarcoma200x

CD34 is a transmembrane surface glycoprotein involved in intercellular adhesion and regulating cell proliferation and differentiation.

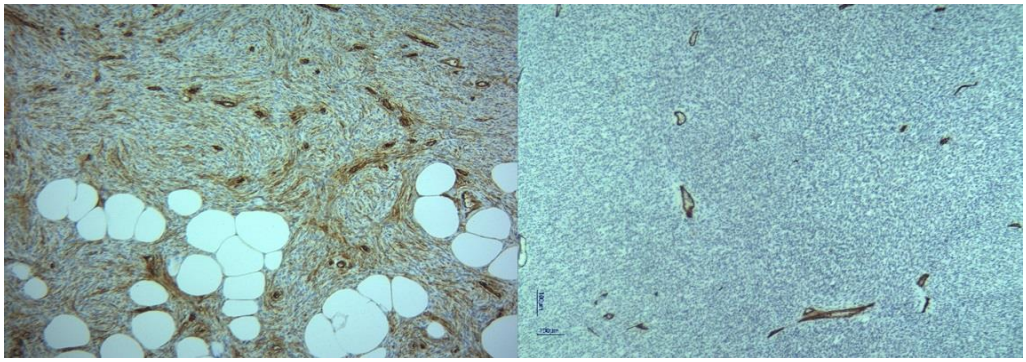


Fig. I.13 CD34 – Liposarcoma 200x Fig. I.14 CD34 – Fibrosarcoma 100x

CD44 is a cell surface adhesion protein that naturally occurs in various isoforms and promotes lymphocyte recruitment during inflammatory responses.

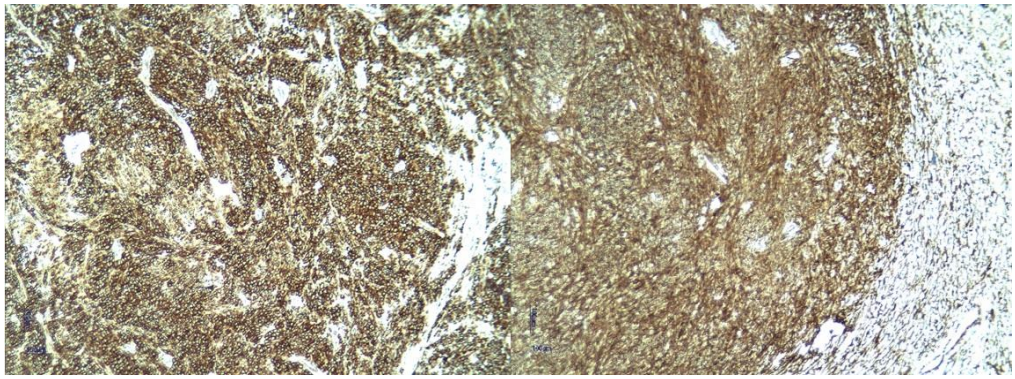


Fig. I.15 CD44 – MPNST 100x Fig. I.16 CD44 – fibrosarcoma 200x

Other Immunohistochemical Markers

CD99 is expressed in most Ewing sarcomas. However, it is expressed in various other tumors: lymphoblastic leukaemia (100%), synovial sarcoma, synovial sarcoma (80-90%), solitary fibrous tumor (90%), desmoplastic small round cell tumor, mesenchymal chondrosarcoma, thymoma, and in rare cases of rhabdomyosarcoma, carcinomas.

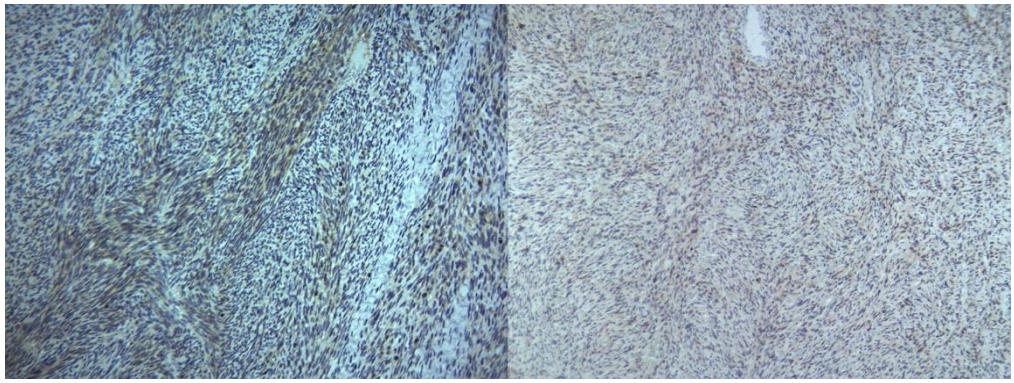


Fig. I.17 CD99 – pleomorhic sarcoma Fig. I.18 CD99 – fibrosarcoma

P53 represents a useful surrogate marker in highlighting the TP53 gene mutation. Strong nuclear positivity or complete absence through immunohistochemistry has a strong correlation with the presence of a mutation, low to intermediate expression.

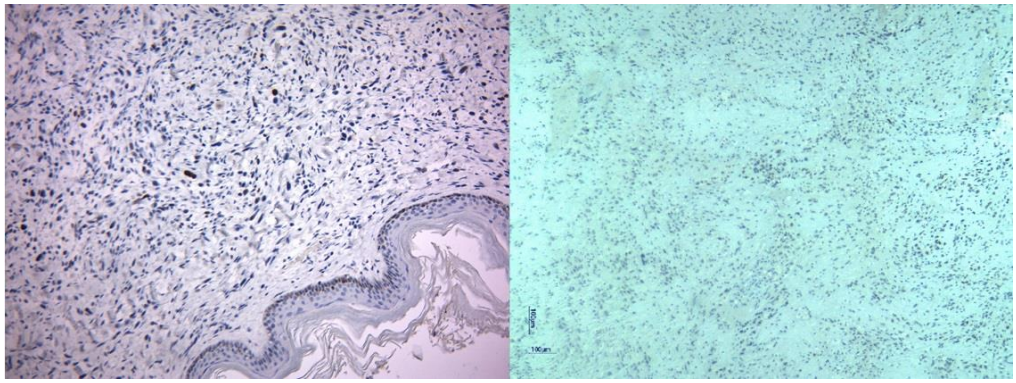
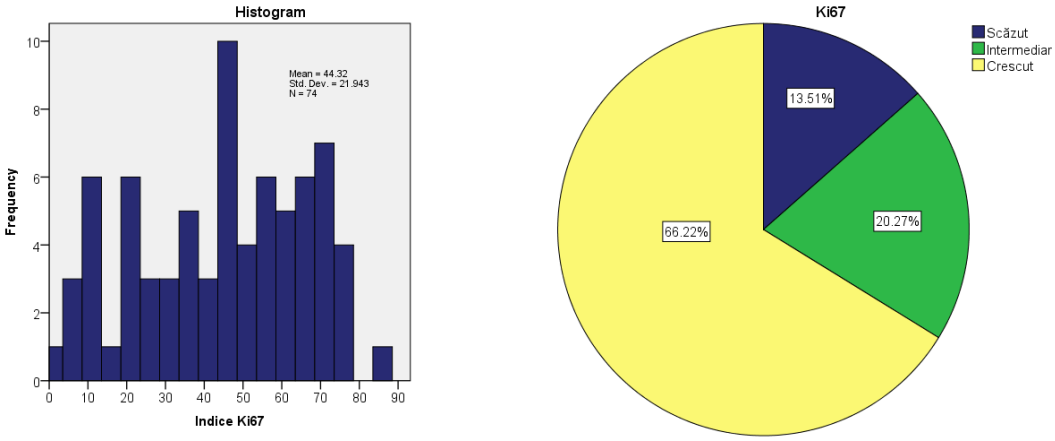


