Int J Clin Exp Med 2015;8(1):890-896
www.ijcem.com /ISSN:1940-5901/IJCEM0003874

Original Article

Role of β-isomerized C-terminal telopeptides (β-CTx) and total procollagen type 1 amino-terminal propeptide (tP1NP) as osteosarcoma biomarkers

Tu Hu[1*], Qingcheng Yang[1*], Jing Xu[2], Zhichang Zhang[1], Nengbin He[1], Yuzhen Du[2]

Departments of [1]Orthopedics, [2]Laboratory Medicine, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China. [*]Equal contributors and co-first authors.

Received November 15, 2014; Accepted January 8, 2015; Epub January 15, 2015; Published January 30, 2015

Abstract: Introduction: Few serum markers are valid and useful for the diagnosis or therapeutic effect monitoring of osteosarcoma. This study aimed to investigate the role of β-isomerized C-terminal telopeptides (β-CTx) and total procollagen type 1 amino-terminal propeptide (tP1NP) as serological biomarkers for osteosarcoma patients. Materials and Methods: A total of 48 patients with osteosarcoma and 55 healthy volunteers were investigated. Serum β-CTx and tP1NP levels were measured by electrochemiluminescence immunoassay. Data were analyzed by t test with Walth's correction and receiver operating characteristic (ROC) curve analysis. Results: The baseline levels of β-CTx and tP1NP were found to be significantly higher in patients with osteosarcoma than the healthy volunteers. The mean areas under the ROC curves were 0.919 (range, 0.864-0.973) for β-CTx and 0.866 (range, 0.792-0.939) for tP1NP. The levels of β-CTx and tP1NP were lower in patients with stable disease after operation than those before operation. Conclusion: These findings support our hypothesis that β-CTx and tP1NP are promising serum biomarkers for diagnosing or monitoring osteosarcoma.

Keywords: Osteosarcoma, bone marker, diagnosis, monitoring, serum

Introduction

Osteosarcoma is the most common primary malignant tumor of the bone in children and young adults. It has an annual incidence of 3 persons per million [1, 2]. Due to its rapid progression, the optimal treatment time for osteosarcoma could be easily missed in case of a misdiagnosis [3, 4]. Nevertheless, even in most cases where a definite diagnosis is made, the patient's illness would have progressed to the advanced stage, resulting in a poor prognosis [5, 6].

The diagnosis of osteosarcoma is mostly based on its clinical presentation and radiological and pathological examinations [7-9]. Although the radiological method is the most reliable, there is a high risk of misdiagnosis with this method. Moreover, due to its low specificity, it is unable to detect the disease in its early stage [10, 11]. Very few common laboratory tests for osteosarcoma are available at present, and several common serum biochemical markers, e.g., alkaline phosphatase and lactate dehydrogenase, are neither sensitive nor specific [12, 13]. The discovery of sensitive and specific serological markers is of great importance for the early detection, treatment, and monitoring of osteosarcoma.

Bone is a metabolically active tissue, and almost 10% of the human adult bone is remodeled every year. Bone remodeling comprises bone resorption and formation. During the remodeling process, the activity of osteoblasts and osteoclasts results in the release of proteins or peptides. The peptide products are either proteins released by the cells, such as bone-specific alkaline phosphatase (BAP) and osteocalcin (OC) or degradation products of cellular activity, such as urinary hydroxyproline, deoxypyridinoline, β-isomerized C-terminal telopeptides (β-CTx), total procollagen type 1 ami-

Table 1. Clinical data of osteosarcoma patients and healthy controls

Parameters	Healthy	Osteosarcoma	*P* value
Gender	55	48	*$P > 0.05$
Male	29	27	
Female	26	21	
Age mean (mix-max)	24.4 (8-49)	20.9 (7-47)	Δ$P = 0.056$
White blood cells (cell/μl) ☆	6821±1478	6745±2589	#$P > 0.05$
Body mass index (kg/m^2)☆	19.1±1.2	18.6±2.5	#$P > 0.05$
Serum creatinine (μmol/L)☆	68.5±15.4	66.9±21.8	#$P > 0.05$
Serum BUN (mmmol/ L)☆	2.9±0.3	3.2±0.6	#$P > 0.05$
AST (IU/L)☆	30.3±7.6	34.8±21.7	#$P > 0.05$
ALT (U/L)☆	19.5±10.6	23.9±21.6	#$P > 0.05$
β-CTx (pg/ml)	426.0 (150.3-1225.0)	1178.4 (269.1-2940.0)	
Mean (mix-max)			Δ$P < 0.001$
tP1NP (ng/ml)	72.5 (17.62-369.0)	259.3 (17.19-1103.0)	
Mean (mix-max)			Δ$P < 0.001$

*By Kruskal Wallis Text, $\chi^2 = 0.127$, P = 0.722. ΔBy t test with Walth's correction. #By t test. ☆Values are the mean ± SD.

