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Original Article

Relationship between Ki-67 and P53 immunohistochemical expression and osteosarcoma chemotherapy sensitivity and long-term prognosis

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Abstract: Objective: To identify biomarkers predictive of chemotherapy sensitivity and prognosis in patients with osteosarcoma. Methods: This retrospective case-control study included 237 primary osteosarcoma patients (aged 13-30 years) treated at Ningbo No.6 Hospital between 2000 and 2019. All patients received neoadjuvant chemotherapy (methotrexate/doxorubicin/cisplatin) followed by tumor resection. Chemotherapy response was assessed using the Response Evaluation Criteria in Solid Tumors (RECIST, version 1.1). Based on RECIST criteria, patients were categorized into a high-sensitivity group (n=120; complete or partial response) or a low-sensitivity group (n=117; stable or progressive disease). Long-term prognosis was defined as good (postoperative survival ≥36) or poor (<36 months). Demographic, clinical, and laboratory variables, including inflammatory markers, tumor biomarkers, and p53/Ki-67 expression, were compared. Independent predictors were identified by multivariate logistic regression for chemotherapy sensitivity and Cox proportional hazards regression for long-term prognosis. Results: In the high-sensitivity group, post-treatment levels of inflammatory markers - including C-reactive protein (CRP), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), platelet-to-lymphocyte ratio (PLR) - as well as isocitrate dehydrogenase 1 (IDH1) mutation frequency, lactate dehydrogenase (LDH), and Ki-67 expression were significantly lower compared to the low-sensitivity group (all $P<0.05$). Multivariate analyses confirmed that lower levels of CRP (Odds Ratio [OR]=0.766; 95% CI: 0.614-0.956), IL-6 (OR=0.889; 95% CI: 0.822-0.961), TNF-α (OR=0.908; 95% CI: 0.833-0.989), PLR (OR=0.975; 95% CI: 0.959-0.992), IDH1 mutation status (OR=0.005; 95% CI: 0.001-0.359), LDH (OR=0.965; 95% CI: 0.937-0.993), and Ki-67 (OR=0.922; 95% CI: 0.861-0.988) were independent predictors of higher chemotherapy sensitivity (all $P<0.05$). Additionally, these factors, along with lower Ki-67 expression, were independently associated with better long-term prognosis by Cox regression analysis. Conclusion: Lower levels of select inflammatory markers, IDH1 mutation status, LDH, and Ki-67 expression were independently associated with increased chemotherapy sensitivity and improved prognosis in osteosarcoma. These biomarkers may aid in risk stratification and therapeutic decision-making for affected patients.

Keywords: Osteosarcoma, chemotherapy sensitivity, prognosis, inflammatory markers, tumor biomarkers

Introduction

Osteosarcoma is the most common primary malignant bone tumor, predominantly affecting children, adolescents, and young adults [1]. Despite advances in multidisciplinary treatments - including neoadjuvant chemotherapy, extensive surgical resection, and adjuvant chemotherapy - the overall survival rates of patients have plateaued over the past decades, with 5-year survival rates remaining at approximately 60% to 70% [2]. A major challenge in clinical management of osteosarcoma is the substantial heterogeneity in chemotherapy response and long-term prognosis, which cannot be fully explained by conventional clinicopathologic data such as tumor size, anatomical location, or histologic subtype [3]. Thus, reliable biomarkers are urgently needed to predict chemotherapy sensitivity and long-term clinical outcome, enabling individualized treatment and optimized patient care [3].

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Immunohistochemical evaluation of tumor tissue provides a practical approach for investigating biologic processes that influence tumor behavior and treatment response [4]. Among the potential biomarkers, Ki-67 and P53 have attracted increasing attention in osteosarcoma research [5, 6]. Ki-67 is a nuclear protein associated with cell proliferation and is expressed during all active phases of the cell cycle, but not in quiescent cells [7]. The Ki-67 labeling index reflects tumor proliferative activity, which is an important feature of tumor aggressiveness [8]. Previous studies have shown that increased Ki-67 expression correlates with higher tumor grade, increased metastatic risk, and inferior survival in several malignancies, including bone tumors [6, 8, 9]. However, its relationship with chemotherapy sensitivity and long-term prognosis in osteosarcoma remains incompletely defined [9].

P53, encoded by the tumor protein P53 (TP53) gene, functions as a critical tumor suppressor by regulating cell cycle arrest, DNA repair, and apoptosis in response to cellular stress [10-12]. Alterations in the P53 pathway, typically through mutation or abnormal expression, represent one of the most frequent genetic aberrations found in human cancers, including osteosarcoma [10, 13]. Dysfunctional or mutant P53 promotes tumor progression, therapeutic resistance, and poor prognosis [13, 14]. While several investigations have examined P53 expression in osteosarcoma, the prognostic significance of P53 immunoreactivity - especially in relation to response to neoadjuvant chemotherapy response - remains controversial [15].

This study introduced an integrated approach that combines immunohistochemical assessment with comprehensive clinical and biochemical data. Specifically, we evaluated Ki-67 and P53 expression levels, alongside inflammatory and tumor-related biomarkers, to elucidate their predictive value for chemotherapy sensitivity and long-term survival in osteosarcoma. By employing rigorous statistical methods, including univariate and multivariate logistic regression analyses, we aim to refine risk stratification and support personalized therapeutic strategies for osteosarcoma. This integrated approach not only provides new insight into the biological mechanisms underlying osteosarcoma progression and therapy response but also holds promise for improving patient outcome through tailored treatment.

