

# sCD30 and sCD40L Detection in Patients with Osteosarcoma, Chondrosarcoma and Ewing Sarcoma

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## ABSTRACT

**Background:** Primary malignant bone tumors are heterogeneous groups of neoplasms, which affect mainly children and adolescents. The most common types are Osteosarcoma, Ewing sarcoma and chondrosarcoma. Elevation of sCD30 and sCD40L has been observed in lymphoma, leukemia and autoimmune disorders. **Objective:** To evaluate serum concentrations of sCD30 and sCD40L in patients with primary malignant bone tumors. **Method:** Fifty-four cases (31 Osteosarcomas, 14 Ewing sarcomas, and 9 Chondrosarcomas) and 54 healthy controls enrolled in this study. Cases with the history of prior treatment (surgery, chemotherapy and radiotherapy) were excluded from the study. Serum levels of sCD30 and sCD40L were detected by an enzyme linked immunosorbent assay (ELISA). **Results:** Mean serum concentration of sCD30 in Ewing sarcoma was significantly higher than that of the control groups ( $p=0.007$ ), but mean serum concentrations of sCD30 in osteosarcoma and chondrosarcoma groups were not significantly different, compared to the controls ( $p=0.41$  and  $p=0.11$ , respectively). Mean serum concentrations of sCD40L in osteosarcoma, Ewing sarcoma and chondrosarcoma groups were significantly higher than that of the control group ( $p<0.0001$ ). In addition, the mean serum level of sCD40L in chondrosarcoma patients was higher than that of both Ewing sarcoma and osteosarcoma groups ( $p<0.001$ ). **Conclusion:** sCD30 and sCD40L increase in primary bone tumors; however the significant of these findings for diagnosis or prognosis of these tumors needs further investigation.

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## INTRODUCTION

Primary malignant bone tumors are rare and account for less than 1% of all malignant tumors. They are also called sarcomas of the bone and bone cancer. They occur more commonly in men, especially in children and during adolescence, but some types are seen in patients aged 35 to 60 years. The most common primary malignant bone tumors are Osteosarcoma, Ewing sarcoma and chondrosarcoma (1).

Osteosarcoma (OS), which usually involves long bones, is the most common bone cancer in childhood and adolescence. It accounts for about 20% of primary malignant sarcomas (2). Osteosarcoma is highly aggressive and metastasizes mainly to the lung (3). In laboratory findings, an increase in alkaline phosphatase and erythrocyte sedimentation rate (ESR) may be observed, as well as an increase in lactic dehydrogenates in 30% of the cases.

The second primary malignant bone tumor in children is Ewing sarcoma. Pelvis and lower extremity are most commonly involved. An elevation in LDH level is reported to be correlated with a worse prognosis in some studies (4). Studies show that Ewing sarcoma constitutes 9% of primary malignant sarcoma (2).

The third most common sarcoma of the bone is chondrosarcoma which constitutes about one-fifth of all primary bone malignancies. According to the previous reports, 0.5-1.5 individuals out of one million populations develop chondrosarcoma (2). It is a malignant tumor forming cartilaginous matrix, but there is no evidence of bone formation directly synthesized by the neoplastic cells (5). The most frequent locations of conventional chondrosarcoma are the pelvis, ribs and proximal extremities (6).

The CD30 molecule is a member of the tumor necrosis nerve growth factor super family. CD4<sup>+</sup> and CD8<sup>+</sup> T-cell clones express CD30 on their surfaces and then release it, as soluble CD30 (sCD30), as a result of a matrix metallo-proteinase activity (7,8). There have been reports that elevated sCD30 levels correlated with severity of autoimmune disorders, lymphomas, leukemia, colorectal cancer, rheumatoid arthritis, and synovitis (9-13). CD30 is not merely released from dying or dead cells but its shedding is an active process of viable CD30<sup>+</sup> cells (14). Expression of CD30 totally depends on activation and proliferation (15). The presence of sCD30 in culture supernatants of CD30<sup>+</sup> cell lines was demonstrated by an ELISA assay in 1989. A proportion of serum samples of patients with CD30-expressing lymphomas (ALCL, HTLV- I-related adult T-cell lymphoma leukemia (ATLL), and angio immunoblastic lymphadenopathy (AILD)-like T-cell lymphomas) also tested positive for sCD30. Normal donors and patients with infectious diseases (except infectious mononucleosis) and tumoral conditions other than those previously mentioned did not have detectable levels of sCD30 in their sera. This gave rise to the idea of using sCD30 as a specific tool for CD30<sup>+</sup> neoplasms (15-17).