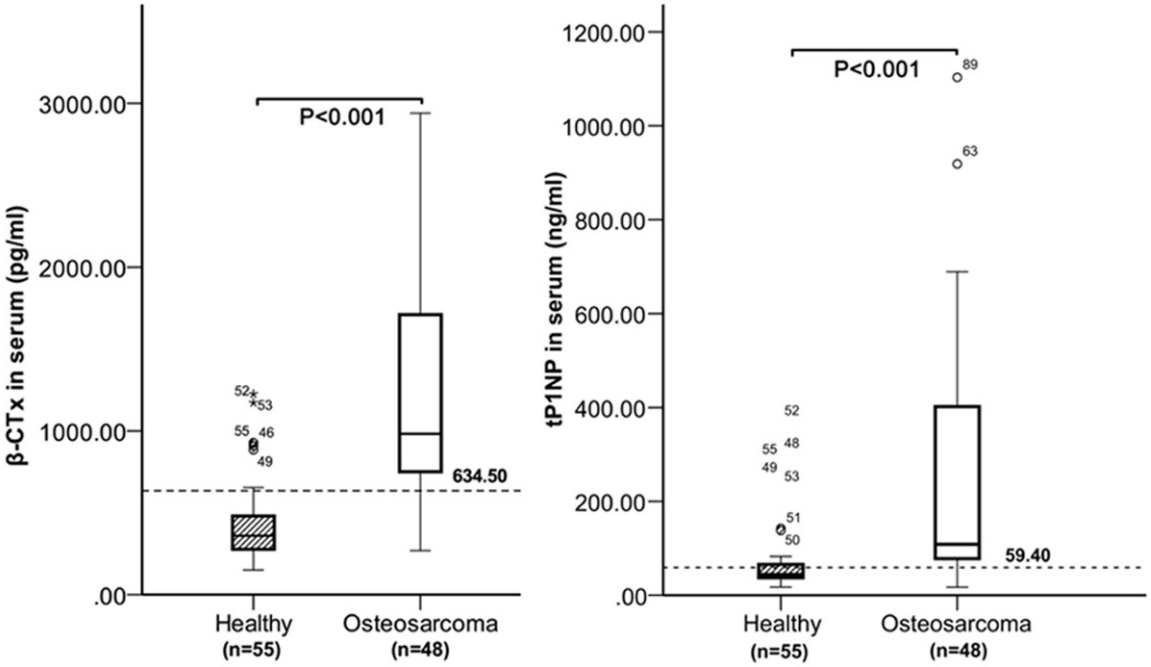

Figure 1. Serum concentrations of the difference. True area = 0.5 and tP1NP on the diagnosis introls (The dotted lines indicate the cutoff values determined using Youden's index. * and ○ are outliners).

no-terminal propeptide (tP1NP), etc [14]. The measurement of these clinical biomarkers, categorized as biochemical bone markers, in serum and urine is widely used as a rapid means to monitor cellular activity of bone [15]. β-CTx and other substances that originate during bone resorption are called bone resorption markers, while tP1NP, BAP, and OC, which are generated from bone reconstruction, are called bone formation markers.

The propagation of tumor cells in bone tissue could hasten bone metabolism or make it unbalanced, altering the levels of serum biochemical bone markers [16, 17]. The applications of serum biochemical bone markers in bone