Materials and methods

Case selection

This retrospective case-control study involved 237 patients with primary osteosarcoma admitted to Ningbo No. 6 Hospital between June 2000 and June 2019. Demographic data of patients were obtained from the hospital case management system. All procedures involving human participants were conducted in accordance with the Declaration of Helsinki. This study was reviewed and approved by the Ethics Committee of Ningbo No. 6 Hospital.

Inclusion and exclusion criteria

Inclusion criteria: (1) Age between 13 and 30 years; (2) Normal cognitive function and ability to cooperate with various treatments and examinations; (3) Pathologically confirmed osteosarcoma [16]; (4) Receipt of standardized neoadjuvant chemotherapy (methotrexate, doxorubicin and cisplatin) after diagnosis; (5) Underwent extensive tumor resection; (6) Availability of complete case records and follow-up data.

Exclusion criteria: (1) Previous history of chemotherapy for malignant tumors; (2) Previous treatment history of osteosarcoma; (3) Neurological disorders or cognitive impairment; (4) Palliative surgery only or presence of distant metastases; (5) Modification of the chemotherapy regimen or combination with other treatments such as targeted therapy.

Grouping

Chemotherapy efficacy was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST) after completion of chemotherapy [17]. Patients with progressive disease (PD) and disease stability (SD) were classified into a low-sensitivity group (n=117), while patients with complete response (CR) and partial response (PR) were classified into a high-sensitivity group (n=120). For survival analysis, patients were divided into two groups based on the patient's survival time: a good prognosis group (n=124; ≥36 months) and a poor prognosis group (n=113; <36 months).

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Treatment methods

The histopathologic diagnosis of all specimens was confirmed by two well-trained pathologists. All medications were administered intravenously. After neoadjuvant chemotherapy, patients underwent extensive tumor resection.

Surgery: The surgical approach was tailored to tumor location and size to ensure adequate exposure. Important neural and vascular structures were carefully dissected and protected. Resection proceeded from surrounding normal tissues, gradually advanced towards the tumor to ensure sufficient safety margins. Bone cutting was performed at predetermined resection lines using a bone saw or other appropriate tools. The tumor was excised along surrounding margins and immediately submitted for pathologic examination to negative margins.

Chemotherapy: The neoadjuvant chemotherapy regimen used a combination of drugs recognized for their efficacy against osteosarcoma, specifically high-dose methotrexate, doxorubicin, and cisplatin, collectively referred to as MAP. Methotrexate was administered intravenously at 12 g/m^2 over 5 hours, once every 3 weeks. Doxorubicin was given intravenously at 75 mg/m^2 over 5 hours, once every 3 weeks. Cisplatin was delivered intravenously at 120 mg/m^2 over 5 hours, once every 3 weeks.

Criteria for chemotherapy sensitivity

After completion of chemotherapy, all patients underwent imaging re-examination. Objective efficacy was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST, version 1.1) [17]. Complete remission (CR): disappearance of all target lesions; Partial remission (PR): ≥30% decrease in the sum of target lesion diameters compared to baseline; Progressive disease (PD): ≥20% increase in the sum of target lesion diameter from baseline or appearance of new lesions; Stable disease (SD): decrease or increase in diameter that did not meet criteria for PR or PD. Tumors evaluated as CR or PR were considered sensitive to chemotherapy, while those evaluated as SD or PD were considered as insensitive.

Detection of Ki-67 and P53 expression

Tumor frozen sections (6 μm thick) were prepared and mounted on silanized glass slides, followed by overnight drying. Sections were fixed in a 1:1 mixture of chloroform and acetone at room temperature for 10 minutes, air-dried for 10 minutes, and incubated with mouse anti-human p53 monoclonal antibody (PAb 1801, Cambridge Research Biochemistry) diluted at 1:1600 or mouse monoclonal Ki-67 antibody (Dako) diluted at 1:25. The working dilution for the p53 antibody was titrated using control human osteosarcoma material to ensure optimal detection. Sections were then counterstained with hematoxylin. All antibody dilutions were prepared in Tris-buffered saline at pH 7.6, containing 1.5% normal pre-immune horse serum. As a control for endogenous peroxidase, the primary antibody was omitted on consecutive sections. Additional consecutive sections were stained with hematoxylin and eosin for histopathologic examination.

Blood testing

Blood testing included the measurement of inflammatory cytokines and tumor-related biomarkers. Fasting venous blood samples (6 ml) were collected at baseline (before the initiation of any treatment) and postoperatively (on the third day after surgery), centrifuged at 3000 rpm for 10 minutes at 4°C, and the supernatant serum was used for subsequent tests.

Inflammatory factors: Serum inflammatory markers, including C-reactive protein (CRP; ab260058, Abcam, USA), interleukin-6 (IL-6; ab178013, Abcam, USA), and tumor necrosis factor-alpha (TNF-α; ab181421, Abcam, USA) were detected using enzyme-linked immunosorbent assay (ELISA). Complete blood counts were performed on EDTA-anticoagulated samples using the Sysmex XN-1000 hematology analyzer (Sysmex, Japan). Neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and systemic immune-inflammation index (SII) were calculated accordingly.