The sCD40L is a functional receptor. Recently activated CD4<sup>+</sup> T-cells rapidly express this molecule on their surface after antigen presentation (12). Elevation of sCD40L in the serum has been observed for autoimmune disorders, chronic lymphatic leukemia, and B-cell lymphomas (12,13). CD40 (CD154) is a protein of the tumor necrosis factor (TNF) gene family. Activated CD4<sup>+</sup> and CD8<sup>+</sup> T-cells, eosinophil, basophils and natural killer (NK) cells express this protein. CD40 may have an important role in the regulation of inflammation. Addition of sCD40L to osteosarcoma cell cultures treated with TNF- $\alpha$  caused an increase in the apoptosis of cells indicating sCD40L can cause apoptosis (18).

The aim of the present study was to evaluate serum concentrations of sCD30 and sCD40L in patients with primary malignant bone tumors and determine whether these serum markers could help in differentiating the tumor type.

## MATERIALS AND METHODS

**Subjects.** A total of 54 patients with osteosarcoma (n=31), Ewing sarcoma (n=14) and chondrosarcoma (n=9) referred to Chamran Hospital in Shiraz, southern Iran from August 2009 to July 2010 were enrolled in the study. The patients were visited by an orthopedic surgeon and a complete physical examination and history taking were performed for each. They were newly diagnosed cases of malignant bone tumor. A questionnaire was completed for each patient as well. It included demographic features, history of other diseases, preoperative treatment (chemotherapy or radiotherapy), distant metastasis and pathological final diagnosis. Pathological reports were considered as definite diagnosis. Cases with autoimmune diseases, chronic infectious diseases, liver diseases, cancers other than bone cancer, and any patient with previously received treatment (surgery, chemotherapy or radiotherapy) were excluded from the study.

A control group consisting of 54 road accident patients with no history and sign and symptoms of autoimmune diseases, rheumatology diseases or malignancies were also selected. This study was approved by the Ethics Committee of Shiraz University of Medical Sciences informed written consents were obtained from the patients.

**ELISA Assay.** Blood samples were collected from both patients and controls. Sera were separated and frozen at  $-70^{\circ}\text{C}$  until examination. Serum soluble CD30 and CD40L were detected by an enzyme linked immunosorbent assay (ELISA) (Bender Med system, Vienna, Austria)