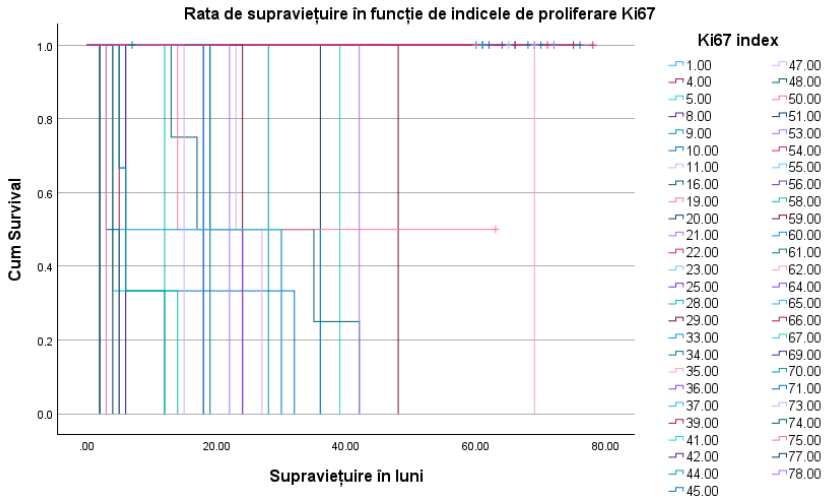
Fig. I.19 P53 –leiomiosarcom 20x Fig. I.20p53 – mixofibrosarcom 200x

Fig. I.25: distribution of cases by Ki67 index



The Ki67 index ranged from 1% to 85%, with a mean value of 44.32% (standard deviation 21.94; CI: [39.24; 49.40]).

Fig. I.26 Survival rate according to Ki67

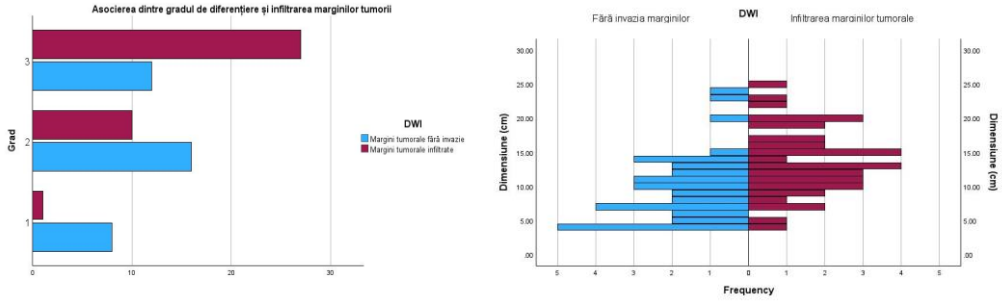


The aim of this study was to clarify the prognostic significance of lymphocytic infiltration in soft tissue sarcomas. To analyze this, we examined the expression of CD4, CD8, CD34, CD44, and CD99 lymphocytes. The immune status at the time of excision is important, but the prognostic significance of tumor-infiltrating lymphocytes is controversial, due to the immune system's different roles during oncogenesis, making it sometimes difficult to clearly distinguish between tumor invasion and the presence of a reactive inflammatory infiltrate.

Positivity for the CD4, CD8, and CD34 markers was associated with a lower grade of aggressiveness in soft tissue sarcomas, without invasion of the tumor margins, thus illustrating a better survival rate among these patients. Patients with the P53 gene mutation are significantly more prone to death and exhibited a higher histological grade, with infiltration of the tumor margins, thus being associated with a lower overall survival rate compared to other patients.

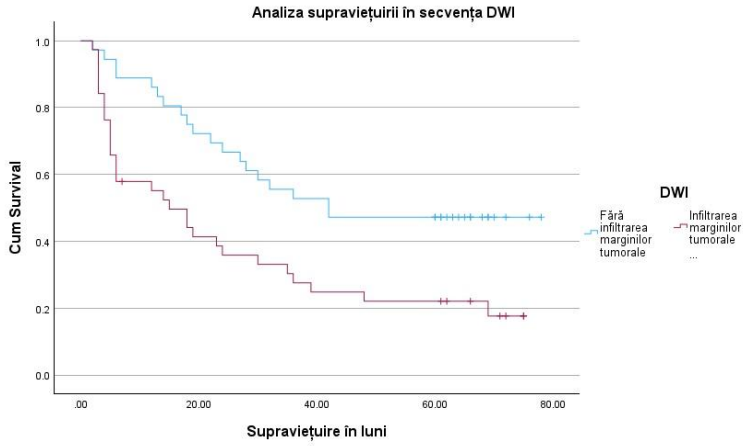
In the study, the tumor margin was classified as infiltrated on DWI (diffusion-weighted imaging) if it was poorly defined or irregular, while well-defined, circumscribed margins were defined as non-infiltrated. The distribution of cases in the studied group showed tumor margin invasion in 51% of cases (N=38) and an association between histological grade and size, indicating that large sarcomas with intermediate and high histological grades were associated with irregular, infiltrative margins and the presence of peritumoral edema.

Fig. I.47 Distribution of cases by grade and size



Additionally, there is an observed association between tumor margin invasion and histological grade, with tumors of intermediate and high malignancy grades more frequently showing margin infiltration. A Kaplan-Meier survival analysis was performed on this cohort of patients to compare the effect of tumor margin invasion on survival.

Fig.I.48 DWI Sequence Survival Analysis



The statistical analysis used in our study reveals a lower overall 5-year survival rate among the cohort of patients who exhibited tumor margin infiltration.

II. Study on the Evaluation of Prognostic Factors, Overall Survival, and Mortality

The effect of age as a prognostic factor on overall survival could be explained only partially by differences in tumor characteristics and the varied, sometimes suboptimal, treatment methods.

The higher mortality rate in older patients could also be attributed to the presence of other associated comorbidities and the body's response to neoadjuvant/adjuvant treatments.

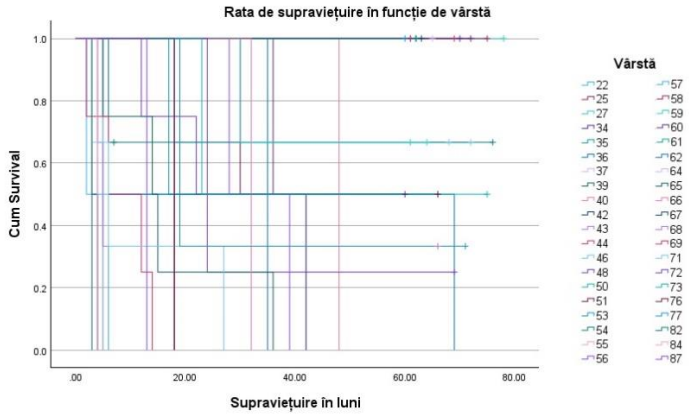
Table II.2 Survival rate by age

Analiza statistică

	Chi-Square	df	Sig.
Log Rank (Mantel-Cox)	104.348	39	<.001
Breslow (Generalized Wilcoxon)	90.215	39	<.001
Tarone-Ware	96.585	39	<.001

Rata de supraviețuire în funcție de vârstă

Fig II.3. Survival rate by age



In the conducted study, advanced age was associated with higher mortality, as indicated by the Kaplan-Meier survival curve. p-values of <0.001, 0.001, and 0.001 suggested a statistically significant correlation between advanced age and overall survival. Certain histological subtypes exhibit different behaviors, indicating that classical staging and prognostic systems may not be applicable to them. The latest WHO classification of soft tissue sarcomas recognizes the importance of accurate histotyping by incorporating immunohistochemistry (IHC) into diagnostic criteria, and in many cases, molecular genetic studies are necessary to achieve the most accurate diagnosis.