Tumor marker detection: Tumor-related markers were assessed, including O-6-methylguanine-DNA methyltransferase (MGMT) promoter methylation, epidermal growth factor receptor (EGFR) amplification, isocitrate dehydrogenase 1 (IDH1) mutation status, lactate dehydrogenase (LDH), SCC Antigen (SCC Ag), and cancer antigen 125 (CA125). MGMT promoter methylation status and EGFR amplification were evaluated by quantitative PCR (qPCR) using the Applied Biosystems 7500 real-time PCR system (Thermo Fisher Scientific, USA). IDH1 mutation status was confirmed through Sanger sequencing using the ABI PRISM 3130 genetic analyzer (Applied Biosystems, USA). LDH activity was determined using a Hitachi 7600 automated biochemical analyzer with a specialized CSF kit (Roche Diagnostics GmbH, Germany). Serum SCC Ag and CA125 were measured by ELISA. Microplates were coated with specific monoclonal antibodies (SCC Ag: PHY-Ab-BZW13, PhyCell, China; CA125: ab253424, Abcam, USA). After incubation with patient samples, antigen-antibody binding was detected with horseradish peroxidase (HRP)-conjugated secondary antibodies (ab6721, Abcam, USA) and developed with tetramethylbenzidine (TMB) substrate (ab171523, Abcam, USA). Absorbance was measured with a microplate reader, and concentrations were calculated accordingly.

Follow-up

Patients were followed up primarily through outpatient visits every three months. For patients who failed to attend scheduled visits, follow-up was conducted by fixed-time telephone contact with family members. The follow-up period ended in March 2025. Overall survival was calculated from the date of surgery to loss to follow-up, death, or last follow-up. For deceased patients, the cause and time of death were recorded in detail.

Statistical method

Continuous variables were expressed as mean ± standard deviation (SD) or median (interquartile range), depending on distribution. Categorical variables were presented as frequencies and percentages, and comparisons between groups were performed using the Chi-square test or Fisher's exact test, as appropriate. Comparisons of continuous variables between two groups were performed using unpaired t-tests. Univariate and multivariate logistic regression analyses were used to identify predictors of chemotherapy sensitivity, reporting odds ratios (ORs) with 95% confidence intervals (CIs). For long-term prognosis, Cox proportional hazards regression analysis was employed, reporting hazard ratios (HRs) and 95% CIs. A two-sided P value <0.05 was considered significant. All statistical analyses were conducted using SPSS 19 software (SPSS Inc., Chicago, IL, USA) and R software package 3.0.2. (Free Software Foundation, Inc, Boston, MA, USA).

Results

Comparison of baseline demographic characteristics between low- and high-sensitivity groups

A total of 237 patients with primary osteosarcoma were included and categorized into low sensitivity (n=117) and high sensitivity (n=120) groups according to chemotherapy response. Comparative analysis of baseline characteristics revealed no significant differences between the two groups in terms of age distribution (13-18 vs 18-30; χ^2=0.223, P=0.637), gender (male vs female; χ^2=0.206, P=0.650), education level (primary or below, secondary, college or above; χ^2=1.019, P=0.601), employment status (employed vs unemployed; χ^2=0.076, P=0.783), or marital status (married, single, divorced; χ^2=1.026, P=0.599). These findings indicate well-balanced baseline demographic and socioeconomic characteristics between the low and high sensitivity groups (**Table 1**).

Comparison of clinical characteristics between low- and high-sensitivity groups

As shown in **Table 2**, there were no significant differences in clinical characteristics between the low sensitivity and high sensitivity groups. Specifically, mean body mass index (BMI) (P=0.467), tumor site distribution (P=0.952), histologic subtype (P=0.317), and proportion of patients with high tumor grade (P=0.243) were comparable between the two groups.

Comparison of chemotherapy sensitivity between two groups of patients

As detailed in **Figure 1**, the high sensitivity group demonstrated favorable responses, with 23 patients (19.17%) achieving complete response and 38 patients (31.67%) partial response. In contrast, the low sensitivity group included 59 patients (49.17%) with stable disease and 58 patients (50.83%) with progressive disease.

Comparison of biochemical indicators between low- and high-sensitivity groups

As shown in **Figure 2**, patients in the low-sensitivity group exhibited significantly higher post-

Table 1. Comparison of general information in the two groups

Data	Low sensitivity group (n=117)	High sensitivity group (n=120)	χ^2	p
Age (years) [n (%)]			0.223	0.637
13-18	52 (44.44%)	57 (47.5%)		
18-30	65 (55.56%)	63 (52.5%)		
Gender [n (%)]			0.206	0.650
Male	57 (48.72%)	62 (51.67%)		
Female	60 (51.28%)	58 (48.33%)		
Education Level (years) [n (%)]			1.019	0.601
Primary or below	21 (17.95%)	19 (15.83%)		
Secondary School	47 (40.17%)	56 (46.67%)		
College or above	49 (41.88%)	45 (37.5%)		
Employment Status [n (%)]			0.305	0.581
Employed	38 (32.48%)	35 (29.17%)		
Unemployed	79 (67.52%)	85 (70.83%)		
Marital Status [n (%)]			1.026	0.599
Married	78 (66.67%)	86 (71.67%)		
Single	35 (29.91%)	29 (24.17%)		
Divorced	4 (3.42%)	5 (4.17%)		

Table 2. Comparison of clinical characteristics between the two groups of patients

Data	Low sensitivity group (n=117)	High sensitivity group (n=120)	t/χ^2	p
BMI [kg/m^2]	24.73±3.30	25.02±3.00	0.729	0.467
Tumor site [n (%)]			0.343	0.952
Femur	66 (56.41%)	67 (55.83%)		
Tibia	18 (15.38%)	21 (17.50%)		
Humeral bone	20 (17.09%)	18 (15.00%)		
Other	13 (11.11%)	14 (11.67%)		
Histologic type [n (%)]			3.528	0.317
Osteoblastic	56 (47.86%)	57 (47.5%)		
Chondroblastic	21 (17.95%)	26 (21.67%)		
Fibroblastic	16 (13.68%)	22 (18.33%)		
Telangiectatic	24 (20.51%)	15 (12.5%)		
Tumor grade [n (%)]			1.365	0.243
Low	21 (17.95%)	15 (12.5%)		
High	96 (82.05%)	105 (87.5%)		

