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Research Paper

# Determinants of tumor necrosis and its impact on outcome in patients with Localized osteosarcoma uniformly treated with a response adapted regimen without high dose Methotrexate– A retrospective institutional analysis

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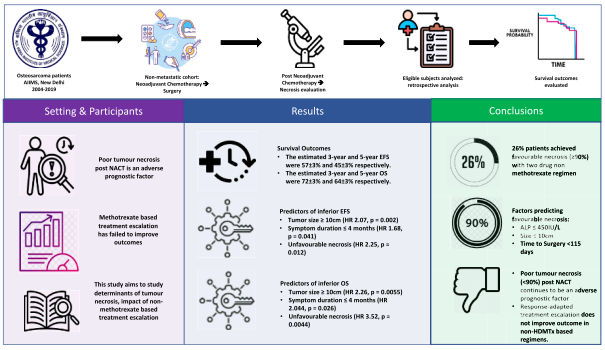
## HIGHLIGHTS

- Early surgery is associated with favourable necrosis.
- Small tumors predict favourable necrosis.
- Low Alkaline phosphatase predicts likelihood of favourable necrosis.
- Favourable necrosis is associated with higher survival rates.
- Response-adapted treatment escalation does not improve outcome in non-HDMTx based regimens.

## GRAPHICAL ABSTRACT

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## ABSTRACT

*Purpose:* Response to neoadjuvant chemotherapy in form of tumor necrosis predicts outcome in osteosarcoma; although response-adapted treatment escalation failed to improve outcome among patients treated with high-dose methotrexate-based (HDMTx) chemotherapy. This study aimed to identify factors predicting tumor necrosis and its impact on survival among patients with non-metastatic osteosarcoma treated with a response-adapted non-HDMTx regimen.

*Methods:* A retrospective single-institutional study was conducted among non-metastatic osteosarcoma patients treated with neoadjuvant therapy between 2004–2019. Patients were treated uniformly with three cycles of

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neoadjuvant cisplatin/doxorubicin. Post-operatively, patients with favourable necrosis (≥90 %) received 3 cycles of cisplatin/doxorubicin, while patients with poor necrosis (<90 %) received escalated treatment with alternating six cycles of cisplatin/doxorubicin and ifosfamide/etoposide. Propensity score matching (PSM) analyses were conducted to ascertain independent impact of necrosis on event-free survival (EFS) and overall survival (OS).

*Results:* Of 594 registered osteosarcoma patients, 280 patients (median age 17 years; male 67.1 %) were included for analysis. 73 patients (26.1 %) achieved favourable necrosis. Patients with smaller tumor size (≤10 cm) (aOR = 2.28; p = 0.030), lower serum alkaline phosphatase (≤450 IU/L) (aOR = 2.10; p = 0.035), and who had surgery earlier (<115 days) (aOR = 2.28; p = 0.016) were more likely to have favourable necrosis. On 1:2 PSM analysis, patients not achieving favourable necrosis demonstrated inferior EFS (HR = 2.68; p = 0.003) and OS (HR = 3.42; p = 0.003).

*Conclusions:* Patients of osteosarcoma with smaller tumor, lower serum alkaline phosphatase and earlier surgery are more likely to achieve favourable necrosis. Tumor necrosis independently predicts outcome in osteosarcoma, and response-adapted treatment escalation fails to overcome the adverse impact of poor necrosis in non-HDMTx based regimen.

## 1. Introduction

The incorporation of multi-modality chemotherapy treatment protocols has resulted in stabilization of the outcomes of osteosarcoma over the last three decades with no further improvement in survival in the western countries.[1] However, the treatment outcomes continue to lag in low-and middle-income countries (LMICs) due to a multitude of challenges, including late diagnosis, lack of access to specialized care, and financial constraints.[2] While high-dose methotrexate (HDMTX)-based protocols have become the standard chemotherapy regimens in high-income countries, the delivery of HDMTX-based regimens entails logistic difficulties in the form of need for inpatient admission and increased supportive care, thus necessitating the use of alternate strategies in settings with resource limitations.[3–8].

Several baseline factors such as large tumor size, raised alkaline phosphatase and most importantly the presence of metastatic disease have been observed to be poor predictors of outcome in osteosarcoma.[4,7,9,10] The percentage of necrosis observed in pathological tumour samples resected post neoadjuvant therapy continues to remain an important parameter predicting long term outcome.[11,12] It is desirable to know the prognostication at diagnosis rather than midway through the treatment protocol. Several attempts have also been made to use non-invasive surrogates of necrosis and angiogenesis, and various imaging modalities prior to surgery may predict necrosis and outcomes in osteosarcoma.[13,14].

Based on the results of the EURAMOS trial, intensification of post-operative chemotherapy among patients with poor necrosis after having received HDMTX-based neoadjuvant therapy, entails no additional benefit.[15] But, the benefit of treatment intensification after non-HDTMX based neoadjuvant chemotherapy regimen and in particular a two-drug neoadjuvant chemotherapy regimen is unclear.[7,16].

It is also interesting to note that attempts at dose intensification of a two-drug neoadjuvant chemotherapy regimen did not make any impact on survival despite increasing the proportions of patients with favourable necrosis.[17] This implies that although necrosis post neoadjuvant chemotherapy is an important prognostic factor yet the intrinsic disease biology may not be altered by achieving higher tumour kill with intensification of therapy.