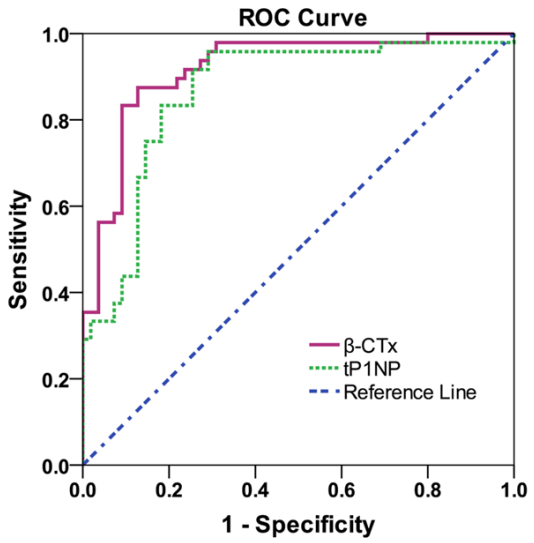

Figure 2. ROC analysis of β-CTx and tP1NP to differentiate osteosarcoma patients from healthy individuals. The areas under the ROC curves for β-CTx and tP1NP are 0.919 and 0.866.

metastases from cancers of the lung, breast, and prostate have been reported [18-20]. Some studies have also reported the use of BAP in the diagnosis of osteosarcoma [21, 22]. A few studies also suggest that the bone resorption marker β-CTx could be used for the diagnosis and treatment of osteosarcoma [23].

In this study, we determined the serum concentrations of β-CTx and tP1NP in patients with osteosarcoma and the healthy volunteers, and compared the diagnostic efficacy of the above markers with receiver operating characteristic (ROC) curve analysis. Additionally, we investigated the levels of the markers in the patients before and after operation.

Materials and methods

General characteristics of study population

Blood samples were collected from 48 osteosarcoma patients between June 2011 and May 2012 at Shanghai Sixth People's Hospital affiliated to Shanghai Jiao Tong University. Osteosarcoma patients who were clinically and histologically diagnosed of primary osteosarcoma by the multidisciplinary musculoskeletal oncology team, and for those whom osteosarcoma was the first diagnosis without prior treatment, were included in the study. All patients received standard treatment and follow-up.

The control group comprised 55 healthy volunteers matched for age and gender. Individuals with a history of musculoskeletal injury within the three years preceding the start of the study, those with abnormal liver and kidney function, those with any pre-existing disease, and those on medication for any reason at the start of the study were excluded. The healthy volunteers were recruited from individuals who underwent the physical examination.

This study was approved by the Research Ethics Committee of Shanghai Sixth People's Hospital, and all participants provided written informed consent before recruitment.

Collection of serum samples

Serum samples of the participants were obtained from venous blood after centrifugation at 6,500 g for 10 min and tested directly or after storing at -80°C. The healthy controls were advised to avoid any vigorous activities, including sports and running, for two days before blood collection. Samples were collected in the morning between 7:30 and 8:30 a.m., from fasting individual.

Eight osteosarcoma patients who were classified at stage III according to the criteria of the Musculoskeletal Tumor Society and received amputation treatment were also followed up. Their serum samples were collected before chemotherapy treatment and for the duration of more than six months after surgery. Four of the eight patients showed stable disease, while the other four had progressive disease.

β-CTx assay

β-CTx was measured in the serum samples by electrochemiluminescence immunoassay (ECLI) using a cobas e601 automated immunoassay system (Roche Co., Mannheim, Germany). A total of 50 μl of serum and a biotinylated monoclonal anti-β-crosslaps antibody were incubated together; the antigen in the sample was liberated from the serum components. The second incubation started with the addition of streptavidin-coated microparticles and a monoclonal β-crosslaps-specific antibody labeled with a ruthenium complex (Tris(2,2'-bipyridyl) ruthenium (II) complex (Ru (bpy)2/3+)). A sandwich complex that bound to the solid phase via the biotin-streptavidin interaction was formed. The reaction mixture was then treated as described above. Instra-assay variation is lower

Table 2. Diagnostic performance of serum bone markers β-CTx and tP1NP on the diagnosis of osteosarcoma

Group	Cutoff value	Sensitivity	Specificity	AUC	*P*-Value*	95% CI#
β-CTx	634.50 pg/ml	87.50%	87.30%	0.919	< 0.001	0.864-0.973
tP1NP	59.40 ng/ml	95.80%	70.90%	0.866	< 0.001	0.792-0.939

*Asymptotic significance, null hypothesis: true area = 0.5. #95% confidence interval of the difference.

than 3% and inter-assay variation is lower than 5%.

tP1NP assay

tP1NP concentration was measured by ECLI using a cobas e601 automated immunoassay system (Roche Co., Mannheim, Germany). A total of 20 μl of serum and a biotinylated monoclonal P1NP-specific antibody were incubated together. After the addition of streptavidin-labeled microparticles and a monoclonal P1NP-specific antibody labeled with a ruthenium complex (Tris(2,2'-bipyridyl)ruthenium (II) complex (Ru(bpy)2/3+)), a sandwich complex was formed, which was treated as described above. Instra-assay variation is lower than 3% and inter-assay variation is lower than 4%.

