Am J Transl Res 2024;16(8):3667-3677
www.ajtr.org /ISSN:1943-8141/AJTR0157080

Original Article

Diagnostic value of combined detection of AKP, TSGF, and LDH for pediatric osteosarcoma: a case-control study

Tao Sun[1], Jin Ma[2], Shumin Zhu[3], Ke Wang[4]

[1]Department of Pediatric Orthopedics, Yantai Yantaishan Hospital, Yantai 264003, Shandong, China; [2]Department of Clinical Laboratory, The Third Hospital of Hebei Medical University, Shijiazhuang 050051, Hebei, China; [3]Department of Clinical Laboratory, The First Hospital of Hebei Medical University, Shijiazhuang 050030, Hebei, China; [4]Department of Bone Disease and Orthopedic Oncology, Yantai Yantaishan Hospital, Yantai 264003, Shandong, China

Received April 8, 2024; Accepted July 9, 2024; Epub August 15, 2024; Published August 30, 2024

Abstract: Objective: To evaluate the diagnostic value of serum alkaline phosphatase (AKP), tumor-supplied growth factor group (TSGF), and lactate dehydrogenase (LDH) for pediatric osteosarcoma. Methods: A retrospective analysis of clinical data from 81 pediatric osteosarcoma patients (osteosarcoma group) and 63 patients with benign bone tumors (benign bone tumor group) admitted to Yantaishan Hospital from February 2023 to November 2023 was conducted. Basic and clinical data differences between the two groups of children were compared. A multivariate regression model was established to determine predictive factors for pediatric osteosarcoma, and the diagnostic value of identified indicators for pediatric osteosarcoma was evaluated. Results: Osteosarcoma group demonstrated significantly higher serum AKP (375.76±73.47 vs 286.12±76.50 U/L), TSGF (69.01±16.30 vs 53.57±16.37 U/mL), and LDH (269.55±66.96 vs 207.46±59.20 U/L) levels as compared to the benign bone tumor group. Correlation analysis suggested significant positive correlations between AKP (rho=0.505), TSGF (rho=406), LDH (rho=0.449) and pediatric osteosarcoma. Multivariate regression analysis showed serum AKP, TSGF, and LDH were independent predictive factor for pediatric osteosarcoma. The AUC value for AKP was 0.794, with a Youden index of 0.459; the AUC value for TSGF was 0.736, with a Youden index of 0.406; and the AUC value for LDH was 0.761, with a Youden index of 0.462. The combined use of these three biomarkers yielded an AUC of 0.886. Conclusion: The combined detection of serum AKP, TSGF, and LDH can enhance the diagnostic accuracy of pediatric osteosarcoma, providing important evidence for clinical treatment.

Keywords: Alkaline phosphatase, tumor-supplied growth factor group, lactate dehydrogenase, pediatric osteosarcoma

Introduction

Osteosarcoma is a common primary malignant bone tumor among children and adolescents, accounting for approximately 5% of pediatric tumors, with up to 90% occurring in the distal femur, proximal tibia, and other limb locations [1]. Clinical symptoms include pain, local swelling, limping caused by pain, or systemic symptoms due to metastasis [2]. Osteosarcoma typically originates from mesenchymal cells within the bone, which directly or indirectly lead to the transformation of cartilage tissue into tumor bone tissue to form bone. Characterized by rapid growth, high metastatic potential, and significant mortality, osteosarcoma poses a substantial threat to the health of affected children [3]. Patients with undiagnosed and untreated osteosarcoma typically develop pulmonary metastases within 6-12 months [4]. Therefore, the early diagnosis of osteosarcoma is of great significance to improve the prognosis of children with subsequent treatment.

However, the currently available diagnostic methods, including imaging modalities and tissue biopsy, have limitations in sensitivity and specificity. Conventional X-rays and CT scans have previously shown limited efficacy due to the lack of early radiographic changes, resulting

https://doi.org/10.62347/IGEA4076

in high misdiagnosis rates and delay in diagnosis [5]. The mean difference of X-ray margins compared to pathology reports was 1.09 cm, with 84% of cases showing little difference in pathological tissue imaging reports. However, in the remaining cases, there was a large difference [6]. While histopathologic examination remains the gold standard for osteosarcoma diagnosis, it is invasive and demands a high level of operative expertise [7]. Consequently, there is a clinical demand for an early, efficient, and highly sensitive diagnostic biomarker for osteosarcoma. Therefore, there is a critical need for novel and reliable biomarkers to aid in the early detection and monitoring of pediatric osteosarcoma.

In recent years, serum biomarkers have garnered increasing attention as possible diagnostic and prognostic tools for various malignancies, including osteosarcoma. With the advancement of tumor pathology research, serum marker detection offers advantages such as low cost, minimal invasiveness, and strong repeatability. It has been applied for early diagnosis of various cancers and is a current research focus [8, 9]. Alkaline phosphatase (AKP), tumor-specific growth factor (TSGF), and lactate dehydrogenase (LDH) have individually shown promise as biomarkers in cancer diagnosis and monitoring [10]. AKP is an enzyme predominantly present in the liver, bone, and placenta, and an elevated level of serum AKP is associated with increased bone turnover, commonly found in osteosarcoma patients [11]. TSGF is a protein expressed specifically by tumor cells and is associated with the progression and metastasis of various cancers [12]. LDH is an enzyme involved in cellular metabolism, and high LDH level is associated with increased tumor burden and poor treatment response in osteosarcoma patients [13]. However, their combined diagnostic utility of these biomarkers in pediatric osteosarcoma remains under-investigated.

This study aims to evaluate the diagnostic potential of serum AKP, TSGF, and LDH levels, both individually and in combination, for pediatric osteosarcoma. By assessing the correlation of these biomarkers with disease status and clinical outcomes, this research seeks to develop a more effective and reliable diagnostic approach for pediatric osteosarcoma. The findings of this study may have significant implication for early detection, risk stratification, and treatment monitoring in pediatric patients with osteosarcoma.

