CANCER DIAGNOSIS & PROGNOSIS *5*: 833-843 (2025)
doi: 10.21873/cdp.10498

Real World Data from Patients With Osteosarcoma Treated in 8 Medical Centers in Greece: Ninety Percent or Greater Tumor Necrosis Is Associated With Disease-free Survival

ANASTASIOS KYRIAZOGLOU[1*], MYRTO MOUTAFI[1*], IOANNIS KOTSANTIS[1], STEPHANIA KOKKALI[2,3], PINELOPI ANTONOPOULOU[1], GERASIMOS ARDAVANIS[2], ELPIDA MAGKOU[2], ELENI GEORGAKI[3], MARIA ANASTASIOU[1], ANASTASIOS PANTAZOPOULOS[1], ANNA BOULOUTA[1], MARIA KYRKASIADOU[1], NIKI GAVRIELATOU[1], EVANGELOS ZAZAS[1], PANAGIOTA ECONOMOPOULOU[1], ALEXANDROS ARDAVANIS[2], VASILEIOS KONTOGEORGAKOS[4], ELEFTHERIA LAKIOTAKI[5], PENELOPE KORKOLOPOULOU[5], PANAYIOTIS J. PAPAGELOPOULOS[4], AMANDA PSYRRI[1] and IOANNIS BOUKOVINAS[6]

[1]Medical Oncology Unit, 2nd Propaedeutic Department of Medicine, School of Medicine, National and Kapodistrian University of Athens, Attikon University Hospital, Athens, Greece;
[2]First Oncology clinic, Agios Savvas Anticancer Hospital, Athens, Greece;
[3]Oncology Unit, 2nd Department of Internal Medicine, National and Kapodistrian University of Athens, Ippokrateion General Hospital, Athens, Greece;
[4]Orthopaedic Surgery & Traumatology Department, School of Medicine, National and Kapodistrian University of Athens, Attikon University Hospital, Athens, Greece;
[5]Department of Pathology, Medical School, National and Kapodistrian University of Athens, Athens, Greece;
[6]Department of Medical Oncology, Bioclinic, Thessaloniki, Greece

Abstract

Background/Aim: Advanced osteosarcomas tend to have poor prognosis with limited therapeutic options beyond first-line therapy. This retrospective, multi-institutional study aimed to evaluate the association between histological response to chemotherapy and survival outcomes, as well as the influence of sex, tumor size, location, and other factors in a Greek cohort.

Patients and Methods: We retrospectively studied the predictive value of distant metastasis, percentage of necrosis, and grade of tumor in 77 cases of sarcoma treated in 8 medical centers in Greece between 2004 and 2022. Median follow up time from the time of diagnosis was 27 months. Statistical analysis was performed using a two-sided significance level of p=0.05.

Results: Our analysis revealed that short bones were affected significantly more frequently in older [median age=43 years, interquartile range (IQR)=30-50] than younger patients (median age=26 years, IQR=18-40). Distant metastasis

continued

[*]These Authors equally contributed to this work.

Dr. Anastasios Kyriazoglou, Attikon University Hospital, Rimini 1, Chaidari, 12462, Athens, Greece. Tel: +30 2105831655/+30 6932234503, e-mail: tassoskyr@gmail.com; anastasios.kyriazoglou@gmail.com

Received March 3, 2025 | Revised April 16, 2025 | Accepted April 29, 2025

was significantly associated with shorter overall survival [OS; HR=3.7, 95% confidence interval (CI)=1.5-9.16, *p*=0.01]. In addition, we found that 90% or greater tumor necrosis was significantly associated with longer disease-free survival (DFS; HR=0.09, 95% CI=0.01-0.09, *p*=0.003) but not with OS (HR=0.62, 95% CI=0.24-1.58, *p*=0.3). Male sex was associated with shorter DFS (HR=5.61, 95% CI=2.12-14.9), *p*<0.001). Grade or bone affected (long *vs.* short) were not significantly associated with survival.

Conclusion: Osteosarcoma patients with 90% or more tumor necrosis were found to have survival advantage. Differences in DFS between sexes highlight the need for tailored treatment approaches and further exploration of biological underpinnings.

Keywords: Osteosarcoma, tumor necrosis, DFS, prognosis.

Introduction

Osteosarcoma is a rare but aggressive primary bone tumor that predominantly arises in adolescence and young adulthood (1). The disease has a strong predilection for the metaphyses of long bones, with the distal femur, proximal tibia, and proximal humerus being the most commonly affected sites. Osteosarcoma accounts for 56% of all malignant bone tumors, followed by Ewing sarcoma (34-36%), and chondrosarcoma (less than 10%) (2). While it represents less than 1% of all cancer cases diagnosed annually in the United States, it contributes to approximately 3% of childhood cancers. In Greece, an estimated 30-50 new cases are diagnosed each year, accounting for approximately 0.05% to 0.08% of all new cancer cases in the country (2). The absence of centralized cancer registries may hinder comprehensive data collection.

When treated with surgery alone, more than 80% of osteosarcoma patients progress despite achieving local tumor control. It is suggested that subclinical metastatic disease is present early in the disease course and systemic chemotherapy can eradicate these tumor deposits when tumor burden is low. Two randomized studies in the 1980s showed that systemic chemotherapy offered survival benefit in osteosarcoma patients in the adjuvant setting. Later neoadjuvant chemotherapy served as a method to increase suitable surgical candidates by diminishing tumor. Moreover, the response to neoadjuvant chemotherapy is a major prognostic factor. With modern therapy, more than 60% of non-metastatic osteosarcoma patients will be long-term survivors (3-7). Various prognostic factors have been linked to survival outcomes, including tumor size, presence of metastases at diagnosis, histological subtype (8, 9), histological grade, response to neoadjuvant chemotherapy, and the adequacy of surgical margins (10-13). The grade of histological response to neoadjuvant chemotherapy appears to correlate significantly with prognosis.

