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## THE EVALUATION OF THE PRIMARY BONE TUMORS BY BONE MARKERS

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**ABSTRACT:** Serum markers reflect a bone cell activity that regulate bone remodeling, bone alkaline phosphatase (PAO), Total Intact Procollagen Type I N-terminal propeptide (TP1NP) for training and tartrate-resistant acid phosphatase (TRAP5), carboxy-terminal collagen crosslinks (Cross Laps) for bone resorption. These markers are sensitive and early tools, Elevated serum levels of these markers is a very good prognostic factor of primary bone tumors as well as monitoring and evaluation tools. We investigated whether biomarkers of bone formation (PAO, P1NP) and bone resorption (CTX and TRAP5) were modified by chemotherapy. The aim of this study was to evaluate the impact of treatment on serum PAO and TP1NP and TP1NP and TRAP5. **Result:** We found a significant correlation between serum levels of training biomarkers (PAO and TP1NP in the group of patients ( $p = 0.0042$ ) and a strong correlation between PAO and TP1NP In the sick and control groups ( $p = 0.00027$ ) and a strong correlation between TP1NP and TRAP5b in the groups of patients and controls ( $p = 0.033$ ). Bone markers can be used in therapeutic monitoring of experimental bone disorders and to assess remodeling during chemotherapy for primary tumors.

**INTRODUCTION:** Bone tumors are rare and constitute about 6-10% of childhood tumors. The biomarkers are useful for the clinical evaluation were but poorly documented during primary tumors<sup>1</sup>. They can be useful in assessing and quantifying followed and remodeling in young carriers of primary tumors Biochemical markers of bone remodeling most sensitive bone formation are (PAO) and (TP1NP) measured in the blood. To assess bone resorption, immunoassays molecules bridging collagen) and associated peptides (CTX, NTX) in blood or urine are currently the most efficient markers<sup>2</sup>. Serum levels of serum alkaline phosphatase (PAO) have been reported as useful for the prognosis of bone tumors<sup>3</sup>.

We know that the evaluation of serum PAO is very difficult in children and adolescents because are generally higher in adults<sup>4</sup>. On the other hand, serum (TRA5b), which has been advocated as a marker of bone resorption are measured at 75-92% of the total acid phosphatase in children<sup>1</sup>. Bone is subject to a permanent physiological renewal through the activities of osteoblasts and osteoclasts. Bone remodeling is well realized by cell implementation of formation and resorption processes that maintain the integrity of the bone under the intricate action systemic hormones and local mediators. However, during various metabolic diseases of the bone, an imbalance between these two processes is observed, causing the more often a loss of bone mass<sup>5</sup>.

Recently, the isolation and characterization of several compounds of the bone matrix have enabled the development of new biochemical markers of bone remodeling<sup>6</sup>. They use either the measurement of enzyme activity related to the

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activity of bone cells, or the measure of a component of bone matrix released into circulation. The markers of formation and resorption activity are differentiated, although some of them (to some degree)<sup>7</sup> are the simultaneous reflection of the two activities. The specificity and sensitivity of these markers have advanced considerably, allowing Exploration reliable, noninvasive, inexpensive, bone turnover. Although this study is difficult because of the complexity of bone metabolism and the required technical<sup>1</sup> implementation is valuable for monitoring primary bone tumors.

The aim of our study was to describe biomarkers of formation and resorption in a population of primary bone tumor patients (osteosarcoma, Ewing and fibrosarcoma tumors) and determine the impact of treatment on the coupling resorption / formation.

**Patients and Methods:** This is a prospective study conducted from January 19, 2014 to January 03, 2015. The patients were enrolled consecutively during hospital visits or were call from the medical records. Inclusion criteria were the presence of documented bone tumor on pathologic analysis. We reviewed the medical records of 17 patients aged between 3 and 60 years. There were, osteosarcoma, Ewing, chondrosarcoma. The number of 17 patients were recruited at the oncology department of Constantine these patients included in our study with proven histologically primitive bone tumors and they were under chemo therapy. Controls are 17 healthy subjects it is composed of students, University employees, hospital and voluntary they are in good health the subject are matched for age and sex.

**Data collection:** A questionnaire was carried to record all the information necessary to our study with the patient himself and by consulting his medical file.

**Blood sample:** Blood sampling shall be effected fasting semi sitting by venipuncture frank position on heparin tubes placed immediately in ice hiding the light sent to the laboratory of biochemistry CHU Constantine in a maximum time of happiness, centrifuged rightly 4000 rpm .the blood plasma is divided into 2 one aliquot for assay and the other is reserved and numbered samples were codified and has retained the shelter of the light.