Statistical analysis

Descriptive statistics were calculated for each parameter. T test was applied to compare the levels of white blood cell count, body mass index, serum creatinine, BUN, AST and ALT levels; T test with Walth's correction was applied to compare the levels of Age, β-CTx, and tP1NP in osteosarcoma patients and healthy volunteers. The Chi-square test was used to determine the proportional difference in gender between the patients and the healthy controls. The diagnostic accuracy was evaluated by ROC curve analysis. The optimal cutoff point for establishing an accuracy score for each biomarker was determined using Youden's index (J), calculated as J = 1 - (false positive rate + false negative rate) = 1 - (1 - sensitivity) + (1 - specificity) = sensitivity + specificity - 1. The statistical calculations were performed using the Statistical Package for the Social Sciences (SPSS) Version 16.0 for Windows (SPSS, Munich, Germany). Two-tailed *P* values < 0.05 were considered statistically significant.

Results

Identification data

The mean age of the 48 osteosarcoma patients (27 male, 21 female) was 20.9 (range, 7-47) years. Of these, 3 patients were below 12 years of age and 45 patients were 12 years or older. Osteosarcoma had affected the femur in 25 patients, the tibia in 14 patients, the humerus in 6 patients, and other body parts in 3 patients. Histologically, all the osteosarcoma patients were classified as conventional subtypes.

The mean age of the 55 healthy volunteers (29 male, 26 female) was 24.4 (range, 8-49) years.

The basic laboratory parameters of the patients, including white blood cell count, body mass index, serum creatinine, BUN (blood urine nitrogen), AST (aspartate aminotransferase) and ALT (alanine aminotransferase) levels were collected (**Table 1**).

Serum concentrations of β-CTx and tP1NP

The baseline levels of β-CTx and tP1NP were found to be significantly higher in patients with osteosarcoma than in healthy volunteers (**Table 1**; **Figure 1**, $P < 0.001$).

Diagnostic values of β-CTx and tP1NP in osteosarcoma patients

ROC curve analysis indicated that the serum bone markers β-CTx and tP1NP had a high diagnostic value (**Figure 2**). The osteosarcoma patients were classified as the positive group, and the healthy volunteers were classified as the negative control group. Areas under the ROC curves (AUC) were 0.919 (0.864-0.973) for β-CTx, and 0.866 (0.792-0.939) for tP1NP. In general, the diagnostic value increased in proportion with AUC, and was thought to be high when AUC was greater than 0.75.

The optimal cutoff point for each biomarker in terms of its diagnostic performance was determined using Youden's index, with β-CTx 634.50 pg/ml and tP1NP 59.40 ng/ml (**Figure 1**), and their corresponding sensitivity and specificity were 87.50% and 87.30%, 95.80% and 70.90%, respectively (**Table 2**).

Changes in serum β-CTx and tP1NP concentrations in osteosarcoma patients before and after operation

In our study, eight osteosarcoma patients were followed up after surgery. We found that the

