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ORIGINAL ARTICLE

Association of Pre-Chemotherapy Serum Alkaline Phosphatase Levels and p53 Protein Expression with Huvos Tumor Necrosis Grading in High-Grade Osteosarcoma: An Indonesian Tertiary Referral Center Experience

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ABSTRACT

Background: High-grade osteosarcoma shows unpredictable chemosensitivity. Whether pre-chemotherapy serum alkaline phosphatase (ALP) and immunohistochemical p53 expression forecast post-neoadjuvant histopathological tumor necrosis remains contested in resource-constrained settings.

Methods: In a hospital-based nested cohort, 14 consecutive patients with primary high-grade extremity osteosarcoma (Palembang, 2023–2025) received three cycles of standardized neoadjuvant chemotherapy before wide resection. Pre-chemotherapy serum ALP and immunohistochemical nuclear p53 were related to Huvos response, dichotomized as poor (<90% necrosis; grades I–II) or good (≥90%; grades III–IV), using Fisher's exact test, risk and odds ratios, and ROC analysis.

Results: The cohort comprised 9 males (64.3%) and 5 females (35.7%), mean age 22.0 ± 10.7 years. Poor histological response occurred in 11 patients (78.6%) and good response in 3 (21.4%). Elevated baseline ALP (>104 U/L) occurred in 92.9%, and p53 immunoreactivity was positive in 78.6%. Baseline serum ALP was not significantly associated with Huvos response (76.9% poor in elevated vs. 100.0% in normal; Fisher's exact p = 1.000; RR = 0.769, 95% CI: 0.571–1.036), nor was p53 (81.8% poor in p53-positive vs. 66.7% in p53-negative; p = 1.000; OR = 2.250, 95% CI: 0.130–38.811; RR = 1.227, 95% CI: 0.526–2.864). ROC analysis for serum ALP yielded an AUC of 0.515 (95% CI: 0.205–0.825, p = 0.938), equivalent to random classification. An empirical cutoff of 176.5 U/L showed 100.0% specificity but only 36.4% sensitivity.

Conclusion: Pre-chemotherapy serum ALP and immunohistochemical p53 expression fail to predict post-neoadjuvant histopathological tumor necrosis in osteosarcoma, and cannot substitute for microscopic Huvos grading in guiding adjuvant treatment.

1. Introduction

Osteosarcoma represents the most prevalent primary malignant neoplasm of the skeletal system in children, adolescents, and young adults, originating from primitive mesenchymal stem cells committed to the osteogenic lineage that aberrantly synthesize immature, unmineralized bone matrix termed osteoid.[1,2] Globally, the incidence of primary osteosarcoma is estimated at 3 to 5 cases per million population annually, exhibiting a classic bimodal age distribution that reaches an initial sharp peak during the second decade of life coinciding with the adolescent pubertal growth spurt, and a secondary, broader peak in individuals aged over 60 years frequently secondary to Paget disease of bone or prior therapeutic irradiation.[1,3] In Indonesia, clinical data from tertiary academic referral hospitals demonstrate that osteosarcoma accounts for the vast majority of primary pediatric and young adult musculoskeletal malignancies, with patients commonly presenting with large-volume, locally advanced extremity tumors predominantly located at the metaphyseal junctions of long bones, including the distal femur, proximal tibia, and proximal humerus.[4,5]

Historically, before the introduction of multi-agent systemic chemotherapy in the 1970s, the management of osteosarcoma relied almost exclusively on definitive surgical ablation or radical limb amputation, yielding dismal 5-year overall survival rates of merely 15% to 20% due to rapid metastatic progression, primarily in the form of occult pulmonary micrometastases already present at diagnosis in up to 80% of patients.[2,6] The advent and integration of neoadjuvant (preoperative) and adjuvant (postoperative) cytotoxic chemotherapy regimens have revolutionized musculoskeletal oncology, shifting the standard of care toward limb-salvage surgery and elevating 5-year event-free and overall survival rates to approximately 65% to 75% for

patients presenting with localized extremity disease.[6,7] Neoadjuvant chemotherapy serves two paramount oncological objectives: first, it provides immediate systemic pharmacological eradication of circulating neoplastic cells and subclinical micrometastatic deposits; second, it induces tumor necrosis, reduces peritumoral soft-tissue edema, and demarcates vascular boundaries, thereby facilitating negative-margin en-bloc surgical resection while sparing major neurovascular structures.[6,8]

The clinical standard of care for evaluating the pharmacodynamic efficacy of neoadjuvant chemotherapy in resected osteosarcoma specimens is the histopathological tumor necrosis assessment established by Huvos and colleagues.[9] Under the Huvos grading system, the entire resected surgical specimen undergoes macroscopic cross-sectional mapping and microscopic histological quantification of the percentage of acellular, necrotic, or fibrotic tumor tissue relative to residual viable neoplastic cells. The response is categorized into four grades: Grade I indicates little or no chemotherapy effect (<50% necrosis); Grade II indicates partial response with 50% to 89% necrosis; Grade III denotes a good response with 90% to 99% necrosis; and Grade IV represents complete histopathological eradication (100% necrosis) with no viable tumor cells detected.[9] In standard clinical oncology practice and international clinical trials, Huvos grading is dichotomized into a poor histological response (Huvos grades I–II; <90% necrosis) and a good histological response (Huvos grades III–IV; ≥90% necrosis).[7,9] Extensive clinical evidence has demonstrated that patients achieving a good histological response have significantly superior 5-year disease-free survival (75% to 85%) compared with poor responders (45% to 55%), making post-chemotherapy necrosis the single most robust prognostic indicator in osteosarcoma.[7,10-12] Furthermore, post-surgical histological necrosis frequently guides postoperative management, dictating whether to maintain identical adjuvant regimens or escalate to second-line salvage therapies.[6,10]

Despite the established clinical utility of post-resection Huvos scoring, this evaluation is inherently retrospective, available only after months of intensive neoadjuvant chemotherapy and major definitive surgery.[7,9] Consequently, identifying reliable, cost-effective baseline circulating biomarkers and pre-treatment molecular tissue indicators capable of predicting histopathological chemotherapy response prior to the initiation of cytotoxic therapy represents an urgent clinical priority.[13,14] Among candidate circulating biomarkers, serum alkaline phosphatase (ALP)—specifically the bone-specific isoform of tissue non-specific alkaline phosphatase (TNAP)—has long been recognized as a biochemical hallmark of osteoblast activity and matrix mineralization.[14,15] Serum ALP is an ectoenzyme anchored to the outer cell membrane via a glycosylphosphatidylinositol (GPI) moiety, where it functions to hydrolyze extracellular inorganic pyrophosphate (PPi), a potent inhibitor of calcification, thereby liberating free phosphate ions and promoting the deposition of hydroxyapatite crystals in osteoid matrix.[14] In osteosarcoma, proliferating malignant osteoblasts characteristically overexpress ALP and secrete matrix metalloproteinases, resulting in marked elevations of circulating enzyme levels in 70% to 90% of newly diagnosed patients.[14,16] Numerous clinical studies have correlated high baseline serum ALP with larger tumor burden, higher histological grade, advanced radiological stage, and an increased risk of pulmonary metastasis.[14-16] However, the capacity of pre-treatment serum ALP to prospectively discriminate between good and poor histopathological chemotherapy response (Huvos grade) remains inconsistent across published series, with conflicting reports failing to achieve diagnostic consensus.[14,17,18]

Concurrently, research into the molecular pathogenesis of osteosarcoma has illuminated the central role of TP53 gene disruptions in driving oncogenesis, genomic instability, and chemotherapeutic resistance.[19,20] The TP53 gene, located on chromosome 17p13.1, encodes the p53 tumor suppressor phosphoprotein, a pivotal transcriptional regulator that orchestrates cellular responses to physiological stress, DNA damage, and oncogenic signaling.[19] Under normal cellular conditions, wild-type p53 maintains genomic integrity by inducing G1/S or G2/M cell cycle arrest via p21/CDKN1A activation to permit DNA repair, or alternatively, triggering irreversible programmed cell death (apoptosis) via transcriptional upregulation of pro-apoptotic effectors including BAX, PUMA, and NOXA if genomic damage is catastrophic.[19-21] The cytotoxic efficacy of standard osteosarcoma chemotherapy agents—specifically the DNA interstrand cross-linking agent cisplatin and the topoisomerase II poison doxorubicin—relies fundamentally on the induction of severe double-strand DNA breaks that activate p53-dependent apoptotic pathways.[19,22] In osteosarcoma, structural somatic mutations, point missense mutations, or deletions in the TP53 gene occur in up to 50% to 80% of cases.[19,23] Missense mutations within the core DNA-binding domain of TP53 frequently yield conformationally altered mutant p53 proteins that escape murine double minute 2 (MDM2)-mediated ubiquitination and proteasomal degradation, resulting in marked intranuclear accumulation and phenotypic overexpression readily detectable by standard immunohistochemistry (IHC) using monoclonal antibodies such as clone DO-7.[19,24] Functionally, mutant p53 not only loses its tumor-suppressive and pro-apoptotic capacities, but frequently acquires oncogenic gain-of-function properties that actively repress

apoptosis, stimulate survival signaling, and promote multidrug resistance.[19,22,25]