**Principle of test:** Blood samples were collected to assess the biomarkers of bone remodeling. The following methods were used to measure the biomarkers (Crosslaps, P1NP, Osteocalcin) on Cobas e601 analyzer (Roche Immunodiagnostics, Meylan, France) ; bone alkaline phosphatase on Ysis analyzer (IDS immunoDiagnostics Systems, Paris, France) and TRAP5Bb levels using Elisa kit (IDS ImmunoDiagnostics Systems).

The normal adult serum PAO level is <15ng/ml and The normal adult serum TRAP5B level is ≤ 2.03ng/ml for Male and ≤ 2.64ng/ml for female. The normal adult serum TP1NP level is 15-75ng/ml for Male and 25-90ng/ml for female, The normal adult serum CROSSLAPS level is <584pg/ml for Male and <570pg/ml for female.

**Statistical analysis:** The statistical analysis performed using Exel Stat software. Quantitative variables are expressed as means ± standard deviation and categorical variables as a percentage of different modalities. The chi 2 test was used to compare differences between groups for qualitative variables. The search for association between continuous variables is done by calculating Spearman and Mann-Whitney correlation coefficient. To determine the effects of those resorption and bone formation, we applied the ANOVA model including variables as covariates. For all tests, the statistical significance was set at 0.05.

**RESULTS:** women, mean age 32 ± 10 years and men, mean age 27 ± 10. Among our patients, 12% were postmenopausal which 12% are premenopause, 53% of them have had chemotherapy. Our population has a three types of bone tumors 47% and 35% Osteosarcoma Ewing's sarcoma and chondrosarcoma 18%. These patients are carried for determination of bone markers in search of an interest, diagnostic and prognostic monitoring. The number is not relatively large because of the rarity of these tumors (**Table 1**).

We evaluate the correlation between (PAO / TP1NP), (PAO / TRAP5b), (PAO /CROSSLAPSS) (TP1NP / TARP5B), (TP1NP / CrossLaps), (TRAP5b / CrossLaps) in patients and controls and (PAO / TRAP5b), (PAO / CROSSLAPSS), (TP1NP / TARP5B), (TP1NP / Cross Laps),

(TRAP5b / CROSSLAP) in patients. There is a significant correlation between the PAO and serum TP1NP in the group of patients ( $P < 0.0042$ ) and ( $0.352 < r < 0.903$ ) and a significant correlation between the PAO and serum TP1NP in the group of patients and controls ( $P < 0.00027$ ) and ( $r = 0.714$ ) and a significant correlation between serum TRAP5b and TP1NP in the group of patients ( $P < 0.033$ ) and ( $0.451 < r < 0.680$ ).

Serum PAO was  $32.7 \pm 28.7$  ng / ml in patients while  $68.5 \pm 50.8$  ng / ml in the controls the rate TP1NP serum was  $83.3 \pm 93.5$  ng / ml in patients while  $400.5 \pm 409.4$  ng / ml in the controls the serum TRAP5b rate is  $2.78 \pm 1.98$  u / l while in patients and  $3.48 \pm 1.11$  u / l in the controls the serum levels Cross Laps was  $2.78 \pm 1.98$  pg / in patients while  $370.6 \pm 255.6$  pg / ml in controls (Table 2).

TABLE 1: DESCRIPTIVE OF 17 PATIENTS WITH BONE TUMORS AND 17 CONTROL POPULATIONS

Pop	Desc	Patient	Mean age±SD	
	Ostéosarcome	8		
	Ewing	6		
	Chondrosarcome	3	Male	27 ± 10
	Tibia localisation 's tumor	29%	Female	32 ± 13
	Fémur localisation 's tumor	59%		
	Humérus localisation 's tumor	12%		
	Controls	17	Male	27 ± 10
			Female	32 ± 13

TABLE 2: BONE REMODELING BIOLOGICAL PARAMETERS

Mark	Pop	Patient	Controls	P-Value <sup>a</sup>
Bone Alkaline phosphatases		32.7 ± 28.7	68.5 ± 50.8	
Median Bone Alkaline Phosphatases (ng/ml) <sup>b</sup>		24.1 (20.4-27.3)	56.3(24.5-74.3)	<0.05
P1NP (ng/ml)		83.3 ± 93.5	400.5 ± 409.4	
Median P1NP (ng/ml) <sup>c</sup>		53.6 (47.5-89.5)	256.6(93.2-571.6)	<10 <sup>-2</sup>
TRAP5b (u/l)		2.78 ± 1.98	3.48 ± 1.11	
Median TRAP5b (u/l) <sup>b</sup>		2.18 (1.70-2.76)	3.52(2.61-4.35)	<0.05
Serum Cross Laps (pg/ml)		311.4 ± 136.8	370.6 ± 255.5	
Median serum Cross Laps (pg/ml) <sup>b</sup>		368.5(182.9-429.8)	242.4(205-424.3)	0.80