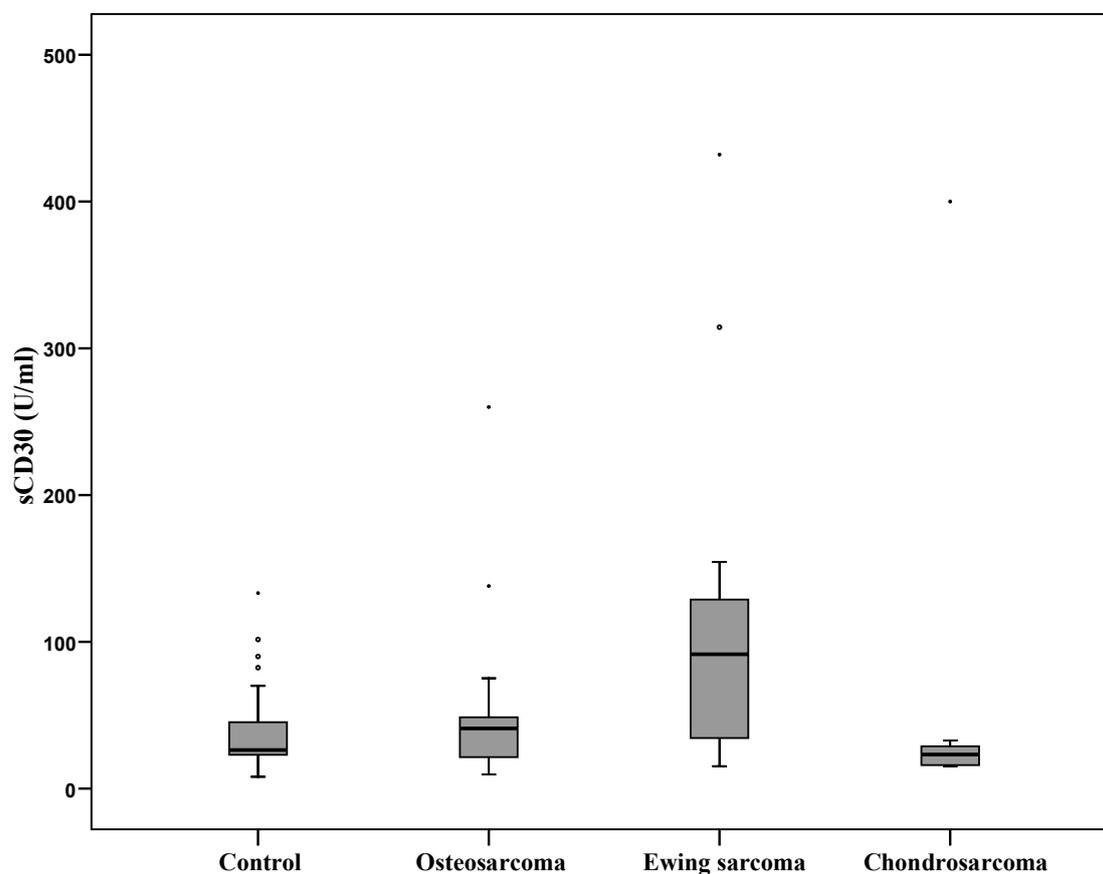
**Statistical Analysis.** Serum concentrations of sCD30 and sCD40L were compared among the four study groups (healthy control, osteosarcoma, Ewing sarcoma, and chondrosarcoma) using Kruskal-Wallis and Mann-Whitney test with Bonferroni correction. Correlation between age and serum levels of sCD30 and sCD40L in the four study groups were evaluated using Spearman rank correlation. Mann-Whitney test was used to compare serum levels of sCD30 and sCD40L between high grade and low grade osteosarcoma and between the two genders. A  $p < 0.05$  was considered significant.

## RESULTS

There were 54 patients with malignant bone tumors (26 females and 28 males with a mean age of  $24.7 \pm 13.9$  years) and 54 healthy controls (28 females, 26 males with mean age  $28.8 \pm 12.9$  years) in this study. The patients were divided into three subgroups; osteosarcoma (n=31), Ewing sarcoma (n=14) and chondrosarcoma (n=9). In healthy controls, the mean serum concentration of sCD30 was 36.7 U/ml (range 8.0-133.2 U/ml) and the mean serum concentration of sCD40L was 1.13 ng/ml (range 0.05-12.0 ng/ml).

The mean serum concentration of sCD30 in Ewing sarcoma was significantly higher than that of the control group, osteosarcoma and chondrosarcoma groups ( $p=0.007$ ,  $p=0.04$ , and  $p=0.04$ , respectively). However, mean serum concentrations of sCD30 in osteosarcoma and chondrosarcoma groups were not significantly different, compared to

that of control group ( $p=0.41$  and  $p=0.11$ , respectively, Figure 1).