The histological response to primary treatment is typically evaluated based on persistence or absence of viable tumor cells (total necrosis). This is often expressed as a percentage of tumor necrosis, which has been correlated with prognosis (14-19). Histological responsiveness to neoadjuvant or induction chemotherapy is a major determinant of prognosis (15, 19-26) . Different grading systems have been used to evaluate the value of histological response. A 4-grade scale (Grade 1: little or no evidence of necrosis; Grade 2: necrosis of 50%-90%; Grade 3: necrosis between 90%-99%; finally, Grade 4: 100% necrosis) is included in Huvos system (27), a 6-grade scale (Grade I: 100% necrosis, complete response to Grade IV: no response, all tumor cells viable) was used by Salzer-Kuntschik (28) ,and a 3-grade scale (Grade I: complete response to Grade IV: poor or no response) by Ayala (29). Several studies (13, 19, 20, 25, 30-32) have used these grading systems and translated these data into percentages of necrotic tissue, thus good responder patients are typically defined as those with necrosis ≥95% (19, 21-23), ≥85-90% (17, 23, 24, 30, 31, 33) or ≥60% (20, 25).

The present study aimed to examine the applicability of established findings and analyze the associations between various clinical and pathological factors within the Greek population. Therefore, this study represents the most comprehensive analysis of osteosarcoma patients in Greece to date, from eight centers across the country. The inclusion of multiple centers allowed for a broad assessment of osteosarcoma management within Greece, capturing variations in treatment protocols, histological assessment practices, and patient outcomes. The findings of this multi-center approach not only reflect the national trends but also contribute to the global understanding of osteosarcoma, particularly in Mediterranean populations.

Patients and Methods

Patient selection. From 2004 to 2022, 77 osteosarcoma patients were treated in 8 medical centers in Greece: Attikon General University Hospital of Athens, General Anticancer Oncological Hospital "Agios Savvas", Ippokrateio General Hospital of Athens, BIOCLINIC General Hospital of Athens, MITERA Hospital, Metropolitan Athens Hospital, Children's Hospital Agia Sophia Athens, General University Hospital of Larissa. Medical records were reviewed retrospectively to assess patient-related, treatment-related, and survival variables. All diagnoses were confirmed by a tissue pathological examination in each center. This study was approved by Attikon University hospital ethics committee (EBΔ210/27-03-2023). Written informed consent was given from each patient to collect and analyze clinical data.

Histological diagnosis. All patients included underwent a biopsy (core needle or surgical) and histological classification was established according to the World Health Organization classification of 2013. Histopathologic slides of patients treated before 2013 were reviewed according to the new classification. Osteosarcomas were classified as classic/conventional, telangiectatic (TOS), osteoblastic (OSS), chondroblastic (COS), fibroblastic,

Table I. *Characteristics of the osteosarcoma patients included in the study (N=77).*

	Characteristic	n (%)
Age*		31 (19, 48)
	Unknown	2
Sex	Female	30 (45%)
	Male	37 (55%)
	Unknown	10
OST	Central	2 (3.6%)
	CONVENTIONAL/CLASSIC	14 (24.8%)
	COS	8 (14%)
	COS/OSS	1 (1.8%)
	CS	1 (1.8%)
	Extraskeletal	3 (5.4%)
	Fibroblastic	3 (5.4%)
	GCRO	1 (1.8%)
	Mixed	2 (3.6%)
	OSS	14 (25%)
	PAR/Periosteal	5(8.9%)
	TOS	1 (1.8%)
	Undifferentiated	1 (1.8%)
	Unknown	21
Location	Long bones	51 (68%)
	Short bones	24 (32%)
	Unknown	2
Surgery	Yes	66 (93%)
	No	4 (7%)
	Unknown	6
Systemic treatment	Neoadjuvant	52 (68%)
	1st line	32 (42%)
	2nd line	16 (21%)
	3rd line	5 (6.5%)
Grade	Grade 1-2	9 (19%)
	Grade 3	39 (81%)
	Unknown	29
Stage	I	4 (7.3%)
	II	30 (54.5%)
	III	11(20%)
	IV	10 (18.2%)
	Unknown	22
Tumor size (cm)	≥10	4 (12%)
	<10	30 (88%)
	Uknown	43
Necrosis ≥90%	Yes	10 (30%)
	No	23 (70%)
	Uknown	44
	OS* (months)	27 (14, 45)
	Uknown	12
	DFS* (months)	19 (8, 37)
	Uknown	12
	1 Median (IQR); n (%)	

*Data presented as mean (interquartile range). OST, Osteosarcoma; TOS, telangiectatic osteosarcoma; OSS, osteoblastic osteosarcoma; COS, chondroblastic osteosarcoma; CS, extraskeletal chondrosarcoma; GCRO, giant cell-rich osteosarcoma; OS, overall survival; DFS, disease free survival; IQR, interquartile range.

extraskeletal, chondrosarcoma (CS), undifferentiated, mixed, periosteal and giant cell-rich osteosarcoma (GCRO). Their characteristics are shown in Table I.

Preoperative evaluation and chemotherapy. Preoperative chemotherapy was based on Rosen regimens (including methotrexate, cisplatin, doxorubicin) or API/AI regimens (cyclophosphamide, doxorubicin, ifosfamide), depending on the age of the patient. Patients aged under 18 years were treated with methotrexate-based regimens. Patients older than 30 years received API/AI chemotherapy. The majority of intermediate aged patients (18-30 years) were treated with methotrexate regimen, according to physician's choice.

Surgery. After preoperative chemotherapy, complete restaging was performed to assess the treatment response. The patients underwent a surgical excision (amputation or limb-sparing surgery) depending on the location and extension of the tumor, neurovascular bundle involvement, and the age and lifestyle of the patient in an expert center. Afterwards they received adjuvant chemotherapy.

Assessment of histological response to chemotherapy. Standardized pathological evaluated protocols, similar to the criteria by Huvos, were used to assess tumor response. Surgical specimens from resections were processed, and the cut surfaces were carefully examined macro- and microscopically, as per classical pathological assessment. Multiple sections were taken from areas most representative of the tumor. Tumor response to neoadjuvant chemotherapy was categorized as either good (≥90% tumor necrosis or minimal viable tumor) or poor (<90% tumor necrosis or significant viable tumor). A good histological response corresponded to the grade III and IV criteria of Huvos, whereas a poor response corresponded to Grades I and II (27).

Postoperative chemotherapy. Patients received postoperative chemotherapy with the same regimens used preoperatively. Postoperative evaluation included clinical evaluation and computed tomography (CT) scans of the chest and operated limb unless otherwise indicated.