Note: BMI: Body Mass Index.

treatment levels of inflammatory markers compared with the high sensitivity group. Specifically, serum CRP (5.56±1.12 mg/L vs 5.07±1.56 mg/L; t=2.821, *P*=0.005), IL-6 (13.55±4.45 pg/mL vs 12.34±3.33 pg/mL; t=2.352, *P*=0.020), TNF-α (11.57±3.87 pg/mL vs 10.35±3.23 pg/mL; t=2.638, *P*=0.009), NLR (2.56±0.67 vs 2.42±0.23; t=2.117, *P*=0.036), PLR (145.67±13.33 vs 138.67±21.67; t=3.004, *P*=0.003), and SII (494.67±20.46 vs 487.57±20.45; t=2.673, *P*=0.008) were all significantly elevated in the low-sensitivity group. These results demonstrate a marked association between reduced chemotherapy sensitivity and heightened systemic inflammation after treatment.

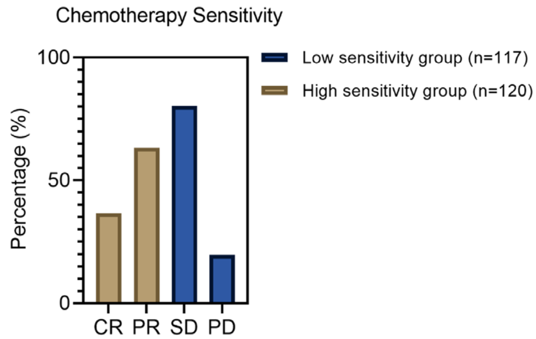

Figure 1. Comparison of chemotherapy sensitivity between both groups of patients. Note: RECIST: Response Evaluation Criteria In Solid Tumors; CR: Complete Response; PR: Partial Response; SD: Stable Disease; PD: Progressive Disease.

As presented in **Table 3**, the low-sensitivity group also demonstrated significantly higher IDH1 mutation status (0.76±0.04% vs 0.75 ± 0.05%; t=2.488, *P*=0.014) and elevated LDH levels (251.57±10.12 U/L vs 247.46±10.23 U/L; t=3.108, *P*=0.002) compared to the high-sensitivity group. No significant intergroup differences were observed in MGMT promoter methylation, EGFR amplification, SCC antigen (SCC Ag), or CA125 (all P>0.05). These results indicate that higher IDH1 mutation frequency and elevated LDH levels are associated with reduced chemotherapy sensitivity in patients with osteosarcoma.

Comparison of P53 and Ki-67 expression levels between low- and high-sensitivity groups

Representative IHC images (**Figure 3**) corroborated these findings, showing intensified nuclear staining for Ki-67 and P53 in tumors from the low-sensitivity group. As shown in **Figure 4**, before treatment, patients in the low-sensitivity group demonstrated significantly higher expression levels of both P53 (15.58±5.54% vs 13.58±5.67%; t=2.748, *P*=0.006) and Ki-67 (15.41±4.56% vs 13.72±4.13%; t=2.989, *P*= 0.003) compared to the high-sensitivity group. These findings indicate that elevated pre-treatment expression of P53 and Ki-67 is associated with reduced chemotherapy sensitivity in patients with osteosarcoma.

Postoperative survival analysis in the two groups

As summarized in **Figure 5**, the high-sensitivity group demonstrated significantly longer overall survival (OS) compared to the low-sensitivity group (53.24±12.37 months vs 32.73±8.46 months; t=14.934, *P*<0.001). There was no significant difference in Progression-free survival (PFS) between the two groups (41.58±9.63 months vs 16.82±5.74 months; t=24.123, *P*<0.001). These results indicate that higher chemotherapy sensitivity is associated with improved overall survival in patients with osteosarcoma.

Logistic regression analysis of chemotherapy sensitivity in patients with osteosarcoma

As shown in **Table 4**, multivariate regression analysis identified lower levels of CRP (OR, 0.766; 95% CI, 0.614-0.956; *P*=0.018), IL-6 (OR, 0.889; 95% CI, 0.822-0.961; *P*=0.003), TNF-α (OR, 0.908; 95% CI, 0.833-0.989; *P*=0.027), PLR (OR, 0.975; 95% CI, 0.959-0.992; *P*=0.004), IDH1 mutation status (OR, 0.005; 95% CI, 0.001-0.359; *P*=0.025), LDH (OR, 0.965; 95% CI, 0.937-0.993; *P*=0.015), and Ki-67 (OR, 0.922; 95% CI, 0.861-0.988; *P*=0.021) as independent predictors of increased chemotherapy sensitivity in patients with osteosarcoma. In contrast, NLR, SII, and P53 expression levels were not independent factors associated with chemotherapy sensitivity by multivariate analysis (P>0.05). These findings indicate that systemic inflammation markers, certain tumor markers, and Ki-67 expression are independently related to chemotherapy response in this population.