At our institute, a two-drug non-HDMTX based neoadjuvant chemotherapy is routinely administered to patients with osteosarcoma, followed by surgery and then treatment intensification of post-operative chemotherapy is done for patients in whom the histopathological necrosis is less than 90 %.[18] The primary objective of this study was to estimate the prevalence of favourable histopathological necrosis (defined as ≥ 90 %) among patients with non-metastatic osteosarcoma who were treated with a uniform two-drug neoadjuvant therapy and underwent surgery at our centre. The secondary objectives included an investigation of the factors predicting favourable necrosis and assessment of the independent prognostic impact of necrosis on long-term survival outcomes among patients treated with a response-adapted treatment protocol at our centre.

## 2. Materials and Methods

### 2.1. Study design

The study was conducted at a single tertiary care cancer centre in India, retrospectively analysing consecutive patients registered in the medical oncology outpatient department from February 2004 to October 2019. Patients with a confirmed diagnosis of osteosarcoma were included. Patients who had metastases at presentation, who underwent upfront surgery, who did not undergo surgery post neoadjuvant chemotherapy or patients for whom tumour necrosis data was not available were excluded from this study. The study was approved by the Institutional Ethics Committee [IEC-454/06.05.2022, RP, 34/2022] and need for informed consent was waived off due to retrospective nature of the study.

### 2.2. Data collection

Data regarding baseline characteristics of all enrolled patients were obtained through an assessment of their medical records. Baseline clinical parameters like the patient's age, gender, duration of symptoms prior to presentation, presence of fever, evidence of neurovascular bundle involvement, tumour size, and disease stage were recorded. The laboratory parameters included complete blood count, liver and renal function tests, and serum alkaline phosphatase.

### 2.3. Treatment administered

Prior to commencing treatment, all patients diagnosed with osteosarcoma at our institution underwent MRI for imaging of the local site. Baseline staging was conducted through either whole-body $^{18}$fluorodeoxyglucose positron emission tomography-computed tomography, or a combination of non-contrast computed tomography of the thorax and a bone scintigraphy using technetium-99 m methylene diphosphonate. These procedures were performed as part of the standard protocol for evaluating the extent and characteristics of the disease in the patients.

All patients were given the two-drug neoadjuvant chemotherapy protocol that did not include HD-MTX. They received three cycles of cisplatin and doxorubicin [Cisplatin 120 mg/m$^2$, Doxorubicin 75 mg/m$^2$; divided over 3 days]. Post neoadjuvant chemotherapy, the patient details were discussed in multi-disciplinary meeting following which patients were taken up for surgery.

### 2.4. Pathological assessment of chemotherapy response

The postoperative specimen was assessed for the amount of necrosis to determine the response to the neoadjuvant therapy. An entire representative slice of the tumour taken through long axis was mapped using a grid pattern diagram. The sum of all necrosed areas measured microscopically were divided by the total cross-sectional area occupied by the tumour to determine percentage necrosis.

### 2.5. Treatment intensification based on necrosis

Patients who had a favourable response to the neoadjuvant therapy (necrosis $\geq$ 90 %) were given further three cycles of adjuvant chemotherapy, consisting of cisplatin and doxorubicin. Subjects with an unfavourable pathological response (necrosis $<$ 90 %) were administered an escalated regimen with total six alternating cycles each of cisplatin/doxorubicin and ifosfamide/etoposide (9 g/m$^2$ of ifosfamide divided over 5 days and 500 mg/m$^2$ of etoposide divided over 5 days) as adjuvant chemotherapy (three cycles each of cisplatin/doxorubicin and ifosfamide/etoposide).

### 2.6. Study outcomes

The primary outcome of the study was the proportion of patients who achieved favourable necrosis in the post-operative specimen. The survival outcomes included event-free survival (EFS) and overall survival (OS). EFS was measured as the time period between treatment initiation and either disease progression/relapse or death from any cause. OS was defined as the time period between treatment initiation and death from any cause. The survival data was censored on 30th November 2022.

### 2.7. Statistical analysis

The statistical analysis of our study was conducted using SPSS (version 26.0; IBM) and R (version 4.4.0). Descriptive statistics were employed to summarize the baseline characteristics of the study population. Continuous variables were presented using the median value along with the corresponding range. Listwise deletion was used for missing variables.

#### 2.7.1. Identification of determinants of necrosis

Multivariable logistic regression analyses were performed to assess the predictive value of variables such as age (categorized as $>$ 18 years or $\leq$ 18 years), sex, site of tumour (appendicular vs axial), tumour size ($\leq$10 cm vs $>$ 10 cm), ECOG-PS (Eastern Cooperative Oncology Group performance status) scores (0–1 vs $\geq$ 2), symptom duration ($\leq$4 months vs $>$ 4 months), haemoglobin levels ($\leq$12 g/dL vs $>$ 12 g/dL), total leukocyte count (TLC) ($\leq$11000/μL vs $>$ 11000/ μL), serum alkaline phosphatase (ALP) levels ($\leq$450 IU/L vs $>$ 450 IU/L), serum albumin levels ($\leq$4.5 g/dL vs $>$ 4.5 g/dL), presence of fever, fracture, and neuromuscular involvement and time to surgery (categorized by median days) on the proportion of favourable necrosis. The variables with a p-value of less than 0.10 in univariable analyses were further subjected to multivariable analysis using forward stepwise manner and factors less than 0.05 were considered as significant across all tests. The results were reported in the form of Odds ratio (OR) and 95 % confidence interval (CI).