After completion of the adjuvant treatment, patients were followed as outpatients every 3 months for 3 years, and then every 6 months. During these evaluations, a plain radiography or CT scanning of the involved limb and of the chest was performed.

Statistical analysis. Continuous variables are presented as median (interquartile range; IQR) and categorical variables as counts and percentages. The Kaplan-Meier method with 95% confidence intervals (CIs) was employed to estimate OS and DFS. OS was defined as the time from the diagnosis to death from any cause and was censored at the date of the latest follow-up. DFS was defined as the time from diagnosis to local recurrence, distant metastasis, or the date of death and the cut-off was made at the date of the latest follow-up. The log-rank test and Cox proportional hazard model were used to assess prognostic factors. Factors were categorized according to previous reports. Log-rank tests were applied for sex, age (<30 years or ≥30 years), primary tumor site (short *vs.* long bone), maximum tumor diameter (<10 cm or ≥10 cm), tumor necrosis (<90% or ≥90%). The Wilcoxon Rank Sum test was used to compare differences in continuous variables (*e.g.*, age) between groups. Significant factors in univariate analyses were entered into a multivariate Cox proportional hazard model. Patients with missing data were excluded from the corresponding analyses. The sample size is clearly reported in the results of each analysis. Statistical analysis was performed using R 3.5.3 (R Foundation for Statistical Computing, Vienna, Austria), with significance level set at $p<0.05$.

Institutional Review Board Statement. Ethical review and approval were waived for this study due to its retrospective design and use of anonymized data, which does not involve direct interaction with human or animal subjects.

Informed consent statement. Informed consent was obtained from all participants involved in the study.

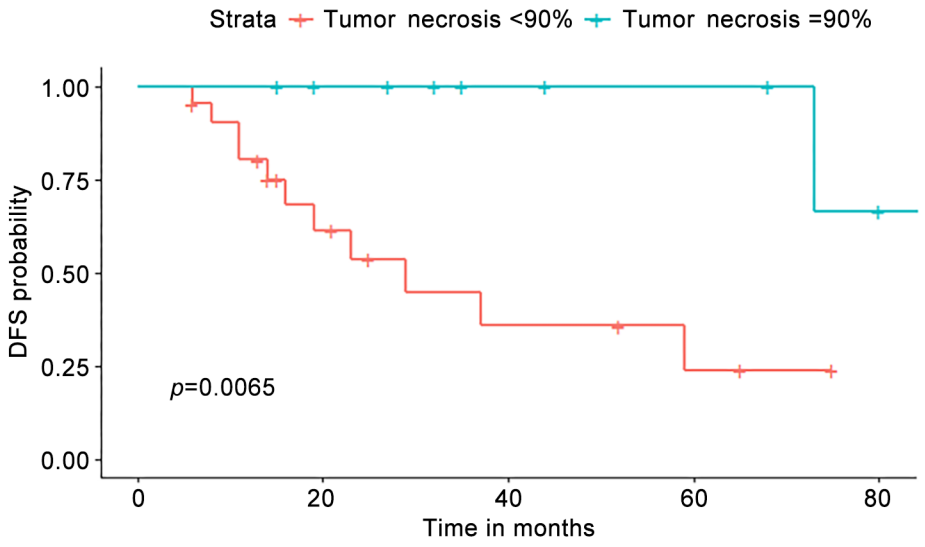

Figure 1. *Kaplan-Meier estimates of disease-free survival (DFS) according to the percentage of tumor necrosis.*

Results

Patients' characteristics. Seventy-seven osteosarcoma patients were included in this study. The median age was 31 (IQR=19-48) (Table I). Thirty patients were female (45%) and 37 were male (55%), aligning with the general male predominance observed in osteosarcoma (male-to-female ratio 1.4:1). Median DFS and OS were 19 and 27 months respectively. The histological subtype was osteoblastic in 14 patients (25%), chondroblastic in 8 patients (14%), fibroblastic in 3 (5.4%) patients and other subtypes in 31 patients (55%). Short bones were affected in 24 patients (32%) and long bones in 51 patients (68%). Overall, 66 (93%) of patients underwent surgery. Metastasis was noted in 10 out of 55 evaluable patients (18%).

Association with outcome. Initial analysis of the data revealed that patients with osteosarcoma involving short bones were significantly older (median age=43 years, IQR=30-50) than those with tumors in long bones (median age=26 years, IQR=18-40; p=0.006, Wilcoxon rank-sum test). Tumor necrosis rate was greater than 90% in 10 out of 32 evaluable patients (30%) according to Huvos criteria. The tumor size was greater than 10 cm in 4 out of 29 evaluable patients (12%). We therefore investigated whether the relationship between sex, age at the time of diagnosis, tumor size, tumor location, histological subtypes, histological response to chemotherapy, is associated with DFS and OS. Our analysis demonstrated that 90% or greater tumor necrosis was significantly associated with longer DFS (HR=0.09, 95% CI=0.09-0.01, p=0.003) but not with OS (HR=0.62, 95% CI=0.24-1.58, p=0.3). Sex also emerged as a significant predictive factor, associated with DFS, with male patients having shorter DFS than female patients (HR=5.61, 95% CI=2.12-14.9, p<0.001) (Figure 1, Figure 2). Age, grade, tumor size or bone affected (long *vs* short) were not significantly associated with survival. Distant metastasis was associated significantly with shorter OS (HR=3.7, 95% CI=1.5-9.16, p=0.01) (Table II, Table III).