Cox regression analysis of long-term prognosis in patients with osteosarcoma

As shown in **Table 5**, multivariate Cox regression analysis demonstrated that lower levels of CRP (HR, 1.241; 95% CI, 1.102-1.397; P=0.007), IL-6 (HR, 1.118; 95% CI, 1.027-1.217; P=0.021), TNF-α (HR, 1.153; 95% CI, 1.041-1.278; P=0.018), PLR (HR, 1.026; 95% CI, 1.008-1.044; P=0.009), IDH1 mutation status (HR, 2.152; 95% CI, 1.302-3.555; P=0.003), LDH (HR, 1.008; 95% CI, 1.002-1.014; P=0.016), and Ki-67 (HR, 1.128; 95% CI, 1.042-1.222; P=0.003) were independent predictors of poor long-term prognosis in patients with osteosarcoma. These findings suggest that certain systemic inflammatory markers, tumor markers, and lower levels of Ki-67 are independently associated with better long-term outcome.

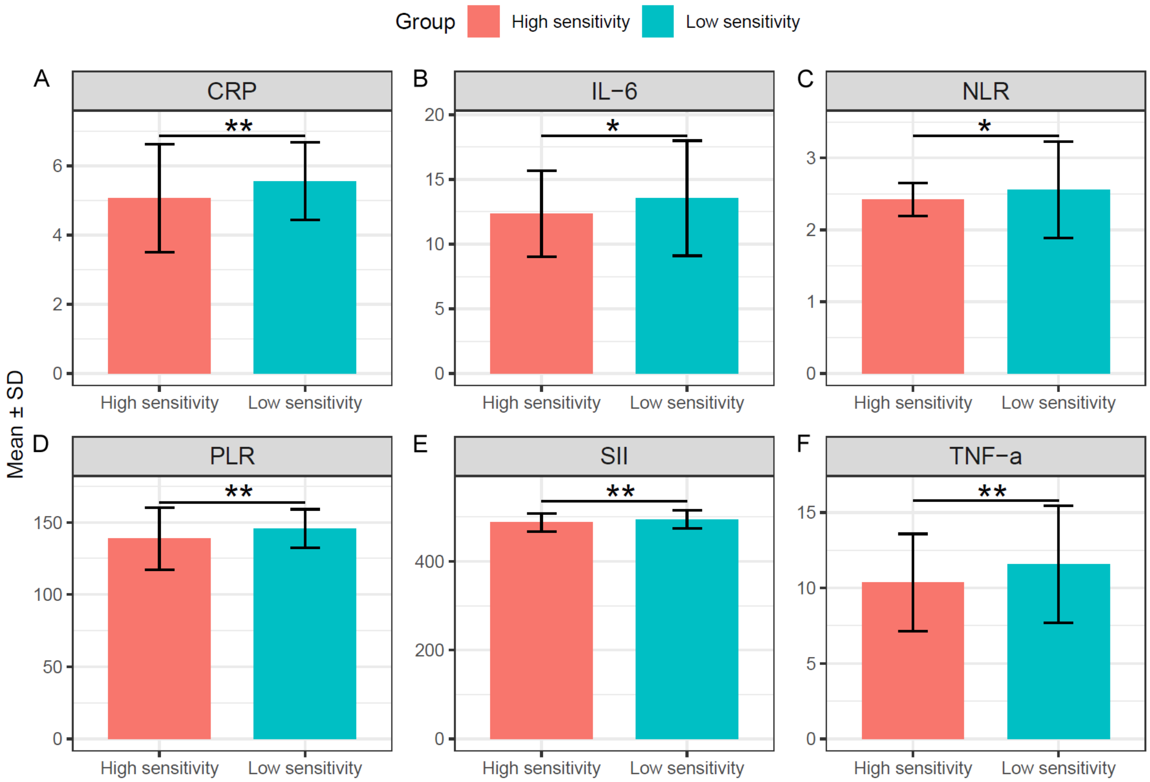

Figure 2. Comparison of inflammatory factors between both groups of patients after treatment. A: CRP; B: IL-6; C: NLR; D: PLR; E: SII; F: TNF-α. Note: CRP: C-Reactive Protein; IL-6: Interleukin-6; TNF-α: Tumor Necrosis Factor alpha; PLR: Platelet-Lymphocyte Ratio; NLR: Neutrophil-Lymphocyte Ratio; SII: Systemic Immune-Inflammation Index. * represents P<0.05 and ** represents P<0.01.

Table 3. Comparison of tumor markers between two groups of patients

Data	Low sensitivity group (n=117)	High sensitivity group (n=120)	t	p
MGMT Promoter Methylation [%]	0.66±0.05	0.65±0.08	0.978	0.329
EGFR Amplification [%]	4.03±0.35	4.02±0.33	0.198	0.843
IDH1 Mutation Status [%]	0.76±0.04	0.75±0.05	2.488	0.014
LDH [U/L]	251.57±10.12	247.46±10.23	3.108	0.002
SCC Ag [ng/mL]	2.53±0.46	2.41±1.02	1.214	0.227
CA125 [IU/mL]	35.57±5.15	34.46±3.72	1.907	0.058

Note: MGMT: O-6-methylguanine-DNA methyltransferase; EGFR: Epidermal Growth Factor Receptor; IDH1: Isocitrate Dehydrogenase 1; LDH: Lactate Dehydrogenase; SCC Ag: SCC Antigen; CA125: Cancer Antigen 125.

Discussion

Our study demonstrated significant associations between lower levels of inflammatory markers (CRP, IL-6, TNF-α, PLR), IDH1 mutation status, LDH, and Ki-67 expression, and increased chemotherapy sensitivity and favorable long-term prognosis in patients with osteosarcoma. These findings underscore the importance of systemic inflammation and proliferative activity as critical determinants of therapeutic response.

