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Bone markers during acute burn care: Relevance to clinical practice?

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ABSTRACT

Objective: Bone changes are increasingly described after burn. How bone markers could help to detect early bone changes or to screen burn patients at higher risk of demineralization is still not made clear. We performed an observational study assessing the changes in serum bone markers after moderate burn.

Methods: Adults admitted in the first 24 h following burn extended on >10% body surface area were included. Serum levels of collagen type 1 cross-linked C-telopeptide (CTX), tartrate-resistant acid phosphatase 5b (TRAP), type 1 procollagen N-terminal (P1NP) and bone alkaline phosphatase (b-ALP) were measured at admission and every week during the first month. Data are expressed as median [min-max].

Results: Bone markers were measured in 20 patients: 18 men, 2 women (including one post-menopausal). Age was 46 [19–86] years old, burn surface area reached 15 [7–85] %. Twelve patients completed the study. All biomarkers mainly remained into normal ranges during evolution. A huge variability was observed regarding biomarkers evolution. Patient's evolution was not linear and could fluctuate from a decrease to an increase of blood concentrations. There was not necessarily a consistency between the two formation or the two resorption markers. Variations observed between two consecutive measurements were lesser than the accepted critical difference in almost one third of the cases.

Conclusions: Considering available data, role and interest of bone markers in management of burn related bone disease remain unclear.

1. Introduction

Burn patients are at risk for bone disease following injury. This is thought to be due to different factors, commonly co-existing after burn: prolonged and sustained inflammation or neuro-endocrine response, immobilization and vitamin D deficiency [1].

A reduced bone turnover and a demineralization have been reported in the burn literature for more than two decades [2].

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These data concern both adults and children. In burn children, these changes had even been associated with an increased risk of bone fractures during the first years following injury [3,4]. In burn adults, such outcome evaluation is currently lacking.

Alterations in some bone markers have been described in a few publications. However, the results were not always consistent, mostly due to differences in the studied population or in the measured bone markers [3,6,7]. A recent study reported changes in a panel of bone turnover markers and regulators of bone metabolism in severe burned males during both early and late phase after injury [8]. These changes seemed to be the expression of an altered bone quality, characterize by a precocious appearance. Yet, from a pragmatic and clinical point of view, choice and use of bone markers to detect early bone changes or to screen burn patients at higher risk of demineralization is still not well defined.

Published data about bone health after burn mainly focused on patients suffering from severe burn. Recently, animal data suggest that non-severe burn can also lead to bone changes as soon as the first month following injury [5]. These findings are disturbing, as the mean BSA of patients admitted in burn centers in Europe has decreased over the past decades, to reach 11–24% [9]. In this context, the present observational study assessed the changes in four serum bone markers in non-severe burned adults during the first month following injury. The objective of the study was to determine the clinical value of the mainly used bone biomarkers in a quite broad spectrum of burn patients.

2. Materials and methods

This cohort study was conducted from March 2012 to January 2013 in a 6-bed burn unit after approval by the local Ethics Committee of our University Hospital (Ref B707201213417, 6th March 2012). Informed consent was obtained from the patients or their relatives prior to enrolment.

Caucasian patients over 18 years, with a burn surface area (BSA) greater than 10% and admitted within the first 24 h following injury were included. Pregnancy, renal or liver failure, prior vitamin D substitution were considered exclusion criteria. They benefited from local standard monitoring and care procedures in term of fluid resuscitation, nutrition and surgery. They daily received vitamin D3 (cholecalciferol, VD3) from oral nutrition (Fresubin 2kcal®, Fresenius-Kabi, Germany, Resource 2.0 fibres®, Nestlé, Switzerland), enteral nutrition (Fresubin HP Energy®, Fresenius-Kabi, Germany) and multivitamin complex supplementation (Supradyn Energy®, Bayer, Germany or Cernevit®, Baxter, USA), reaching a daily dose of 600–800 UI.

For each patient, assessment of global bone health at admission was performed using the FRAX tool (Fracture Risk Assessment tool) (https://www.shef.ac.uk/FRAX/tool.aspx?country=18). The University of Sheffield, in association with the World Health Organization, developed this instrument. FRAX uses validated clinical risk factor to provide a prediction of individual's risk of fracture in the next 10 years. The risk is expressed in percentages.