In the Indonesian healthcare setting, the landscape of musculoskeletal oncology presents unique challenges characterized by geographic disparities, diagnostic referral delays, high rates of advanced local presentation, and variable socioeconomic resources.[4,5] At Dr. Mohammad Hoesin General Hospital in Palembang, South Sumatra—the premier tertiary academic oncology referral center serving a multi-provincial population—osteosarcoma patients are routinely managed through a multimodal protocol based on the European pediatric oncology guidelines of the Werkgroep Kindertumoren Emma Kinderziekenhuis Academic Medical Center (WKT-EKZ-AMC, 1989), comprising three cycles of neoadjuvant cisplatin, doxorubicin, and ifosfamide followed by surgical resection and three adjuvant cycles.[4,5] However, despite aggressive systemic administration, a substantial proportion of patients experience suboptimal histological necrosis, disease recurrence, or metastatic progression.[4] To date, no documented clinical investigation in South Sumatra has evaluated the associative and predictive relationships between baseline serum ALP levels, immunohistochemical p53 protein expression, and post-neoadjuvant histopathological tumor necrosis graded by the Huvos system.

To address this critical knowledge gap, the present study was conducted to comprehensively investigate the association between pre-chemotherapy serum ALP levels and immunohistochemical p53 protein expression with histopathological tumor necrosis (Huvos score) in patients with primary high-grade extremity osteosarcoma undergoing standardized neoadjuvant chemotherapy at Dr. Mohammad Hoesin General Hospital Palembang. By evaluating the empirical predictive accuracy, relative risk estimates, and receiver operating characteristic (ROC) discriminative performance of these baseline biomarkers, this study seeks to clarify their translational utility as non-invasive stratification tools and provide evidence-based insights into the complex pathophysiology of chemoresistance in Indonesian osteosarcoma cohorts.

2. Methods

Study Design and Clinical Setting

This investigation was designed and executed as a hospital-based observational analytical nested cohort study utilizing secondary medical record data and archival pathological specimen archives. The study was conducted at the Department of Orthopaedic and Traumatology, the Division of Musculoskeletal Oncology, the Department of Anatomical Pathology, and the Central Medical Record Installation of Dr. Mohammad Hoesin General Hospital in Palembang, South Sumatra, Indonesia. Dr. Mohammad Hoesin General Hospital functions as the top-tier tertiary academic referral center and principal oncology care hub for South Sumatra and neighboring southern provinces of the Indonesian archipelago, serving a catchment population exceeding 15 million individuals. The research protocol, retrospective data retrieval, specimen re-examination, and manuscript compilation were carried out across the study period extending from January 2023 through December 2025.

Ethical Approval and Research Governance

The study protocol conformed strictly to the ethical principles outlined in the Declaration of Helsinki (1964) and its subsequent amendments, and was conducted in full compliance with the International Ethical Guidelines for Health-related Research Involving Humans established by the Council for International Organizations of Medical Sciences (CIOMS) and the World Health Organization (WHO). Ethical clearance and formal exemption were officially granted by the Health Research Ethics Committee of Dr. Mohammad Hoesin General Hospital and the Faculty of Medicine, Universitas Sriwijaya, Palembang, under approval letter number DP.04.03/D.XVIII.06.08/ETIK/219/2025. In accordance with institutional research governance regulations governing retrospective observational research utilizing existing, anonymized clinical data and archived pathological blocks, a formal waiver of individual patient written informed consent was granted. Strict data confidentiality protocols were enforced throughout all phases of data curation; all personal patient identifiers were expunged and replaced with unique alphanumeric research codes stored on password-encrypted, firewall-protected digital institutional servers.

Study Population and Eligibility Criteria

The study population comprised all consecutive patients presenting to Dr. Mohammad Hoesin General Hospital with newly diagnosed, histopathologically confirmed primary high-grade extremity osteosarcoma between January 1, 2023, and December 31, 2025. Given the relatively low annual population incidence of primary bone sarcomas and the specialized tertiary nature of the clinical setting, a total sampling strategy was adopted to eliminate selection bias and capture the full clinical heterogeneity of patients managed under the standardized institutional treatment protocol during the defined three-year observation window.

To establish a rigorous, homogeneous clinical cohort, strict inclusion and exclusion criteria were defined a priori. The inclusion criteria required: (1) patient presentation to the Orthopaedic Oncology Clinic at Dr. Mohammad Hoesin General Hospital between January 2023 and December 2025; (2) primary anatomical tumor localization involving the extremities (long tubular bones of the upper or lower appendicular skeleton); (3) definitive histological diagnosis of high-grade osteosarcoma established by core needle biopsy or open incisional biopsy prior to systemic therapy; (4)

documented availability of baseline, pre-chemotherapy venous blood biochemical assays including serum ALP and lactate dehydrogenase (LDH); (5) documented availability of diagnostic, pre-treatment formalin-fixed paraffin-embedded (FFPE) tissue blocks with adequate cellularity for immunohistochemical evaluation; (6) complete administrative and clinical adherence to three consecutive cycles of standardized neoadjuvant systemic chemotherapy; and (7) successful completion of definitive en-bloc surgical resection (limb salvage surgery or radical amputation) at the institution, with subsequent full macroscopic and microscopic pathological evaluation of the resected surgical specimen.

The exclusion criteria were formulated to eliminate confounding systemic conditions and non-standard therapeutic interventions: (1) secondary osteosarcoma arising from underlying pre-existing bone disease (e.g., Paget disease of bone, fibrous dysplasia) or secondary to prior external beam ionizing radiation therapy; (2) primary craniofacial, spinal, pelvic, or axial skeletal osteosarcoma, which exhibit divergent surgical resectability, biological behaviors, and drug delivery dynamics; (3) pre-existing chronic hepatobiliary disease, cholestasis, liver cirrhosis, active viral hepatitis, or chronic renal failure, which independently alter systemic circulating alkaline phosphatase clearance; (4) concomitant metabolic bone disorders known to distort osteoblastic turnover, including primary hyperparathyroidism, osteomalacia, rickets, or active healing of concurrent non-neoplastic skeletal fractures; (5) prior administration of cytotoxic chemotherapy, immunotherapy, or targeted kinase inhibitors at outside facilities prior to baseline institutional assessment; (6) premature discontinuation, failure to complete, or protocol deviation during neoadjuvant chemotherapy; and (7) surgical resection performed at external institutions or missing resected pathology specimens precluding standardized Huvos necrosis assessment.

Neoadjuvant Chemotherapy Protocol

All included patients were treated according to the standardized institutional clinical osteosarcoma protocol established at Dr. Mohammad Hoesin General Hospital, which is adapted from the European pediatric oncology guidelines of the Werkgroep Kindertumoren Emma Kinderziekenhuis Academic Medical Center (WKT-EKZ-AMC, 1989) and aligned with the Indonesian National Clinical Practice Guidelines (PNPK) for Osteosarcoma.[5,26-28] The protocol mandates three intensive preoperative (neoadjuvant) cycles administered at 21-day intervals, followed by definitive surgical resection and three identical postoperative (adjuvant) cycles. Each 21-day cycle comprises: (1) Cisplatin administered at a dose of 100 mg/m^2 intravenously over 24 hours on Day 1, with aggressive pre- and post-hydration (mannitol diuresis) to mitigate nephrotoxicity; (2) Doxorubicin (Adriamycin) administered at a dose of 25 mg/m^2/day as a continuous intravenous infusion on Days 1 and 2 (cumulative dose 50 mg/m^2 per cycle), with cardiac monitoring; and (3) Ifosfamide administered at a dose of 2,000 mg/m^2/day intravenously on Days 1 through 3 (cumulative dose 6,000 mg/m^2 per cycle), accompanied by sodium 2-mercaptoethanesulfonate (mesna) uroprotection (administered at 120% of the total ifosfamide dose in divided intravenous doses) and hyperhydration to prevent hemorrhagic cystitis. Supportive care included standardized serotonin 5-HT3 receptor antagonists, dexamethasone, and prophylactic granulocyte colony-stimulating factor (G-CSF) support as clinically indicated.

