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Tartrate-resistant acid phosphatase 5b, but not periostin, is useful for assessing Paget's disease of bone

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ABSTRACT

Background: Periostin is a matricellular protein with a preferential location in cortical bone and periosteal tissue, and tartrate-resistant acid phosphatase 5b (TRAP5b) is a marker of osteoclast numbers. In Paget's disease of bone (PDB), there is increased cortical thickening and probably increased periosteal apposition, along with increased osteoclast numbers.

Objectives: To analyse if circulating periostin is a biomarker for PDB, and if it is associated with disease activity and involvement of long bones that represent major cortical contribution. Also, to analyse whether TRAP5b, a scarcely explored bone resorption marker, is useful in the assessment of PDB.

Patients and methods: We recruited 42 patients with PDB (13F/29M; 71 ± 11.6 yrs). 71.4% had active disease, 66.6% had polyostotic disease and 54.8% had long bone involvement. Blood and urine samples were taken between 8:00 and 10:00 A.M. after an overnight fast. Periostin and TRAP5b were measured in serum, using commercial ELISA assays (Biomedica and IDS, respectively). Serum total ALP, PINP, CTX, bone ALP and urinary NTX were measured. Reference values for periostin and TRAP5b were obtained from 45 healthy subjects.

Results: Serum periostin did not differ between patients and controls (989.4 ± 173.2 vs. 966.9 ± 195.4 pMol/L, $p = 0.572$). No significant differences were observed between patients with and without active disease (964.5 ± 168.8 vs.1051.6 ± 175.6 pMol/L, $p = 0.143$), involvement or not of long bones (1022.2 ± 145.8 vs 949.7 ± 198.2 pMol/L, $p = 0.181$) and monostotic or polyostotic disease (963.8 ± 198.7 vs 1002.2 ± 161.4 pMol/L, $p = 0.505$). There were significant correlations between serum periostin and all bone turnover markers (bone ALP, PINP, uNTX, sCTX and TRAP5b) in PDB patients with active disease, but not in the inactive PDB group. Serum TRAP5b was significantly higher in PDB patients than in controls (4.43 ± 1.76 vs. 3.21 ± 1.02 U/L, $p < 0.001$), in those with active disease (4.98 ± 1.76 vs. 3.07 ± 0.72 U/L, $p < 0.001$) and in patients with polyostotic disease than in those with monostotic disease (4.81 ± 1.79 vs 3.68 ± 1.5 U/L, $p = 0.005$). TRAP5b levels were not influenced by previous bisphosphonate treatment (4.14 ± 1.42 vs. 4.84 ± 2.02 U/L, $p = 0.206$).

Conclusions: Periostin is not useful for assessing PDB, whilst TRAP5b, which has been a scarcely explored bone turnover marker until now, may be useful in the analysis of this disease, providing new information on the resorption process. In addition, periostin levels correlate with all classical BTMs in active PDB, suggesting that this marker may reflect periosteal and cortical metabolism in accelerated bone turnover states.

1. Introduction

Bone remodelling is increased in active Paget's disease of bone (PDB). Indeed, bone formation and resorption markers are related to the activity of the disease, analysed by radionuclide bone scintigraphy [1]. A recent meta-analysis, which included 17 observational studies, showed that PDB is best assessed by measuring procollagen type I amino-terminal propeptide (PINP) before and after bisphosphonates.

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Nevertheless, total alkaline phosphatase (ALP) activity, bone specific alkaline phosphatase (bone ALP) and urinary amino-terminal telopeptide of type I collagen (NTX) are adequate alternatives in naïve patients, as are total ALP and urinary NTX for monitoring treatment [2].