Discussion

In line with the evolving use of limb-sparing surgery, the introduction of neoadjuvant chemotherapy for osteosarcoma, coupled with the preoperative evaluation of tumor response, provides critical insights into tumor

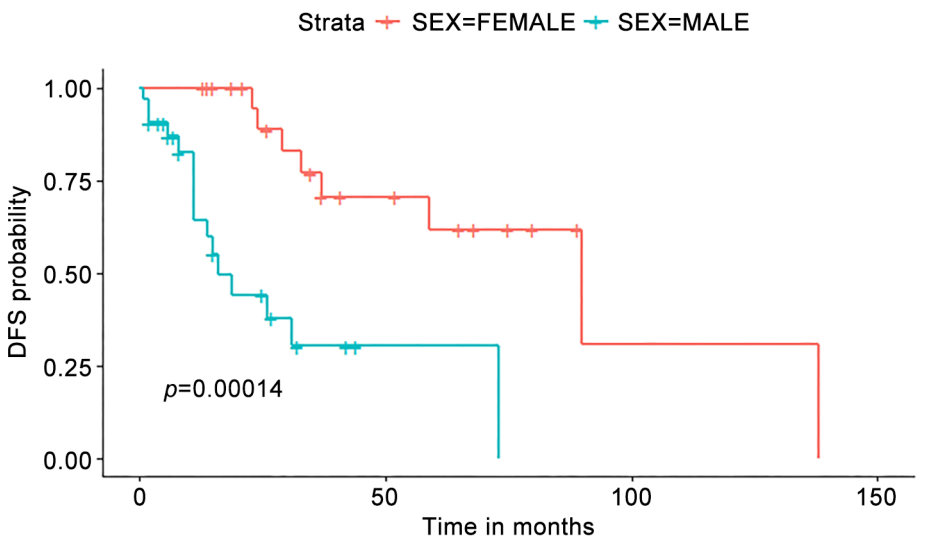

Figure 2. *Kaplan-Meier estimates of disease-free survival (DFS) according to sex.*

Table II. *Univariate analysis of overall survival (OS) and disease-free survival (DFS).*

Variable	Group	N	OS		DFS	
			HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value
Location	Short bones	65	–	0.3	–	0.7
	Long bones		0.72 (0.38-1.37)		0.83 (0.37-1.86)	
Grade	Low-intermediate	44	–	0.3	–	0.6
	High		0.66 (0.29-1.47)		1.51 (0.34-6.69)	
Tumor Size (cm)	≥10	29	–	0.9	–	0.1
	<10		1.11 (0.25-4.88)		0 (0.00-Inf)	
Stage	I-III	49	–	**0.01**	–	0.089
	IV		3.7 (1.50-9.16)		3.02 (0.94-9.67)	
Sex	Female	56	–	0.3	–	**<0.001**
	Male		1.47 (0.75-2.87)		5.61 (2.12-14.90)	
Age		64	1.01 (0.99-1.03)	0.4	1 (0.98-1.03)	0.8
Tumor necrosis	≥90%	32	–	0.3	–	**0.003**
	<90%		0.62 (0.24-1.58)		0.09 (0.01-0.75)	

Statistically significant *p*-values are shown in bold. HR: Hazard ratio; CI: confidence interval.

drug sensitivity and disease prognosis. Our study demonstrated a significant difference in survival outcomes between osteosarcoma patients achieving more than 90% tumor necrosis and those with less than 90% necrosis, as evaluated using the Huvos grading system. Consistent with previous findings from a U.S. study (14), multivariate analysis revealed that 90% and higher tumor necrosis rate is an independent predictor for DFS.

Tumor necrosis threshold has been debatable across different populations and healthcare settings. In a retrospective investigation comprising 438 osteosarcoma patients, individuals exhibiting less than a 50% necrosis rate demonstrated inferior OS compared to cohorts with varying grades of necrosis (50-75%, 75-98% or 98-100%) (34). Due to the limited sample size in our study group, it is challenging to definitively conclude that a tumor

Table III. *Multivariate Cox regression analysis for disease-free survival.*

Variable	HR (95% CI)	*p*-Value
Sex		**<0.001**
Female	–	
Male	23.8 (2.71- 209)	
Tumor necrosis (%)		**<0.001**
≥90%	–	
<90%	0.02 (0.00-0.29)	

Statistically significant *p*-values are shown in bold. HR: Hazard ratio; CI: confidence interval.

necrosis rate of 90% or higher is the optimal cutoff for predicting disease-free survival (DFS). However, when considered alongside the findings of previous studies (14, 17, 23, 24, 30, 31, 33), using 90% necrosis as a surrogate outcome measure appears to be a reasonable approach.

Recently a deep-learning model (DLM)-based evaluation of viable tumor cell density suggested alternative approaches to overcome limitations of traditional histological tumor necrosis assessment that often relies on pathologists' "eyeball assessment" of viable *vs.* necrotic tumor areas across tissue sections (35). The researchers found that in grade II cases (necrosis rate: 50-90%) DLM could better predict prognosis than traditional pathologist assessment. In these low necrosis rate osteosarcomas with incomplete tumor cell necrosis that would be falsely identified as viable, the DLM would more accurately assess and reflect cell death (35). However, the tumor cell density did not show association with prognosis. Advanced artificial intelligence (AI) techniques may address heterogeneity in assessment of tumor necrosis and improve prognostic accuracy (36).

Only 30-50% of patients are reported to respond well to neoadjuvant chemotherapy (14, 37), with the rest experiencing worse outcomes and high risk of recurrence or metastasis (8, 26). Poor response to neoadjuvant treatment in osteosarcoma is still a topic of interest both translationally and clinically. A phase 3 trial comparing MAP (methotrexate, cisplatin, doxorubicin) with MAPIE (methotrexate, cisplatin, doxorubicin, ifosfamide and etoposide) did not manage to show any benefit for patients who had a poor histological response to chemotherapy (3). Event-free survival was not better for the poor responders who received the addition of ifosfamide and etoposide, underscoring the complexity of management of chemo-resistant osteosarcomas. The poor response to neoadjuvant chemotherapy, particularly to key agents like cisplatin and doxorubicin might indicate both biological and therapeutic causes. Patient-derived cell lines show different expression and mutational profiles in cisplatin and doxorubicin resistant models (38). Resistance to neoadjuvant treatment remains an unsolved problem and highlights the need for biomarkers to identify the best candidates for preoperative management, allowing treatment stratification and alternative therapeutic strategies. Patients with poor response to neoadjuvant treatment tend to recur/metastasize after the end of adjuvant chemotherapy. Therapeutic options for these patients remain limited, demonstrating modest clinical benefit, with the majority of studies to show a median progression-free survival (mPFS) below 7 months (39). Targeted therapies and immunotherapy have shown controversial success in improving the outcome (40).