#### 2.7.2. Assessment of the prognostic impact of necrosis

Kaplan Meier analyses were performed to report survival outcomes in form of EFS and OS outcomes. The median follow-up of the cohort was calculated by reverse Kaplan Meier analysis. Multivariable cox regression analyses were carried out to determine the independent impact of favourable necrosis on EFS and OS, adjusted for confounders. Furthermore, to account for the imbalance of confounders between the two groups (favourable vs unfavourable necrosis), a propensity score matched analysis was conducted using *matchit* package in R. A propensity score was estimated by logistic regression model for each variable – age, sex, symptom duration, size of tumor, hemoglobin, total leucocyte count and serum alkaline phosphatase. Pairs between groups of favourable and unfavourable necrosis were matched 2:1 with nearest neighbour matching and the balance of the matched cohort was analysed using standardized mean difference. Finally, the prognostic impact of necrosis on EFS and OS in the propensity matched cohort was estimated by multivariable cox regression analyses.

## 3. Results

### 3.1. Baseline patient characteristics

A total of 594 patients with osteosarcoma were registered during the study period. Out of these 204 patients had metastases at initial presentation and were excluded. Of the remaining 390 patients, 28 subjects underwent upfront surgery while in 62 subjects, surgery was not performed. Thus, a total of 300 patients underwent surgery after neoadjuvant chemotherapy; of these, necrosis data were available for 280 patients, who were then included for the final analysis.(Fig. 1).

The baseline socio-demographic and clinical characteristics of the cohort are summarized in Table 1. The median age in our study population was 17 years and nearly two thirds of the patients were male. The median size of the tumour was 9 cm whereas the median duration of symptoms prior to presentation was 4 months. Patients underwent surgery after a median of 114 days from the date of initiation of treatment.

### 3.2. Determinants of tumour necrosis

In the cohort of 280 analysable patients, 73 (26.1 %) attained favourable necrosis in the post-operative resected specimen after neoadjuvant chemotherapy. On the multivariable analysis, patients with tumor size $\leq$ 10 cm (aOR 2.28, 95 %CI 1.08–4.81, p = 0.030); lower serum ALP levels (aOR 2.10; 95 %CI 1.05–4.18; p = 0.035) and who underwent surgery earlier ($<$115 days) (aOR 2.28; 1.16–4.47; p = 0.016) were more likely to attain favourable necrosis (Table 2).

### 3.3. Determinants of survival outcome

At a median follow-up of 84.1 months, there were 88 (31.4 %) mortalities in the cohort. The estimated 3-year and 5-year EFS were 57 $\pm$ 3 % and 45 $\pm$ 3 % respectively, while the estimated 3-year and 5-year

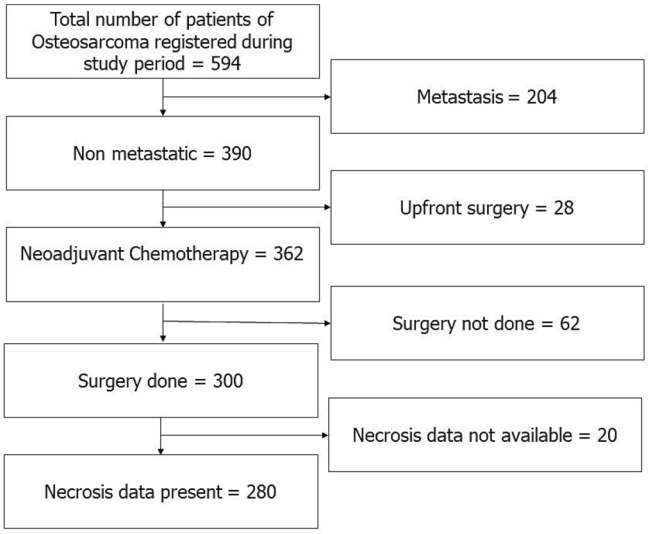


**Fig. 1.** Flow diagram of the study.

**Table 1**
Clinical and demographic characteristics of non-metastatic osteosarcoma cases (n = 280).

| Demographics/ Clinical Parameters | | Median (with range)/ n (%) |
|---|---|---|
| Age (years) (n = 280) | | 17 (2–71) |
| Size (cm) (n = 238) | | 9 (1–48) |
| Site (n = 278) | Axial | 8 (2.9) |
| | Appendicular | 270 (97.1) |
| Sex (n = 280) | Male | 188 (62.1) |
| | Female | 92 (37.9) |
| Fever (n = 280) | Yes | 28(10) |
| | No | 252 (90) |
| Symptom duration (months) (n = 255) | | 4 (1–36) |
| Hemoglobin (g/dL) (n = 275) | | 11.9 (4–16.2) |
| Total Leukocyte Count (/μL) (n = 274) | | 8000 (3200–42800) |
| Serum Albumin (g/dL) (n = 258) | | 4.5 (2.1–5.9) |
| Serum Alkaline Phosphate (IU/L) (n = 262) | | 405 (97–9121) |
| ECOG-PS (n = 184) | 0,1 | 151 (82.1) |
| | >=2 | 33 (17.9) |
| Fracture (n = 280) | Yes | 16 (5.7) |
| | No | 264 (94.3) |

ECOG-PS: Eastern Cooperative Oncology Group Performance Status.