There have been, however, few attempts to look for more efficient new bone markers in monitoring disease activity, or even in identifying PDB patients with involvement of a certain skeletal compartment. In this regard, periostin is a matricellular protein secreted by osteocytes and osteoblasts, with a preferential location in cortical bone and periosteal tissue [3]. Since in PDB there is increased cortical thickening and perhaps an enhanced periosteal apposition, there is rationale for exploring this novel marker of cortical and periosteal metabolism [3,4]. Indeed, circulating periostin has been reported to predict the risk of fracture, particularly non-vertebral fractures in postmenopausal French women [5], and a positive correlation with cortical thickness in healthy subjects has been found [6]. However, circulating periostin may be highly influenced by sources other than bone, since it is expressed in collagen-rich tissues subjected to mechanical strain, as well as in other locations such as the aorta, breast or the gastrointestinal tube [3,4]. For this reason, a specific assay for measuring the circulating bone isoform is advisable for assessing metabolic bone diseases such as PDB.

Periostin has been studied in a number of extraskeletal disorders, mostly related to cancer and bone metastasis [7], inflammatory disorders [8], and in bone diseases, including fibrous dysplasia of bone [9], juvenile Paget's disease [10], postmenopausal women at risk of osteoporotic fractures [5] and in postmenopausal women with previous bisphosphonate treatment [11].

Because PDB is characterised by both increased resorption activity and number of osteoclasts, the analysis of a bone turnover marker (BTM) that indirectly reflects their number seems appropriate. Tartrate-resistant acid phosphatase 5b (TRAP5b) is not based on degradation of type I collagen; it is a marker of the number of osteoclasts [12]. TRAP5b has been scarcely analysed in PDB, and when so, most former assays were of limited usefulness due to the relative instability on storage of TRAP5b [13,14]. Bone resorption markers such as serum carboxi-terminal telopeptide of type I collagen (CTX), and particularly NTX, have demonstrated to be sensitive in PDB [2], but TRAP5b could offer additional information. Thus, we were able to indirectly assess not only the resorption activity, but indirectly estimate the number of involved osteoclasts as well.

For all these reasons, we have analysed whether circulating periostin is a biomarker for PDB, and if it is associated with disease activity and major cortical involvement, and secondly, whether TRAP5b is useful in the assessment of the resorption process in PDB.

2. Patients and methods

We included 42 patients diagnosed with PDB (13F/29M, 71 ± 11.6 years). Thirty patients had active disease, defined as total ALP activity higher than the upper normal range, 23 patients had long bone involvement (predominance of cortical component) and 28 patients had polyostotic disease, assessed by radionuclide bone scintigraphy (Tc^{99m}methylenediphosphonate) performed at a median of 7.1 (1.1–25.8) months prior to the biochemical assessment. Patients were excluded if they had additional diseases or medications other than bisphosphonates, known to influence bone metabolism. Particular care was applied in the exclusion of patients with severe osteoarthritis and cancer. Previous bisphosphonate treatment and fragility fractures, bone pain and body mass index were recorded.

Blood and urine samples were taken between 8:00 and 10:00 A.M., after an overnight fast. Total ALP was measured with the Advia 2400 analyser (Siemens, Tarrytown, NY, USA), bone ALP by ELISA (Immunodiagnostic Systems, Boldon, England), serum PINP and CTX were measured with the Cobas e601 analyser (Roche Diagnostics, Mannheim, Germany) and NTX by ELISA (Osteomark NTX Alegre, Scarborough, ME, USA). PTH in plasma and serum 25OHD were measured by chemiluminescent assays in Advia Centaur (Siemens, Tarrytown, NY, USA) and Liaison XL (Diasorin, Saluggia, Italy), respectively.

In order to measure serum periostin and TRAP5b, we used serum obtained by centrifugation at 2200 rpm during 15 min. Haemolysis was discarded visually by checking the tubes. Serum samples were stored at −80 °C until assayed. Serum periostin and TRAP5b were measured by ELISA. The ELISA assay for periostin was from Biomedica (Vienna, Austria) and the TRAP5b ELISA assay was from Immunodiagnostic Systems (Boldon, England). Reference values for periostin and TRAP5b were obtained from 45 healthy subjects (29F/16M, 56 ± 8.8 years).

All patients provided written informed consent to participate, and the study was approved by the Ethics Committee of the Hospital Clínic.