Surgical Management

Following completion of the third neoadjuvant cycle and complete hematological recovery (absolute neutrophil count >1,500/μL, platelet count >100,000/μL), patients underwent clinical re-staging, including plain radiography, regional contrast-enhanced magnetic resonance imaging (MRI) of the involved limb segment, and chest computed tomography (CT) to assess local tumor demarcation and systemic pulmonary status.[29] Definitive surgical resection was scheduled between 3 and 4 weeks following the final neoadjuvant chemotherapy infusion. Surgical decision-making was conducted by a dedicated multidisciplinary musculoskeletal tumor board. Wide en-bloc surgical resection was executed by board-certified orthopaedic oncology surgeons, with the primary objective of achieving clear, tumor-free margins (R0 resection) according to the Enneking oncological staging criteria.[8,30] Depending on anatomical neurovascular proximity, intra-articular tumor extension, soft-tissue contamination, and pathological fracture status, patients underwent either limb salvage surgery (LSS) involving wide resection and endoprosthetic reconstruction with custom modular megaprostheses, or radical limb ablation (amputation or disarticulation).[8,30]

Measurement of Circulating Serum Alkaline Phosphatase

Baseline venous blood samples (5 mL) were obtained from each patient prior to the initiation of any cytotoxic chemotherapy, typically collected on the morning of admission for diagnostic biopsy or during pre-treatment baseline staging. Blood was collected into standard gel-barrier vacutainer tubes without anticoagulants, allowed to clot at room temperature for 30 minutes, and centrifuged at 3,000 rpm for 10 minutes to separate serum. Serum alkaline phosphatase (ALP) activity was quantified spectrophotometrically using the standard International Federation of Clinical Chemistry (IFCC) reference method on an automated clinical chemistry analyzer (Cobas c501/c702 module; Roche Diagnostics GmbH, Mannheim, Germany) in the central laboratory of Dr. Mohammad Hoesin General Hospital. The assay measures the catalytic rate of conversion of p-

nitrophenyl phosphate to p-nitrophenol in the presence of 2-amino-2-methyl-1-propanol (AMP) buffer at pH 10.4 and 37°C, with absorbance monitored at 405 nm. In accordance with the manufacturer's calibrated specifications and clinical laboratory diagnostic standards at our institution, circulating serum ALP levels were categorized as normal (≤104 U/L) or elevated (>104 U/L).[14,17] Additionally, baseline serum lactate dehydrogenase (LDH) was measured on the same platform, with levels categorized as normal (≤250 U/L) or elevated (>250 U/L).[17]

Immunohistochemical Assessment of p53 Protein Expression

Immunohistochemical staining for p53 protein was performed on representative 4-µm-thick formalin-fixed paraffin-embedded (FFPE) tissue sections obtained from pre-treatment diagnostic core needle biopsy specimens. Tissue sections were deparaffinized in xylene and rehydrated through descending graded ethanol solutions to distilled water. Heat-induced epitope retrieval (HIER) was executed in 10 mM sodium citrate buffer (pH 6.0) using an automated pressurized decloaking chamber at 95°C for 20 minutes. Endogenous peroxidase activity was quenched by incubating sections in 3% hydrogen peroxide for 10 minutes at room temperature, followed by incubation with 5% normal goat serum for 30 minutes to block non-specific background binding. Sections were subsequently incubated with a primary mouse monoclonal anti-p53 antibody (Clone DO-7, ready-to-use; Dako/Agilent Technologies, Glostrup, Denmark) for 60 minutes at room temperature. Clone DO-7 recognizes an epitope residing between amino acids 19 and 26 of both human wild-type and missense-mutant p53 protein, primarily detecting the accumulated, conformationally stabilized mutant protein resulting from prolonged half-life.[19,22] Secondary detection was accomplished using the horseradish peroxidase (HRP)-conjugated polymer detection system (EnVision+ System-HRP; Dako/Agilent Technologies), followed by visualization with 3,3'-diaminobenzidine (DAB) chromogen and light nuclear counterstaining with Mayer's hematoxylin. High-grade serous ovarian carcinoma tissue known to harbor strong TP53 missense mutations served as a positive control, while omission of the primary antibody served as a negative control.

Immunohistochemical scoring was independently performed by two certified anatomical pathologists blinded to patient clinical outcomes, baseline laboratory values, and final Huvos necrosis grades. Nuclear staining was evaluated in representative high-power fields (400× magnification) containing viable malignant osteoblasts, avoiding areas of biopsy-induced crush artifact, hemorrhage, or spontaneous necrosis. In accordance with established international consensus guidelines for TP53 biomarker evaluation in sarcomas, p53 immunoreactivity was quantified as the percentage of neoplastic cell nuclei exhibiting distinct chromogenic DAB positivity among at least 500 viable tumor cells. Tumors displaying distinct nuclear staining in ≥10% of neoplastic cells were scored as positive (indicative of mutant p53 protein accumulation), whereas tumors exhibiting <10% nuclear reactivity were classified as negative.[19,22] Inter-observer concordance was evaluated, and discordant cases were resolved by simultaneous re-evaluation at a multi-headed microscope to reach definitive consensus.

Histopathological Tumor Necrosis Quantification (Huvos Grading)

Histopathological evaluation of chemotherapy response was conducted on the definitive surgical resection specimens (limb salvage or amputation) following completion of neoadjuvant therapy, adhering strictly to the macroscopic mapping and microscopic quantification protocol established by Huvos and colleagues at Memorial Sloan Kettering Cancer Center.[9] Upon surgical resection, the specimen was immediately transferred to the Department of Anatomical Pathology, oriented, radiographed, and fixed in 10% neutral buffered formalin for 48 to 72 hours. The bone tumor was sectioned longitudinally in the coronal or sagittal plane using an electric bone band saw into continuous 5-mm-thick whole-organ slices. Slices were photographed, mapped onto a standardized grid diagram, decalcified in 10% ethylenediaminetetraacetic acid (EDTA) or formic acid solution, and systematically sampled to encompass the entire tumor cross-section, tumor-host bone interfaces, neurovascular margins, and surrounding soft-tissue components. A minimum of one tissue block per centimeter of maximum tumor diameter was embedded in paraffin, sectioned at 4 µm, and stained with hematoxylin and eosin (H&E).

Microscopic evaluation was conducted by certified anatomical pathologists to determine the percentage of chemotherapy-induced tumor necrosis across all mapped whole-mount slides. Chemotherapy response was characterized morphologically by acellular ghost tumor cells, extensive coagulative necrosis, pyknosis, karyorrhexis, osteoid matrix homogenization, cystic degeneration, foamy histiocyte infiltration, and dense reparative fibrosis, contrasted against areas of viable, proliferating atypical osteoblasts with intact nuclear chromatin and mitotic activity. The global histological tumor necrosis was calculated as: Chemotherapy Necrosis Percentage = (Total Surface Area of Acellular Necrotic/Fibrotic Tumor / Total Tumor Surface Area Evaluated) × 100%. The resulting percentage was classified into four discrete Huvos grades: Grade I, little or no effect of chemotherapy (<50% necrosis); Grade II, partial response with 50% to 89% necrosis; Grade III, good response with 90% to 99% necrosis; and Grade IV, complete response with 100% necrosis (no viable histological tumor). In accordance with standard oncological practice and clinical trial design, Huvos grades were dichotomized into poor histological

response (Huvos grades I–II; <90% necrosis) and good histological response (Huvos grades III–IV; ≥90% necrosis).[7,9-11]

Statistical Analysis

All statistical analyses were executed using IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism, Version 9.5.0 (GraphPad Software, San Diego, CA, USA). Continuous variables were tested for normality using the Shapiro-Wilk test and visual inspection of Q-Q plots. Normally distributed continuous variables were expressed as mean ± standard deviation (SD), whereas non-normally distributed continuous variables were presented as median and range (minimum–maximum). Categorical variables were summarized as absolute frequencies (n) and relative percentages (%). Bivariate associations between categorical baseline variables (serum ALP normal vs. elevated; p53 IHC negative vs. positive) and dichotomized Huvos response (poor vs. good) were examined using Pearson's Chi-Square test (χ^2) with continuity correction where appropriate. Because of the modest sample size (n = 14) and the occurrence of contingency cells with expected counts <5, two-tailed Fisher's Exact Test was evaluated as the primary confirmatory test of association. Effect sizes were quantified by calculating Odds Ratios (OR) and Relative Risks (RR) for poor histological response, accompanied by 95% confidence intervals (95% CI) computed via the Woolf logit method. To evaluate whether continuous pre-treatment serum ALP levels exhibited discriminative diagnostic accuracy independent of the clinical cutoff, non-parametric Receiver Operating Characteristic (ROC) curve analysis was conducted. The Area Under the Curve (AUC) was calculated along with standard error (SE), asymptotic two-tailed p-values, and 95% confidence intervals. The optimal empirical threshold for serum ALP was determined using Youden's J statistic (J = Sensitivity + Specificity - 1). A two-sided p-value <0.05 was considered statistically significant across all tests.