In our cohort, elevated pre-treatment Ki-67 expression was significantly associated with reduced chemotherapy sensitivity and shorter overall survival. This finding aligns with previous studies suggesting that high proliferative activity may confer resistance to cytotoxic agents [7, 18]. Elevated Ki-67 labeling implies

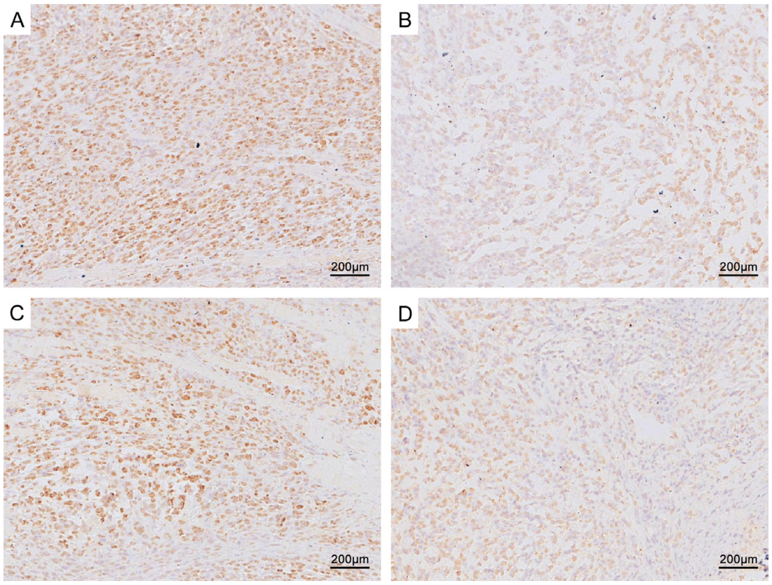

Figure 3. Representative immunohistochemical staining of Ki-67 and P53 in pre-treatment osteosarcoma samples stratified by chemotherapy sensitivity (200× magnification). A: Low sensitivity group (Ki-67 high expression); B: High sensitivity group (Ki-67 low expression); C: Low sensitivity group (P53 aberrant expression); D: High sensitivity group (P53 normal expression).

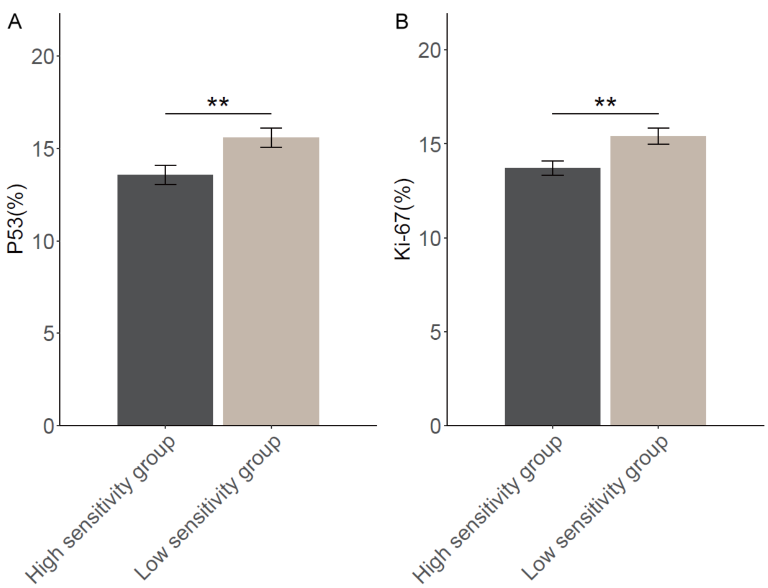

Figure 4. Comparison of P53 and Ki-67 expression levels between both groups of patients before treatment. A: P53; B: Ki-67. Note: ** represents P<0.01.

a substantial fraction of tumor cells actively cycling, which may render these cells initially more susceptible to cytotoxic agents targeting rapid proliferation [18, 19]. However, paradoxically, in osteosarcoma, higher Ki-67 expression has frequently been correlated with inferior responses to chemotherapy and shorter survival [8]. This apparent contradiction may stem from several latent mechanisms.

First, a high Ki-67 index is reflective not only of tumor cell proliferative capacity but also intrinsic genomic instability and clonal heterogeneity [20]. Rapid turnover increases the probability of acquiring mutations conferring chemoresistance, such as those affecting drug efflux, DNA damage repair, and apoptotic signaling pathways [21]. Consequently, although some fraction of rapidly dividing cells is eradicated by chemotherapy, resistant subclones swiftly repopulate the tumor, leading to early progression or relapse [21]. Moreover, a robust proliferative drive can indicate dysregulated cell cycle checkpoints, which may compromise the effectiveness of chemotherapeutic agents that rely on functional checkpoint-mediated apoptosis [22]. Additionally, tumors with high proliferative activity often exhibit a hypoxic, nutrient-deprived microenvironment, which promotes selection of aggressive phenotypes, upregulates anti-apoptotic proteins, and impairs the delivery of chemotherapeutic drugs due to aberrant vasculature [23, 24]. Therefore, elevated Ki-67 may not only indicate high proliferative activity but also a biologically aggressive, therapy-resistant tumor milieu. Similar findings have been reported by Zeng et al. [8] whose meta-analysis confirmed that Ki-67 positive expression is significantly associated with reduced overall survival in osteosarcoma patients, and the association between high Ki-67 expression and poor prognosis in sarco-