Materials and methods

Study subjects

A retrospective analysis was conducted on the clinical data of 81 pediatric osteosarcoma patients admitted to Yantaishan Hospital from February 2023 to November 2023, designated as the osteosarcoma group. Simultaneously, analysis was also performed on 63 patients with benign bone tumors admitted to Yantaishan Hospital during the same time frame, designated as the benign bone tumor group (**Figure 1**). Inclusion criteria: Ages <18; Confirmed diagnosis of osteosarcoma or benign bone tumor through pathological examination, with Enneking stage I for osteosarcoma; Normal cognitive ability and mental status; Complete clinical data (Demographic information can be found in past medical records and blood samples can be found and analyzed in clinical sample banks). Exclusion criteria: Significant dysfunction of major organs such as the liver, kidneys, or heart; Presence of concomitant malignancies in other locations; Dysfunction of the hematologic, endocrine, or immune systems. This study adhered to the principles outlined in the Declaration of Helsinki and was approved by the Clinical Trial Ethics Committee of Yantaishan Hospital (No. 2023091). Patients' informed consent was waived due to the retrospective nature.

Methods

Data collection: General information for all patients, including gender, age, place of residence, primary caregiver, weight, as well as clinical data such as disease course, tumor size, and tumor location, was obtained through retrospective analysis of medical records.

Detection methods: Upon admission, fasting venous blood samples of 5 mL were collected from all patients within one day. After layering and centrifugation at 2800 r/min for 10 minutes with a centrifugal radius of 10 cm, the supernatant was collected for measurement. The residual blood samples were stored in the clinical sample bank at -80°C for future testing

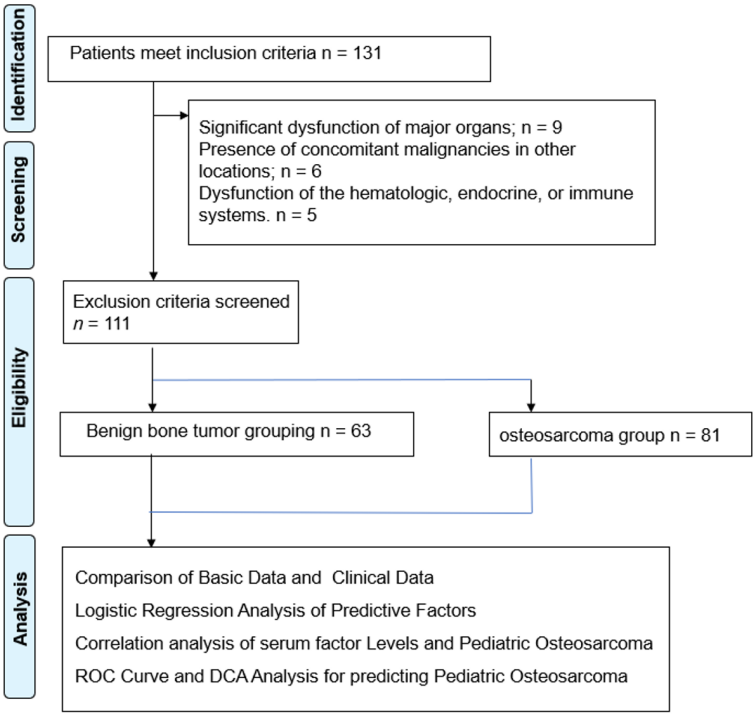

Figure 1. Flow chart for this study.

when needed. Serum levels of AKP, TSGF, and LDH were detected using a fully automated biochemical analyzer (20162400396, Shenzhen PuKang Electronics Co., Ltd., China) and immunoturbidimetric assay kits (produced by Roche Diagnostics GmbH, 20162404197). Measurements were performed in strict accordance with the instructions provided with the assay kits. Each data point was measured in triplicate, and the average was taken as the final result. The normal range for serum AKP level was <350 U/L; for TSGF level, <64 U/mL; and for LDH level, 109-245 U/L [14].

Outcome measures

Differences in basic and clinical data between the two groups of patients were observed. A multivariate regression model was established to determine the predictive factors for pediatric osteosarcoma. The correlation between various indicators and pediatric osteosarcoma was analyzed. Receiver operating characteristic (ROC) curves were plotted to determine the area under the curve (AUC), optimal threshold, sensitivity, specificity, and Youden's index, to assess the diagnostic value of individual or combined indicators for pediatric osteosarcoma.

Statistical methods

SPSS 25.0 statistical software was used for data analysis. Counted data were presented as n (%), and analyzed using Chi-square test or correction for continuity Chi-square test as appropriate. Normally distributed metric data were represented as (x±s) and compared between groups using a t-test. For non-normally distributed data, statistical analysis was performed after variable transformation to achieve normal distribution.

Spearman analysis was performed to analyze the relationship between continuous variables AKP/TSGF/LDH and pediatric osteosarcoma occurrence. Significant indicators with statistical differences between the two groups were selected for binary logistic regression analysis, using pediatric osteosarcoma as the dependent variable and serum AKP, TSGF, and LDH levels as independent variables. On the basis of multi-factor regression analysis, multiple prediction indicators were integrated, and nomogram plot was drawn to predict the results. ROC curves were constructed, and the AUC, optimal threshold, sensitivity, specificity, and Youden index for each indicator were determined to analyze the diagnostic value of serum AKP, TSGF, LDH alone and in combination. Besides, "rmda" package in R software was used to make decision curve analysis (DCA). The AUC values of multi-factor prediction model and single factor prediction model were compared to judge the diagnostic value. A P<0.05 was considered significant.