3. Results

Cohort Demographics and Clinical Characteristics

During the three-year investigation period from January 2023 through December 2025, a total of 14 consecutive patients with primary high-grade extremity osteosarcoma met all eligibility criteria and completed the study protocol. The baseline demographic and clinical characteristics of the study cohort are detailed in Table 1 and Table 2, and visually illustrated in Figure 1. Among the 14 patients, there was a predominance of males (n = 9; 64.3%) over females (n = 5; 35.7%), establishing a male-to-female ratio of 1.8:1. The age of the cohort ranged from 9.0 to 45.0 years, with a mean ± SD of 22.00 ± 10.74 years and a median age of 18.00 years, reflecting the characteristic predilection of osteosarcoma for adolescents and young adults during periods of skeletal growth. The primary tumor predilection was located exclusively in the appendicular skeleton, with the distal femur being the most frequent site (n = 8; 57.1%), followed by the proximal tibia (n = 4; 28.6%), and the proximal humerus (n = 2; 14.3%). Histopathological subtyping revealed that conventional osteoblastic osteosarcoma was the predominant morphological variant (n = 10; 71.4%), followed by chondroblastic osteosarcoma (n = 3; 21.4%) and fibroblastic osteosarcoma (n = 1; 7.1%). Regarding definitive surgical intervention following neoadjuvant chemotherapy, 8 patients (57.1%) underwent limb salvage surgery (LSS) with modular megaprosthetic reconstruction, whereas 6 patients (42.9%) required radical limb ablation (amputation) due to major neurovascular encasement or extensive soft-tissue invasion.

Table 1. Baseline Demographic and Clinical Characteristics of the Osteosarcoma Cohort (n = 14).

Variable / Characteristic	Frequency (n)	Percentage (%)
Age Group (years)	7	50.0%
• Pediatric / Adolescent (<18 years)	5	35.7%
• Young Adult (18–30 years)	2	14.3%
• Adult (>30 years)		
Sex	9	64.3%
• Male	5	35.7%
• Female		
Primary Anatomical Location	8	57.1%
• Distal Femur	4	28.6%
• Proximal Tibia	2	14.3%
• Proximal Humerus		
Histopathological Subtype	10	71.4%
• Osteoblastic Osteosarcoma	3	21.4%
• Chondroblastic Osteosarcoma	1	7.1%
• Fibroblastic Osteosarcoma		
Definitive Surgical Intervention	8	57.1%
• Limb Salvage Surgery (LSS)	6	42.9%
• Radical Amputation / Disarticulation		
Clinical Follow-up Status	6	42.9%
• Alive without evidence of disease	5	35.7%
• Disease progression / Metastasized	3	21.4%
• Deceased		

Data presented as absolute numbers (n) and relative percentages (%). Abbreviation: LSS, limb salvage surgery.

Table 2. Distribution of Baseline Biomarkers and Post-Chemotherapy Histopathological Response (n = 14).

Biomarker / Histological Parameter	Classification	Frequency (n)	Percentage (%)
Pre-Chemotherapy Serum Alkaline Phosphatase (ALP) (Mean ± SD: 301.79 ± 243.68 U/L; Median: 217.50 U/L)	Elevated (> 104 U/L) Normal (≤ 104 U/L)	13 1	92.9% 7.1%
Pre-Chemotherapy Serum Lactate Dehydrogenase (LDH) (Mean ± SD: 650.07 ± 386.44 U/L; Median: 512.00 U/L)	Elevated (> 250 U/L) Normal (≤ 250 U/L)	13 1	92.9% 7.1%
Immunohistochemical p53 Protein Expression (Clone DO-7, Monoclonal Antibody)	Positive (≥ 10% nuclear staining) Negative (< 10% nuclear staining)	11 3	78.6% 21.4%
Huvos Histopathological Tumor Necrosis Grade (Dichotomized Clinical Response)	Poor Response (Grades I–II; <90% necrosis) • Huvos Grade I (<50% necrosis) • Huvos Grade II (50%–89% necrosis) Good Response (Grades III–IV; ≥90% necrosis) • Huvos Grade III (90%–99% necrosis) • Huvos Grade IV (100% complete necrosis)	11 2 9 3 3 0	78.6% 14.3% 64.3% 21.4% 21.4% 0.0%

Cutoff thresholds: Serum ALP >104 U/L; Serum LDH >250 U/L; p53 immunoreactivity ≥10% nuclear positivity; Huvos necrosis ≥90% good response.
Abbreviations: ALP, alkaline phosphatase; LDH, lactate dehydrogenase; SD, standard deviation.

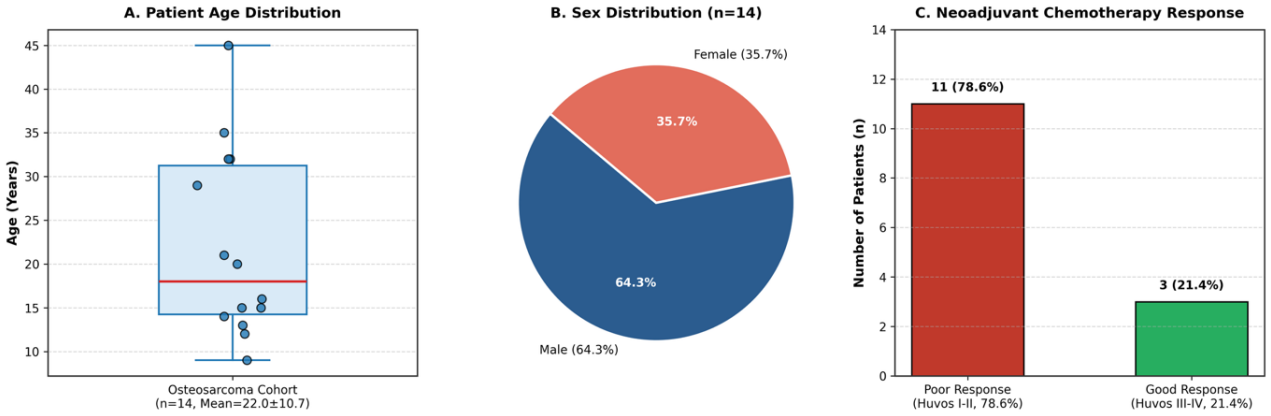

Figure 1. *Baseline demographic and histopathological response distribution in the osteosarcoma cohort (n = 14). (A) Age distribution boxplot with individual patient data points demonstrating adolescent predilection (mean 22.0 ± 10.7 years). (B) Sex distribution pie chart showing male predominance (64.3%). (C) Neoadjuvant chemotherapy histopathological response classified by the Huvos grading system, showing a predominance of poor histological responders (78.6%).*

Bivariate Association of Serum ALP and p53 with Huvos Histological Response

To address the primary research objectives, bivariate contingency analyses were conducted cross-tabulating pre-chemotherapy serum ALP levels and immunohistochemical p53 protein expression against the dichotomized Huvos histopathological response, as summarized in Table 3 and illustrated in Figure 2. Among the 13 patients presenting with elevated baseline serum ALP (>104 U/L), 10 patients (76.9%) exhibited a poor histological response (Huvos grades I–II), while 3 patients (23.1%) achieved a good histological response (Huvos grades III–IV). The single patient presenting with a normal baseline serum ALP level (91.0 U/L) demonstrated a poor histological response (Huvos grade II, 100.0%). Crosstabulation analysis yielded a Pearson Chi-Square value of χ^2 = 0.294 (df = 1, asymptotic p = 0.588). Given the presence of expected cell frequencies below 5, Fisher's Exact Test was evaluated and demonstrated a two-sided p-value of 1.000, confirming that baseline serum ALP elevation was not significantly associated with post-neoadjuvant tumor necrosis. Risk analysis revealed a Relative Risk (RR) of 0.769 (95% CI: 0.571–1.036) for poor histological response in patients with elevated baseline ALP compared to those with normal levels. The fact that the 95% confidence interval spans unity underscores the absence of a statistically significant association between pre-treatment serum ALP and chemotherapy-induced tumor necrosis.