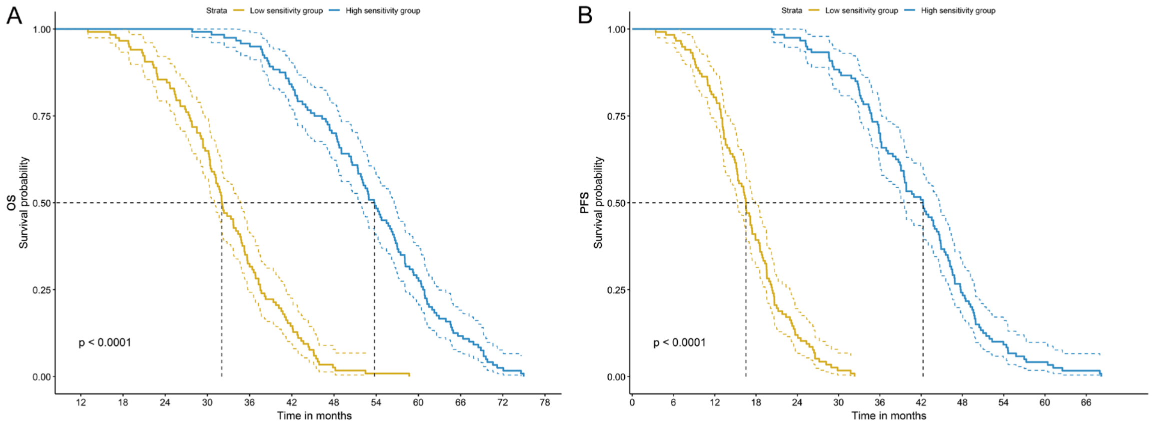

Figure 5. Postoperative survival analysis between the two groups. A: OS; B: PFS. Note: OS: Overall Survival; PFS: Progression-Free Survival.

Table 4. Logistic regression analysis of chemotherapy sensitivity in patients with osteosarcoma

Data	Univariate analysis			Multivariate analysis		
	P	OR	95% CI	P	OR	95% CI
CRP (mg/L)	0.006	0.764	0.627-0.923	0.018	0.766	0.614-0.956
IL-6 (pg/mL)	0.021	0.924	0.863-0.987	0.003	0.889	0.822-0.961
TNF-α (pg/mL)	0.010	0.907	0.841-0.976	0.027	0.908	0.833-0.989
NLR	0.036	0.566	0.327-0.953	0.132	0.636	0.353-1.146
PLR	0.004	0.978	0.963-0.993	0.004	0.975	0.959-0.992
SII	0.010	0.983	0.970-0.996	0.189	0.990	0.975-1.005
IDH1 Mutation Status (%)	0.015	0.000	0.000-0.207	0.025	0.005	0.001-0.359
LDH (U/L)	0.003	0.961	0.936-0.986	0.015	0.965	0.937-0.993
P53 (%)	0.007	0.938	0.894-0.982	0.142	0.960	0.908-1.014
Ki-67 (%)	0.004	0.914	0.858-0.970	0.021	0.922	0.861-0.988

Note: CRP: C-Reactive Protein; IL-6: Interleukin-6; TNF-α: Tumor Necrosis Factor alpha; NLR: Neutrophil-Lymphocyte Ratio; PLR: Platelet-Lymphocyte Ratio; SII: Systemic Immune-Inflammation Index; IDH1: Isocitrate Dehydrogenase 1; LDH: Lactate Dehydrogenase.

Table 5. Cox regression analysis of long-term prognosis in patients with osteosarcoma

Data	Univariate analysis		Multivariate analysis	
	P	HR (95% CI)	P	HR (95% CI)
CRP (mg/L)	0.001	1.283 (1.104-1.492)	0.007	1.241 (1.102-1.397)
IL-6 (pg/mL)	0.013	1.162 (1.032-1.309)	0.021	1.118 (1.027-1.217)
TNF-α (pg/mL)	0.003	1.194 (1.063-1.342)	0.018	1.153 (1.041-1.278)
NLR	0.287	1.087 (0.932-1.268)	0.226	0.924 (0.812-1.051)
PLR	0.004	1.034 (1.011-1.058)	0.009	1.026 (1.008-1.044)
SII	0.092	1.018 (0.997-1.039)	0.402	1.007 (0.991-1.023)
IDH1 Mutation Status (%)	<0.001	2.843 (1.632-4.952)	0.003	2.152 (1.302-3.555)
LDH (U/L)	0.001	1.012 (1.005-1.019)	0.016	1.008 (1.002-1.014)
P53 (%)	0.104	1.062 (0.987-1.142)	0.218	1.037 (0.978-1.099)
Ki-67 (%)	<0.001	1.184 (1.072-1.308)	0.003	1.128 (1.042-1.222)

Note: CRP: C-Reactive Protein; IL-6: Interleukin-6; TNF-α: Tumor Necrosis Factor alpha; NLR: Neutrophil-Lymphocyte Ratio; PLR: Platelet-Lymphocyte Ratio; SII: Systemic Immune-Inflammation Index; IDH1: Isocitrate Dehydrogenase 1; LDH: Lactate Dehydrogenase.

ma was validated through the TCGA dataset. Additionally, Hernandez-Rodriguez et al.'s study showed that Ki-67 overexpression was directly associated with a higher incidence of lung metastasis and increased mortality [25].