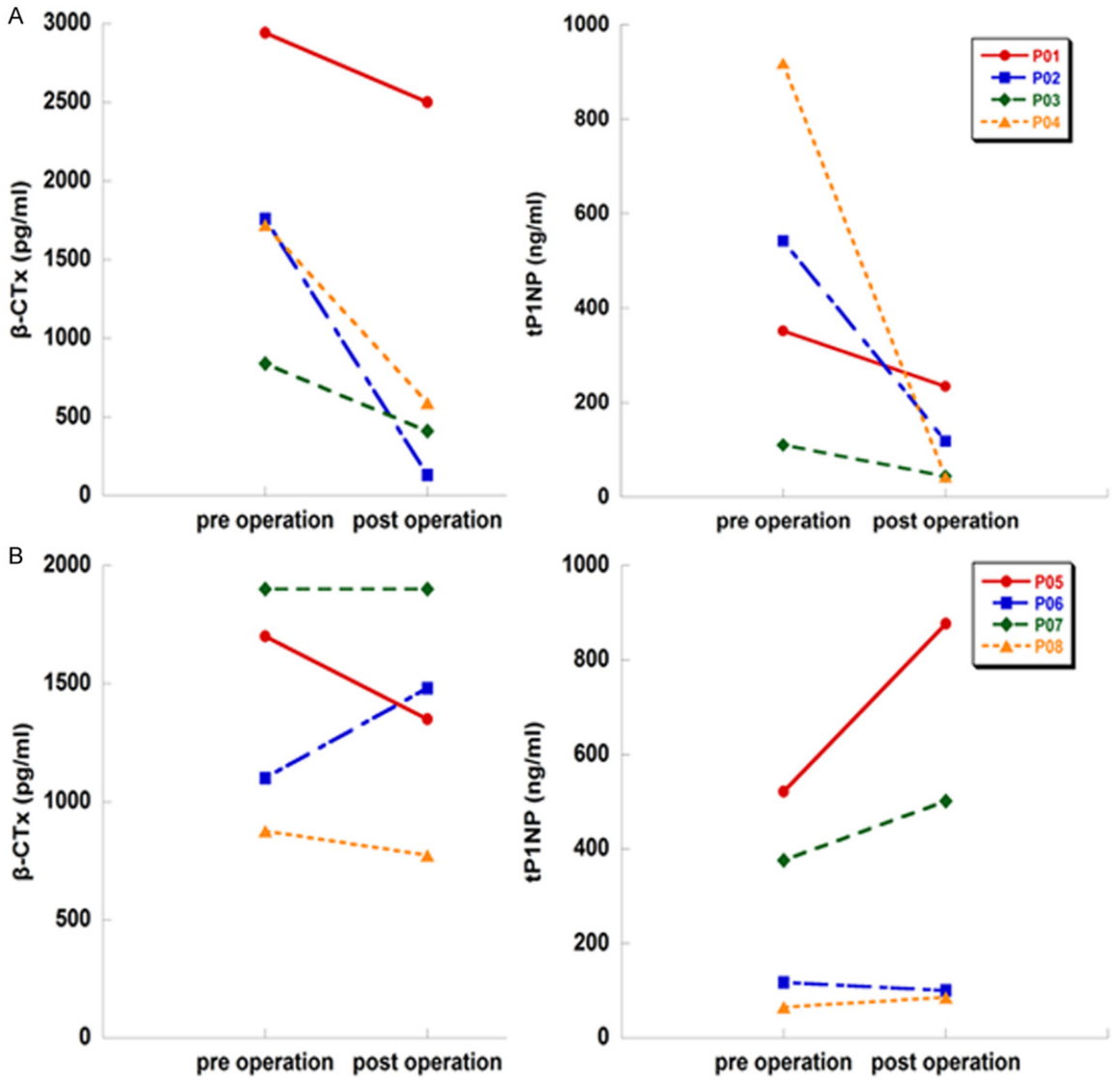

Figure 3. Comparison of serum concentrations of β-CTx and tP1NP in osteosarcoma patients before and after operation. Variation of serum β-CTx and tP1NP levels in patients with (A) good postoperative assessment and (B) poor postoperative assessment.

changes in the concentrations of serum β-CTx and tP1NP before and after surgery were accord with the postoperative clinical presentation in these patients (**Figure 3A**). The levels of serum biochemical bone markers were significantly decreased in patients with good postoperative assessment. However, patients with poor postoperative assessment showed elevated levels of osteogenic or osteolytic markers (**Figure 3B**). The identification data and data of β-CTx, tP1NP of every participant were shown in the Supplementary File.

Discussion

We evaluated two biochemical markers of bone metabolism in serum as potential biomarkers for identifying osteosarcoma patients. This study shows that β-CTx and tPINP determination can discriminate healthy individuals from patients with osteosarcoma. So, our results confirm the data from other investigators, who support the use of biochemical bone formation and resorption markers as valuable tools to confirm the presence or absence of osteosarcoma [23, 24].

Aminoterminal propeptide of type I procollagen (P1NP) reflects bone formation. Two different antigens exist in human serum, as a trimeric form and a monomeric form. The total P1NP (tP1NP) assay measures both forms. In children, bone formation is very active, which might be effected on the specificity of tP1NP in diag-

nosis of osteosarcoma. The C-terminal telopeptides (CTx) reflect bone resorpation. In the C-terminal telopeptides, the α-isomerized CTx presents converts to the β-isomerized CTX as the bone ages [25]. β-CTx shows a significant correlation with tumor, while the α-CTx is relevant to pathologic situations of high bone remodeling, quantification of degradation products from the newly synthesized collagen [26]. So, β-CTx shows better specificity (87.30%) than tP1NP (70.90%) in diagnosis of osteosarcoma in our findings.