2.1. Statistical analysis

This study is exploratory, and thus the sample size has not led to testing a formal confirmatory hypothesis because of logistic availability issues. However, with the gathered sample size (i.e. 42 patients and 45 controls), this study was expected to detect an effect size (i.e. differences between groups divided by the standard deviation) of ≥0.611 [15], and also to be discriminative for odds ratios of ≥3.5 [16]. Finally, for the patients' group, it was expected that Pearson's correlations of ≥0.42 would be detected. In all three cases, the sample size calculations were conducted using a 5% two-sided level of significance and a 80% statistical power.

Data are expressed as frequencies and percentages for categorical variables and as mean (SD). Fisher's exact test was used for categorical variables and student *t*-test for continuous variables. To study correlations, we used the Pearson's method. All analyses were performed using SAS 9.4 software (SAS Institute, Cary, NC, USA) and the level of significance was established at the two-sided 5% level.

3. Results

The clinical and demographic characteristics of the patients are included in Table 1. Serum periostin did not differ between patients and controls (989.4 ± 173.2 vs. 966.9 ± 195.4 pMol/L, $p = 0.572$) (Fig. 1). No significant differences were observed between patients with and without active disease (964.5 ± 168.8 vs.1051.6 ± 175.6 pMol/L, $p = 0.143$), involvement or not of long bones (1022.2 ± 145.8 vs 949.7 ± 198.2 pMol/L, $p = 0.181$), monostotic or polyostotic disease (963.8 ± 198.7 vs 1002.2 ± 161.4 pMol/L, $p = 0.505$) and previous bisphosphonate treatment or not (990.2 ± 200.5 vs 977.7 ± 140.6 pMol/L, $p = 0.819$) (Fig. 2).

Serum periostin levels did not differ between men and women when both PDB patients and controls were assessed (men: 979.6 ± 159.9 vs

Table 1
Demographic and clinical features.

	Patients $N = 42$
Age (years)	71.0 ± 11.6
Male n (%)	29 (69.0)
Female n (%)	13 (30.9)
Monostotic disease n (%)	14 (33.3)
Polyostotic disease n (%)	28 (66.6)
Number of involved bones	2.85 ± 2.04
Long bone involvement n (%)	23 (54.8)
High total ALP n (%)	30 (71.4)
Normal total ALP n (%)	12 (28.6)
Previous bisphosphonates n (%)	21/41 (51.2)
Previous fracture n (%)	8 (19.0)
Bone pain n (%)	16/39 (41.0)
Osteoarthritis n (%)	20 (47.6)
Body mass index (kg/cm^2)	28.3 ± 5.5

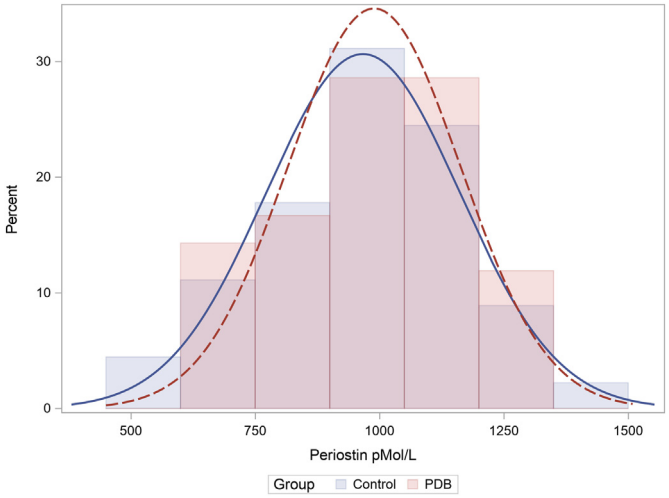


Fig. 1. Histogram of serum periostin levels in PDB patients and controls ($p = 0.572$).