Similarly, evaluation of immunohistochemical p53 protein expression revealed no statistically significant correlation with Huvos tumor necrosis. Among the 11 patients exhibiting positive p53 nuclear

immunoreactivity (≥10% positive nuclei), 9 patients (81.8%) were classified as poor histological responders, whereas 2 patients (18.2%) achieved a good response. In the p53-negative group (n = 3), 2 patients (66.7%) exhibited a poor response and 1 patient (33.3%) achieved a good response. Pearson Chi-Square analysis yielded χ^2 = 0.321 (df = 1, asymptotic p = 0.571), and two-sided Fisher's Exact Test demonstrated p = 1.000. Risk estimation for mutant p53 expression yielded an Odds Ratio (OR) of 2.250 (95% CI: 0.130–38.811) and a Relative Risk (RR) of 1.227 (95% CI: 0.526–2.864) for poor histological response. The extremely wide 95% confidence interval and p = 1.000 confirm that pre-treatment p53 immunoreactivity does not reliably differentiate between poor and good histological responders in this clinical cohort.

Table 3. Bivariate Association of Baseline Biomarkers with Huvos Tumor Necrosis Response (n = 14).

Baseline Biomarker Variable	Category	Poor Response (I–II) (n = 11, 78.6%)	Good Response (III–IV) (n = 3, 21.4%)	Total (n = 14)	Risk Estimates (95% CI)	Statistical Test (p-value)*
Serum Alkaline Phosphatase (ALP)	Elevated (> 104 U/L) Normal (≤ 104 U/L)	10 (76.9%) 1 (100.0%)	3 (23.1%) 0 (0.0%)	13 1	RR = 0.769 (0.571–1.036) Reference	Pearson χ^2 = 0.294 (p = 0.588) Fisher's Exact Test p = 1.000
Immunohistochemical p53 Protein	Positive (≥ 10%) Negative (< 10%)	9 (81.8%) 2 (66.7%)	2 (18.2%) 1 (33.3%)	11 3	OR = 2.250 (0.130–38.811) RR = 1.227 (0.526–2.864)	Pearson χ^2 = 0.321 (p = 0.571) Fisher's Exact Test p = 1.000
Serum Lactate Dehydrogenase (LDH)	Elevated (> 250 U/L) Normal (≤ 250 U/L)	10 (76.9%) 1 (100.0%)	3 (23.1%) 0 (0.0%)	13 1	RR = 0.769 (0.571–1.036) Reference	Pearson χ^2 = 0.294 (p = 0.588) Fisher's Exact Test p = 1.000

**Statistical significance assessed at alpha = 0.05. Fisher's exact test reported as two-sided. Continuity correction χ^2: ALP = 0.000 (p = 1.000); p53 = 0.000 (p = 1.000). Abbreviations: ALP, alkaline phosphatase; LDH, lactate dehydrogenase; OR, odds ratio; RR, relative risk; CI, confidence interval.*

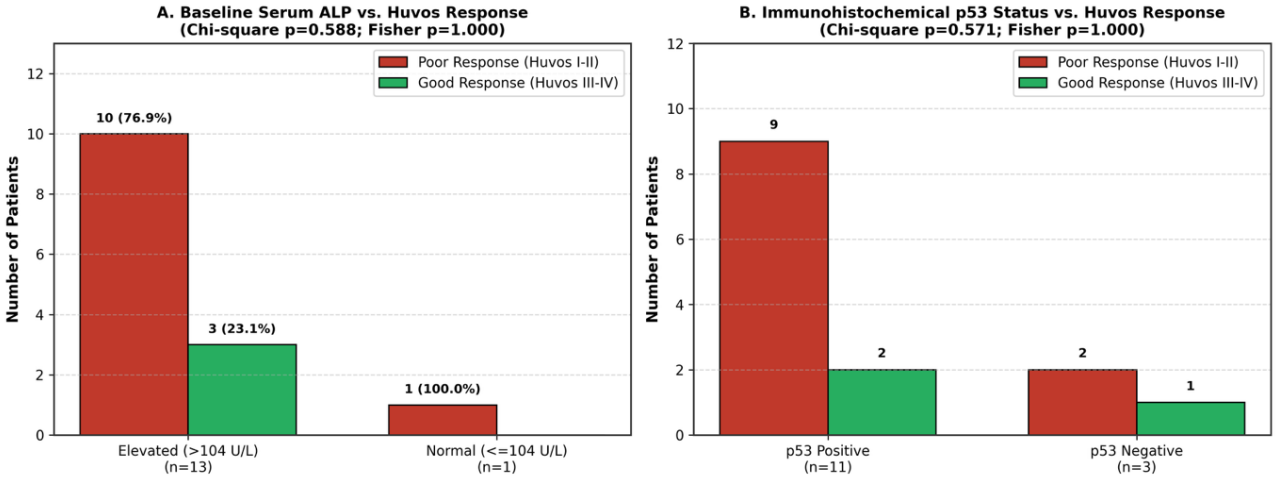

Figure 2. *Bivariate distribution of Huvos histopathological response according to baseline biomarker status. (A) Proportions of poor (Huvos I–II) and good (Huvos III–IV) responders stratified by baseline serum ALP status (>104 U/L vs. ≤104 U/L; Fisher's exact p = 1.000). (B) Proportions of poor and good responders stratified by pre-treatment immunohistochemical p53 protein expression (positive vs. negative; Fisher's exact p = 1.000).*

Receiver Operating Characteristic (ROC) Curve Analysis of Baseline Serum ALP

To determine whether continuous baseline serum ALP levels possessed diagnostic discriminatory capacity for predicting chemotherapy-induced histological necrosis that was obscured by the conventional clinical reference threshold (104 U/L), a non-parametric Receiver Operating Characteristic (ROC) curve analysis was conducted with poor histological response (Huvos grades I–II) designated as the positive actual state. The resulting empirical ROC curve is illustrated in Figure 3, with corresponding diagnostic coordinate matrices detailed in Table 4. The area under the curve (AUC) for baseline serum ALP was calculated at 0.515 (Standard Error = 0.158; asymptotic two-tailed p = 0.938; 95% CI: 0.205–0.825). Because an AUC of 0.500 denotes complete diagnostic equivalence to random chance, this finding indicates that pre-treatment circulating ALP has no intrinsic capacity to distinguish between patients who will achieve extensive histological tumor necrosis (≥90%) and those who will experience poor necrosis (<90%).

Table 4. Receiver Operating Characteristic (ROC) Diagnostic Performance and Selected Coordinate Cutoffs for Pre-Chemotherapy Serum ALP (n = 14).

Serum ALP Cutoff Threshold	Sensitivity (True Positive Rate)	1 - Specificity (False Positive Rate)	Specificity (True Negative Rate)	Youden's Index (J)*
90.0 U/L	1.000 (100.0%)	1.000 (100.0%)	0.000 (0.0%)	0.000
104.0 U/L (Clinical Reference)	0.909 (90.9%)	1.000 (100.0%)	0.000 (0.0%)	-0.091
150.0 U/L	0.636 (63.6%)	0.667 (66.7%)	0.333 (33.3%)	-0.031
176.5 U/L (Optimal Empirical Cutoff)	0.364 (36.4%)	0.000 (0.0%)	1.000 (100.0%)	0.364
250.0 U/L	0.364 (36.4%)	0.000 (0.0%)	1.000 (100.0%)	0.364
500.0 U/L	0.182 (18.2%)	0.000 (0.0%)	1.000 (100.0%)	0.182

**Area Under the Curve (AUC) = 0.515 (SE = 0.158; asymptotic p = 0.938; 95% CI: 0.205–0.825). Youden's Index J = Sensitivity + Specificity - 1. Abbreviations: ALP, alkaline phosphatase; ROC, receiver operating characteristic; AUC, area under the curve; SE, standard error; CI, confidence interval.*