OS were 72 ± 3 % and 64 ± 3 %.

On multivariable analysis, tumour size ≥ 10 cm (HR 2.07, 95 % CI 1.30—3.32, p = 0.002), symptom duration ≤ 4 months (HR 1.68, 95 % CI 1.02—2.77, p = 0.041) and unfavourable necrosis (HR 2.25, 95 % CI 1.22—4.14, p = 0.012) were significant predictors of inferior EFS (Table 3, Fig. 2).

Similarly, size ≥ 10 cm (HR 2.26, 95 % CI 1.27—4.028, p = 0.0055), symptom duration ≤ 4 months (HR 2.044, 95 % CI 1.089—3.831, p = 0.026) and unfavourable necrosis (HR 3.52, 95 % CI 1.48—8.39, p = 0.0044) significantly predicted inferior OS (Table 4, Fig. 2).

On a 1:2 propensity score matched cohort, adjusted for other confounders, patients not achieving favourable necrosis demonstrated inferior EFS (HR = 2.68; 95 % CI: 1.39–5.16; p = 0.003) and OS (HR = 3.42; 95 % CI: 1.48–7.87; p = 0.003). (Fig. 3).

## 4. Discussion

In this study, the patient cohort was predominantly males which is reflective of the sex disparity in favour of males for health seeking that is prevalent in India; and more than one third of the subjects had a symptom duration of more than 4 months which may be related to the barriers in the journey of the families to access timely diagnosis.[19,20] We observed that the proportion of patients achieving favourable necrosis was 26 % when two drugs were used for neoadjuvant therapy. This estimate is notably lower than the proportion of favourable necrosis observed in 63 % of the subjects when a 3-drug HDMTX based neoadjuvant chemotherapy was used in the EURAMOS trial.[21].

HDMTX is generally omitted in many resource-constrained settings and alternative 3-drug regimens, mainly with the addition of ifosfamide on a cisplatin-doxorubicin backbone, are commonly used. Various small-scale studies using similar 3-drug neoadjuvant treatment reports the proportion of favourable necrosis between 37–63 %.[22–26] On the other hand, the use of a 4-drug intensified protocol in a cohort of metastatic osteosarcoma showed a proportion of favourable necrosis in 54 % of subjects, perhaps suggesting no additional benefit of fourth drug.[27].

Hence, the findings suggest that the proportion of favourable necrosis achieved with 2-drug neoadjuvant protocol is uniformly lower compared to 3-drug regimens with or without HDMTX. However, the uniform adoption of 3-drug neoadjuvant regimen with or without HDMTX in osteosarcoma remains challenging in many resource-constrained settings, often due to concerns regarding toxicities and availability of limited healthcare resources.

Furthermore, our results suggest that patients with smaller tumor size and lower baseline serum ALP were more likely to attain favorable necrosis, similar to findings in breast cancer, where smaller tumor size is associated with more significant pathological response.[28] However, in osteosarcoma, the relationship between tumor size and necrosis has