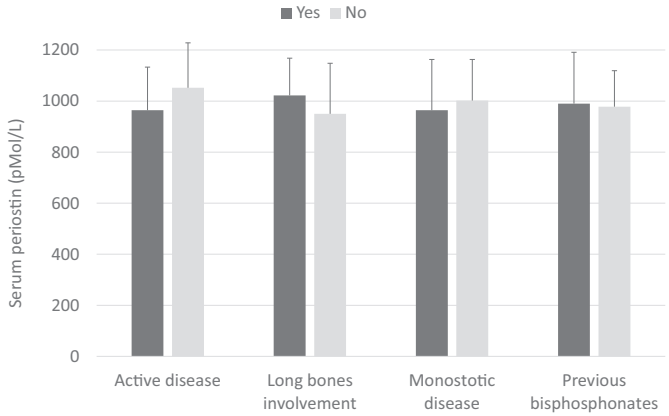


Fig. 2. Mean (SD) serum periostin levels according to different features in patients with PDB: active disease or not (p = n.s.), long bone involvement or not (p = n.s.), monostotic or polyostotic disease (p = n.s.) and previous bisphosphonate treatment or not (p = n.s.).

women: 975.7 ± 209.3 pMol/L, $p = 0.923$). A slight correlation was observed between periostin levels and age ($r = 0.220$, $p = 0.040$).

Serum TRAP5b was significantly higher in PDB patients than in controls (4.43 ± 1.76 vs. 3.21 ± 1.02 U/L, $p < 0.001$) (Fig. 3). Moreover, TRAP5b was significantly higher in patients with polyostotic disease than in those with monostotic disease (4.81 ± 1.79 vs 3.68 ± 1.5 U/L, $p = 0.005$), and in those with active disease (4.98 ± 1.76 vs. 3.07 ± 0.72 U/L, p < 0.001). TRAP5b levels were not influenced by previous bisphosphonate treatment (4.14 ± 1.42 vs. 4.84 ± 2.02 U/L, $p = 0.206$), even in the 16 patients treated in the last 6.5 years (duration of time when < 1% of PDB patients have a relapse after a single infusion of zoledronic acid) [17] (> 6.5 years: 5.20 ± 1.79 vs. < 6.5 years: 3.80 ± 1.16 U/L, $p = 0.052$).

Serum TRAP5b levels did not differ between men and women when both PDB patients and controls were assessed (men: 3.92 ± 1.64 vs. women: 3.68 ± 1.45 U/L, $p = 0.473$), and no correlation with age was found ($r = 0.160$, $p = 0.311$).

All classical BTMs (bone ALP, PINP, uNTX and sCTX) correlated among themselves when PDB patients were analysed as a group (Fig. 4), without significant correlations between periostin and any of the assessed BTMs, except for TRAP5b ($r = 0.305$, $p = 0.05$). However, when PDB patients were classified according to the disease activity, there were significant correlations between serum periostin and all BTMs (bone ALP, PINP, uNTX, sCTX and TRAP5b) in PDB patients with active

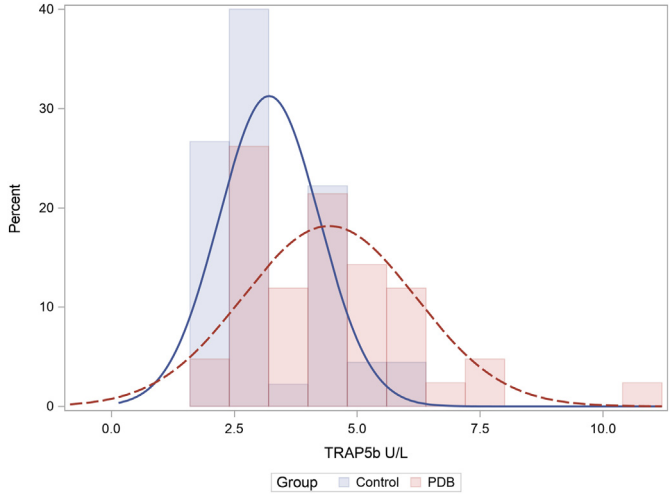


Fig. 3. Histogram of serum TRAP5b levels in PDB patients and controls ($p < 0.001$).