Results

Comparison of basic data between the two groups of patients

As shown in **Table 1**, the osteosarcoma group consisted of 42 male and 39 female patients, with a mean age of (4.67±1.02) years and a mean weight of (17.91±1.38) kg. Patients were almost evenly distributed between urban (39

Table 1. Comparison of basic data between the two groups of patients

Item	Osteosarcoma Group (n=81)	Benign Bone Tumor Group (n=63)	χ^2/t	P
Gender [n (%)]				
Male	42 (51.85)	31 (49.21)	0.099	0.753
Female	39 (48.15)	32 (50.79)		
Age (years)	4.67±1.02	4.54±1.09	0.736	0.463
Residence [n (%)]				
Urban	39 (92.86)	30 (47.62)	0.004	0.950
Rural	42 (7.14)	33 (52.38)		
Primary Caregiver [n (%)]				
Father	13 (16.05)	10 (15.87)	0.056	0.972
Mother	58 (71.60)	46 (73.02)		
Other	10 (12.35)	7 (11.11)		
Weight (kg)	17.91±1.38	18.03±1.46	0.505	0.615

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

Item	Osteosarcoma Group (n=81)	Benign Bone Tumor Group (n=63)	χ^2/t	P
Duration of Disease (months)	4.33±2.17	4.25±2.26	0.216	0.830
Tumor Size (cm)	4.12±0.38	4.20±0.45	1.156	0.250
Tumor Location [n (%)]				
Limbs	70 (86.42)	56 (88.89)	0.198	0.567
Non-limbs	11 (13.58)	7 (11.11)		
Serum AKP (U/L)	375.76±73.47	286.12±76.50	7.133	0.001
Serum TSGF (U/mL)	69.01±16.30	53.57±16.37	5.628	0.001
Serum LDH (U/L)	269.55±66.96	207.46±59.20	5.804	0.001

AKP: Alkaline phosphatase; TSGF: tumor-specific growth factor; LDH: lactate dehydrogenase.

cases) and rural (42 cases) areas. The primary caregivers were mostly mothers (58 cases), followed by fathers (13 cases), and others (10 cases). The benign bone tumor group included 31 male and 32 female patients, with a mean age of (4.54±1.09) years, a mean weight of (18.03±1.46) kg, and also a balanced distribution between urban (30 cases) and rural (33 cases) areas. The primary caregivers were also mothers (46 cases), followed by fathers (10 cases), and others (7 cases). There were no significant differences in basic data, including gender, age, place of residence, primary caregiver, and weight between the two groups (all P>0.05).

Comparison of clinical data between the two groups of patients

As shown in **Table 2**, the osteosarcoma group had a disease course of (4.33±2.17) months, tumor size of (4.12±0.38) cm, with tumors located in the limbs in 70 cases and non-limb locations in 11 cases. The serum levels were (375.76±73.47) U/L for AKP, (69.01±16.30) U/mL for TSGF, and (269.55±66.96) U/L for LDH. The benign bone tumor group had a disease course of (4.25±2.26) months, tumor size of (4.20±0.45) cm, with tumors located in the limbs in 56 cases and non-limb locations in 7 cases. The serum levels were (286.12±76.50) U/L for AKP, (53.57±16.37) U/mL for TSGF, and (207.46±59.20) U/L for LDH. There were no significant differences in disease course (P=0.830), tumor size (P=0.250), or tumor location (P=0.567) between the two groups (all P>0.05). However, there were significant differences in the comparison of clinical data such as serum AKP (P=0.001), TSGF (P=0.001), and LDH levels (P=0.001), indicating that serum AKP, TSGF, and LDH levels may be predictive factors for pediatric osteosarcoma.

Correlation analysis

As shown in **Figure 2**, Spearman's rho coefficients revealed moderate positive correlations between AKP (rho=0.505, P<0.001), TSGF

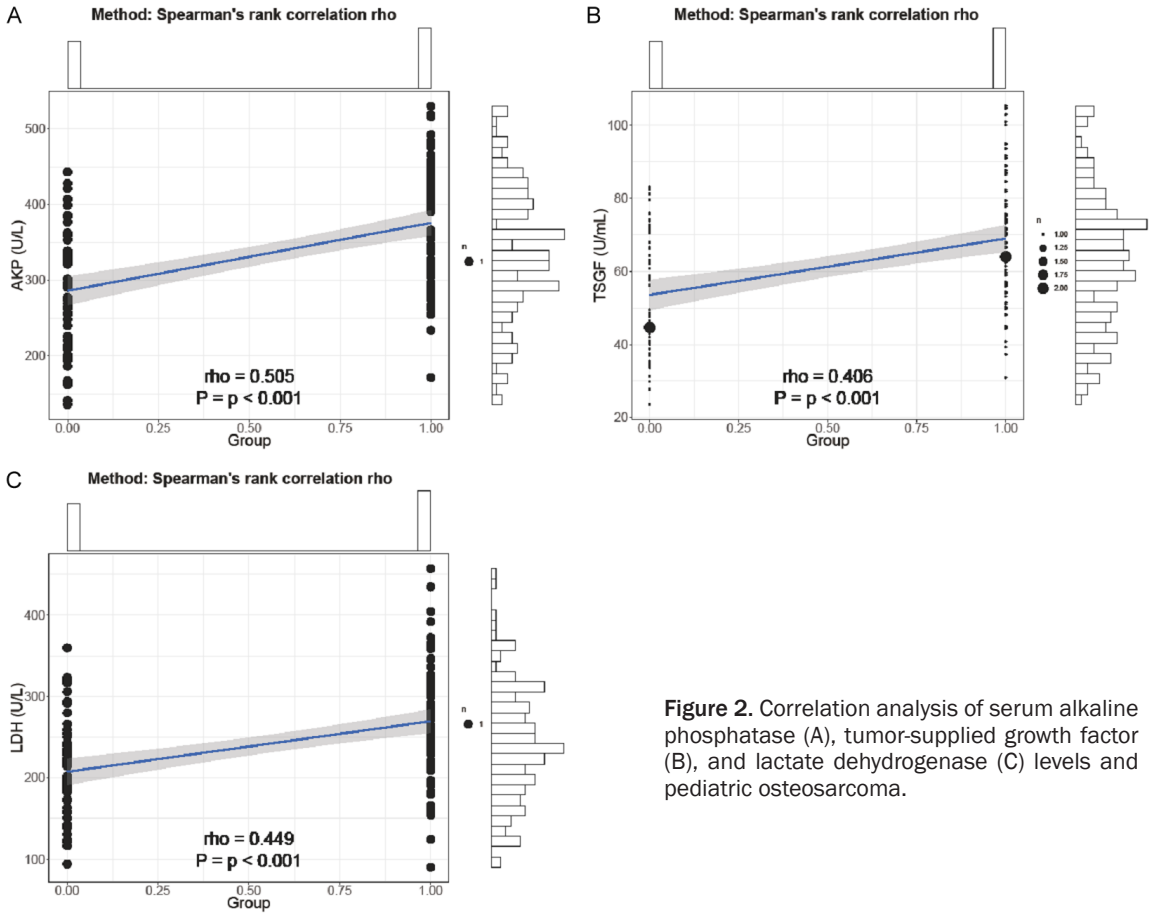

Figure 2. Correlation analysis of serum alkaline phosphatase (A), tumor-supplied growth factor (B), and lactate dehydrogenase (C) levels and pediatric osteosarcoma.