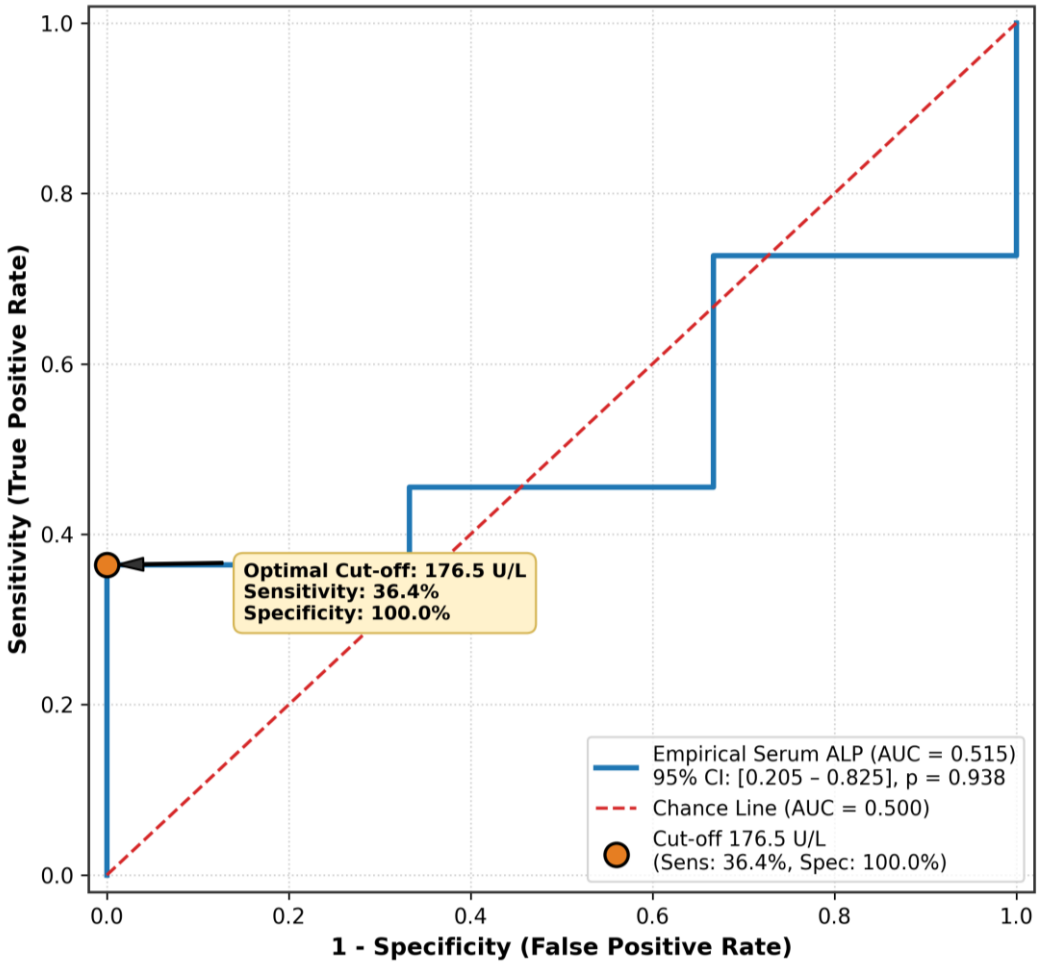

Figure 3. *Empirical Receiver Operating Characteristic (ROC) curve of pre-chemotherapy serum alkaline phosphatase (ALP) for discriminating post-neoadjuvant histopathological poor response (Huvos I–II). Blue line denotes empirical ALP curve (AUC = 0.515, 95% CI: 0.205–0.825, p = 0.938); red dashed line denotes diagonal reference of chance (AUC = 0.500); orange point indicates the optimal empirical Youden cutoff at 176.5 U/L (sensitivity 36.4%, specificity 100.0%).*

Stratified Contingency Analysis of the Empirical Serum ALP Threshold (176.5 U/L)

Evaluation of the ROC coordinates identified an empirical optimal cutoff value at 176.5 U/L, which maximized Youden's index (J = 0.364). At this cutoff point, baseline serum ALP demonstrated a specificity of 100.0% but an extremely poor sensitivity of 36.4%. To evaluate whether stratifying patients by this empirical cutoff yielded prognostic separation, a 2×2 contingency table was generated, as detailed in Table 5. Among patients with serum ALP >176.5 U/L (n = 9), 6 patients (66.7%) exhibited a poor histological response and 3 patients (33.3%) achieved a good response. Among patients with serum ALP ≤176.5 U/L (n = 5), all 5 patients (100.0%) exhibited a poor histological response. The Pearson Chi-Square test yielded χ^2 = 2.121 (df = 1, p = 0.145), and Fisher's Exact Test yielded p = 0.258. The Relative Risk for poor histological response in patients with ALP >176.5 U/L was 0.667 (95% CI: 0.420–1.058). Thus, even when utilizing an

optimized empirical threshold, pre-chemotherapy serum ALP failed to demonstrate a statistically significant association with post-chemotherapy tumor necrosis. Forest plot visualization of all calculated risk estimates (OR and RR) across the evaluated markers is depicted in Figure 4, confirming that all 95% confidence intervals cross the line of null effect (1.0).

Table 5. Stratified Contingency Analysis of the Empirical Serum ALP Threshold (176.5 U/L) Against Huvos Necrosis Outcome (n = 14).

ALP Empirical Cutoff (176.5 U/L)	Poor Response (I–II)	Good Response (III–IV)	Total (n)	Relative Risk (95% CI)	Statistical Significance*
> 176.5 U/L ≤ 176.5 U/L	6 (66.7%) 5 (100.0%)	3 (33.3%) 0 (0.0%)	9 5	RR = 0.667 (0.420–1.058) Reference	Pearson χ^2 = 2.121 (p = 0.145) Fisher's Exact Test p = 0.258
Total Cohort	11 (78.6%)	3 (21.4%)	14	—	Likelihood Ratio = 3.091 (p = 0.079)

**Statistical significance assessed at alpha = 0.05. Continuity correction χ^2 = 0.603 (p = 0.437); Linear-by-linear association χ^2 = 1.970 (p = 0.160).*
Abbreviations: ALP, alkaline phosphatase; RR, relative risk; CI, confidence interval.

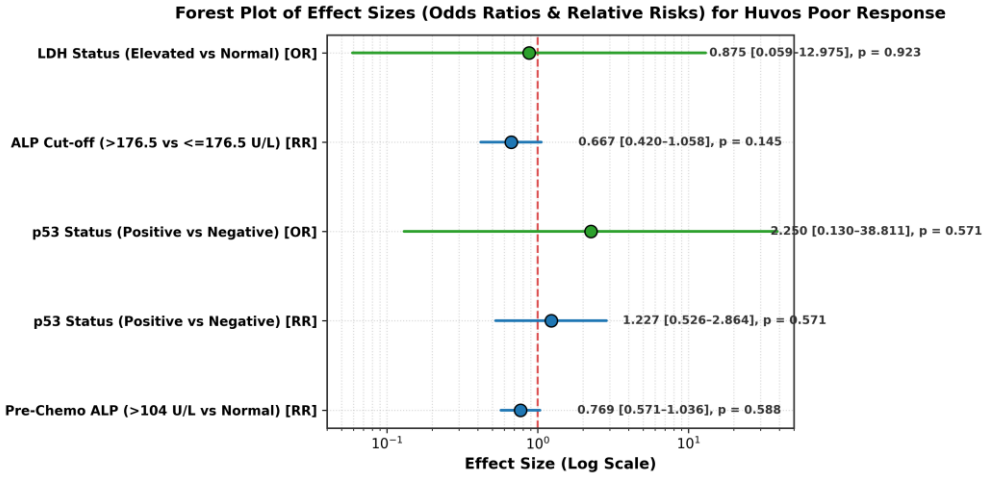

Figure 4. *Forest plot of calculated effect sizes (Odds Ratios and Relative Risks) with 95% Confidence Intervals for pre-chemotherapy serum ALP, p53 immunohistochemical expression, empirical ALP cutoff, and serum LDH in predicting Huvos poor histological response. The red vertical dashed line indicates the line of null effect (1.0). All evaluated parameters demonstrate 95% confidence intervals crossing the null boundary.*