The distribution of cases based on diagnosis was as follows:

- Pleomorphic sarcoma: 20.27% of cases (n=15)
- Liposarcoma: 16.22% of cases (n=12)
- Synovial sarcoma: 13.51% of cases (n=10)
- Malignant fibrous histiocytoma: 12.16% of cases (n=9)
- Leiomyosarcoma: 12.16% of cases (n=9)
- Fibrosarcoma: 6.76% of cases (n=5)
- Myxofibrosarcoma: 5.41% of cases (n=4)
- Rhabdomyosarcoma: 4.05% of cases (n=3)
- Angiosarcoma: 4.05% of cases (n=3)
- Neurofibrosarcoma: 2.70% of cases (n=2)
- Epithelioid sarcoma: 2.70% of cases (n=2)

Fig. II.7: distribution of cases according to diagnosis

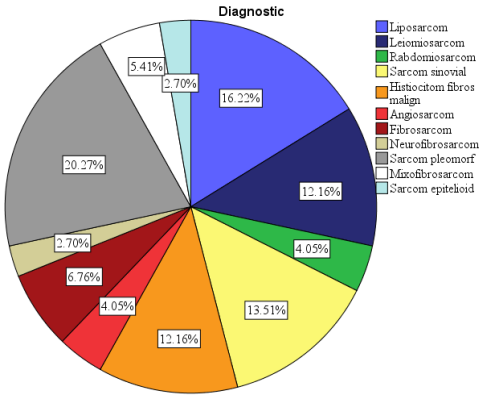


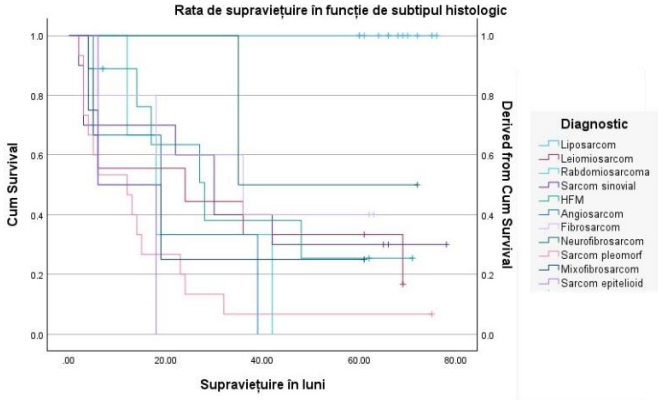
Table II.1 Statistical analysis of the diagnosis

Analiza statistică

	Chi-Square	df	Sig.
Log Rank (Mantel-Cox)	32.964	10	<.001
Breslow (Generalized Wilcoxon)	28.149	10	.002
Tarone-Ware	30.860	10	<.001

Rata de supraviețuire în funcție de diagnostic

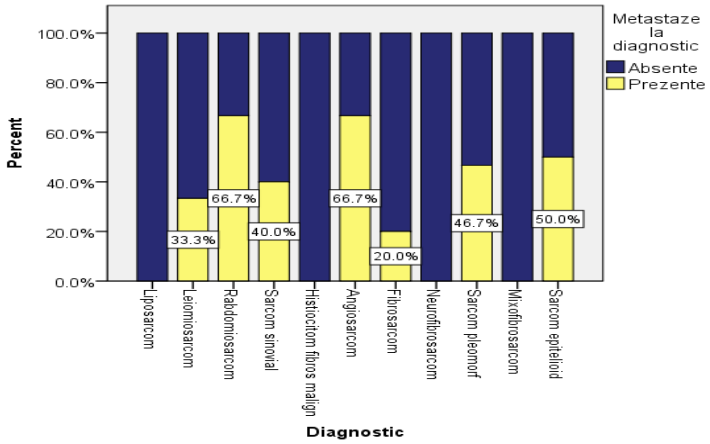
Fig.II.8 Survival rate according to histological subtype



In the conducted study, the most frequently encountered histological type was pleomorphic sarcoma (20.3%), followed by liposarcoma (16.2%) and synovial sarcoma (13.6%). The histological subtype of the tumor appears to be one of the most influential factors on prognosis, as observed through the application of the Kaplan-Meier survival curve.

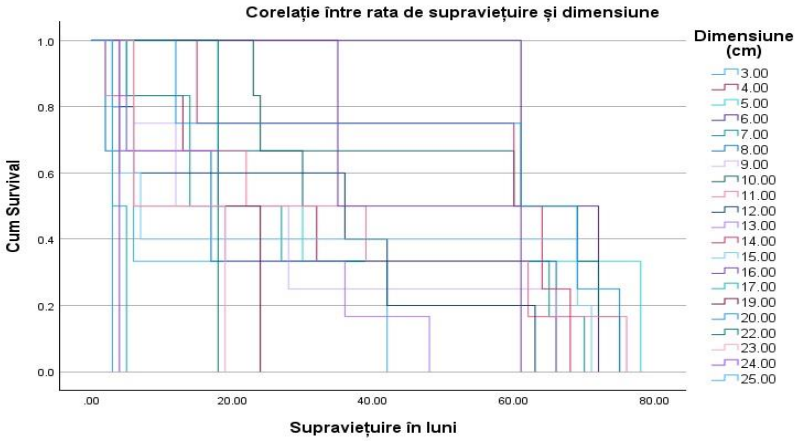
Association of diagnosis with metastases

Fig. II.9 distribution of cases according to diagnosis and presence of metastases



It was noted that certain histological subtypes were associated with a higher incidence of metastases. Regarding tumor size, 83% of patients were diagnosed with a tumor exceeding five centimeters at the time of diagnosis. The overall survival study indicates that a lower 5-year survival rate and a higher-grade sarcoma were associated with tumor size. Tumor size is an independent prognostic factor reported in the literature. A size greater than 5 cm is associated with poorer overall survival. The Kaplan-Meier statistical analysis indicates a lower 5-year survival rate for tumors larger than 5 cm.

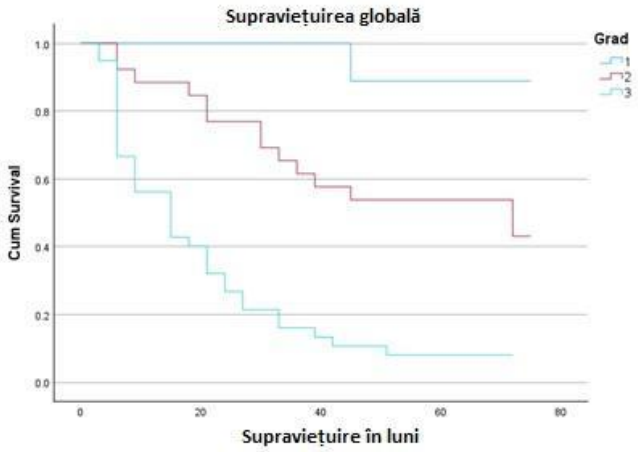
Fig. II.12 Correlation between survival rate and size



Degree of malignancy:

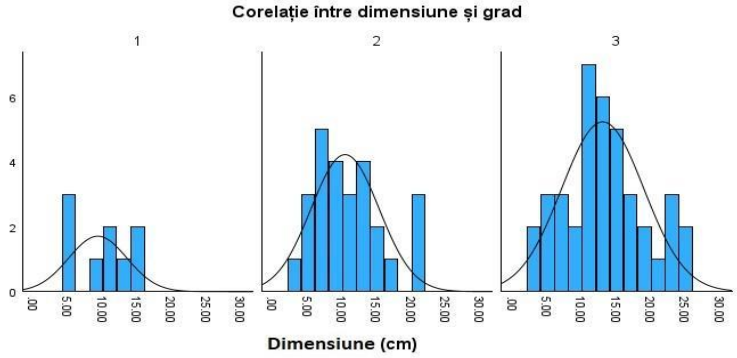
The most significant prognostic factor of survival and metastasis rate in adult soft tissue sarcomas is the degree of histological aggressiveness. Overall survival among patients diagnosed with low-grade soft tissue sarcomas is markedly superior compared to patients who experienced intermediate and high-grade sarcomas.