Table 3. Univariate logistic regression analysis of predictive factors for pediatric osteosarcoma

Item	Coefficient	Std Error	Wald	*P* Value	OR	95% CI
AKP≥303.29 (U/L)	2.140	0.399	5.368	<0.001	8.500	3.986-19.155
TSGF≥49.705 (U/mL)	2.326	0.469	4.961	<0.001	10.241	4.291-27.560
LDH≥235.59 (U/L)	2.003	0.380	5.268	<0.001	7.408	3.587-16.000

AKP: Alkaline phosphatase; TSGF: tumor-specific growth factor; LDH: lactate dehydrogenase.

(rho=406, P<0.001), LDH (rho=0.449, P< 0.001) and the occurrence of pediatric osteosarcoma. These findings suggest the diagnostic utility of these serum biomarkers in the identification and assessment of pediatric osteosarcoma.

Logistic regression analysis of factors for pediatric osteosarcoma

As shown in **Tables 3**, **4**, both univariate and multivariate logistic regression analysis of serum AKP, TSGF, and LDH levels revealed OR values greater than 1, indicating serum AKP, TSGF, and LDH levels as an independent risk factor for pediatric osteosarcoma.

Diagnostic value of serum AKP, TSGF, LDH levels alone or in combination for pediatric osteosarcoma

As illustrated in **Figure 3**, the ROC curve analysis revealed an AUC value of 0.794 for AKP, with a Youden index of 0.459, and an optimal threshold of 303.29 U/L. Using 303.29 U/L as the optimal diagnostic cutoff, AKP demonstrated a sensitivity of 0.84 and specificity of 0.619 for diagnosing pediatric osteosarcoma. TSGF exhibited an AUC value of 0.736, a Youden index of 0.406, and an optimal threshold of 49.705 U/mL, resulting in a sensitivity of 0.914 and specificity of 0.492 for diagnosing pediatric osteosarcoma. LDH showed an AUC value of

Table 4. Multifactorial logistic regression analysis of predictive factors for pediatric osteosarcoma

Influencing Factor	Coefficient	Std Error	Wald	*P* Value	OR	95% CI
AKP≥303.29 (U/L)	2.946	0.596	4.940	<0.001	19.030	5.913-61.251
TSGF≥49.705 (U/mL)	2.250	0.611	3.680	<0.001	9.487	2.862-31.443
LDH≥235.59 (U/L)	2.405	0.560	4.297	<0.001	11.073	3.698-33.156

AKP: Alkaline phosphatase; TSGF: tumor-specific growth factor; LDH: lactate dehydrogenase.

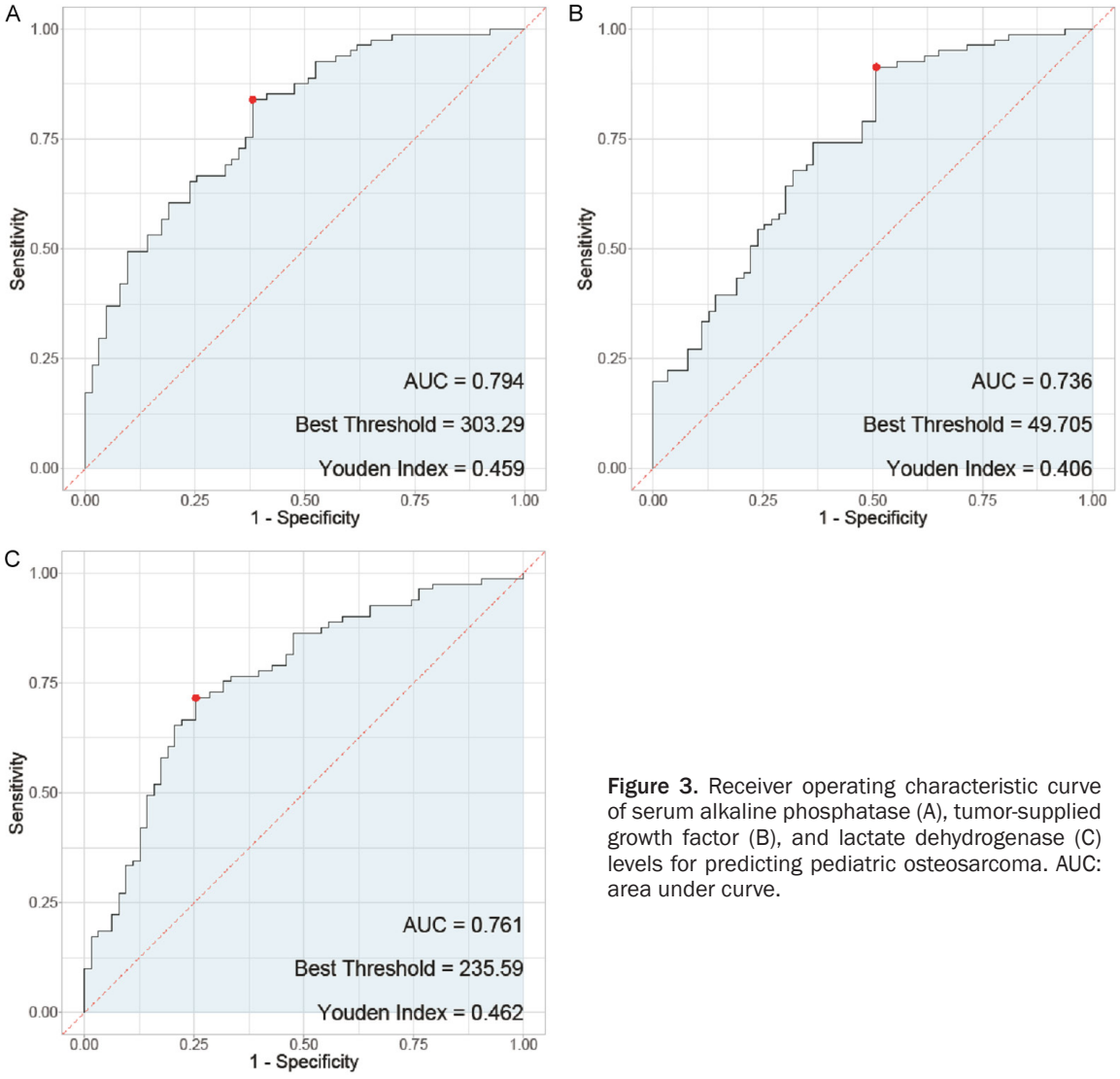

Figure 3. Receiver operating characteristic curve of serum alkaline phosphatase (A), tumor-supplied growth factor (B), and lactate dehydrogenase (C) levels for predicting pediatric osteosarcoma. AUC: area under curve.