**Table 2**
Predictors of Favourable Tumour Necrosis post neoadjuvant chemotherapy in the study population.

| Parameter | Categories | Necrosis>=90 % (n = 73) | Necrosis < 90 % (n = 207) | Univariable Odds Ratio | 95 % CI | P value | Multivariable Odds Ratio | 95 % CI | P value |
|---|---|---|---|---|---|---|---|---|---|
| 1. Age (years) (n = 280) | ≤ 18 (n = 170) | 44 (25.8 %) | 126 (74.2 %) | 0.97 | 0.56–1.68 | 0.929 | | | |
| | >18 (n = 110) | 29 (26.3 %) | 81 (73.7 %) | 1 | | | | | |
| 2. Sex (n = 280) | Male (n = 188) | 47 (25 %) | 141 (75 %) | 1 | 0.67–2.07 | 0.56 | | | |
| | Female (n = 92) | 26 (28.2 %) | 66 (71.8 %) | 1.18 | | | | | |
| 3. Site (n = 278) | Appendicular (n = 270) | 72 (26.7 %) | 198 (73.3 %) | 2.54 | 0.31–21.05 | 0.386 | | | |
| | Axial (n = 8) | 1 (12.5 %) | 7 (87.5 %) | 1 | | | | | |
| 4. Size (cm) (n = 238) | ≤ 10 cm (n = 149) | 46 (30.9 %) | 103 (69.1 %) | 2.39 | 1.23–4.67 | 0.01 | 2.28 | 1.08–4.81 | 0.030 |
| | >10 cm (n = 89) | 14 (15.7 %) | 75 (84.3 %) | 1 | | | 1 | | |
| 5. ECOG- PS (n = 184) | 0–1(n = 151) | 39 (25.8 %) | 112 (74.2 %) | 0.80 | 0.35–1.83 | 0.59 | | | |
| | ≥ 2 (n = 33) | 10 (30.3 %) | 23 (69.7 %) | 1 | | | | | |
| 6. Symptom Duration (months) (n = 255) | ≤ 4 (n = 150) | 48 (32 %) | 102 (68 %) | 2.13 | 1.16–3.89 | 0.014 | | | |
| | >4 (n = 105) | 19 (18.1 %) | 86 (81.9 %) | 1 | | | | | |
| 7. Hemoglobin (g/dL) (n = 275) | ≤12 (n = 129) | 35 (27.1 %) | 94 (72.9 %) | 1.06 | 0.55–1.61 | 0.836 | | | |
| | >12 (n = 146) | 38 (26 %) | 108 (74 %) | 1 | | | | | |
| 8. Total Leukocyte Count (per cumm) (n = 274) | ≤ 11,000 (n = 236) | 58 (24.6 %) | 178 (75.4 %) | 0.56 | 0.27–1.15 | 0.114 | | | |
| | >11000 (n = 38) | 14 (36.8 %) | 24 (63.2 %) | 1 | | | | | |
| 9. Serum Alkaline Phosphate (IU/L) (n = 262) | ≤ 450 (n = 147) | 49 (33.3 %) | 98 (66.7 %) | 2.24 | 1.25–4.01 | 0.007 | 2.10 | 1.05–4.18 | 0.035 |
| | >450 (n = 115) | 21 (18.2 %) | 94 (81.2 %) | 1 | | | 1 | | |
| 10. Fever (n = 280) | Yes (n = 28) | 8 (28.6 %) | 20 (71.4 %) | 1 | | 0.751 | | | |
| | No (n = 252) | 65 (25.8 %) | 187 (74.2 %) | 0.87 | 0.36–2.07 | | | | |
| 11. Fracture (n = 280) | Yes (n = 16) | 2 (12.5 %) | 14 (87.5 %) | 1 | | 0.218 | | | |
| | No (n = 264) | 71 (26.9 %) | 193 (73.1 %) | 2.58 | 0.51–11.62 | | | | |
| 12. Albumin (g/dL) (n = 258) | ≤3.5 (n = 22) | 8 (36.3 %) | 14 (63.6 %) | 1.64 | 0.66–4.10 | 0.290 | | | |
| | >3.5 (n = 236) | 61 (25.8 %) | 175 (74.1 %) | 1 | | | | | |
| 13. Time to surgery (n = 269) | <115 days (n = 137) | 47 (34.3 %) | 90 (65.7 %) | 2.35 | 1.33–4.14 | 0.003 | 2.28 | 1.16–4.47 | 0.016 |
| | ≥115 days (n = 132) | 24 (18.2 %) | 108 (81.8 %) | 1 | | | 1 | | |

ECOG-PS: Eastern Cooperative Oncology Group Performance Status.

**Table 3**
Predictor of event-free survival in non-metastatic osteosarcoma undergoing neoadjuvant chemotherapy and surgery (n = 280).

| | | | | Univariable | | | | Multivariable | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Parameter | Categories | 3 yr EFS (Standard error) | 5 yr EFS (Standard error) | Median (months) | Hazards Ratio | 95 % CI | P value | Hazards Ratio | 95 % CI | P value |
| 1. Age (years) (n = 280) | ≤ 18 (n = 170) | 51 % (±4%) | 46 % (±4%) | 41.3 | 1 | | 0.588 | | | |
| | >18 (n = 110) | 50 % (±5%) | 45 % (±5%) | 32.03 | 1.09 | | | | | |
| 2. Sex (n = 280) | Male (n = 188) | 49 % (±4%) | 44 % (±4%) | 30.67 | 1.119 | 0.787–1.589 | 0.532 | | | |
| | Female (n = 92) | 53 % (±5%) | 49 % (±5%) | 43.3 | 1 | | | | | |
| 3. Site (n = 278) | Appendicular (n = 270) | 50 % (±3%) | 45 % (±3%) | 37.533 | 1 | | 0.173 | | | |
| | Axial (n = 8) | 30 % (±17 %) | 30 % (±17 %) | 12.167 | 1.86 | 0.761–4.545 | | | | |
| 4. Size (cm) (n = 238) | ≤ 10 (n = 149) | 58 % (±4%) | 53 % (±4%) | Not reached | 1 | | 0.002 | 1 | | 0.002 |
| | >10 (n = 89) | 39 % (±5%) | 33 % (±5%) | 22.9 | 1.765 | 1.24–2.511 | | 2.074 | 1.296–3.320 | |
| 5. ECOG- PS (n = 184) | 0–1(n = 151) | 54 % (±4%) | 51 % (±4%) | 69.3 | 1.00603 | 0.569–1.736 | 0.984 | | | |
| | ≥ 2 (n = 33) | 58 % (±9%) | 49 % (±9%) | Not reached | 1 | | | | | |
| 6. Symptom Duration (months) (n = 255) | ≤ 4 (n = 150) | 47 % (±4%) | 43 % (±4%) | 29.267 | 1.412 | 0.491—1.021 | 0.064 | 1.683 | 1.02–2.77 | 0.041 |
| | >4 (n = 105) | 63 % (±5%) | 56 % (±5%) | Not reached | 1 | | | 1 | | |
| 7. Hemoglobin (g/dL) (n = 275) | ≤12 (n = 129) | 52 % (±5%) | 50 % (±5%) | 47 | 1.24 | 0.579–1.123 | 0.203 | | | |
| | >12 (n = 146) | 49 % (±4%) | 42 % (±4%) | 25.8 | 1 | | | | | |
| 8. Total leukocyte count (per μL) (n = 274) | ≤ 11,000 (n = 236) | 50 % (±3%) | 46 % (±3%) | 32.5 | 1.052 | 0.586–1.541 | 0.836 | | | |
| | >11000 (n = 38) | 57 % (±8%) | 47 % (±9%) | 45.233 | 1 | | | | | |
| 9. Serum Alkaline Phosphate (IU/L) (n = 262) | ≤ 450 (n = 147) | 57 % (±4%) | 51 % (±4%) | 69.333 | 1 | | 0.167 | | | |
| | >450 (n = 115) | 47 % (±5%) | 44 % (±5%) | 25.8 | 1.274 | 0.904–1.796 | | | | |
| 10. Fever (n = 280) | Yes (n = 28) | 50 % (±9%) | 50 % (±9%) | 26.33 | 1.14 | 0.506–1.522 | 0.641 | | | |
| | No (n = 252) | 50 % (±3%) | 45 % (±3%) | 37.53 | 1 | | | | | |
| 11. Fracture (n = 280) | Yes (n = 264) | 50 % (±3%) | 45 % (±3%) | not reached | 1 | | 0.893 | | | |
| | No (n = 16) | 51 % (±13 %) | 51 % (±13 %) | 32.5 | 1.053 | 0.444–2.028 | | | | |
| 13. Necrosis (n = 280) | ≥90 % (n = 73) | 66 % (±6%) | 61 % (±6%) | Not reached | 1 | | 0.001 | 1 | | 0.012 |
| | <90 % (n = 207) | 44 % (±4%) | 39 % (±4%) | 25.633 | 1.994 | 1.319–3.015 | | 2.245 | 1.218–4.138 | |
| 14. Albumin (g/dL) (n = 258) | >3.5 (n = 236) | 49 % (±3%) | 46 % (±3%) | 30.667 | 1.291 | 0.678—2.461 | 0.437 | | | |
| | ≤3.5 (n = 22) | 62 % (±11 %) | 51 % (±11 %) | Not reached | 1 | | | | | |