Fig. II.15 Overall survival by degree



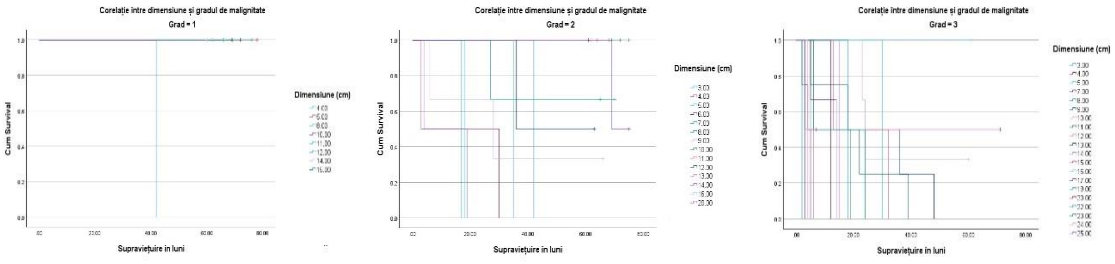
In the study, 52.70% of the sarcomas were grade 3, 35.14% grade 2 and 12.16% grade 1, thus highlighting a direct association between tumors with a high degree of aggressiveness and large tumors, as well as a lower 5-year survival rate.

Fig. II.16 Correlation between size and grade



Kaplan Meier's analysis of survival showed a more reserved prognosis in the case of sarcomas with a high degree of aggression (G3), with death occurring in some cases less than 6 months after diagnosis.

Fig. II.17: correlation between size and degree of malignancy



27% of patients had metastatic disease at the time of admission to the hospital due to poor tumor control or incomplete oncological treatment. Most lung metastases originated from tumors with a high degree of malignancy. However, some of the patients with low-grade sarcomas included in this study developed lung metastases. These lesions showed the ability to disseminate early, which denotes a similarity between their biological behavior and high-grade lesions.

Table II.13. Distribution of cases according to the presence of metastases at diagnosis

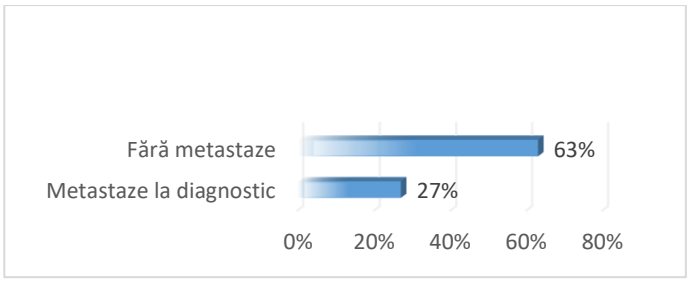


Table II.14. Statistical analysis of overall survival by metastatic disease

Analiza statistică

	Chi-Square	df	Sig.
Log Rank (Mantel-Cox)	29.279	1	<.001
Breslow (Generalized Wilcoxon)	22.822	1	<.001
Tarone-Ware	26.000	1	<.001

Rata de supraviețuire în funcție de boala metastatică

Fig. II.32: Overall survival in metastatic disease

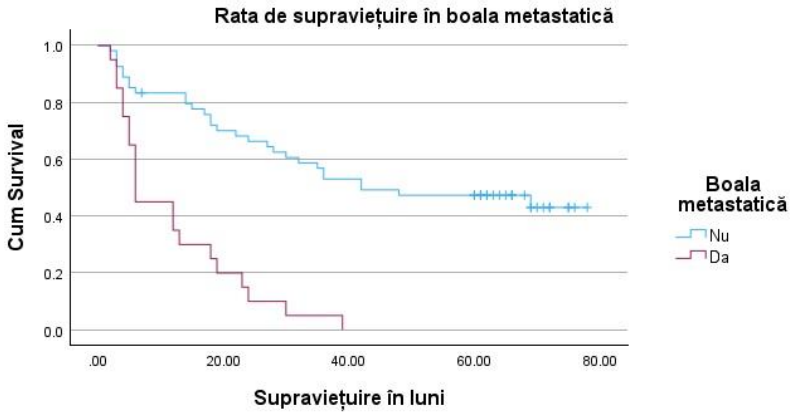
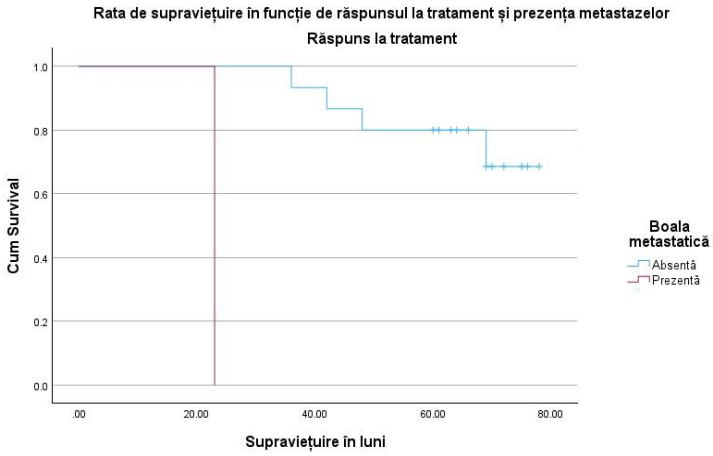
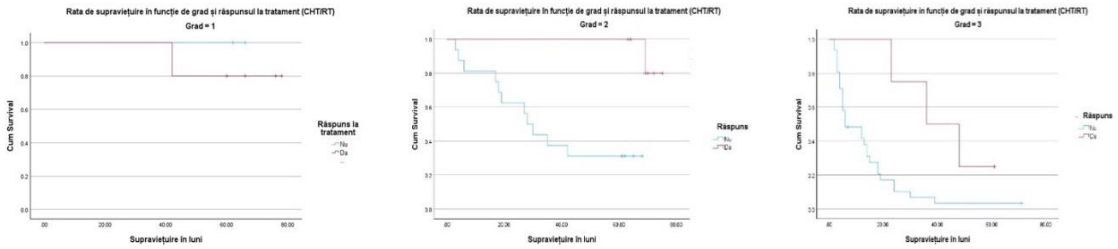


Fig.II.33 Survival rate according to response to treatment



The data supporting the use of neoadjuvant chemotherapy in soft tissue sarcomas largely comes from small retrospective series with very few randomized controlled trials. Results vary between studies. In the present study, the neoadjuvant chemotherapy used did not show benefits on survival rates in patients with disseminated disease.