In addition, we followed up eight osteosarcoma patients after amputation surgery and found that the concentrations of β-CTx and tP1NP declined in four patients with stable disease. On the other hand, patients with progressive disease showed irregular changes in the concentrations of these markers after surgery, with the levels of some markers elevated and some, reduced. Therefore, we conclude that β-CTx and tP1NP may serve as the detection markers of choice for monitoring osteosarcoma. Our findings are consistent with other reports on the possible role of biochemical bone markers in prognosis of osteosarcoma [24].

In normal circumstances, there is extensive biological variation in bone markers as a result of variations in gender, age, race, hormone levels, and rhythm changes [27, 28]; this variation restricts their diagnostic value as serum markers to a certain few diseases. In this study, the criteria for the selection of healthy volunteers were comparable to the general conditions of the patients; thus, the influence of these confounding factors could be eliminated.

In conclusion, the findings of this study support our hypothesis that β-CTx and tP1NP are promising serological markers for the detection and diagnosis of osteosarcoma. However, to confirm our findings, further large-scale studies with well-characterized patient samples are warranted.

Acknowledgements

We are thankful to the teams in our bone tumor wards and outpatient departments for sample collection. We are also grateful to the Department of Laboratory Medicine, without whose inputs, this paper would not have been complete.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Yuzhen Du, Department of Laboratory Medicine, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, 600 Yishan Road, Shanghai 200233, China. Tel: +86 21 38297226; Fax: +86 21 38297000; E-mail: duyuzhen2005@163.com

References

[1] Chou AJ, Geller DS and Gorlick R. Therapy for osteosarcoma: where do we go from here? Paediatr Drugs 2008; 10: 315-327.

[2] Picci P. Osteosarcoma (osteogenic sarcoma). Orphanet J Rare Dis 2007; 2: 6.

[3] Kumar H, Buch AC, Sawlani VM and Chandanwale SS. Diaphyseal osteosarcoma with varying histomorphologic patterns. Adv Biomed Res 2014; 3: 33.

[4] Sugalski AJ, Jiwani A, Ketchum NS, Cornell J, Williams R, Heim-Hall J, Hung JY and Langevin AM. Characterization of localized osteosarcoma of the extremity in children, adolescents, and young adults from a single institution in South Texas. J Pediatr Hematol Oncol 2014; 36: e353-358.

[5] Luetke A, Meyers PA, Lewis I and Juergens H. Osteosarcoma treatment - where do we stand? A state of the art review. Cancer Treat Rev 2014; 40: 523-532.

[6] Ta HT, Dass CR, Choong PF and Dunstan DE. Osteosarcoma treatment: state of the art. Cancer Metastasis Rev 2009; 28: 247-263.

[7] Biermann JS, Adkins DR, Agulnik M, Benjamin RS, Brigman B, Butrynski JE, Cheong D, Chow W, Curry WT, Frassica DA, Frassica FJ, Hande KR, Hornicek FJ, Jones RL, Mayerson J, McGarry SV, McGrath B, Morris CD, O'Donnell RJ, Randall RL, Santana VM, Satcher RL, Siegel HJ, von Mehren M, Bergman MA and Sundar H. Bone cancer. J Natl Compr Canc Netw 2013; 11: 688-723.

[8] Hogendoorn PC, Athanasou N, Bielack S, De Alava E, Dei Tos AP, Ferrari S, Gelderblom H, Grimer R, Hall KS, Hassan B, Jurgens H, Paulussen M, Rozeman L, Taminiau AH, Whelan J and Vanel D. Bone sarcomas: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2010; 21 Suppl 5: v204-213.

[9] Wang TI, Wu PK, Chen CF, Chen WM, Yen CC, Hung GY, Liu CL and Chen TH. The prognosis of patients with primary osteosarcoma who have undergone unplanned therapy. Jpn J Clin Oncol 2011; 41: 1244-1250.