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been inconsistent as evidenced by a study which showed no correlation between these two variables.[29] Despite this, ALP remains an indicator of osteoblastic activity, potentially serving as a marker for tumor bulk.[30] Lower ALP levels might reflect smaller tumor size, which could result in a higher rate of necrosis. Symptom duration was not predictive of treatment response in form of necrosis, which aligns with our previous study, where diagnostic delay was not predictive of outcome in bone sarcomas.[31] However, we observed that delay in surgery was associated with increased proportion of unfavourable necrosis, which highlights the importance and challenges of multidisciplinary coordination in osteosarcoma especially in LMICs.[6] A previous study assessing the impact of delay in surgery at our centre, has reported increased risk of local recurrence with delay in surgery, although impact of necrosis on local recurrence was unclear in that study due to limited sample size [32].

We observed that patients not achieving favourable necrosis in the post-operative specimen after two-drug neoadjuvant therapy, demonstrated inferior survival outcomes compared to patients who had favourable necrosis, despite treatment intensification. Even after adjustment for potential confounders using propensity matched analysis, unfavourable necrosis continued to be an independent predictor of inferior outcome. Therefore, treatment escalation was unable to overcome the effect of poor tumour biology. We know from the findings of the EURAMOS trial, additional treatment intensification for patients with poor chemotherapy response post 3-drug HDMTX based neoadjuvant therapy failed to improve outcome.[21] Hence, the underlying tumor biology plays a crucial role in determining treatment outcome and whether treatment intensification post two drug non-methotrexate neoadjuvant chemotherapy has an impact on improving survival outcomes needs to be evaluated in prospectively designed randomized controlled trials. This also underscores the importance of exploring alternate treatment targets in osteosarcoma for development of targeted therapies.

This study is the largest study from a LMIC which specifically analysed the impact of pathological necrosis post 2-drug non-HDMTX based neoadjuvant therapy on the outcome of osteosarcoma; the study adds value to the literature as this underscores the importance of disease biology in the outcome of osteosarcoma regardless of treatment intensity. The study is limited by its retrospective nature which introduces inherent constraints in data collection and potential biases.

## 5. Conclusion

In conclusion, this study reports that the proportion of the patients achieving favourable necrosis post 2-drug non-HDMTx based neoadjuvant regimen in osteosarcoma is 26 %. Patients with smaller tumor size, lower baseline serum ALP and who underwent earlier surgery are more likely to attain favourable necrosis. Unfavourable necrosis continues to be an independent prognostic factor for survival outcomes despite treatment intensification.