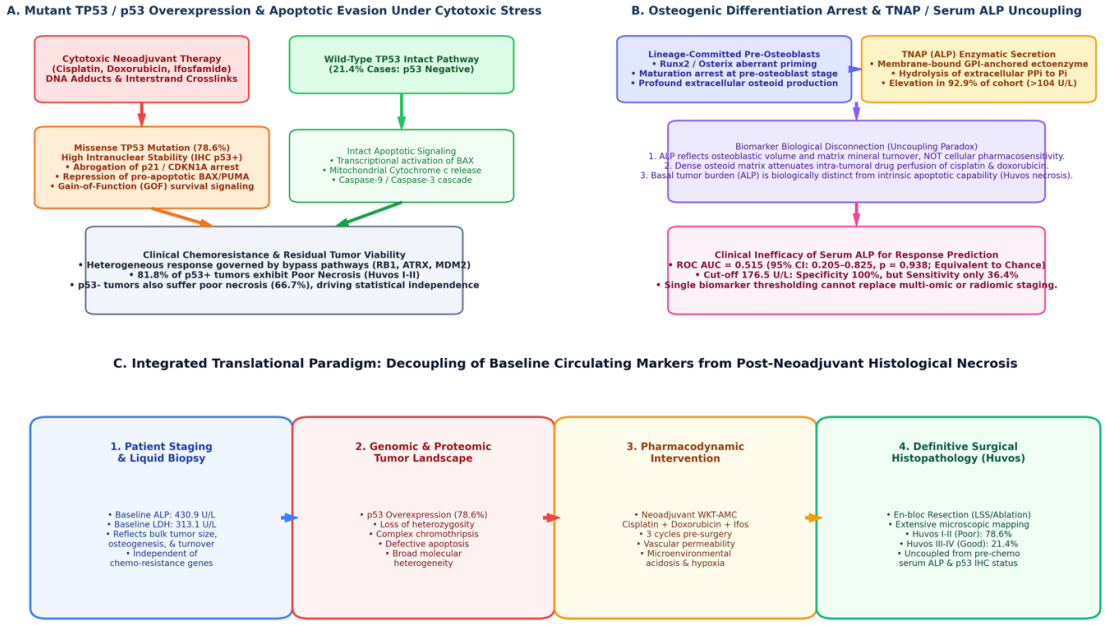

Figure 5. *Translational biomolecular mechanism diagram illustrating the uncoupling paradox of pre-chemotherapy biomarkers and post-neoadjuvant histological necrosis in osteosarcoma. (A) Molecular cascade of mutant TP53 overexpression leading to escape from MDM2 degradation, loss of p21-mediated cell cycle arrest, repression of BAX/PUMA, and attenuation of DNA damage-induced apoptosis under cisplatin, doxorubicin, and ifosfamide therapy. (B) Aberrant pre-osteoblast lineage arrest driving high TNAP (ALP) ectoenzyme expression and massive osteoid matrix deposition, creating elevated interstitial fluid pressure and a physical diffusion barrier that decouples baseline metabolic turnover from cellular chemosensitivity (ROC AUC = 0.515). (C) Integrated translational schema illustrating the biological discordance between circulating liquid biopsy staging and definitive microscopic Huvos tumor necrosis in tertiary referral practice.*

4. Discussion

The primary clinical objective of neoadjuvant chemotherapy in high-grade extremity osteosarcoma is to achieve extensive microscopic tumor necrosis (≥90%; Huvos grades III–IV), which serves as the premier benchmark of systemic chemosensitivity and the most reliable surrogate of long-term event-free survival.[7,9,11,12] However, identifying reliable, non-invasive pre-treatment clinical, biochemical, or molecular biomarkers capable of predicting this critical histopathological endpoint prior to initiating toxic multi-agent therapy remains an elusive goal in translational oncology.[13,14] In this prospective nested cohort study conducted at Dr. Mohammad Hoesin General Hospital Palembang, we performed the first dedicated clinical evaluation in South Sumatra examining the predictive utility of baseline pre-chemotherapy serum alkaline phosphatase (ALP) levels and immunohistochemical p53 protein expression in relation to definitive post-resection Huvos necrosis scores.

The principal finding of our investigation is that neither baseline serum ALP activity nor immunohistochemical p53 protein expression demonstrated a statistically significant association with post-neoadjuvant histopathological tumor necrosis (all $p > 0.05$). Furthermore, Receiver Operating Characteristic (ROC) curve analysis for baseline serum ALP yielded an area under the curve (AUC) of 0.515 (95% CI: 0.205–0.825, $p = 0.938$), a value virtually indistinguishable from 0.500, confirming that circulating baseline ALP functions at the level of pure chance in discriminating between poor and good histological responders. Even when patients were stratified using an empirically optimized Youden cutoff of 176.5 U/L, which provided 100% specificity, sensitivity remained profoundly deficient at 36.4%, and contingency testing showed no significant difference in necrosis outcomes ($p = 0.258$). These empirical findings establish that pre-chemotherapy circulating ALP and static p53 immunoreactivity cannot serve as reliable baseline predictive biomarkers for chemotherapy-induced tumor necrosis.

To elucidate why baseline serum ALP fails to predict post-chemotherapy necrosis, it is necessary to examine the biological role of this ectoenzyme within the osteogenic lineage—a phenomenon we designate as the 'Uncoupling Paradox' of circulating ALP (schematized in Figure 5B).[14,15,31] Serum ALP, predominantly represented in osteosarcoma by the bone isoform of tissue non-specific alkaline phosphatase (TNAP), is an ectoenzyme tethered to the outer cell membrane of osteoblasts by a glycosylphosphatidylinositol (GPI) anchor.[14,15] In normal physiology and osteosarcoma pathogenesis, TNAP functions enzymatically to cleave inorganic pyrophosphate (PPi)—a physiological inhibitor of calcification—thereby liberating inorganic phosphate (Pi) to promote the deposition of hydroxyapatite crystals in the newly formed osteoid matrix.[14,31] Consequently, circulating serum ALP directly reflects the total volume of osteoblastically active tumor tissue, the degree of extracellular bone matrix formation, and gross disease burden.[14-16] However, our findings demonstrate that macroscopic metabolic bone turnover and total neoplastic bulk are biologically uncoupled from intrinsic cellular pharmacosensitivity. A massive, highly osteoblastic tumor producing immense quantities of ALP may possess exquisite intrinsic cellular sensitivity to the DNA-damaging effects of cisplatin and doxorubicin, resulting in extensive post-chemotherapy necrosis (Huvos IV). Conversely, a smaller, osteolytic or telangiectatic tumor with only moderate ALP elevation may harbor intrinsic or acquired multidrug resistance mechanisms, resulting in absolute chemoresistance and minimal necrosis (Huvos I).

Furthermore, the dense unmineralized osteoid and fibrotic matrix characteristic of ALP-rich osteoblastic osteosarcoma forms a dense biophysical barrier that impairs vascular drug delivery.[8,14,31] Dense extracellular matrix deposition combined with disorganized tumor neoangiogenesis generates elevated interstitial fluid pressure (IFP) within the tumor core, physically attenuating the transcapillary transport, convection, and diffusion of hydrophilic cytotoxic agents such as cisplatin and ifosfamide into deep neoplastic compartments.[8,31] Thus, baseline ALP elevation may capture contradictory pathophysiological forces: while reflecting active tumor turnover, it simultaneously signifies a microenvironment prone to impaired drug delivery. This biological dissonance explains why static pre-treatment ALP levels fail to correlate with subsequent histological necrosis in our cohort, echoing the observations of Bramer et al.[18] and Wang et al.,[14] who demonstrated that baseline ALP correlates with overall disease burden and metastatic risk, but not with local histological response.

The second major biological parameter evaluated in this study was the immunohistochemical expression of the p53 tumor suppressor protein. The TP53 gene orchestrates the canonical DNA damage response pathway, serving as the critical molecular guardian that executes cell cycle arrest or initiates apoptosis following chemotherapy-induced double-strand breaks (schematized in Figure 5A).[19,20] In wild-type cells exposed to DNA adducts from cisplatin or topoisomerase II inhibition by doxorubicin, p53 accumulates rapidly, transcriptionally upregulating p21/CDKN1A to halt cell cycle progression, while concurrently transactivating pro-apoptotic effectors including BAX, PUMA, and NOXA to trigger mitochondrial outer membrane permeabilization (MOMP), cytochrome c release, and caspase-dependent apoptosis.[19-21] In osteosarcoma, however, TP53 is disrupted in up to 80% of cases through complex

structural rearrangements, copy number losses, or point mutations.[19,24]

In our cohort, p53 nuclear immunoreactivity was detected in 78.6% of patients, yet positive expression showed no statistically significant association with poor Huvos necrosis (81.8% in p53-positive vs. 66.7% in p53-negative; p = 1.000; OR = 2.250). This apparent paradox stems from the biological limitations of static immunohistochemistry in capturing the multi-dimensional genomic spectrum of TP53 disruptions.[19,21,32] Standard diagnostic immunohistochemistry using monoclonal antibody clone DO-7 reliably detects high levels of missense-mutant p53 protein, which resists MDM2-mediated degradation and accumulates within tumor cell nuclei.[19,24] Many of these missense mutations exert dominant-negative or gain-of-function (GOF) effects that promote genomic instability, drug efflux pump expression, and survival signaling, consistent with chemotherapeutic resistance.[19,22,33] However, an equally significant proportion of aggressive osteosarcomas harbor TP53 deletions, frameshift mutations, nonsense mutations, or promoter hypermethylations that completely abolish protein expression, resulting in a 'null phenotype' that is completely negative on immunohistochemical staining.[19,21] These p53-null tumors lack wild-type apoptotic defense mechanisms and are notoriously chemoresistant, yet they are categorized as 'p53-negative' by standard IHC.[19,34]

Consequently, dichotomizing osteosarcoma biopsy specimens by IHC immunoreactivity groups together intrinsically resistant p53-null tumors with genuinely functional wild-type p53 tumors in the 'negative' category, severely eroding the discriminative power of the assay.[19,21,32] Furthermore, cytotoxic chemotherapy can induce p53-independent alternative cell death pathways, including necroptosis, ferroptosis, autophagy-dependent cell death, and mitotic catastrophe, allowing some tumors harboring mutant p53 to still undergo extensive necrosis when exposed to multi-agent regimens.[20,32,35] These multifaceted biological mechanisms demonstrate that a static pre-treatment IHC assessment cannot reliably forecast the dynamic, multifactorial pharmacodynamic response occurring across multiple months of neoadjuvant therapy.