[10] Sadoghi P, Leithner A, Clar H, Glehr M, Wibmer C, Bodo K, Quehenberger F and Windhager R. The threat of misdiagnosis of primary osteosarcoma over the age of 60: a series of seven cases and review of the literature. Arch Orthop Trauma Surg 2010; 130: 1251-1256.
[11] Suresh S and Saifuddin A. Radiological appearances of appendicular osteosarcoma: a comprehensive pictorial review. Clin Radiol 2007; 62: 314-323.
[12] Savitskaya YA, Rico-Martinez G, Linares-Gonzalez LM, Delgado-Cedillo EA, Tellez-Gastelum R, Alfaro-Rodriguez AB, Redon-Tavera A and Ibarra-Ponce de Leon JC. Serum tumor markers in pediatric osteosarcoma: a summary review. Clin Sarcoma Res 2012; 2: 9.
[13] Tomer G, Cohen IJ, Kidron D, Katz K, Yosipovitch Z, Meller I and Zaizov R. Prognostic factors in non-metastatic limb osteosarcoma: A 20-year experience of one center. Int J Oncol 1999; 15: 179-185.
[14] Rachner TD, Khosla S and Hofbauer LC. Osteoporosis: now and the future. Lancet 2011; 377: 1276-1287.
[15] Coates P. Bone turnover markers. Aust Fam Physician 2013; 42: 285-287.
[16] Matsuo K and Irie N. Osteoclast-osteoblast communication. Arch Biochem Biophys 2008; 473: 201-209.
[17] Suva LJ, Washam C, Nicholas RW and Griffin RJ. Bone metastasis: mechanisms and therapeutic opportunities. Nat Rev Endocrinol 2011; 7: 208-218.
[18] Lumachi F, Santeufemia DA, Del Conte A, Mazza F, Tozzoli R, Chiara GB and Basso SM. Carboxy-terminal telopeptide (CTX) and amino-terminal propeptide (PINP) of type I collagen as markers of bone metastases in patients with non-small cell lung cancer. Anticancer Res 2013; 33: 2593-2596.
[19] Pollmann D, Siepmann S, Geppert R, Wernecke KD, Possinger K and Luftner D. The amino-terminal propeptide (PINP) of type I collagen is a clinically valid indicator of bone turnover and extent of metastatic spread in osseous metastatic breast cancer. Anticancer Res 2007; 27: 1853-1862.
[20] Yavropoulou MP, van Lierop AH, Hamdy NA, Rizzoli R and Papapoulos SE. Serum sclerostin levels in Paget's disease and prostate cancer with bone metastases with a wide range of bone turnover. Bone 2012; 51: 153-157.
[21] Limmahakhun S, Pothacharoen P, Theera-Umpon N, Arpornchayanon O, Leerapun T, Luevitoonvechkij S and Pruksakorn D. Relationships between serum biomarker levels and clinical presentation of human osteosarcomas. Asian Pac J Cancer Prev 2011; 12: 1717-1722.
[22] Liu PP, Leung KS, Kumta SM, Lee KM and Fung KP. Bone-specific alkaline phosphatase in plasma as tumour marker for osteosarcoma. Oncology 1996; 53: 275-280.
[23] Ambroszkiewicz J, Gajewska J, Klepacka T, Chelchowska M, Laskowska-Klita T and Wozniak W. Clinical utility of biochemical bone turnover markers in children and adolescents with osteosarcoma. Adv Med Sci 2010; 55: 266-272.
[24] Ambroszkiewicz J, Gajewska J, Laskowska-Klita T and Wozniak W. [Biochemical bone resorption markers in children with osteosarcoma]. Med Wieku Rozwoj 2004; 8: 225-233.
[25] Bonde M, Qvist P, Fledelius C, Riis BJ and Christiansen C. Immunoassay for quantifying type I collagen degradation products in urine evaluated. Clin Chem 1994; 40: 2022-2025.
[26] Houze P, Bellik B, Extra JM, Bouro F and Bousquet B. Urinary carboxyterminal telopeptide of collagen I as a potential marker of bone metastases chemotherapy monitoring in breast cancer. Clin Chim Acta 1999; 281: 77-88.
[27] Hlaing TT and Compston JE. Biochemical markers of bone turnover - uses and limitations. Ann Clin Biochem 2014; 51: 189-202.
[28] Meier C, Seibel MJ and Kraenzlin ME. Use of bone turnover markers in the real world: are we there yet? J Bone Miner Res 2009; 24: 386-388.