Sex was also associated with prognosis in our study, emphasizing the potential role of biological factors such as immune response differences or sex-specific osteosarcoma biology. Female patients were more likely to have longer DFS than male patients. Various studies, highlight sex differences in immunity (37, 41, 42). Females exhibit more robust innate and adaptive immune responses than males, evidenced by fewer early childhood infections and higher autoimmune diseases prevalence as adults (41, 43-45). When considering sex-specific treatment strategies, differences in gene expression and immune related pathways have been noted in osteosarcoma. These genes include *CDK4* (46), *LCK* (47, 48), ROS1 (47, 48), *FLT3* (47) and *TP53* (46), which are targeted by therapies either approved or under clinical investigation in sarcomas and other cancers (41).

Interestingly, tumor size, histological subtype, and bone location were not significantly associated with survival in the Greek cohort. This might be due to sample size, population characteristics or different treatment strategies

and warrants further investigation. In addition, the observation that older patients are more likely to have short bone involvement is an intriguing finding of age-related patterns in osteosarcoma presentation (49). In addition, the presence of metastasis had a negative impact on OS in our cohort, as expected, in line with previous studies (50).

Although not explicitly analyzed in our study, regional and socioeconomic factors have been shown to play an important role in histological response and survival (14, 37). Previous study in the U.S. has demonstrated that patients with lower socioeconomic status had significantly lower rates of achieving more than 90% tumor necrosis (14). These findings suggest that non-biological factors, including disparities in access to care, treatment timing, and healthcare infrastructure, may influence treatment outcomes. In our cohort 30% of the patients achieved 90% or greater necrosis, compared to 35% of osteosarcoma patients in the U.S. National Cancer Database cohort (14). The slightly lower rate in the Greek cohort might reflect differences in regional treatment practices and healthcare access.

Our study has several caveats mainly due to its retrospective character. The patients included did not receive the same regimen; the use of methotrexate was not decided with universal criteria but according to physician's choice. Furthermore, histological assessment was not performed by the same pathologist. Thus, grading and the exact subtype of osteosarcoma in each case may be a matter of discrepancies. Additionally, this is a study of 77 cases only, which makes the extraction of conclusions weak. While acknowledging these constraints, our study provides valuable insights for the management of osteosarcoma in Greece and establishes a foundation for future prospective investigations to validate and expand upon these findings, such as optimizing chemotherapy regimens or incorporating novel agents targeting key molecular pathways.

Conclusion

This study reinforces the value of achieving a cutoff of 90% necrosis as a treatment goal as it might serve as an independent prognostic value for patients with osteosarcoma undergoing chemotherapy. Poor response to neoadjuvant chemotherapy remains a challenge highlighting the need for innovative therapeutic approaches. Molecularly targeted therapies, immune checkpoint inhibitors, and advanced drug delivery systems, could provide new avenues for improving response rates and overall survival. Furthermore, in our study population, male osteosarcoma patients were associated with worse DFS compared to female patients. The mechanisms responsible for this association may be linked to variations in tumor biology or immune response. A thorough understanding of these mechanisms is crucial in developing tailored strategies that can optimize outcomes for both males and females with osteosarcoma.

While our study provides valuable insights into the management of osteosarcoma, it is important to acknowledge the need for further larger, multi-institutional studies, to strengthen the generalizability of these findings. Additionally, integrating emerging technologies such as AI in pathological tumor assessment will be crucial in enhancing diagnostic accuracy and improving treatment outcomes across diverse populations.

Conflicts of Interest

The Authors declare no conflicts of interest.

Authors' Contributions

AK and MM: Conceptualization, Methodology, Software, Writing-Original draft preparation. MM, AK, AP: Supervision. IK, PO, SK, PA, GA, EM, EG, MA, AP, AB, MK, NG, EZ, AA, VK, EL, PK, PJP, IB, AP: Writing-Reviewing and Editing.

Funding

This study was funded by the Hellenic Group of Sarcomas and rare tumors.

Artificial Intelligence (AI) Disclosure

During the preparation of this manuscript, a large language model (ChatGPT, OpenAI) was used solely for language editing and stylistic improvements in select paragraphs. No sections involving the generation, analysis, or interpretation of research data were produced by generative AI. All scientific content was created and verified by the authors. Furthermore, no figures or visual data were generated or modified using generative AI or machine learning-based image enhancement tools.

References

1 Choi JH, Ro JY: The 2020 WHO Classification of Tumors of Soft Tissue: Selected changes and new entities. Adv Anat Pathol 28(1): 44-58, 2021. DOI: 10.1097/pap.0000000000000284

2 Howlader N, Noone AM, Krapcho M, Miller D, Brest A, Yu M, Ruhl J, Tatalovich Z, Mariotto A, Lewis DR, Chen HS, Feuer EJ, Cronin KA: Seer cancer statistics review, 1975-2018, National Cancer Institute. SEER Cancer Statistics Review, 1975-2018, National Cancer Institute. Bethesda, MD, USA. Available at: https://seer.cancer.gov/csr/1975_2018/, based on November 2020 SEER data submission, posted to the SEER web site, April 2021 [Last accessed on April 29, 2025]

3 Marina NM, Smeland S, Bielack SS, Bernstein M, Jovic G, Krailo MD, Hook JM, Arndt C, van den Berg H, Brennan B, Brichard B, Brown KLB, Butterfass-Bahloul T, Calaminus G, Daldrup-Link HE, Eriksson M, Gebhardt MC, Gelderblom H, Gerss J, Goldsby R, Goorin A, Gorlick R, Grier HE, Hale JP, Hall KS, Hardes J, Hawkins DS, Helmke K, Hogendoorn PCW, Isakoff MS, Janeway KA, Jürgens H, Kager L, Kühne T, Lau CC, Leavey PJ, Lessnick SL, Mascarenhas L, Meyers PA, Mottl H, Nathrath M, Papai Z, Randall RL, Reichardt P, Renard M, Safwat AA, Schwartz CL, Stevens MCG, Strauss SJ, Teot L, Werner M, Sydes MR, Whelan JS: Comparison of MAPIE *versus* MAP in patients with a poor response to preoperative chemotherapy for newly diagnosed high-grade osteosarcoma (EURAMOS-1): an open-label, international, randomised controlled trial. Lancet Oncol 17(10): 1396-1408, 2016. DOI: 10.1016/S1470-2045(16)30214-5