A striking clinical observation in our Indonesian cohort is the exceptionally high rate of poor histological response (78.6%; 11 of 14 patients), with only 21.4% (3 of 14 patients) achieving good histological necrosis (≥90%; Huvos III–IV). In major international clinical trials from North America and Western Europe, such as the EURAMOS-1, COSS, and INT-0133 protocols, the reported proportion of good histological responders typically ranges between 50% and 65%.[7,10,33] The substantially lower rate of favorable necrosis in our cohort mirrors findings from other resource-limited healthcare environments in Southeast Asia, including reports from Cipto Mangunkusumo General Hospital in Jakarta (good response rates of 20% to 30%)[4] and tertiary oncology centers in Vietnam and the Philippines.[28]

This marked disparity in therapeutic efficacy is attributable to several intersecting clinical, socio-demographic, and systemic factors inherent to cancer care delivery in developing countries.[4,26,28] First, diagnostic referral delay is pervasive in our setting. In South Sumatra, patients with persistent musculoskeletal extremity pain frequently seek traditional treatments, bone-setters, or primary community clinics for several months before being referred to an orthopaedic oncology service. By the time of tertiary referral, tumors frequently attain massive dimensions, exhibiting extensive extraskeletal soft-tissue extension, pathological cortical fractures, and extensive intratumoral central necrosis and hypoxia.[4,29] Massive primary tumor volume is an established negative prognostic factor that diminishes drug penetrance and fosters dense microenvironmental immunosuppression.[18,35] Second, nutritional compromise and systemic inflammation are common among our patient cohort, frequently precluding the administration of full-dose chemotherapy without dose reductions or scheduling delays, thereby lowering the cumulative dose intensity required to achieve maximal tumor eradication.[26,27] Third, logistical constraints and chemotherapy stock outages occasionally lengthen the intervals between cycles beyond the ideal 21-day window, permitting drug-resistant neoplastic subclones to repopulate.[26]

From a translational perspective, our findings provide a compelling rationale for reconsidering the role of static baseline biomarkers in osteosarcoma management (illustrated in Figure 5C).[13,31] While baseline serum ALP and pre-treatment p53 IHC status provide valuable initial diagnostic, staging, and biological classification data, they cannot substitute for the definitive macroscopic and microscopic histopathological assessment provided by the post-resection Huvos grading system.[9,11] Clinicians must exercise caution and avoid altering neoadjuvant chemotherapy protocols or making definitive prognostic commitments based solely on pre-treatment ALP levels or p53 staining intensity.

To transcend the inherent limitations of static pre-treatment biomarkers, future translational research must pivot toward dynamic, longitudinal, and multi-modal assessment paradigms.[13,16,36] First, longitudinal monitoring of serum ALP kinetics—evaluating the percentage reduction or clearance rate (delta-ALP) during neoadjuvant cycles rather than a single baseline value—has emerged as a far more reliable indicator of active in-vivo cytoreduction.[16] A rapid decline of serum ALP toward normal physiological levels following the first two cycles reflects effective suppression of viable osteoblasts and has been shown to correlate significantly with favorable Huvos necrosis.[16] Second,

advanced functional imaging modalities, particularly diffusion-weighted MRI (DWI-MRI) with apparent diffusion coefficient (ADC) mapping and 18F-FDG PET/CT, offer non-invasive, spatial quantification of cellularity changes and metabolic shutdown during neoadjuvant therapy, overcoming the biophysical diffusion barriers of dense osteoid matrix.[17,36] Third, liquid biopsy technologies interrogating circulating tumor DNA (ctDNA) and tumor-derived extracellular vesicles offer real-time tracking of minimal residual disease, clonal evolution, and specific TP53 somatic mutation clearance in peripheral blood, bridging the gap between benchside molecular genomics and bedside oncology practice.[37,41,42]

This study possesses several notable strengths. It represents the first dedicated clinical investigation in South Sumatra to systematically evaluate the associative and diagnostic predictive performance of baseline circulating ALP and immunohistochemical p53 expression in relation to standardized Huvos tumor necrosis. All patients were managed under a uniform, standardized neoadjuvant chemotherapy protocol (WKT-EKZ-AMC) and received definitive surgical care at a single tertiary academic referral center, eliminating inter-institutional treatment protocol heterogeneity. Histopathological evaluations were conducted on complete surgical resection specimens using rigorous whole-organ macroscopic mapping and microscopic quantification by board-certified pathologists. Furthermore, the evaluation of effect sizes with relative risks, odds ratios, and non-parametric ROC curves with Youden index optimization provides an objective, mathematically rigorous assessment of diagnostic utility.

Nevertheless, several methodological limitations must be acknowledged. First, the sample size of 14 patients, although reflecting a complete 3-year census of consecutive eligible high-grade osteosarcoma patients completing the full multimodal protocol at our institution, is relatively modest. This limitation reflects the rarity of primary malignant bone tumors and the clinical realities of patient attrition in resource-constrained environments, where advanced metastatic disease at presentation or non-compliance reduces the number of patients completing three neoadjuvant cycles and definitive resection. Second, the small sample size and small number of good responders (n = 3) precluded the application of multivariable logistic regression modeling, adhering strictly to the statistical rule of thumb requiring at least 10 outcome events per variable to prevent model overfitting. Third, p53 status was assessed using immunohistochemistry rather than full-exon next-generation sequencing (NGS). While IHC is widely accessible, cost-effective, and standardized in clinical pathology laboratories throughout developing countries, it cannot distinguish between different functional classes of TP53 missense mutations, nor can it identify p53-null deletions without concurrent molecular sequencing. Future multi-center prospective studies incorporating larger cohorts, longitudinal biomarker sampling, functional DWI-MRI radiomics, and comprehensive genomic profiling are warranted to develop validated, multimodal predictive nomograms tailored to pediatric and young adult osteosarcoma populations in Southeast Asia.[36,38-40,43-45]

5. Conclusion

In conclusion, baseline pre-chemotherapy serum alkaline phosphatase (ALP) levels and immunohistochemical p53 protein expression are not significantly associated with post-neoadjuvant histopathological tumor necrosis (Huvos score) in patients with primary high-grade extremity osteosarcoma. Receiver operating characteristic analysis confirms that baseline circulating ALP activity has negligible discriminative capacity (AUC = 0.515), functioning at the level of random chance. The lack of predictive correlation reflects the biological uncoupling between baseline osteoblastic matrix turnover and intrinsic cellular pharmacosensitivity, as well as the complex, non-linear mechanisms of TP53-mediated chemoresistance. Clinicians must not rely on baseline ALP or static p53 status to anticipate chemotherapy response or modify preoperative protocols. Standardized macroscopic and microscopic histopathological evaluation using the Huvos grading system remains the indispensable clinical benchmark for determining chemotherapeutic efficacy and dictating postoperative therapeutic strategies.

Declarations

Ethics Approval and Consent to Participate

This study was approved by the Health Research Ethics Committee of Dr. Mohammad Hoesin General Hospital / Faculty of Medicine, Universitas Sriwijaya (Ethical Exemption No. DP.04.03/D.XVIII.06.08/ETIK/219/2025). A waiver of informed consent was granted due to the retrospective analysis of de-identified archival records and specimens.

Consent for Publication

Not applicable.

Availability of Data and Materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable academic request.

Competing Interests

The authors declare that they have no competing financial or non-financial interests.

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Authors' Contributions

DCS conceptualized the study, performed clinical data acquisition, coordinated histological re-examinations, executed statistical analyses, and drafted the original manuscript. PR supervised the clinical oncology investigations, performed surgical resections, and critically revised the manuscript for intellectual content. DHH supervised the pharmacological and biomolecular analyses, guided biomarker interpretations, and revised the final manuscript. All authors read and approved the final manuscript.

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Generative AI disclosure

The authors declare that no generative artificial intelligence tools were used in the design, analysis, or writing of this manuscript.

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