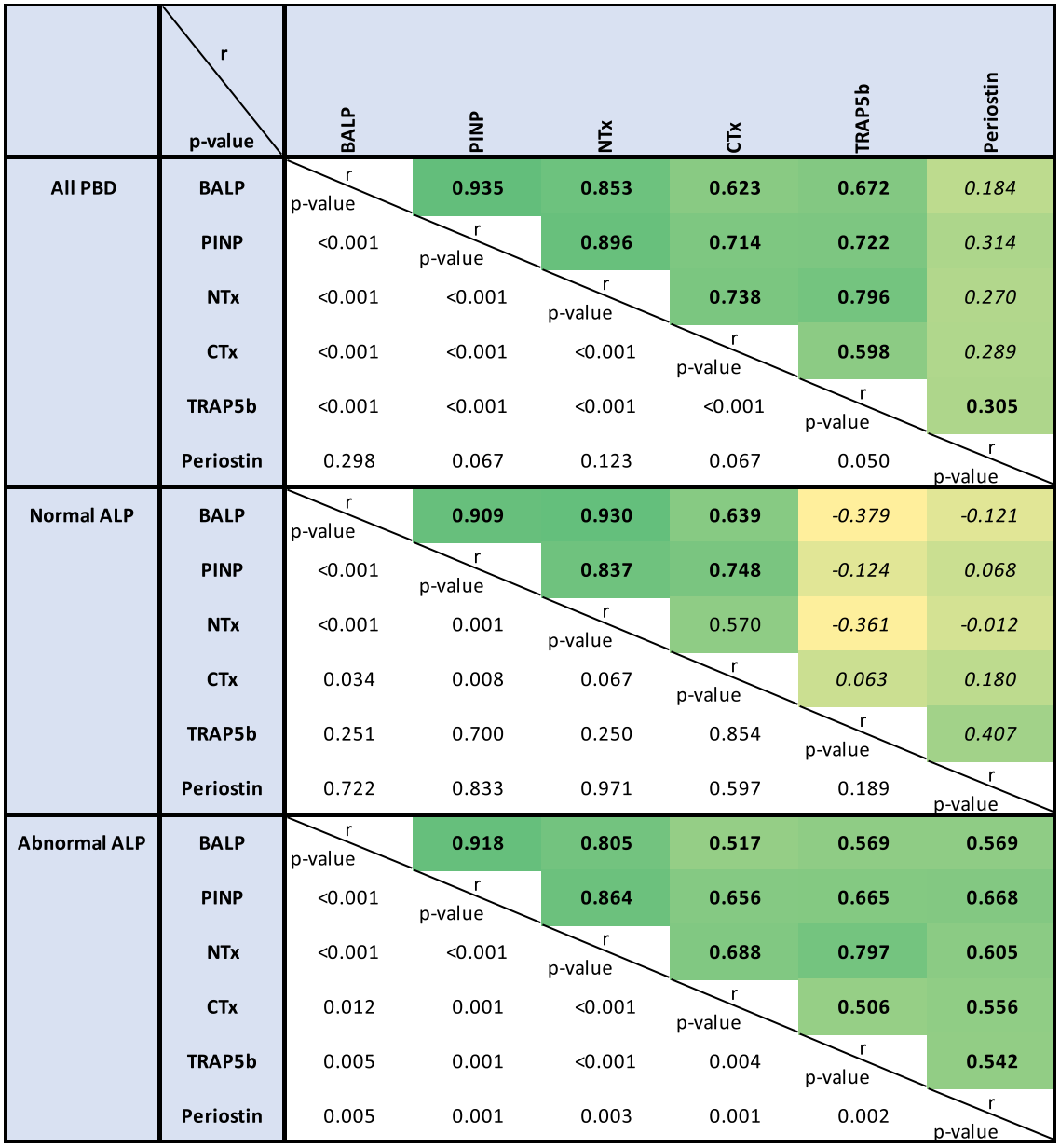
disease, but not in the inactive PDB group. TRAP5b correlated with all classical BTMs in PDB patients analysed as a group and in those with active disease (Fig. 4).