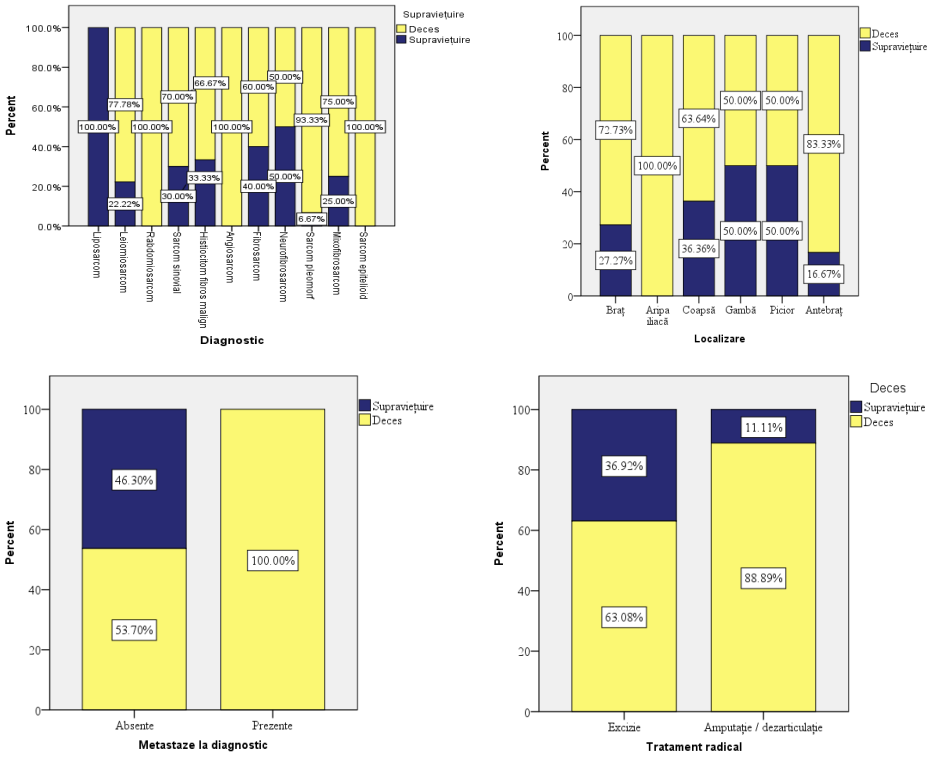
Fig.II.35 Survival by grade and response to treatment

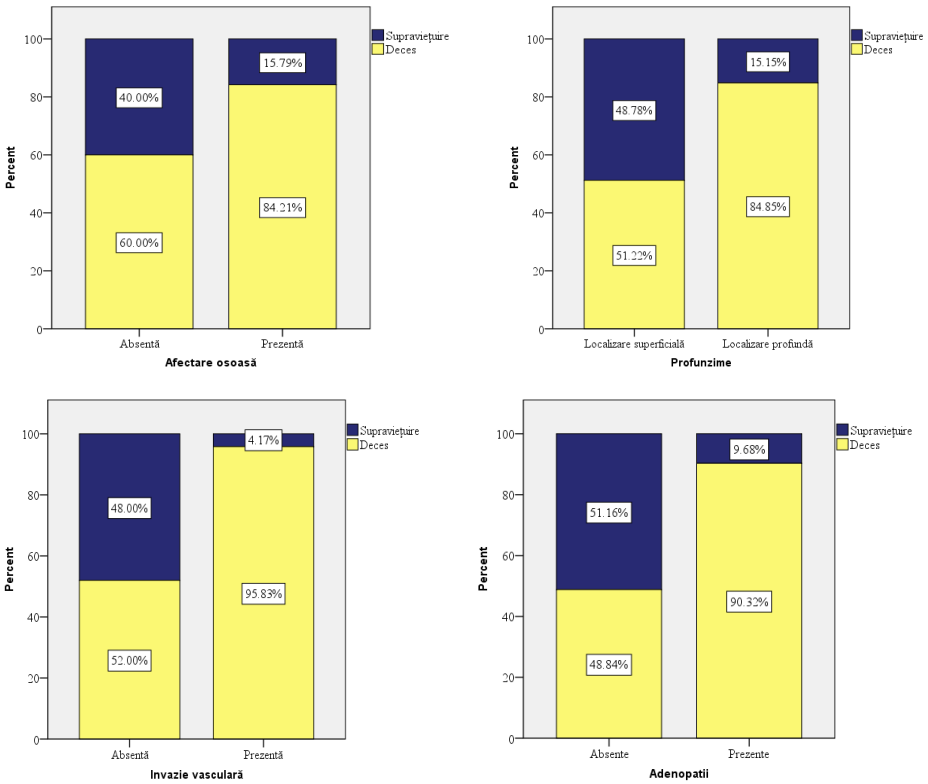


The Kaplan-Meier statistical analysis highlighted a correlation between the grade of aggressiveness and survival rate in the studied cohort. Results for low-grade tumors (G1) did not show significant differences in prognosis. However, significant survival differences were observed in moderate-grade (G2) and high-grade (G3) sarcomas with respect to the response to neoadjuvant treatment.

By the time the study was completed, only 33.78% of patients had survived (n=25), while 66.22% had died (n=49). The majority of deaths occurred within less than 3 years from diagnosis/intervention. Specifically, 40.82% of patients died within 6 months (n=20), 48.98% died within 1-3 years (n=24), and only 10.20% died after more than 3 years (n=5).

Fig, II.49: distribution of deaths/survivors according to other parameters



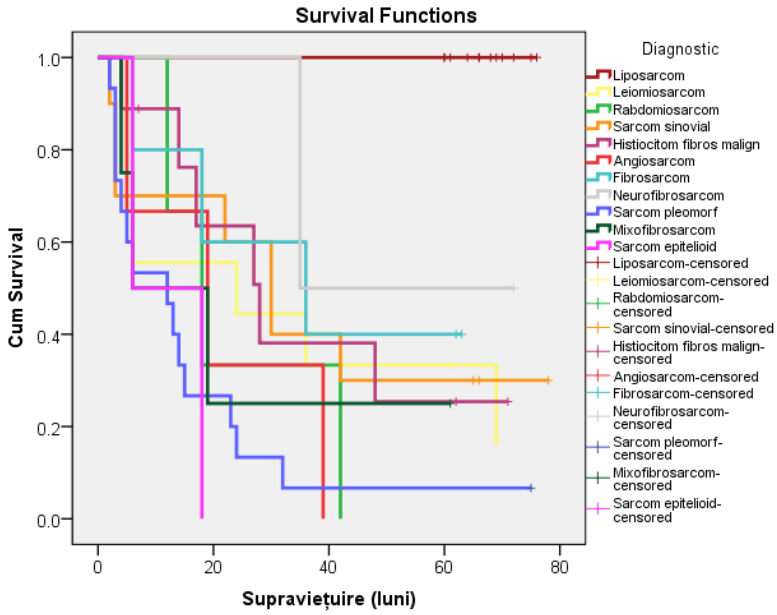


At the end of the study, the following proportions of patients had died:

- 35.14% of women and 32.43% of men;
- 100% of patients who had presented with metastases vs. 53.70% of those without metastases;
- 88.89% of patients who required radical treatment vs. 63.08% of those who had undergone excisions;
- 84.21% of patients with bone involvement vs. 60% of those without bone involvement;
- 84.85% of patients with deep tumor localization vs. 51.22% of those with superficial localization;
- 95.83% of patients with vascular invasion vs. 52% of those without vascular invasion;
- 90.32% of patients with adenopathy vs. 48.84% of those without adenopathy;
- 87.76% of patients with high Ki67, 40% with intermediate Ki67, vs. 0% with low Ki67;
- 76.92% of patients with areas of necrosis vs. 54.29% of those without necrosis;
- 78.18% of patients who had undergone chemotherapy vs. 23.53% of those who had not received chemotherapy;
- 81.63% of patients with an unfavorable response to chemotherapy vs. 31.25% of those with a good response to chemotherapy;
- 62.50% of patients with local recurrence vs. 65.22% of those without recurrence;
- 89.74% of patients with histological grade III, 50% of those with grade II, and 11.11% of those with grade I.