4 Bruland ØS, Høifødt H, Sæter G, Smeland S, Fodstad Ø: Hematogenous micrometastases in osteosarcoma patients. Clin Cancer Res 11(13): 4666-4673, 2005. DOI: 10.1158/1078-0432.Ccr-05-0165

5 Cade S: Osteogenic sarcoma; a study based on 133 patients. J R Coll Surg Edinb 1(2): 79-111, 1955.

6 Isakoff MS, Bielack SS, Meltzer P, Gorlick R: Osteosarcoma: Current treatment and a collaborative pathway to success. J Clin Oncol 33(27): 3029-3035, 2015. DOI: 10.1200/JCO.2014.59.4895

7 Yonemoto T, Tatezaki S, Ishii T, Satoh T, Kimura H, Iwai N: Prognosis of osteosarcoma with pulmonary metastases at initial presentation is not dismal. Clin Orthop Relat Res 349: 194-199, 1998. DOI: 10.1097/00003086-199804000-00024

8 Bacci G, Bertoni F, Longhi A, Ferrari S, Forni C, Biagini R, Bacchini P, Donati D, Manfrini M, Bernini G, Lari S: Neoadjuvant chemotherapy for high-grade central osteosarcoma of the extremity. Cancer 97(12): 3068-3075, 2003. DOI: 10.1002/cncr.11456

9 Hauben EI, Weeden S, Pringle J, Van Marck EA, Hogendoorn PC: Does the histological subtype of high-grade central osteosarcoma influence the response to treatment with chemotherapy and does it affect overall survival? A study on 570 patients of two consecutive trials of the European Osteosarcoma Intergroup. Eur J Cancer 38(9): 1218-1225, 2002. DOI: 10.1016/s0959-8049(02)00037-0

10 Eleutério SJ, Senerchia AA, Almeida MT, Da Costa CM, Lustosa D, Calheiros LM, Barreto JH, Brunetto AL, Macedo CR, Petrilli AS: Osteosarcoma in patients younger than 12 years old without metastases have similar prognosis as adolescent and young adults. Pediatr Blood Cancer 62(7): 1209-1213, 2015. DOI: 10.1002/pbc.25459

11 Xin S, Wei G: Prognostic factors in osteosarcoma: A study level meta-analysis and systematic review of current practice. J Bone Oncol 21: 100281, 2020. DOI: 10.1016/j.jbo.2020.100281

12 Letaief F, Khrouf S, Yahiaoui Y, Hamdi A, Gabsi A, Ayadi M, Mezlini A: Prognostic factors in high-grade localized osteosarcoma of the extremities: the Tunisian experience. J Orthop Surg 28(3): 2309499020974501, 2020. DOI: 10.1177/2309499020974501

13 Lindner NJ, Scarborough MT, Spanier SS, Enneking WF: Local host response in osteosarcoma after chemotherapy referred to radiographs, CT, tumour necrosis and patient survival. J Cancer Res Clin Oncol 124(10): 575-580, 1998. DOI: 10.1007/s004320050217

14 Richardson SM, Wurtz LD, Collier CD: Ninety percent or greater tumor necrosis is associated with survival and social determinants of health in patients with osteosarcoma in the National Cancer Database. Clin Orthop Relat Res 481(3): 512-522, 2023. DOI: 10.1097/CORR.0000000000002380

15 Bacci G, Mercuri M, Longhi A, Ferrari S, Bertoni F, Versari M, Picci P: Grade of chemotherapy-induced necrosis as a predictor of local and systemic control in 881 patients with non-metastatic osteosarcoma of the extremities treated with neoadjuvant chemotherapy in a single institution. European Journal of Cancer 41(14): 2079-2085, 2005. DOI: 10.1016/j.ejca.2005.03.036

16 LiBrizzi CL, Wang Z, Sulam J, James AW, Levin AS, Morris CD: The use of weakly supervised machine learning for necrosis assessment in patients with osteosarcoma: A pilot study. J Orthop Res 42(2): 453-459, 2024. DOI: 10.1002/jor.25693