**Figure 1.** Comparison of mean serum levels of sCD30 between four study groups.

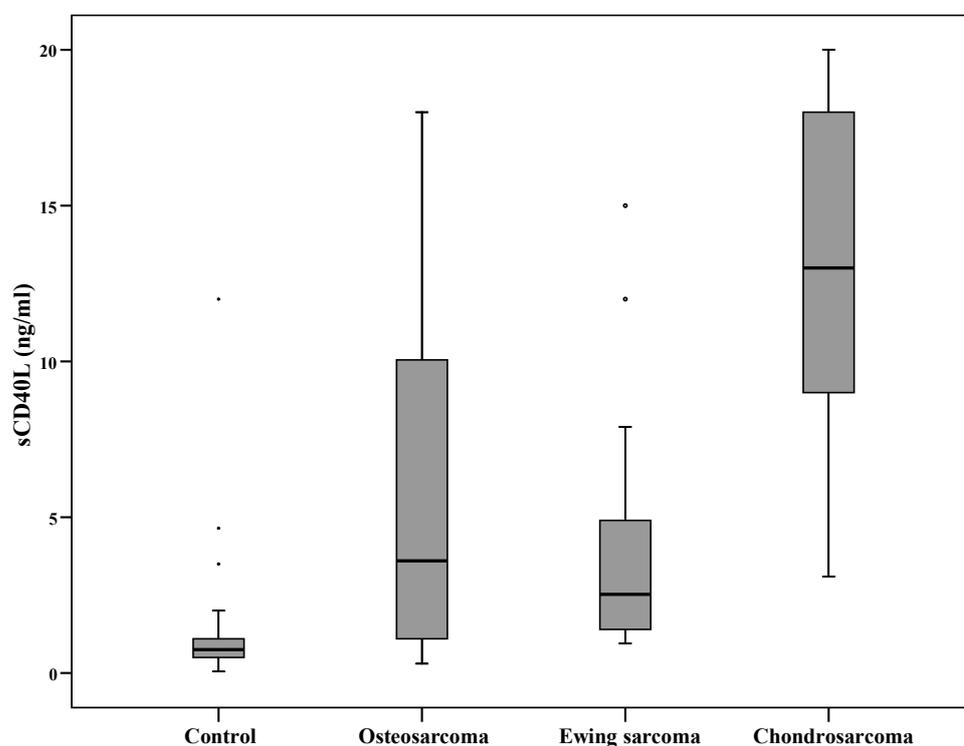
Mean serum concentrations of sCD40L in osteosarcoma, Ewing sarcoma and chondrosarcoma groups were significantly higher than that the control group ( $P < 0.0001$ ). In addition, the mean serum level of sCD40L in patients with chondrosarcoma was higher than that of both Ewing sarcoma and osteosarcoma groups ( $p < 0.001$ ), but there was no significant difference between osteosarcoma and Ewing sarcoma (Figure 2).

The maximum serum level of sCD30 was detected in Ewing sarcoma (432 U/ml) while the maximum serum level of sCD40L was observed in chondrosarcoma (20 ng/ml). Patients with osteosarcoma were classified according to grade and there was no significant difference in serum levels of sCD30 ( $p=0.28$ ) and sCD40L ( $p=0.95$ ) between low grade and high grade groups (Table 1).

**Table 1. Comparison of serum levels of sCD30 and sCD40L in osteosarcoma group in terms of grade.**

Serum Protein	Low grade (Osteosarcoma) Median (Min-Max)	High grade (Osteosarcoma) Median (Min-Max)	P Value
sCD30 (U/ml)	46.8 (18.8-75.2)	40.5 (9.6-260)	0.28
sCD40L (ng/ml)	4.7 (0.3-13)	3.38 (0.6-18)	0.95

When Table 2 shows the correlation between age and serum levels of sCD30 and sCD40L in each study group. As revealed, there was a linear, reverse and intermediate correlation between age and serum level of sCD30, in the controls and osteosarcoma patients ( $p=0.001$  and  $\gamma \approx -0.442$ ;  $p=0.048$  and  $\gamma \approx -0.357$ , respectively).

**Figure 2.** Comparison of mean serum levels of sCD40L between four study groups.

**Table 2. Correlation between age and serum levels of sCD30 and SCD40L in the study groups.**

Serum Protein	sCD30		sCD40L	
	P Value	$\gamma^*$	P Value	$\gamma$
HealthyControl (n:54)	0.001	-0.442	0.445	0.106
Osteosarcoma (n: 31)	0.048	-0.357	0.842	0.037
Ewingsarcoma (n:14)	0.193	-0.370	0.886	0.420
Chondrosarcoma (n:9 )	0.486	0.268	0.651	-0.176

\*spearman rank

When serum levels of sCD30 and sCD40L were compared between males and females in each study group, no significant difference was observed between the control, osteosarcoma, Ewing sarcoma, and chodrosarcoma groups (Table 3).