<sup>a</sup>: Mann-Whitney test

<sup>b</sup>:median (25th through 75th percentile)

<sup>c</sup>:Procollagen type 1 N-terminal Propeptide

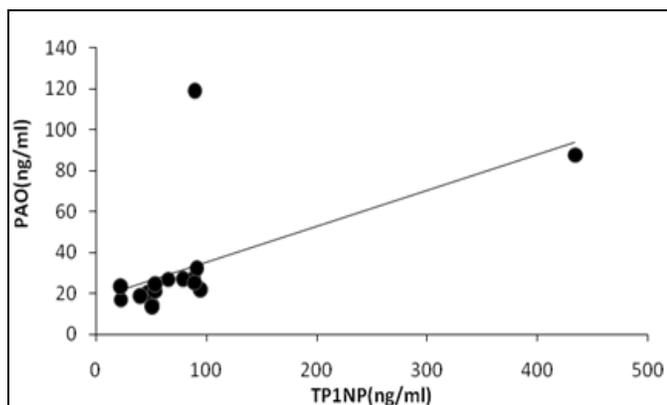


FIG. 1: CORRELATIONS BETWEEN PAO / TP1NP IN (PATIENTS).

Result of non-parametric test for linkage analysis between two quantitative variables: Test Spearman So there is a positive link between 2 quantitative variables studied. The coefficient of determination

determines the percentage of variance explained by the linear regression model. So there is a significant correlation between serum and PAO TP1NP in the group of patients ( $P < 0.0042$ ) and ( $0.352 < r < 0.903$ ).

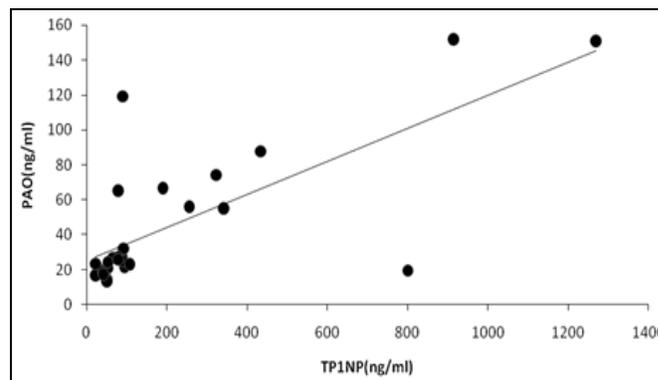


FIG. 2: CORRELATION BETWEEN PAO/TP1NP IN PATIENTS AND CONTROLS

Result of non-parametric test for linkage analysis between two quantitative variables: Test Spearman So there is a positive link between 2 quantitative variables studied.

The coefficient of determination determines the percentage of variance explained by the linear regression model. So there is a significant correlation between serum and PAO /TP1NP in the group of patients and controls ( $P < 0.00027$ ) and ( $r = 0.714$ ).

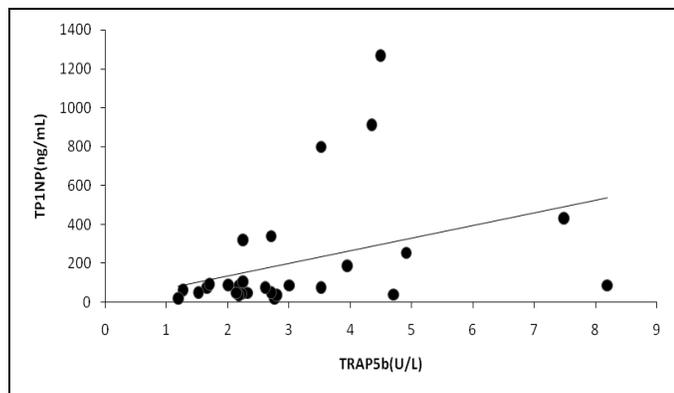


FIG. 3: CORRELATION BETWEEN TP1NP/TRAP5B IN PATIENTS AND CONTROLS

Result of linkage analysis test between two quantitative variables: Pearson correlation so there is a positive link between 2 quantitative variables studied.