0.761, a Youden index of 0.462, and an optimal threshold of 235.59 U/L, yielding a sensitivity of 0.716 and specificity of 0.746 for diagnosing pediatric osteosarcoma. Then, the DCA plot confirmed the diagnostic performance of serum AKP, TSGF, LDH levels for pediatric osteosarcoma (**Figure 4**).

By integrating these factors, a multifactor predicting nomogram model for pediatric osteosarcoma was drawn (**Figure 5A**). When combined (**Figure 5C**), the three parameters yielded an AUC value of 0.886, higher than the AUCs of each single. At the optimal threshold, the diagnostic sensitivity was 0.951, and the specificity was 0.635, significantly higher than that of the individual values, indicating that the combination of the three values could enhance the diagnostic value for pediatric osteosarcoma. The DCA curve suggested high application value of

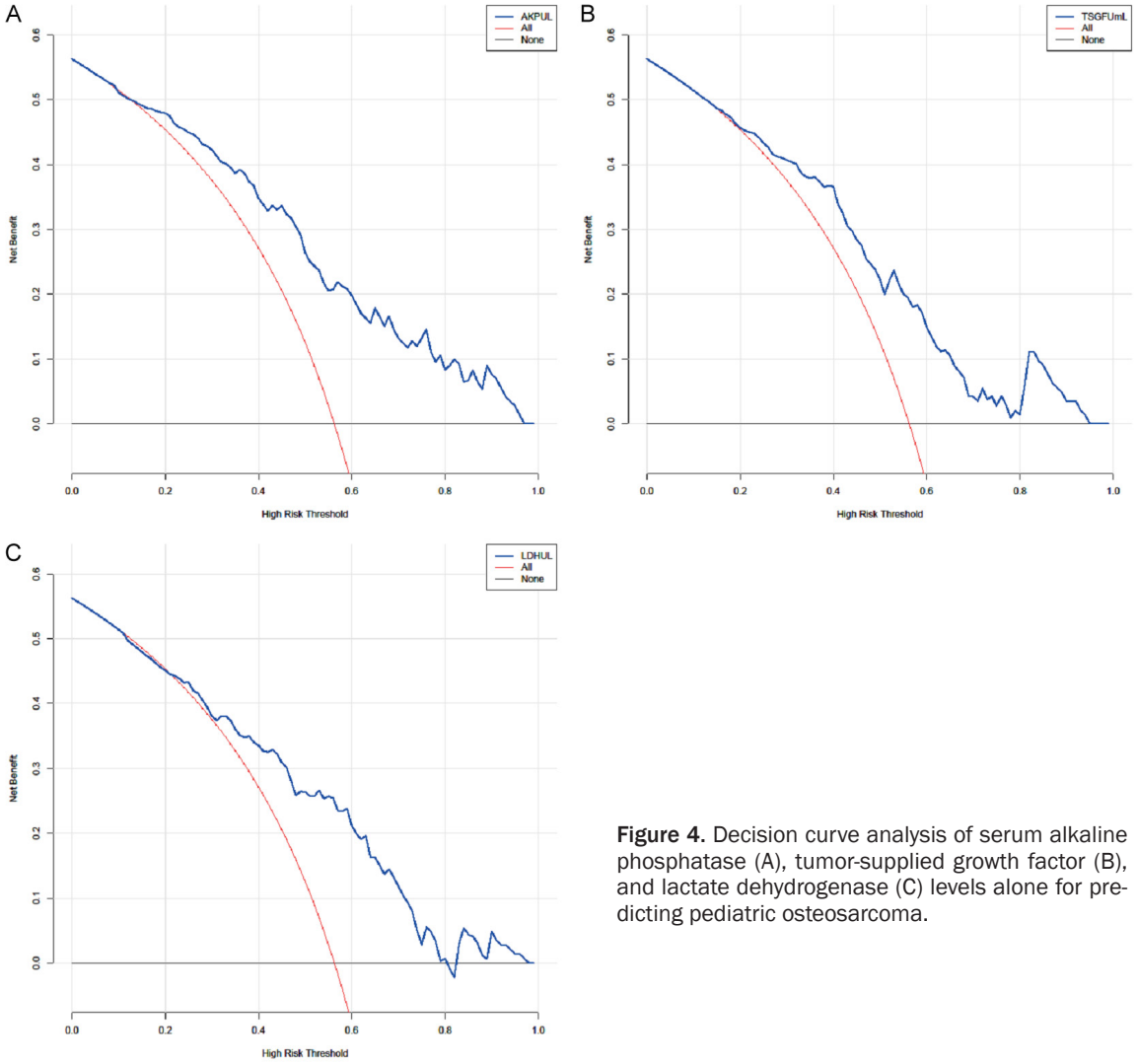

Figure 4. Decision curve analysis of serum alkaline phosphatase (A), tumor-supplied growth factor (B), and lactate dehydrogenase (C) levels alone for predicting pediatric osteosarcoma.

this nomogram model (**Figure 5B**). Besides, the calibrate plot of the nomogram model suggested a high stability (**Figure 5D**).