17 Li X, Ashana AO, Moretti VM, Lackman RD: The relation of tumour necrosis and survival in patients with osteosarcoma. Int Orthop 35(12): 1847-1853, 2011. DOI: 10.1007/s00264-011-1209-7
18 Samimi MA, Mirkheshti N, Pazouki A: Assessing the percent of necrosis after neoadjuvant chemotherapy with 24hr infusional cisplatin/3 days doxorubicin intermittent with ifosfamide-doxorubicin for osteosarcoma. Int J Hematol Oncol Stem Cell Res 8(1): 5-8, 2014.
19 Rosen G, Caparros B, Huvos AG, Kosloff C, Nirenberg A, Cacavio A, Marcove RC, Lane JM, Mehta B, Urban C: Preoperative chemotherapy for osteogenic sarcoma:Selection of postoperative adjuvant chemotherapy based on the response of the primary tumor to preoperative chemotherapy. Cancer 49(6): 1221-1230, 1982. DOI: 10.1002/1097-0142(19820315)49:6<1221::aid-cncr2820490625>3.0.co;2-e
20 Winkler K, Beron G, Kotz R, Salzer-Kuntschik M, Beck J, Beck W, Brandeis W, Ebell W, Erttmann R, Göbel U: Neoadjuvant chemotherapy for osteogenic sarcoma: results of a Cooperative German/Austrian study. J Clin Oncol 2(6): 617-624, 1984. DOI: 10.1200/jco.1984.2.6.617
21 Bacci G, Picci P, Ferrari S, Ruggieri P, Casadei R, Tienghi A, Brach del Prever A, Gherlinzoni F, Mercuri M, Monti C: Primary chemotherapy and delayed surgery for nonmetastatic osteosarcoma of the extremities. Results in 164 patients preoperatively treated with high doses of methotrexate followed by cisplatin and doxorubicin. Cancer 72(11): 3227-3238, 1993. DOI: 10.1002/1097-0142(19931201)72:11<3227::aid-cncr2820721116>3.0.co;2-c
22 Saeter G, Alvegård TA, Elomaa I, Stenwig AE, Holmström T, Solheim OP: Treatment of osteosarcoma of the extremities with the T-10 protocol, with emphasis on the effects of preoperative chemotherapy with single-agent high-dose methotrexate: a Scandinavian Sarcoma Group study. J Clin Oncol 9(10): 1766-1775, 1991. DOI: 10.1200/jco.1991.9.10.1766
23 Kalifa C, Razafindrakoto H, Vassal G, Contesso G, Vanel D, Edeline V, Valteau D, Lemerle J: Chemotherapy in osteogenic sarcoma: The experience of the Pediatric Department of the Gustave Roussy Institute. Cancer Treat Res 62: 347-349, 1993. DOI: 10.1007/978-1-4615-3518-8_42
24 Benjamin RS, Chawla SP, Carrasco CH, Raymond AK, Murray JA, Armen T, Patel S, Wallace S, Ayala A, Papadopoulos NE, Plager C, Romsdahl MM, Martin RG: Preoperative chemotherapy for osteosarcoma with intravenous adriamycin and intra-arterial cis-platinum. Ann Oncol 3(Suppl 2): S3-S6, 1992. DOI: 10.1093/annonc/3.suppl_2.s3
25 Wunder JS, Paulian G, Huvos AG, Heller G, Meyers PA, Healey JH: The histological response to chemotherapy as a predictor of the oncological outcome of operative treatment of Ewing sarcoma. J Bone Joint Surg Am 80(7): 1020-33, 1998. DOI: 10.2106/00004623-199807000-00011
26 Bielack SS, Kempf-Bielack B, Delling G, Exner GU, Flege S, Helmke K, Kotz R, Salzer-Kuntschik M, Werner M, Winkelmann W, Zoubek A, Jürgens H, Winkler K: Prognostic factors in high-grade osteosarcoma of the extremities or trunk: an analysis of 1,702 patients treated on Neoadjuvant Cooperative Osteosarcoma Study Group protocols. J Clin Oncol 20(3): 776-790, 2002. DOI: 10.1200/jco.2002.20.3.776
27 Huvos AG, Rosen G, Marcove RC: Primary osteogenic sarcoma: Pathologic aspects in 20 patients after treatment with chemotherapy en bloc resection, and prosthetic bone replacement. Arch Pathol Lab Med 101(1): 14-18, 1977.
28 Salzer-Kuntschik M, Delling G, Beron G, Sigmund R: Morphological grades of regression in osteosarcoma after polychemotherapy - Study COSS 80. J Cancer Res Clinl Oncol 106(S1): 21-24, 1983. DOI: 10.1007/BF00625047
29 Ayala AG, Raymond AK, Jaffe N: The pathologist's role in the diagnosis and treatment of osteosarcoma in children. Human Pathol 15(3): 258-266, 1984. DOI: 10.1016/S0046-8177(84)80189-6
30 Bacci G, Picci P, Ruggieri P, Mercuri M, Avella M, Capanna R, Brach Del Prever A, Mancini A, Gherlinzoni F, Padovani G, Leonessa C, Biagini R, Ferraro A, Ferruzzi A, Cazzola A, Manfrini M, Campanacci M: Primary chemotherapy and delayed surgery (neoadjuvant chemotherapy) for osteosarcoma of the extremities. The Istituto Rizzoli Experience in 127 patients treated preoperatively with intravenous methotrexate (high *versus* moderate doses) and intraarterial cisplatin. Cancer 65(11): 2539-2553, 1990. DOI: 10.1002/1097-0142(19900601)65:11<2539::aid-cncr2820651125>3.0.co;2-m
31 Goorin AM, Schwartzentruber DJ, Devidas M, Gebhardt MC, Ayala AG, Harris MB, Helman LJ, Grier HE, Link MP: Presurgical chemotherapy compared with immediate surgery and adjuvant chemotherapy for nonmetastatic osteosarcoma: Pediatric Oncology Group Study POG-8651. J Clin Oncol 21(8): 1574-1580, 2003. DOI: 10.1200/jco.2003.08.165
32 Kim MS, Lee SY, Cho WH, Song WS, Koh JS, Lee JA, Yoo JY, Jeon DG: Tumor necrosis rate adjusted by tumor volume change is a better predictor of survival of localized osteosarcoma patients. Ann Surg Oncol 15(3): 906-914, 2008. DOI: 10.1245/s10434-007-9779-8
33 Tsuda Y, Tsoi K, Parry MC, Stevenson JD, Fujiwara T, Sumathi V, Jeys LM: Impact of chemotherapy-induced necrosis on event-free and overall survival after preoperative MAP chemotherapy in patients with primary high-grade localized osteosarcoma. Bone Joint J 102-B(6): 795-803, 2020. DOI: 10.1302/0301-620X.102B6.BJJ-2019-1307.R1
34 Harting MT, Lally KP, Andrassy RJ, Vaporciyan AA, Cox CS Jr, Hayes-Jordan A, Blakely ML: Age as a prognostic factor for patients with osteosarcoma: an analysis of 438 patients. J Cancer Res Clin Oncol 136(4): 561-570, 2010. DOI: 10.1007/s00432-009-0690-5