When PDB patients were classified according to the involvement of long bones, all classical BTMs (bone ALP, PINP, uNTX and sCTX) as well as TRAP5b correlated among themselves in both groups, except sCTX and TRAP5b in the group without long bone involvement. Serum periostin was positively correlated with PINP ($r = 0.627$, $p = 0.009$), uNTX ($r = 0.508$, $p < 0.044$) and TRAP5b ($r = 0.464$, $p = 0.026$), but not with bone ALP and sCTX in the group with long bone involvement. There were no significant correlations between periostin and any BTMs in the group without long bone involvement.

4. Discussion

The results of this study suggest that periostin is not a sensitive bone marker of PDB, whilst TRAP5b is a promising BTM in the identification and assessment of PDB. Thus, periostin levels were similar in PDB patients and controls, as well as in PDB patients with active and inactive disease. An interesting and unexpected finding is that there were no differences in periostin levels between PDB patients with and without involvement of long bones. Given that periostin is a marker of cortical and periosteal metabolism [3,4], our hypothesis was that periostin would allow identify patients with greater cortical involvement, as occurs in PDB with the classical thickening of the cortex. However, this hypothesis was not confirmed in our series, despite the fact that the group with long bone involvement constituted the 55% of patients.

Periostin correlated with all classical BTMS (PINP, bone ALP, uNTX, sCTX) and with TRAP5b in PDB patients with active disease, but not in those with inactive disease and neither with most BTMs when long bones were involved. These results suggest that periostin may be a marker of bone metabolism when bone turnover is highly increased. Walsh et al. reported that periostin was higher at age 16–18 than at other years of age less than age 70+, when the skeleton is under modelling and cortical consolidation, along with high bone turnover [18]. In active PDB there is an increase in both local bone modelling and remodelling [19]. Also, these authors found that at over 70 years of age, levels of periostin were similar to those of patients in their thirties, probably reflecting the increase in periosteal circumference associated with aging [18]. We didn't find differences in age according to the activity of the disease, probably because PDB is a disease of elderly people. Recently, Bonnet et al. described that periostin is a substrate of catepsin K at the periosteum, being periosteal modelling a process independent of osteoclasts [20]. Given the correlation of periostin with



	r / p-value	BALP	PINP	NTx	CTx	TRAP5b	Periostin
All PBD	BALP	r	**0.935**	**0.853**	**0.623**	**0.672**	*0.184*
	PINP	<0.001	r	**0.896**	**0.714**	**0.722**	*0.314*
	NTx	<0.001	<0.001	r	**0.738**	**0.796**	*0.270*
	CTx	<0.001	<0.001	<0.001	r	**0.598**	*0.289*
	TRAP5b	<0.001	<0.001	<0.001	<0.001	r	**0.305**
	Periostin	0.298	0.067	0.123	0.067	0.050	r
Normal ALP	BALP	r	**0.909**	**0.930**	**0.639**	*-0.379*	*-0.121*
	PINP	<0.001	r	**0.837**	**0.748**	*-0.124*	*0.068*
	NTx	<0.001	0.001	r	0.570	*-0.361*	*-0.012*
	CTx	0.034	0.008	0.067	r	*0.063*	*0.180*
	TRAP5b	0.251	0.700	0.250	0.854	r	*0.407*
	Periostin	0.722	0.833	0.971	0.597	0.189	r
Abnormal ALP	BALP	r	**0.918**	**0.805**	**0.517**	**0.569**	**0.569**
	PINP	<0.001	r	**0.864**	**0.656**	**0.665**	**0.668**
	NTx	<0.001	<0.001	r	**0.688**	**0.797**	**0.605**
	CTx	0.012	0.001	<0.001	r	**0.506**	**0.556**
	TRAP5b	0.005	0.001	<0.001	0.004	r	**0.542**
	Periostin	0.005	0.001	0.003	0.001	0.002	r