Discussion

Osteosarcoma is one of the most prevalent bone tumors, originating from primitive mesenchymal cells capable of multidirectional differentiation. Consequently, osteosarcoma cells often exhibit pleomorphic features, posing significant challenges for disease diagnosis and treatment [15-17]. Osteosarcoma primarily affects individuals under the age of 20, and the main site of onset is the lower limbs, especially the knee joint, manifested as unexplained joint pain. Pain caused by tumor can easily be mistaken for normal growing pain or sprain, leading to missed opportunities for timely diagnosis and treatment [18-20].

Serum factor testing, which assesses disease by detecting levels of factors in the blood to indicate disease presence, serves as an important diagnostic tool. Yang et al. established a nomogram to predict the cancer-specific survival (CSS) and overall survival rate of osteosarcoma. It showed that age, stage, grade, surgery, primary site, and tumor size were independent risk factors for osteosarcoma [21]. Lu et al. established a nomogram to predict the risk of distant metastasis of osteosarcoma [22]. Compared to previous studies, this study proposed the predictive effect of serum markers on osteosarcoma in children. A more accurate prediction method is proposed, which has clinical value. Conventional X-rays and CT scans have previously shown limited efficacy due to the lack of early radiographic changes, resulting in high misdiagnosis rates and delay in diag-

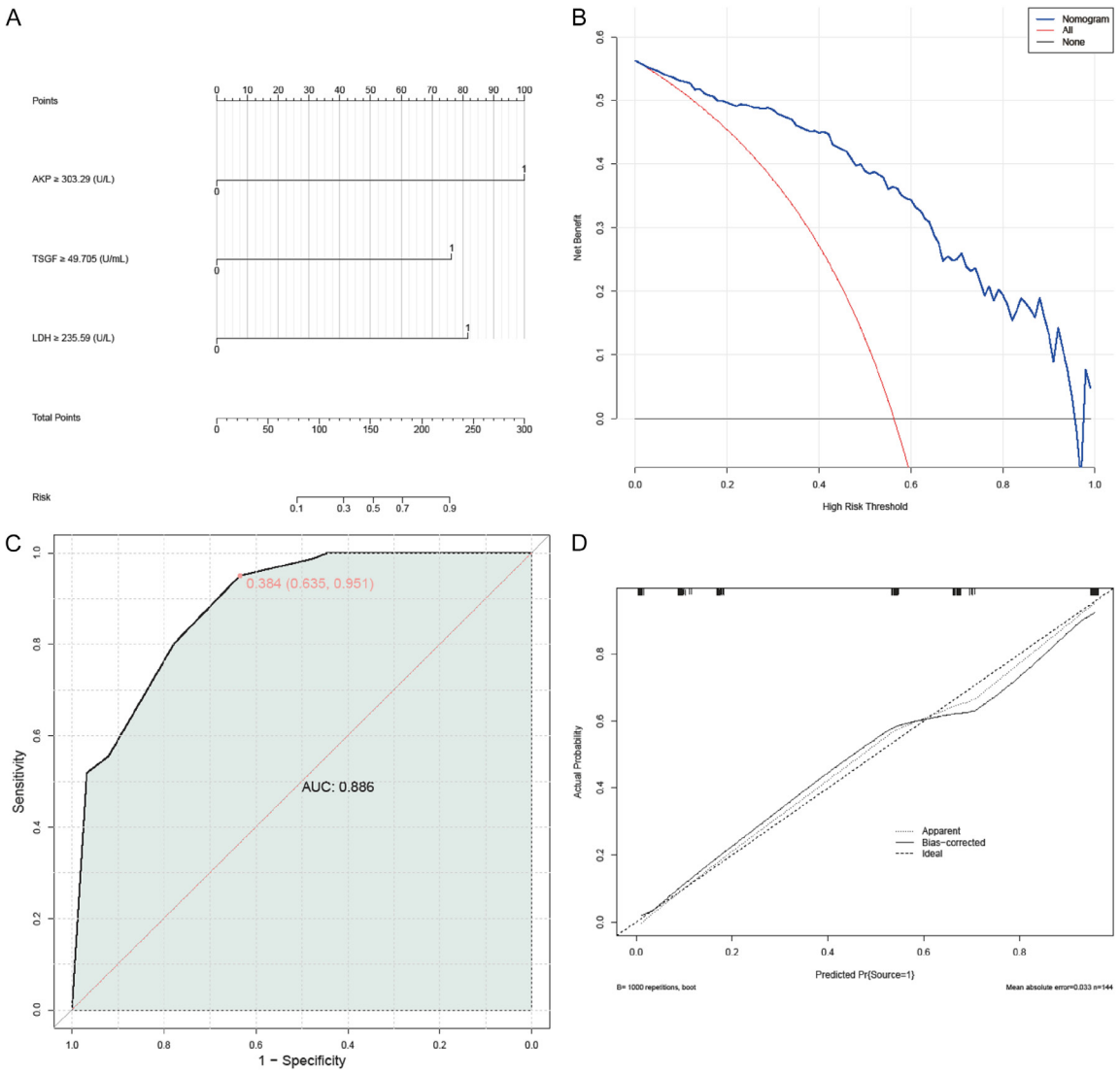

Figure 5. The nomogram (A), decision curve analysis (B), receiver operating characteristic curve (C), and calibrate plot (D) of multifactor predicting model for predicting pediatric osteosarcoma.

nosis. However, patient's disease condition can be reflected in the blood index more quickly, and the delayed diagnosis rate and misdiagnosis rate of serum factor detection are lower than that of imaging examination. Although needle biopsy remains the gold standard for diagnosing osteosarcoma, it can be distressing and painful for young patients. Therefore, an alternative early detection protocol for pediatric osteosarcoma is needed.