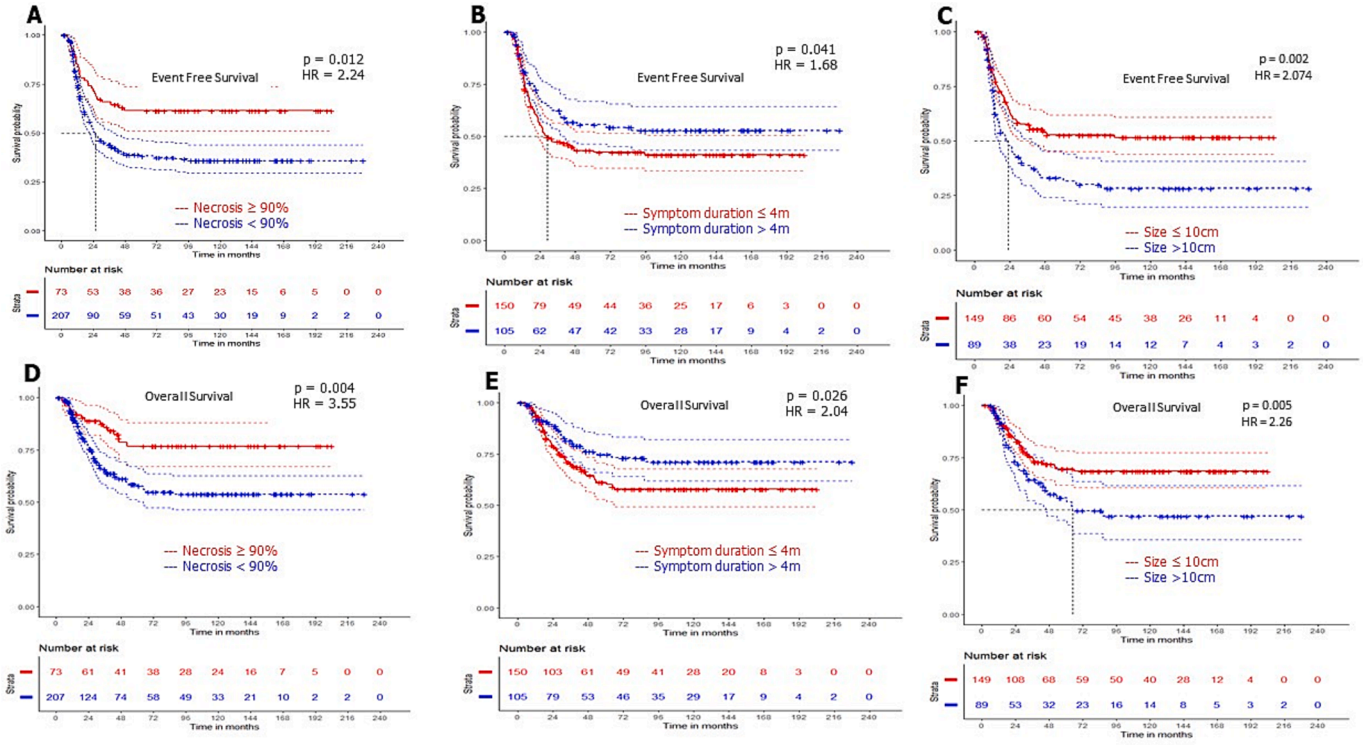


**Fig. 2.** Impact of necrosis (A), symptom duration (B) and tumor size (C) on event free survival; Impact of necrosis (D), symptom duration (E), and tumor size (F) on overall survival of the cohort.

**Table 4**
Predictors of overall survival in non-metastatic osteosarcoma undergoing neoadjuvant chemotherapy and surgery (n = 280).

| Parameter | Categories | 3 year OS (Standard error) | 5 year OS (Standard error) | Univariable Hazards Ratio | 95 % CI | P value | Multivariable Hazards Ratio | 95 % CI | P value |
|---|---|---|---|---|---|---|---|---|---|
| 1. Age (years) (n = 280) | ≤ 18 (n = 170) | 72 % (±4%) | 63 % (±4%) | 1 | | 0.629 | | | |
| | >18 (n = 110) | 73 % (±5%) | 67 % (±5%) | 1.114 | 0.727—1.733 | | | | |
| 2. Sex (n = 280) | Male (n = 188) | 70 % (±4%) | 61 % (±4%) | 1.279 | 0.805–2.034 | 0.297 | | | |
| | Female (n = 92) | 75 % (±5%) | 70 % (±5%) | 1 | | | | | |
| 3. Site (n = 278) | Appendicular (n = 270) | 72 % (±3%) | 65 % (±3%) | 1 | | 0.043 | | | |
| | Axial (n = 8) | 44 % (±19 %) | 44 % (±19 %) | 2.83 | 1.034–7.743 | | | | |
| 4. Size (cm) (n = 238) | ≤ 10 (n = 149) | 75 % (±4%) | 69 % (±4%) | 1 | | 0.015 | 1 | | 0.005 |
| | >10 (n = 89) | 65 % (±6%) | 56 % (±6%) | 1.75 | 1.115–2.746 | | 2.262 | 1.271–4.028 | |
| 5. ECOG- PS (n = 184) | 0–1(n = 151) | 75 % (±4%) | 63 % (±4%) | 1.043 | 0.526–2.068 | 0.905 | | | |
| | ≥ 2 (n = 33) | 73 % (±8%) | 64 % (±9%) | 1 | | | | | |
| 6. Symptom Duration (months) (n = 255) | ≤ 4 (n = 150) | 70 % (±4%) | 61 % (±4%) | 1.628 | 1.003—2.645 | 0.0483 | 2.044 | 1.089—3.831 | 0.026 |
| | >4 (n = 105) | 82 % (±4%) | 75 % (±5%) | 1 | | | 1 | | |
| 7. Hb (g/dL) (n = 275) | ≤12 (n = 129) | 70 % (±4%) | 67 % (±5%) | 1.12 | 0.735—1.718 | 0.588 | | | |
| | >12 (n = 146) | 72 % (±4%) | 62 % (±5%) | 1 | | | | | |
| 8. TLC (per cumm) (n = 274) | ≤ 11,000 (n = 236) | 73 % (±3%) | 65 % (±4%) | 1 | | 0.576 | | | |
| | >11000 (n = 38) | 69 % (±8%) | 64 % (±9%) | 1.183 | 0.655–2.137 | | | | |
| 9. S.ALP (IU/L) (n = 262) | ≤ 450 (n = 147) | 79 % (±4%) | 70 % (±4%) | 1 | | 0.016 | | | |
| | >450 (n = 115) | 65 % (±5%) | 58 % (±5%) | 1.705 | 1.104–2.635 | | | | |
| 10. Fever (n = 280) | Yes (n = 28) | 84 % (±7%) | 84 % (±7%) | 1 | | 0.093 | | | |
| | No (n = 252) | 70 % (±3%) | 62 % (±3%) | 2.169 | 0.879—5.347 | | | | |
| 11. Fracture (n = 280) | Yes (n = 264) | 72 % (±3%) | 64 % (±3%) | 1.447 | 0.457—4.587 | 0.529 | | | |
| | No (n = 16) | 75 % (±13 %) | 75 % (±13 %) | 1 | | | | | |
| 13. Necrosis (n = 280) | >90 % (n = 73) | 86 % (±4%) | 77 % (±5%) | 1 | | 0.003 | 1 | | 0.004 |
| | ≤90 % (n = 207) | 66 % (±4%) | 59 % (±4%) | 2.339 | 1.340–4.083 | | 3.522 | 1.479–8.389 | |
| 14. Albumin (g/dL) (n = 258) | >3.5 (n = 236) | 71 % (±3%) | 62 % (±4%) | 1.668 | 0.675–4.121 | 0.267 | | | |
| | ≤3.5 (n = 22) | 79 % (±9%) | 74 % (±10 %) | 1 | | | | | |