Fig. 4. Heat-map table of bone turnover markers (bone ALP, PINP, uNTX, sCTX and TRAP5b) and periostin Pearson correlations.
Cells are colored according to the intensity of the correlation and significant correlations are marked in bold. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

all bone formation and resorption markers in active PDB, our hypothesis is that in addition to being a modelling marker, periostin is a marker of bone remodelling only when this process is greatly increased. Indeed, Polyzos et al. reported in a relatively inactive period of the disease that there was a marked increase in periostin levels in juvenile Paget's disease [10], a rare genetic bone disorder characterised by accelerated bone turnover due to inactivating mutations in the gene encoding osteoprotegerin [21]. By contrast, periostin was not correlated with bone formation and resorption markers in a large series of French women; both in women who did not develop fractures and in those who developed fractures in a follow-up of 7 years. It should be noted that levels of BTMs (PINP, osteoclacin and sCTX) were not higher at baseline in women who developed fractures [5], reinforcing our hypothesis that correlations only exist when bone remodelling is highly accelerated. Periostin levels were increased during teriparatide therapy in postmenopausal women with osteoporosis, however, no correlation was found with BTMs including PINP [22]. These results could suggest that pagetic bone and osteoporotic bone under teriparatide have different patterns of cortical and periosteal tissue in both situations. Nevertheless, a disregulation of synthesis or secretion of periostin by pagetic cells or different periostin immunoreactivity cannot be ruled out.

It should be pointed out that in recent years different commercial ELISA assays have been developed in order to improve the measurement of circulating periostin specific of bone tissue. In this study, we used the recently developed ELISA from Biomedica that recognises all the 7 known isoforms of the human periostin molecule [18]. The USCN assay, believed to evaluate total circulating periostin plus potential fragments, was used in several clinical studies from French and Swiss research groups [5,23]. In addition, there are other ELISA assays from different companies such as Adipogen, Roche and Abbott, which recognise a number of different isoforms of the periostin molecule, and it is unknown what isoforms some of these assays recognise [18]. Recently, a new ELISA for a periostin fragment resulting from cathepsin-K digestion has been developed, aimed at being a bone-specific assay

[23,24].

TRAP5b has been scarcely analysed in PDB, and it was done using an assay that measures enzyme activity. TRAP5b was measured in 10 and 51 PDB patients, respectively, > 20 years ago, and higher levels than in controls were found [14], particularly in patients with severe disease [13]. Indeed, when using the modified Hillmann method, TRAP5b was less sensitive than uNTX and only 41% of PDB patients had increased levels [13]. In the current study using an ELISA assay, we have found that TRAP5b levels were higher in PDB patients than in controls, and in those with active and polyostotic disease compared with those with inactive and monostotic disease, respectively. TRAP5b correlated with all BTMs in PDB patients as a group and in those with active disease. The lack of effect of age and gender in its serum levels, in addition to its low biological variability [25] reinforces the utility of TRAP5b measurement as a marker of bone resorption, or in a strictus sensus as an indirect marker of osteoclast number. To assess resorption in PDB combining markers that provide information on bone resorption activity, such as NTX, and on resorbing cell numbers, such as TRAP5b, is compelling.

Mori et al. developed a mathematical model to predict bone mineral density changes after zoledronic acid treatment using early response of TRAP5b [26]. However, an attenuated response to antiresorptive therapy in comparison with other markers of bone resorption was reported some years ago [25–27]. Perhaps this is the reason that TRAP5b levels were not lower in the patients from our series previously treated with bisphosphonates.

Our study has several limitations. We used an assay for circulating periostin that measures the seven known isoforms of periostin, although the specific identification of the bone isoform is at present unknown [18]. Secondly, our control group was not age and sex-matched. However, both periostin and TRAP5b were not influenced by gender, and only a slight correlation was observed between periostin levels and age, without correlation of TRAP5b with age. The strength of our study is the careful analysis of the PDB disease according to the clinical and biochemical characteristics such as active vs. inactive disease, monostotic vs polyostotic disease and long bone involvement or not, among other parameters.

In conclusion, serum periostin analysed by the ELISA Biomedica assay is not useful for assessing PDB. By contrast, TRAP5b is a useful marker of bone resorption in PDB assessment. The low biological variability and the different magnitude of its levels according to the disease, whether or not it is active and polyostotic or monostotic, reinforces its use in this metabolic bone disease.

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