**Table 3. Comparison of mean serum levels of sCD30 and sCD40L between two genders.**

Serum protein	Group	N (F/M)	Male Median(Min-Max)	Female Median(Min-Max)	P
sCD30 (U/ml)	Control	54 ( 28/26 )	30.6 (8-133.2)	25.3 (17.3-82.4)	0.42
	Osteosarcoma	31 (13 /18 )	40.5 (16.4-260)	42.0 (9.6-75.2)	0.95
	Ewing sarcoma	14 (8 /6 )	116.4 (15.2-432)	75 (16-154.4)	0.41
	Chondrosarcoma	9 (5 /4 )	24.9 (15.2-400)	23.2 (16-32.8)	0.91
sCD40L (ng/ml)	Control	54 ( 28/26 )	0.73 (0.15-4.7)	0.75 (0.05-12)	0.60
	Osteosarcoma	31 (13 /18 )	5.95 (0.3-17.5)	3.1 (0.55-18)	0.89
	Ewing sarcoma	14 (8 /6 )	2.13 (0.95-12)	3.05 (1.15-15)	0.35
	Chondrosarcoma	9 (5 /4 )	10.8 (3.1-20)	15 (8-20)	0.41

## DISCUSSION

The present study is among the very few ones that investigated the serum levels of sCD30 and sCD40L in patients with primary malignant bone tumors. The number of cases evaluated here was higher than the previous studies (19).

We found that the mean serum concentration of sCD30 was significantly elevated in patients with Ewing sarcoma, compared with that of the healthy controls, but there was no significant difference between sCD30 levels in osteosarcoma and chondrosarcoma groups and healthy controls. This is in contrast with the findings of Holzer and colleagues (19), who observed significant elevations in sCD30 levels in all patients with malignant bone tumors (Ewing sarcoma, osteosarcoma and chondrosarcoma), compared with the control group. The difference between their findings and ours may be due to the smaller number of patients and controls in Holzer et al study (19). We observed that mean sCD30 level in Ewing sarcoma patients was significantly higher than that in the osteosarcoma group which is in agreement with the findings of Holzer and colleagues (19).

Studies on other tumors including lymphoma and adult T-cell leukemia have also demonstrated an increase in serum sCD30 level. Moreover, the increase in sCD30 level was higher in patients with relapse, in comparison to those who had responded to treatment (20).

We also noticed that mean serum concentrations of sCD40L in osteosarcoma, Ewing sarcoma and chondrosarcoma groups were significantly higher than that of the control group. Similarly, Holzer et al. (18) reported that serum levels of sCD40L were significantly elevated in all patients with malignant bone tumors (Ewing sarcoma, osteosarcoma and chondrosarcoma) compared with healthy controls. There was also an increase in serum concentration of CD40L in patients with B cell lymphoma (21).

While we found that mean serum level of sCD40L in chondrosarcoma patients were significantly higher than those in Ewing sarcoma and osteosarcoma groups, Holzer et al. (19) reported no significant difference. As shown in previous reports, various biological activities of tumor cells, such as cell adhesion, motility, differentiation and death may be influenced by CD40 and CD40L (8).

Similar to Holzer et al. study (19), we found no correlation between serum levels of sCD30 and sCD40L and age and sex. In our osteosarcoma group, there was no correlation between serum concentrations of these proteins and grade of tumor, either. However, van den Oord (22) showed that in metastatic melanoma, prognosis was worse for tumors with CD40-positive cells when compared to CD40-negative tumors.

The present findings suggest that serum levels of sCD30 and sCD40L may be useful biomarkers for diagnosis of malignant bone tumors. sCD30 may even have the potential to differentiate Ewing sarcoma from the other types while sCD40L may help distinguish chondrosarcoma. Since these proteins are involved in the inflammation and regulation of the immune system, they could also be used as indicators for response to immunotherapy of these tumors. Larger studies on correlation of these serum markers with malignant bone tumors are recommended in which patients are investigated at different stages of the disease and followed up after treatment.

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