P53, known as the "guardian of the genome", is a master regulator of cellular responses to DNA damage and oncogenic stress [11, 12]. Functional p53 triggers cell cycle arrest or apoptosis in response to genotoxic insults, such as those delivered by chemotherapy [10, 26]. In osteosarcoma, TP53 mutations and aberrant P53 protein expression are highly prevalent and have been linked to both tumorigenesis and therapeutic resistance [27]. Immunohistochemically, increased P53 staining may indicate either overexpression of mutant, non-functional protein, or less commonly, increased expression of wild-type protein in response to cellular stress [13]. In the context of most sarcomas, however, strong P53 expression is typically inferred as a surrogate for TP53 mutation.

The functional consequence of mutant P53 is abrogation of its tumor suppressor activity, resulting in impaired cell cycle checkpoints, defective apoptosis, and increased tolerance for DNA damage and chromosomal instability - all features that promote both tumor progression and chemoresistance [28]. Cells harboring dysfunctional P53 may bypass mitotic catastrophe and remain viable following cytotoxic insults, surviving and propagating resistant clones [29]. Furthermore, mutant P53 can acquire gain-of-function properties, further driving tumor cell invasion, metastasis, and drug resistance by modulating transcriptional programs unrelated to canonical tumor suppression [13].

An important intersection between Ki-67 and P53 lies in their joint regulation of cell cycle control and DNA damage response [18, 30]. In normal physiology, P53 activation due to DNA damage results in cell cycle arrest, facilitating time for repair or, failing that, apoptosis [13, 26]. In tumors with P53 dysfunction, unchecked cycling persists despite DNA insult [13, 26]. As a result, chemotherapeutic regimens reliant on P53-mediated cell death may be rendered substantially less effective [13]. Siontis et al. [31] also observed that in 47 resected specimens of osteosarcoma after neoadjuvant chemotherapy, high expression of p53 was associated with poorer overall survival. This aligns with our findings. Our multivariate analysis identified lower Ki-67 expression as an independent predictor of chemotherapy sensitivity and favorable prognosis, whereas P53 expression did not retain significance. This underscores Ki-67's role in reflecting tumor proliferative activity and genomic instability, which may drive chemoresistance through clonal selection of aggressive subpopulations. In contrast, while univariate analysis associated elevated P53 with reduced sensitivity, its lack of independence by multivariate models suggests confounding interactions with other biomarkers.

Moving beyond intrinsic tumor biology, the tumor microenvironment and host systemic inflammatory response have emerged as critical modulators of chemotherapeutic response and outcome [32]. Notably, systemic inflammation markers, including CRP, IL-6, TNF-α, and PLR, were robust independent predictors of both chemotherapy response and survival. This aligns with evidence that chronic inflammation fosters chemoresistance by cytokine-mediated upregulation of multidrug resistance proteins and suppression of cytotoxic immunity [32]. Similarly, IDH1 mutation status and elevated LDH signify metabolic reprogramming toward glycolysis, further promoting therapy-resistant phenotypes. Chronic inflammation can facilitate genomic instability, promote angiogenesis, and support the emergence of chemoresistant clones through cytokine-mediated survival signaling pathways [33]. Furthermore, pro-inflammatory cytokines have been shown to upregulate multidrug resistance-related proteins and attenuate cytotoxic responses, creating a hostile environment for effective chemotherapeutic intervention [32, 34].

Consistent with our findings, patients with higher IDH1 mutation status and elevated LDH levels exhibited reduced chemotherapy sensitivity and poorer long-term prognosis [35]. These observations support the notion that metabolic alterations and glycolytic activity play crucial roles in determining the aggressiveness and chemoresistance of osteosarcoma. Mutations in metabolic enzymes alter the epigenetic landscape and metabolic programming of osteosarcoma cells, conferring drug resistance through altered energy utilization or redox homeostasis

[1, 36]. LDH, as a marker of glycolytic activity, further supports the notion that metabolic adaptation underpins aggressive and resilient tumor phenotypes - hallmarks that are frequently observed in malignancies with high Ki-67 and abnormal P53 expression [35].

It is also necessary to consider methodologic factors. The semi-quantitative assessment of Ki-67 and P53 by immunohistochemistry, though widely employed, is inherently subject to inter-observer variability, technical thresholds, and tumor heterogeneity [37, 38]. Despite these limitations, when carefully performed and interpreted, these markers provide actionable biologic information [37, 38]. Standardization in scoring, use of digital image analysis, and integration with molecular diagnostics could further refine their prognostic and predictive utility, thereby guiding personalized treatment strategies.

Clinically, these findings have tangible implications. Early identification of patients with high Ki-67 and P53 expression could prompt consideration of alternative or intensified systemic therapies, closer surveillance, or enrollment in clinical trials exploring novel agents targeting cell cycle, apoptosis, or inflammation. Additionally, adjuncts that re-sensitize tumors to chemotherapy, such as inhibitors of the MDM2-P53 axis, immune modulators, or anti-inflammatory agents, could be integrated into multi-modal protocols for patients at elevated risk of resistance. It also opens the door to developing composite prognostic models incorporating immunohistochemical and biochemical markers - offering a multidimensional characterization of tumor biology and host response.

In summary, the interplay of Ki-67 and P53 expression with chemotherapy sensitivity and prognosis in osteosarcoma reflects the complex biological tapestry of tumor proliferation, genomic instability, cell death regulation, and a permissive pro-inflammatory microenvironment. High proliferative activity and dysregulated apoptosis underpin chemoresistance and unfavorable outcome, while systemic inflammation amplifies these effects. Recognizing these relationships helps explain disease pathophysiology and also enables novel precision oncology therapy for the management of osteosarcoma. Continued research integrating immunohistochemical, molecular, and clinical findings holds promise for transforming patient care and improving long-term outcome in this challenging malignancy.

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Disclosure of conflict of interest

None.

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