ECOG-PS: Eastern Cooperative Oncology Group Performance Status.

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None.

## CRediT authorship contribution statement

**Prabhat Gautam Roy:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data

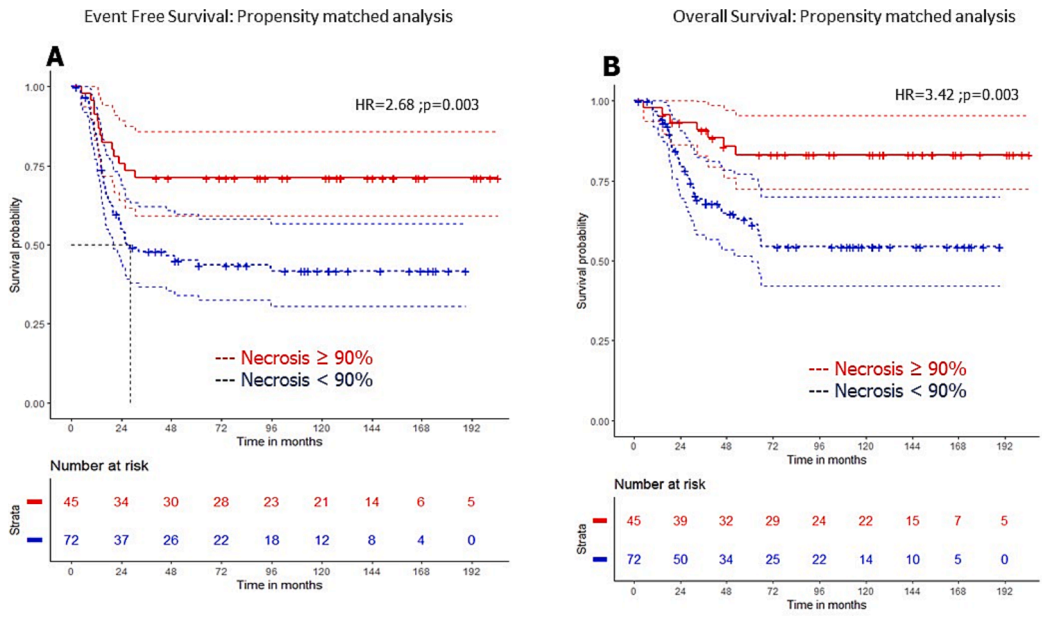


**Fig. 3.** Impact of necrosis on event-free survival (A) and overall survival (B) in a 1:2 propensity score matched cohort.

curation. **Shuvadeep Ganguly:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Archana Sasi:** Writing – review & editing, Visualization, Supervision, Formal analysis, Data curation, Conceptualization. **Vivek Kumar:** Writing – review & editing, Validation, Methodology, Formal analysis, Data curation. **Adarsh Barwad:** Writing – review & editing, Supervision, Project administration, Methodology. **Asit Ranjan Mridha:** Writing – review & editing, Supervision, Project administration, Investigation. **Shah Alam Khan:** Writing – review & editing, Supervision, Resources, Project administration, Investigation. **Venkatesan Sampath Kumar:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Data curation. **Love Kapoor:** Writing – review & editing, Visualization, Resources, Project administration, Methodology. **Deepam Pushpam:** Writing – review & editing, Validation, Supervision, Data curation, Conceptualization. **Sameer Bakhshi:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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