Blood samples were collected in all patients at admission (D0) and then in the early morning of the 7th, 14th, 21st and 28th day of in-hospital follow-up. Blood was then centrifugated (3500 rpm, 15 min, 4 °C) and supernatant was finally frozen and stored at −80 °C.

Serum collagen type 1 cross-linked C-telopeptide (CTX), serum type 1 procollagen N-terminal (P1NP) and serum bone alkaline phosphatase (b-ALP) were assayed using chemoluminiscence (iSYS automate, IDS, Boldon, UK). CTX concentration <695 ng/l and b-ALP concentration <21 μg/l were considered normal. Normal range for P1NP was 7.5–95.4 ng/ml with changes according to age and gender [10]. Serum tartrate-resistant acid phosphatase 5b (TRAP) was measured using ELISA (IDS, Boldon, UK): NR was 1.5–4.7 U/l. CTX and TRAP are bone resorption markers while P1NP and b-ALP are bone formation markers.

Intra-individual coefficient of variation (CV_i) for the used bone markers was collected via the website https://www.westgard.com/biodatabase1.htm, excepting for TRAP [11]. CV_i is essential to calculate the critical difference (CD), which is the value above (or under) which a variation between two consecutive biological measurements may be considered as biologically significant, with a probability of 95%. CD depends also on the analytical coefficient of variation (CV_a) of the parameter and can be calculated according the following formula: $CD = 2^{1/2} \times 1.96 \times (CV_a^2 + CV_i^2)^{1/2}$ [12]. In daily practice, since CV_a is much lower than CV_i, CD is usually estimated as $3\times$ CV_i.

Serum level of 25OH-D and 3rd generation PTH were determined with Liaison® (DiaSorin, Stillwater, MN, USA). The normal ranges were respectively 30–100 ng/ml and 4–26 pg/ml. 1,25$(OH)_2$-D was assayed with iSYS® automate (IDS, Boldon, UK): normal range was <85 pg/ml. Serum levels of albumin (ALB) was assayed using spectrophotometry (Cobas automate, Roche, Mannheim, Germany): NR was 38–49 g/l. Levels of calcium (Ca), phosphate (P) and creatinine were assayed with Cobas automate (Roche, Mannheim, Germany): NR were respectively 2.15–2.6 mmol/l, 0.74–1.51 mmol/l and 7.2–11.8 mg/l. Serum total calcium (Ca Tot) level was corrected for albumin level according to the following formula: Corr Ca Tot (mml/l) = serum Ca Tot − 0.025 (serum albumin − 40).

Statistical analysis was performed using Graphpad Prism (version 6.0 for Mac OSX, Graphpad Inc., San Diego, CA, USA). Data were tested for normality using the Shapiro-Wilk test. Results are expressed as medians and ranges (min-max). Unpaired data were compared using Mann and Whitney test. Paired data were analyzed using Wilcoxon test or Friedman test with multiple comparisons, as required. Correlation between bone markers and baseline data were assessed using nonparametric Spearman test. A p value <0.05 was considered to be statistically significant.

3. Results

Biological data were measured in 20 patients: 18 men, 2 women (including a post-menopausal). Median age was 46 [19–86] years old. Definitive median BSA, as evaluated by a senior intensivist, reached 15 [7–85] %. Frequency distribution

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of BSA is shown in Fig. 1. Most of the patients suffered from non-severe burned. Median Abbreviated Burn Severity Index (ABSI) score was 5 [4–12]. Of the included patients, twelve remained hospitalized during at least one month and completed the study.

Median FRAX risk of major osteoporotic fracture was 1.85 [1.4–16] %. Median FRAX risk of hip fracture was 0.2 [0–13] %. The highest risks were observed in the 3 patients ≥70 y.

Evolution of the bone markers is detailed in Fig. 2. From a global point of view, all bone markers seemed to increase after injury, while mainly remaining into normal range. Statistically, paired data were not different (except for P1NP at D28 and CTX at D21). However, from an individual point of view, a huge variability was observed regarding bone markers evolution. Patient's evolution was not linear and could fluctuate from a decrease to an increase of blood concentrations between two consecutive time points. More importantly, there was not necessarily a consistency between the two formation or the two resorption markers.

Of note, the variations observed between two consecutive measurements were lesser than the accepted critical difference in almost one third of the cases. This is report in Table 1. Fluctuations of TRAP were the less clinically significant.