From the time of diagnosis/study entry until death, patients had lived between 2 and 69 months, with a median survival duration of 17.57 months.

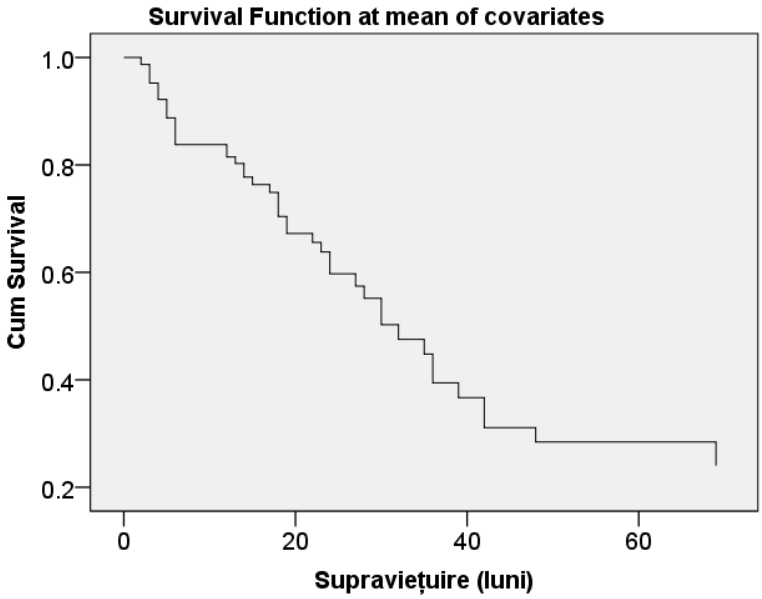
Fig. II.63: Survival curves by diagnosis



A different percentage of censored cases (survivors) were present in the groups with pleomorphic sarcoma (6.7%), liposarcoma (100%), synovial sarcoma (30%), malignant fibrous histiocytoma (33.3%), leiomyosarcoma (22.2%), fibrosarcoma (40%), myxofibrosarcoma (25%), rhabdomyosarcoma (0%), angiosarcoma (0%), neurofibrosarcoma (50%), epithelioid sarcoma (0%).

A Cox regression analysis was conducted to examine the impact of various predictive factors/parameters on the survival time of patients in the studied cohort. The included predictive factors were histological grade, depth, and Ki67 index. The regression model was statistically significant, $\chi^2 = 54.438$, $p \leq 0.001$.

Fig. II.64 – Statistical analysis – Cox regression

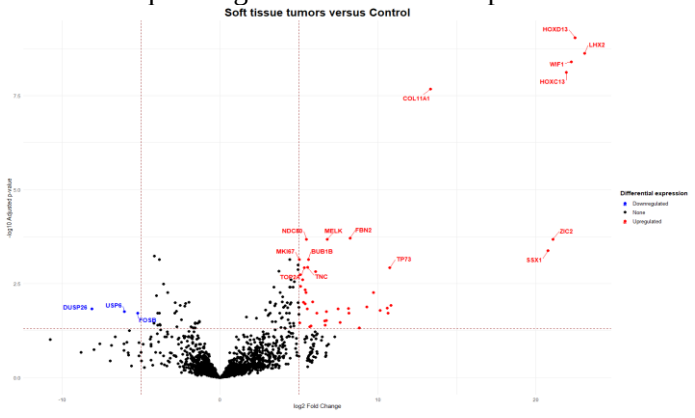


Regression analysis showed that parameters were significantly associated with survival time.

I. Study on the Molecular Analysis of Soft Tissue Sarcomas through Next-Generation Sequencing

Next-generation sequencing (NGS) can provide additional insight into the many gene modifications. To date, there is very little data in the literature describing the use of this technique in the diagnostic/prognostic management of soft tissue sarcomas. This study aims to test the usefulness of next-generation sequencing for identifying mutations that can be targeted within these tumors.
Sequencing data were manually analyzed using DESeq2 after which we developed 3 types of Vulcan diagram and Heat map analysis (minus C analysis – the sample lot analyzed was not comprehensive enough to isolate different genes between the analyzed variants).
Vulcan analysis results display gene expression according to statistical significance of expression differences, as follows:
- X axis (Log2 Fold Change): Represents the degree of change in gene expression between the two compared groups (tumors vs. control). Positive values indicate upregulated genes (expressed more in tumors) and negative values indicate genes downregulated (expressed less in tumors).
- Y-axis (Log10 Adjusted p-value): Represents the statistical significance of expression changes. The higher the value, the more statistically significant the change in expression.
- The dotted lines determine some quadrants that we can use as zones to determine the statistical significance as well as the level of expression. All genes above the horizontal dashed line are sufficiently significant. statistically ($p<0.05$) and all genes located laterally from the vertical dashed lines are sufficiently differently expressed between the two compared groups (FoldChange < -5 or > 5). Genes located at the extremes of the graph (top left and right) are the most relevant, being both statistically significantly different and with a large change in expression.

Fig. III.3 Genomic Sequencing of the Control Group and Soft Tissue Sarcomas

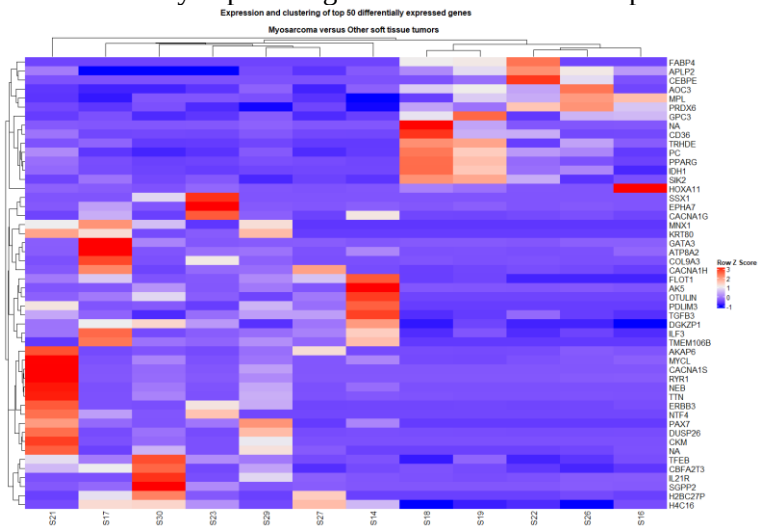


Thus, the most overexpressed genes following analysis A (sarcoma vs. control lot) were:
TOP2A, TNC, BUB1B, MKI67, NDC80, MELK, FBN2, TP73, SSX1, ZIC2, COL11A1, HOXC13, WIF1, LHX2, HOXD13.

Heat map analysis results display gene expression levels in a matrix format, where each cell of the matrix represents the expression level of a gene in a specific sample. Red indicates higher gene expression, while blue indicates lower gene expression. The intensity of the color reflects the level of expression.

- It should be noted that this graph displays gene-by-gene expression, for all samples, but only after the comparison was made by differential analysis (Tumors vs Control)
- The lines above and to the left of the graph attempt to group samples and genes based on their similarity.
- Helps to visualize expression differences between multiple samples simultaneously. It allows the identification of groups of genes with similar expression and the observation of expression patterns specific to certain conditions or tumor types.