The findings of this study underscore the diagnostic potential of three serum factor levels - AKP, TSGF, and LDH - in pediatric osteosarcoma. Elevated levels of these indicators are strongly associated with the presence of the disease, serving as predictive biomarkers. Studies by Zhang et al. [23] have revealed a significant increase in serum AKP levels in osteosarcoma patients compared to benign bone tumor patients, which is consistent with the results of this study. The serum AKP level consistently balances with the amount produced in bone tissue; however, in cases such as osteosarcoma and other malignant tumors, the extensive proliferation and destruction of bone tissue by tumor cells significantly increase the activity of AKP, leading to elevated serum AKP levels [24, 25]. Wang et al. [26] found that serum TSGF level is an important reference indicator for distinguishing between benign bone tumors and osteosarcoma, aligning with

the conclusions of this study. TSGF, a growth factor specifically expressed in tumor cells, is generally absent or minimally expressed in normal cells. However, in osteosarcoma, tumor cells augment the production and release of TSGF to activate cell proliferation signaling pathways, promoting the proliferation and survival of osteosarcoma cells [27, 28]. Studies by Sittiju et al. [29] found that the serum LDH level in osteosarcoma patients was higher compared to patients with benign bone tumors, also mirroring the findings of this study. Under normal circumstances, LDH is typically present in tissues such as the liver, myocardium, muscles, and red blood cells. However, with the occurrence of osteosarcoma, LDH level increases due to the proliferation and destruction of tumor cells, leading to released LDH into the bloodstream and causing elevated serum LDH level [30, 31]. Elevated serum levels of AKP, TSGF, and LDH are common in pediatric osteosarcoma, and these three indicators serve as important auxiliary markers in differentiating pediatric osteosarcoma from benign bone tumors [32].

The results of this study indicate that serum factors AKP, TSGF and LDH can facilitate the diagnosis of osteosarcoma in children. The innovation of this study lies in the construction of the multifactor predicting model using serum AKP, TSGF, and LDH for pediatric osteosarcoma diagnosis. The rationale behind this is that the combination of the three values can synergistically aid in diagnoses, providing more comprehensive diagnostic information and effectively reducing or avoiding potential issues with misdiagnosis or missed diagnosis associated with single serum markers. This improves the diagnostic efficacy for the disease, offering a more accurate and objective basis for clinical disease diagnosis and treatment.

While this study highlights the diagnostic potential of serum AKP, TSGF, and LDH levels for pediatric osteosarcoma, it is essential to acknowledge several limitations that could impact the generalizability and robustness of the findings. Firstly, the retrospective nature of the analysis may have introduced inherent biases and confounding factors, potentially influencing the results. Additionally, the relatively modest sample size of 81 pediatric osteosarcoma patients and 63 benign bone tumor patients from a single hospital may limit the ability to draw definitive conclusions applicable to a broader population. The exclusion criteria implemented in this study, such as excluding patients with significant organ dysfunction or concomitant malignancies, could have led to a selection bias that might not fully represent the diversity of pediatric osteosarcoma cases encountered in clinical practice. Furthermore, the use of serum biomarkers as diagnostic tools alone may not provide a comprehensive assessment of pediatric osteosarcoma, as the disease is multifactorial in nature and may require a more integrated approach for accurate diagnosis and monitoring.

Future research efforts could address these limitations by conducting prospective studies to attain a more comprehensive understanding of the diagnostic value of these serum markers in pediatric osteosarcoma. Prospective controlled trials with larger sample sizes and longer follow-up durations are warranted to further validate the findings.

Conclusions

Serum AKP, TSGF, and LDH levels hold significant diagnostic value for pediatric osteosarcoma. These markers were substantially increased in pediatric osteosarcoma patients compared to those with benign bone tumors, indicating their potential as predictive markers.

Disclosure of conflict of interest

None.

Address correspondence to: Ke Wang, Department of Bone Disease and Orthopedic Oncology, Yantai Yantaishan Hospital, No. 10087 Keji Avenue, Laishan District, Yantai 264003, Shandong, China. E-mail: Wangk126yx@126.com

References

[1] Yoshida A. Osteosarcoma: old and new challenges. Surg Pathol Clin 2021; 14: 567-583.
[2] Yan Y, Liu X, Li Y, Yan J, Zhao P and Yang L. EPB41L4A-AS1 and UNC5B-AS1 have diagnostic and prognostic significance in osteosarcoma. J Orthop Surg Res 2023; 18: 261.
[3] Chen B, Zeng Y, Liu B, Lu G, Xiang Z, Chen J, Yu Y, Zuo Z, Lin Y and Ma J. Risk factors, prognostic factors, and nomograms for distant metastasis in patients with newly diagnosed osteosarcoma: a population-based study. Front Endocrinol (Lausanne) 2021; 12: 672024.