The coefficient of determination determines the percentage of variance explained by the linear regression model so there is a significant correlation between serum and TRAP5b TP1NP in the group of patients ( $P < 0.033$ ) and ( $0.451 < r < 0.680$ )

**DISCUSSION:** We evaluated the levels of PAO and TP1NP and CrossLaps and TRAP 5b these markers are specific to bone formation and bone resorption. TRAP 5b, serum and TP1NP CROSSLAPSS and PAO are widely used in biochemical tests and routine and may be carried out in almost every laboratory.

TRAP5b is secreted by osteoclasts is advocated as a biomarker of osteoclast activity and bone resorption<sup>1</sup>. The total activity PAO can often be attributed to TRAP5b activity, accounting for about 90-92% of total activity PAO.

Children up to 12 years, 80% in children aged 12-18 years and 67% in adults.

The fraction of TRAP5b represents about 75% of total activity DTP in children up to 14 years for girls and 19 for boys and 64% among adults<sup>1</sup>.

In this study, there is a strong correlation between serum PAO and TP1NP in the group of patients ( $p = 0.0042$ ) and a strong correlation between PAO and TP1NP in the diseased and control groups ( $p = 0.00027$ ) and a strong correlation between TP1NP and TRAP5b in the groups of patients and controls ( $p = 0.033$ ). The serum PAO tetraphasic show a similar model<sup>8</sup> Serum levels of average PAO obtained in this study showed a similar pattern tetraphasic these references.

Our results differ from the literature that the average concentration of PAO is  $14.2 \text{ mg / L}$ <sup>9</sup>. This difference may be due to the choice of study population including gender and age and treatment, and the relatively small number of patients in our study population.

It is interesting to note that the reports of PAO and TP1NP showed a strong correlation in all tumor types. Several studies have shown that markers of bone turnover increased with age. A positive association between age and bone turnover markers (PAO, P1NP, CrossLaps, TRAP5b serum) in a population<sup>15</sup>. As well Relate an increase CrossLaps) with age<sup>10</sup>. In our study, all markers of bone turnover are higher. Our results show a significant elevation of serum concentrations of CTX<sup>10</sup>.

Several studies<sup>11</sup> had found a significant increase in both markers of formation (PAO, TP1NP) than those of bone resorption (CrossLaps, TRAP5b). In our patients receiving prolonged, high-dose chemotherapy, there was a significant decrease in concentrations). Our results showed an early decrease in serum important PAO chemotherapy explained by a reduction in osteoblast activity and show a prognostic evaluation and remodeling during chemotherapy for primary tumors. Sectional and longitudinal studies have shown significant correlations, but weak between bone loss and concentrations of bone markers measured at the start of the observation period<sup>12</sup>. Bone turnover levels and bone density. Increased bone remodeling was highlighted<sup>13</sup>. It is considered difficult to determine the specific reference biological markers

in a hospital. If a high level of bone marker in serum is in an adolescent patient, it is difficult to determine whether it should be suspected osteosarcoma or Ewing because normal people can also reach such a high level because of a thrust pubertal<sup>14</sup>.

The most relevant result in this study is the evaluation and monitoring of the primitive bone tumors golds dune chemotherapy because it is a decrease in biochemical marker rates with high correlations.

**CONCLUSION:** Nowadays the biochemical study of the bone is under development, the use of biochemical markers of bone metabolism allows to directly evaluate bone remodeling. Concentrations in bone alkaline phosphatase and TRAP5b in serum are sensitive markers, currently used in the biochemical study of bone remodeling, development of all of these biochemical markers requires remodeling evaluation interest during chemotherapy for Primary tumors and prognostic.

In this study, levels of (PAO / TP1NP) and (TP1NP / TRAP5b) serum strongly correlated with each other, this correlation is useful for evaluation of osteosarcoma and Ewing and chondrosarcoma, novel presented is an evaluation method may be clinically useful in assessing whether or PAO TRAP5b or elevated serum TP1NP for confirmation of bone tumors these markers allow an evaluation of the bone activity but they are often limited for the moment looking clinical and are not yet widely used in the treatment of bone tumors. The following proper dosing conditions is mandatory to limit the pre-analytical variability and further studies are needed to determine optimal threshold. However, practical recommendations on the use of these markers of bone turnover are needed.

**CONFLICT OF INTEREST:** The manuscript is an original article which has not been submitted for publication elsewhere. The author Kabouche samy reports that: I have no conflict of interest and all Authors in this manuscript declares that they have no conflict of interest. This article contain studies with human serum.

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