Fig. III.4 top 50 most differently expressed genes of the Control Group and Soft Tissue Sarcomas



Expression and clustering of the top 50 differentially expressed genes highlights the presence of BCL6, CNBP, NA, MACROD1, CLTCL1, TPM3, SSX1, SPP1, COL1A1, LHX2, PIMREG, FANCA, MELK, CDC25C, HOXD13, MKI67.

Fig. III. 5 Leiomyosarcoma vs. Group control

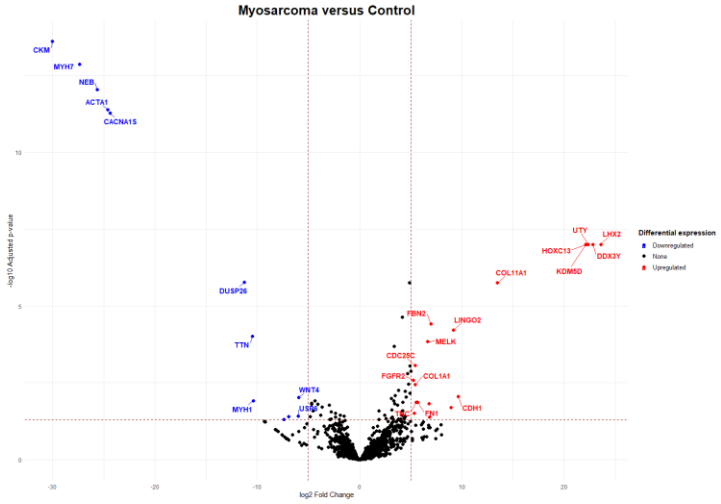
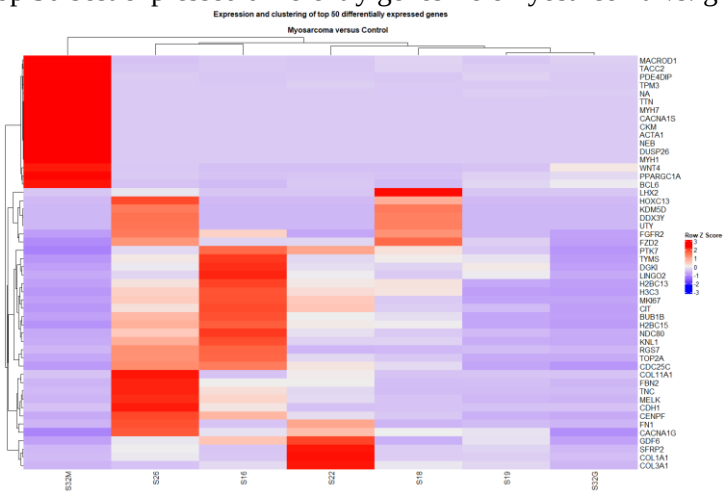
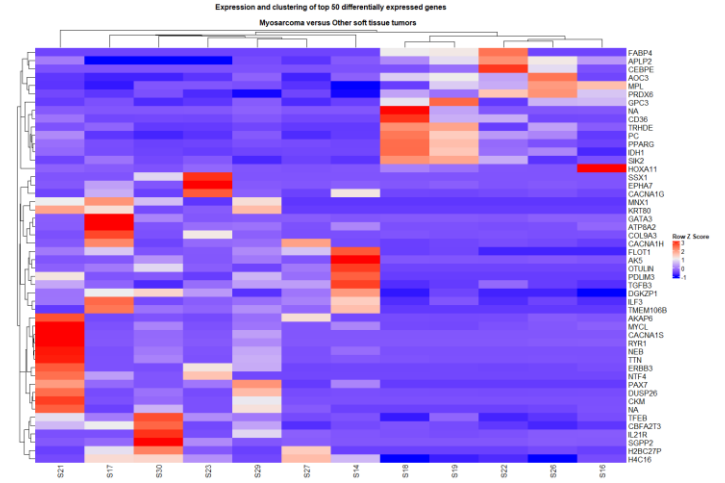


Fig. III.6 Top 50 best expressed differently genes Leiomyosarcoma vs. group control



B analysis results – leiomyosarcoma vs. control lot shows overexpression of FN1, CDH1, FGFR2, CDC25C, MELK, LINGO2, FBN2, COL11A1, KDM50, HOXC13, UTY, LHX2, DDX3Y, HOXC13. The overexpression of the most different genes was highlighted by the presence of MACROD1, TACC2, PDE4DIP, TPM3, NA, TTN, MYH7, CACNA1S, CKM, ACTA1, UTY, FGFR2, PTK7, TOP2A, KNL1.

Fig. III. 7 Top 50 best expressed differently genes – leiomyosarcoma vs. other STS



While the literature has associated COL1A::PDGFB with dermatofibrosarcoma protuberans (DFSP), our findings identified the COL1A2::COL1A1 mutation in a patient with desmoid-type fibromatosis. This discovery suggests a potential link between different collagen gene fusions and distinct types of fibromatosis, which is a precursor lesion to fibrosarcoma. Additionally, we observed the presence of the SS18::SSX1 mutation in a patient diagnosed with synovial sarcoma. The tumor sample was obtained from a 34-year-old woman who was diagnosed with a large palpable mass (123/101/86 mm) located in the posterior compartment of the thigh. Histopathological and immunohistochemical examinations revealed the presence of a biphasic synovial sarcoma, for which the treatment approach included neoadjuvant chemotherapy/radiotherapy followed by surgical excision of the tumor and subsequent adjuvant chemotherapy.

Conclusions and Personal Contributions

Conclusions of the pHd Thesis

This study established the theoretical foundations of research on soft tissue sarcomas from the perspective of modern oncology. The motivation behind choosing this topic is related to the need for a deeper understanding of the pathogenic mechanisms and the clinical, imaging, histopathological, genetic, and molecular diversity of these neoplasms, aiming to develop innovative therapies and optimize patient prognosis.

The research study concluded with the presentation of results and conclusions based on statistical data processing. The scientific research objectives were achieved, allowing us to develop a predictive model for the behavior of certain soft tissue sarcomas. Data analysis highlighted the most important prognostic factors involved in overall survival while assessing the risk of metastasis or secondary developments.

The immunohistochemical markers analyzed were correlated with prognosis as follows:

- Ki67: Evaluation of the proliferative index was statistically correlated with a poor prognosis.
- Positivity of CD4, CD8, and CD34 markers was associated with a lower degree of aggression in soft tissue sarcomas, without tumor margin invasion, thus illustrating a better survival rate among these patients.
- Patients with P53 gene mutations were more frequently predisposed to death and exhibited higher histological grades with tumor margin infiltration, leading to a lower overall survival compared to other patients.

The statistical analysis in our study revealed a lower 5-year overall survival rate among patients with tumor margin infiltration.

Regarding prognostic factors based on patient characteristics, the descriptive analysis of the studied cohort did not reveal a statistically significant correlation with certain characteristics (BMI, AHC, history of local trauma, or presence of associated comorbidities) and 5-year survival due to the limited number of patients. Advanced age was associated with higher mortality.

Histological subtype appears to be one of the most significant factors influencing prognosis. In our study, the prognosis for liposarcoma was generally better. Certain histological subtypes were associated with a higher incidence of metastases, with rhabdomyosarcoma and angiosarcoma being the most frequent, both presenting metastases in 66.7% of cases. There was a statistically significant association between histological subtype and the presence of metastases. The global survival study indicated that a lower 5-year survival rate and a higher-grade sarcoma were associated with tumor size.

At the time of hospital admission, 27% of patients had metastatic disease. Survival analysis highlighted a poor prognosis for patients at advanced stages and differing responses to treatment for each tumor subtype. Most patients, 76.39%, received neoadjuvant chemotherapy. Significant survival differences were found in moderate (G2) and high-grade (G3) sarcomas concerning the response to neoadjuvant treatment. Radical treatment was performed more frequently in patients with bone involvement (21.05%).