35 Kawaguchi K, Miyama K, Endo M, Bise R, Kohashi K, Hirose T, Nabeshima A, Fujiwara T, Matsumoto Y, Oda Y, Nakashima Y: Viable tumor cell density after neoadjuvant chemotherapy assessed using deep learning model reflects the prognosis of osteosarcoma. NPJ Precis Oncol 8(1): 16, 2024. DOI: 10.1038/s41698-024-00515-y
36 Hagi T, Nakamura T, Yuasa H, Uchida K, Asanuma K, Sudo A, Wakabayahsi T, Morita K: Prediction of prognosis using artificial intelligence-based histopathological image analysis in patients with soft tissue sarcomas. Cancer Med 13(10): e7252, 2024. DOI: 10.1002/cam4.7252
37 Whelan JS, Jinks RC, McTiernan A, Sydes MR, Hook JM, Trani L, Uscinska B, Bramwell V, Lewis IJ, Nooij MA, van Glabbeke M, Grimer RJ, Hogendoorn PC, Taminiau AH, Gelderblom H: Survival from high-grade localised extremity osteosarcoma: combined results and prognostic factors from three European Osteosarcoma Intergroup randomised controlled trials. Ann Oncol 23(6): 1607-1616, 2012. DOI: 10.1093/annonc/mdr491
38 Tippett VL, Tattersall L, Ab Latif NB, Shah KM, Lawson MA, Gartland A: The strategy and clinical relevance of *in vitro* models of MAP resistance in osteosarcoma: a systematic review. Oncogene 42(4): 259-277, 2023. DOI: 10.1038/s41388-022-02529-x
39 Gazouli I, Kyriazoglou A, Kotsantis I, Anastasiou M, Pantazopoulos A, Prevezanou M, Chatzidakis I, Kavourakis G, Economopoulou P, Kontogeorgakos V, Papagelopoulos P, Psyrri A: Systematic review of recurrent osteosarcoma systemic therapy. Cancers (Basel) 13(8): 1757, 2021. DOI: 10.3390/cancers13081757
40 Tawbi HA, Burgess M, Bolejack V, Van Tine BA, Schuetze SM, Hu J, D'Angelo S, Attia S, Riedel RF, Priebat DA, Movva S, Davis LE, Okuno SH, Reed DR, Crowley J, Butterfield LH, Salazar R, Rodriguez-Canales J, Lazar AJ, Wistuba II, Baker LH, Maki RG, Reinke D, Patel S: Pembrolizumab in advanced soft-tissue sarcoma and bone sarcoma (SARC028): a multicentre, two-cohort, single-arm, open-label, phase 2 trial. Lancet Oncol 18(11): 1493-1501, 2017. DOI: 10.1016/S1470-2045(17)30624-1
41 Mills LJ, Spector LG, Largaespada DA, Williams LA: Sex differences in expression of immune elements emerge in children, young adults and mice with osteosarcoma. Biol Sex Differ 12(1): 5, 2021. DOI: 10.1186/s13293-020-00347-y
42 Pan M, Zhou MY, Jiang C, Zhang Z, Bui NQ, Bien J, Siy A, Achacoso N, Solorzano AV, Tse P, Chung E, Thomas S, Habel LA, Ganjoo KN: Sex-dependent prognosis of patients with advanced soft tissue sarcoma. Clin Cancer Res 30(2): 413-419, 2024. DOI: 10.1158/1078-0432.CCR-23-1990
43 Libert C, Dejager L, Pinheiro I: The X chromosome in immune functions: when a chromosome makes the difference. Nat Rev Immunol 10(8): 594-604, 2010. DOI: 10.1038/nri2815
44 Klein SL, Flanagan KL: Sex differences in immune responses. Nat Rev Immunol 16(10): 626-638, 2016. DOI: 10.1038/nri.2016.90
45 Piccini P, Montagnani C, de Martino M: Gender disparity in pediatrics: a review of the current literature. Ital J Pediatr 44(1): 1, 2018. DOI: 10.1186/s13052-017-0437-x
46 Perry JA, Kiezun A, Tonzi P, Van Allen EM, Carter SL, Baca SC, Cowley GS, Bhatt AS, Rheinbay E, Pedamallu CS, Helman E, Taylor-Weiner A, McKenna A, DeLuca DS, Lawrence MS, Ambrogio L, Sougnez C, Sivachenko A, Walensky LD, Wagle N, Mora J, de Torres C, Lavarino C, Dos Santos Aguiar S, Yunes JA, Brandalise SR, Mercado-Celis GE, Melendez-Zajgla J, Cárdenas-Cardós R, Velasco-Hidalgo L, Roberts CW, Garraway LA, Rodriguez-Galindo C, Gabriel SB, Lander ES, Golub TR, Orkin SH, Getz G, Janeway KA: Complementary genomic approaches highlight the PI3K/mTOR pathway as a common vulnerability in osteosarcoma. Proc Natl Acad Sci U.S.A. 111(51): E5564-E5573, 2014. DOI: 10.1073/pnas.1419260111
47 Scott MC, Temiz NA, Sarver AE, LaRue RS, Rathe SK, Varshney J, Wolf NK, Moriarity BS, O'Brien TD, Spector LG, Largaespada DA, Modiano JF, Subramanian S, Sarver AL: Comparative transcriptome analysis quantifies immune cell transcript levels, metastatic progression, and survival in osteosarcoma. Cancer Res 78(2): 326-337, 2018. DOI: 10.1158/0008-5472.CAN-17-0576
48 Mirabello L, Zhu B, Koster R, Karlins E, Dean M, Yeager M, Gianferante M, Spector LG, Morton LM, Karyadi D, Robison LL, Armstrong GT, Bhatia S, Song L, Pankratz N, Pinheiro M, Gastier-Foster JM, Gorlick R, de Toledo SRC, Petrilli AS, Patino-Garcia A, Lecanda F, Gutierrez-Jimeno M, Serra M, Hattinger C, Picci P, Scotlandi K, Flanagan AM, Tirabosco R, Amary MF, Kurucu N, Ilhan IE, Ballinger ML, Thomas DM, Barkauskas DA, Mejia-Baltodano G, Valverde P, Hicks BD, Zhu B, Wang M, Hutchinson AA, Tucker M, Sampson J, Landi MT, Freedman ND, Gapstur S, Carter B, Hoover RN, Chanock SJ, Savage SA: Frequency of pathogenic germline variants in cancer-susceptibility genes in patients with osteosarcoma. JAMA Oncol 6(5): 724-734, 2020. DOI: 10.1001/jamaoncol.2020.0197
49 Araki Y, Yamamoto N, Hayashi K, Takeuchi A, Miwa S, Igarashi K, Higuchi T, Abe K, Taniguchi Y, Yonezawa H, Morinaga S, Asano Y, Nojima T, Tsuchiya H: Primary osteosarcoma in patients over 75 years of age: a case series and literature review. In Vivo 36(6): 2899-2909, 2022. DOI: 10.21873/invivo.13031
50 Pollack SM, Redman MW, Baker KK, Wagner MJ, Schroeder BA, Loggers ET, Trieselmann K, Copeland VC, Zhang S, Black G, McDonnell S, Gregory J, Johnson R, Moore R, Jones RL, Cranmer LD: Assessment of doxorubicin and pembrolizumab in patients with advanced anthracycline-naive sarcoma: a phase 1/2 nonrandomized clinical trial. JAMA Oncol 6(11): 1778-1782, 2020. DOI: 10.1001/jamaoncol.2020.3689