[4] Liu R and Shao J. Research progress on risk factors related to intracranial artery, carotid artery, and coronary artery stenosis. Front Cardiovasc Med 2022; 9: 970476.
[5] Moukengue B, Lallier M, Marchandet L, Baud'huin M, Verrecchia F, Ory B and Lamoureux F. Origin and therapies of osteosarcoma. Cancers (Basel) 2022; 14: 3503.
[6] Katz TH, Hasan OH and Miller BJ. Accuracy of X-ray and magnetic resonance imaging in defining the tumor margin in primary bone sarcoma. Iowa Orthop J 2021; 41: 27-33.
[7] Regan DP, Chow L, Das S, Haines L, Palmer E, Kurihara JN, Coy JW, Mathias A, Thamm DH, Gustafson DL and Dow SW. Losartan blocks osteosarcoma-elicited monocyte recruitment, and combined with the kinase inhibitor toceranib, exerts significant clinical benefit in canine metastatic osteosarcoma. Clin Cancer Res 2022; 28: 662-676.
[8] Shoaib Z, Fan TM and Irudayaraj JMK. Osteosarcoma mechanobiology and therapeutic targets. Br J Pharmacol 2022; 179: 201-217.
[9] Shu H, Zhao L, Li X, Gong J, Yin G and Chen H. Silica nanoparticle-modified microcomb electrode for voltammetry detection of osteopontin with high sensitivity. Biotechnol Appl Biochem 2022; 69: 1733-1740.
[10] Zhang C, He J, Qi L, Wan L, Wang W, Tu C and Li Z. Diagnostic and prognostic significance of dysregulated expression of circular RNAs in osteosarcoma. Expert Rev Mol Diagn 2021; 21: 235-244.
[11] Pu F, Guo H, Shi D, Chen F, Peng Y, Huang X, Liu J, Zhang Z and Shao Z. The generation and use of animal models of osteosarcoma in cancer research. Genes Dis 2023; 11: 664-674.
[12] Smrke A, Anderson PM, Gulia A, Gennatas S, Huang PH and Jones RL. Future directions in the treatment of osteosarcoma. Cells 2021; 10: 172.
[13] Liu WF, Huang Z, Gong LH, Yang YK, Jin T, Deng ZP, Li Y, Hao L, Zhang Q, Ding Y and Niu XH. Synchronous multicentric osteosarcoma: treatment and prognostic factor analysis. Zhonghua Yi Xue Za Zhi 2019; 99: 2897-2902.
[14] Zhang J, He X, Yu W, Ying F, Cai J and Deng S. Primary exophytic extraskeletal osteosarcoma of the liver: a case report and literature review. Risk Manag Healthc Policy 2021; 14: 1009-1014.
[15] Huang Q, Liang X, Ren T, Huang Y, Zhang H, Yu Y, Chen C, Wang W, Niu J, Lou J and Guo W. The role of tumor-associated macrophages in osteosarcoma progression - therapeutic implications. Cell Oncol (Dordr) 2021; 44: 525-539.
[16] Lei J, He MY, Li J, Li H, Wang W, Gopinath SCB and Xu LZ. miRNA identification by nuclease digestion in ELISA for diagnosis of osteosarcoma. Biotechnol Appl Biochem 2022; 69: 1365-1372.
[17] Li R, Fan L, Ma F, Cao Y, Gao J, Liu H and Li Y. Effect of etomidate on the oxidative stress response and levels of inflammatory factors from ischemia-reperfusion injury after tibial fracture surgery. Exp Ther Med 2017; 13: 971-975.
[18] Kim MS, Bolia IK, Iglesias B, Sharf T, Roberts SI, Kang H, Christ AB and Menendez LR. Timing of treatment in osteosarcoma: challenges and perspectives - a scoping review. BMC Cancer 2022; 22: 970.
[19] Aran V, Devalle S, Meohas W, Heringer M, Cunha Caruso A, Pinheiro Aguiar D, Leite Duarte ME and Moura Neto V. Osteosarcoma, chondrosarcoma and Ewing sarcoma: clinical aspects, biomarker discovery and liquid biopsy. Crit Rev Oncol Hematol 2021; 162: 103340.
[20] Belayneh R, Fourman MS, Bhogal S and Weiss KR. Update on osteosarcoma. Curr Oncol Rep 2021; 23: 71.
[21] Yang QK, Lai QY, Wang Y, Wang Y, Yao ZX and Zhang XJ. Establishment and validation of prognostic nomograms to predict overall survival and cancer-specific survival for patients with osteosarcoma. Neoplasma 2021; 68: 434-446.
[22] Lu S, Wang Y, Liu G, Wang L, Wu P, Li Y and Cheng C. Construction and validation of nomogram to predict distant metastasis in osteosarcoma: a retrospective study. J Orthop Surg Res 2021; 16: 231.
[23] Zhang F, Lu SX, Hu KS, Gan YH, Chen Y, Ge NL, Yang BW, Zhang L, Chen RX, Ren ZG and Yin X. Albumin-to-alkaline phosphatase ratio as a predictor of tumor recurrence and prognosis in patients with early-stage hepatocellular carcinoma undergoing radiofrequency ablation as initial therapy. Int J Hyperthermia 2021; 38: 1-10.
[24] Takagi S, Sasaki Y, Koike S, Takemoto A, Seto Y, Haraguchi M, Ukaji T, Kawaguchi T, Sugawara M, Saito M, Funauchi Y, Ae K, Matsumoto S, Fujita N and Katayama R. Platelet-derived lysophosphatidic acid mediated LPAR1 activation as a therapeutic target for osteosarcoma metastasis. Oncogene 2021; 40: 5548-5558.
[25] Tan B, Wu Y, Wu Y, Shi K, Han R, Li Y, Qian Z and Liao J. Curcumin-microsphere/IR820 hybrid bifunctional hydrogels for in situ osteosarcoma chemo-co-thermal therapy and bone reconstruction. ACS Appl Mater Interfaces 2021; 13: 31542-31553.
[26] Wang Y, Zhang L, Zhao G, Zhang Y, Zhan F, Chen Z, He T, Cao Y, Hao L, Wang Z, Quan Z and Ou Y. Homologous targeting nanoparticles for enhanced PDT against osteosarcoma HOS cells and the related molecular mechanisms. J Nanobiotechnology 2022; 20: 83.

[27] Wu X, Zhang X, Feng W, Feng H, Ding Z, Zhao Q, Li X, Tang N, Zhang P, Li J and Wang J. A targeted erythrocyte membrane-encapsulated drug-delivery system with anti-osteosarcoma and anti-osteolytic effects. ACS Appl Mater Interfaces 2021; 13: 27920-27933.
[28] Xu Z, Zheng K, Du Z, Xin J, Luo M and Wang F. Colorimetric identification of miRNA-195 sequence for diagnosing osteosarcoma. Biotechnol Appl Biochem 2022; 69: 974-980.
[29] Sittiju P, Chaiyawat P, Pruksakorn D, Klangjorhor J, Wongrin W, Phinyo P, Kamolphiwong R, Phanphaisarn A, Teeyakasem P, Kongtawelert P and Pothacharoen P. Osteosarcoma-specific genes as a diagnostic tool and clinical predictor of tumor progression. Biology (Basel) 2022; 11: 698.
[30] He X, Tang F, Zou C, Li L, Wang Y, Kenmegne GR, Zhou Y, Lu M, Min L, Luo Y and Tu C. Prognostic significance of modified lung immune prognostic index in osteosarcoma patients. Front Genet 2022; 13: 972352.
[31] Zhang H, Wang T, Gong H, Jiang R, Zhou W, Sun H, Huang R, Wang Y, Wu Z, Xu W, Li Z, Huang Q, Cai X, Lin Z, Hu J, Jia Q, Ye C, Wei H and Xiao J. A novel molecular classification method for osteosarcoma based on tumor cell differentiation trajectories. Bone Res 2023; 11: 1.
[32] Zheng D, Liu W, Xie W, Huang G, Jiang Q, Yang Y, Huang J, Xing Z, Yuan M, Wei M, Li Y, Yin J, Shen J and Shi Z. AHA1 upregulates IDH1 and metabolic activity to promote growth and metastasis and predicts prognosis in osteosarcoma. Signal Transduct Target Ther 2021; 6: 25.