Local recurrence was diagnosed in 34.29% of patients (n=24). Notably, about 25% of patients presented to our clinic with already diagnosed local recurrence in other services. A significantly higher incidence of vascular invasion and adenopathy's was observed in patients with local recurrence.

By the end of the study, only 33.78% of patients had survived (n=25), with most deaths occurring within less than 3 years of diagnosis/intervention. At the study's conclusion, deaths were observed among all patients with metastases (100%) compared to 53.70% of those without metastases; 88.89% of patients who required radical treatment; 87.76% of patients with high Ki67, compared to 40% with intermediate Ki67 (vs. 0% with low Ki67). Mortality increased with a higher tumor grade. Patients with liposarcoma differed significantly from other patients, surviving considerably longer. Although the differences were not statistically significant, the graph shows that certain diagnoses had notable survival distributions compared to others, e.g., epithelioid sarcoma had 0% survivors, with a very short time to death compared to other patients (under 20 months).

Data generated by Illumina DRAGEN RNA identified a significant number of variants across all samples, most of which were SNPs (95.4%). The workflow also highlighted several homozygous and heterozygous insertions/deletions (4.6%). The COL1A2::COL1A1, SF3B1::NOM1, and SS18::SSX1 fusions were identified by both DRAGEN and the RNA-Seq alignment pipeline, suggesting high confidence in detecting these fusion events.

Research Directions

Identification and Utilization of New Immunohistochemical Markers: There is a need to identify and utilize new immunohistochemical markers that can significantly impact prognosis and enhance the accuracy of patient outcome predictions.

Assessment of Soft Tissue Sarcoma Extent: Evaluating the extent of sarcomas using advanced imaging techniques such as Diffusion-Weighted Imaging (DWI), Apparent Diffusion Coefficient (ADC), and Dynamic Contrast-Enhanced Imaging (DCE) and analyzing their impact on overall survival.

Next-Generation Genomic Sequencing: Advanced genomic sequencing will be particularly valuable for better stratifying sarcoma subtypes with high genomic complexity and heterogeneity, including undifferentiated sarcomas.

Future of Malignancy Grading: The development and implementation of molecular grading systems for assessing levels of malignancy.

Combining Current Biomarkers with Emerging Discoveries: Integrating existing NGS-based biomarkers with emerging discoveries, such as Tumor Lysis Scores (TLS), will be necessary for more accurate predictions of responses to immune checkpoint inhibitors in sarcomas. Molecular profiling is already routinely used as a predictive factor in evaluating prognosis for epithelial cancers (e.g., breast cancer), where genetic expression tests help to stratify patient risk and guide adjuvant therapy.

Targeted Therapy for Advanced and Metastatic Disease: Currently, chemotherapy for patients with advanced local or metastatic disease has limited efficacy. Identifying molecular abnormalities for which small molecule drugs (targeted therapies) are available or can be developed is essential for improving outcomes in these patients.

Personal Contributions

My personal contributions to this research were crucial in achieving the proposed objectives and advancing knowledge in the treatment of soft tissue sarcomas. The results obtained in this thesis will play a role in designing future clinical studies aimed at optimizing diagnostic and therapeutic management, as well as predicting the behavior of certain tumor entities to improve survival rates and prognosis in soft tissue sarcomas.

Given that statistical data on the incidence, prevalence, survival rates, and mortality for this pathology have not been studied nationally, the goal of this research was to develop a unique database considering the growing patient population in the Orthopedics – Traumatology Clinic at the Bucharest Emergency University Hospital. (Subchapter – Evaluation of Prognostic Factors Based on Patient Characteristics).

By studying certain lymphocytic immunohistochemical markers (CD4, CD8, CD34), I demonstrated that their positivity represented a positive prognostic factor and could also predict an increased survival rate among these patients, thereby opening new research directions for other markers. (Chapter – Study on the Association of Immunohistochemical Markers with Prognosis).

As a result of developing this database, I was able to analyze survival and death rates based on the prognostic factors studied, thereby creating a diagnostic protocol (imaging, histopathological, and

immunohistochemical) that optimized individualized therapeutic management, specific to each tumor entity and adapted to patient characteristics. (Subchapter – Survival and Mortality Rates).

The PhD research also included a molecular analysis of patients from the studied cohort, a unique national-level study with promising results concerning diagnostic and therapeutic management in this pathology. Funding was primarily obtained from personal funds and a research grant won within the doctoral school. (Chapter – Study on the Molecular Analysis of Soft Tissue Sarcomas through Next-Generation Sequencing).

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List of published articles:

Enhancing Diagnosis and Prognosis by Assessing the Clinical, Morphological, and Behavior Aspects in Soft Tissue Sarcomas

– **Serban B**, Popa M, Cursaru A, et al. (July 07, 2024) Enhancing Diagnosis and Prognosis by Assessing the Clinical, Morphological, and Behavior Aspects in Soft Tissue Sarcomas. Cureus 16(7): e64025. doi:10.7759/cureus.64025 (https://www.cureus.com/articles/265485-enhancing-diagnosis-and-prognosis-by-assessing-the-clinical-morphological-and-behavior-aspects-in-soft-tissue-sarcomas#!/) Impact factor 1.2

Practical management of renal cell carcinoma: integrating current approaches with advances in bone metastasis treatment (Eremia, I. A., **Serban, B**., Popa, M., Iancu, A., Nica, S., & Cirstoiu, C. (2024). Practical management of renal cell carcinoma: integrating current approaches with advances in bone metastasis treatment. *EFORT open reviews*, *9*(6), 488–502. https://doi.org/10.1530/EOR-23-0178) Impact Factor: 4.3 (https://eor.bioscientifica.com/view/journals/eor/9/6/EOR-23-0178.xml)

Implications of oral dysbiosis and HPV infection in head and neck cancer: from molecular and cellular mechanisms to early diagnosis and therapy Constantin, M., Chifiriuc, M. C., Mihaescu, G., Vrancianu, C. O., Dobre, E. G., Cristian, R. E., Bleotu, C., Bertesteanu, S. V., Grigore, R., **Serban, B**., & Cirstoiu, C. (2023). Implications of oral dysbiosis and HPV infection in head and neck cancer: from molecular and cellular mechanisms to early diagnosis and therapy. *Frontiers in oncology*, *13*, 1273516. https://doi.org/10.3389/fonc.2023.1273516 https://www.frontiersin.org/journals/oncology/articles/10.3389/fonc.2023.1273516/full Impact factor 3.5

Local recurrence management of extremity soft tissue sarcoma **Serban B**, Cretu B, Cursaru A, Nitipir C, Orlov-Slavu C, Cirstoiu C. Local recurrence management of extremity soft tissue sarcoma. EFORT Open Rev. 2023 Aug 1;8(8):606-614. doi: 10.1530/EOR-23-0095. PMID: 37526250; PMCID: PMC10441250. (https://eor.bioscientifica.com/view/journals/eor/8/8/EOR-23-0095.xml) Impact factor 4.3

Survival prognostic factors and molecular aspects in extremity soft tissue sarcoma"***Journal of Mind and Medical Sciences*** *(JMMS, J Mind Med Sci)* **Bogdan Serban**, Eugen Radu, Adrian Cursaru, Bogdan Stefan Cretu, Sergiu Andrei Iordache, Madalina Cirnu, Cosmin Florentin Niculae, Cătălin Florin Cîrstoiu – în curs de publicare – oct. 2024 (https://scholar.valpo.edu/jmms/) – Impact factor 1.6