Evolution of bone markers was also analyzed according to BSA (BSA ≤20% or >20%), with a focus on evolution between inclusion and D28, reported as the ratio between D28 and D0 and expressed in percentage. Ratio were not statistically different in case of BSA >20% when compared to lesser BSA. This analysis is detailed on Fig. 3. When regarding specifically on b-ALP and TRAP, no correlation was found for none of the two biomarkers between the ratio and BSA or FRAX risks.

Phospho-calcic metabolism related parameters are reported in Table 2, focusing on the 12 patients who completed the study. Vitamin D levels did not significantly changed during the month of follow-up. PTH was slightly increased at D0 and returned into normal ranges at D28. Albumin level was lower than the normal ranges both at D0 and D28. Corr Ca Tot levels significantly increased at D28, remaining however within respective normal ranges. Phosphate levels were stable, into normal ranges.

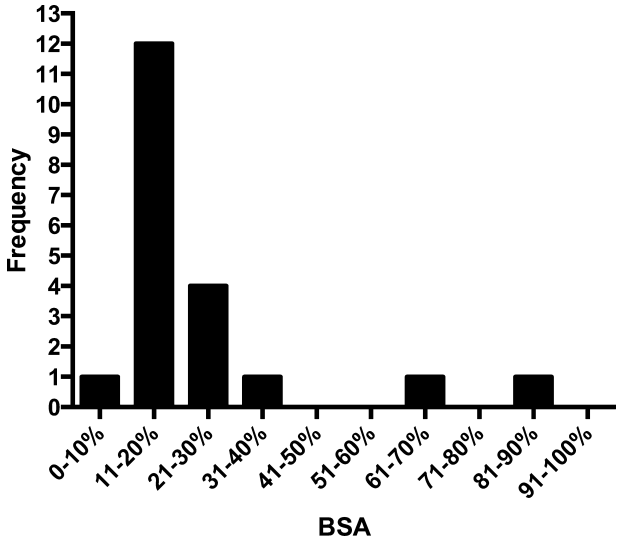

Fig. 1 – Histogram of BSA among included patients.

4. Discussion

Despite the low number of included patients, the present results constitute a supplementary outlook on bone markers, describing their evolution in a context of non-severe burn, from an individual point of view and not only as a cohort. Clearly, evolution of bone markers was not similar between patients, with non-linear fluctuations. The observed variations in bone markers could not be explained by heterogeneity in burn surface area or bone frailty. It seems thus hard to define a global profile regarding bone formation or resorption. From these results and the previously published data, ruling on interest of bone markers measurement at the bedside would be premature.

Ideally, bone markers screening could help to detect alteration in bone metabolism earlier than the observed consequences on mineral density and finally than the occurrence of fractures. Such detection could allow specific prevention targeting frail patients, especially as some prevention strategies have already shown beneficial effects. Pamidronate has been studied in severe burn children: its administration during acute phase preserved bone mass during the two first years after injury [13,14]. Vitamin D supplementation, again in burn children, reduced the incidence of bone fractures at 22 months after injury [4]. Oxandrolone, a testosterone-derived anabolic agent, is one of the pharmacologic strategies to counteract burn related hypermetabolism and hypercatabolism. Its beneficial effects on maintaining lean body mass have been demonstrated. However, a one year administration in severe burn children failed to influence blood osteocalcin levels [15]. On the contrary, recombinant human growth hormone administered during 12 months after injury resulted in an increase in blood osteocalcin levels in severe burn children [16]. To date, all these results cannot be transposed in burn adults.

Admittedly, the population recruited in the present study is heterogeneous. It is well known that gender and age influence bone health. However, evaluation covered only one month and data was analyzed as paired data, thus limiting the influence of these two parameters. Excepting PTH levels decreasing after 28 days, phospho-calcic metabolism might be considered stable, and thus of poor influence on the observed bone markers changes.

In the context of serial measurements, paired data analysis should ideally taking into account the principle of critical difference (CD) between two consecutive measures in the same patient. The CD is a way to quantify the significance and the relevance of observed changes. Relevance is based on the concepts of analytical variability (CV_a), related to the assay itself used in a define lab, and on intra-individual variability (CV_i), corresponding to the random variation of the observed result around the patient's own homeostasic point [12]. The critical difference is the value above (or under) which it can be stated that the observed difference between two consecutive measures is significantly higher that the inherent variability, and thus not due to chance. In the present study, fluctuations

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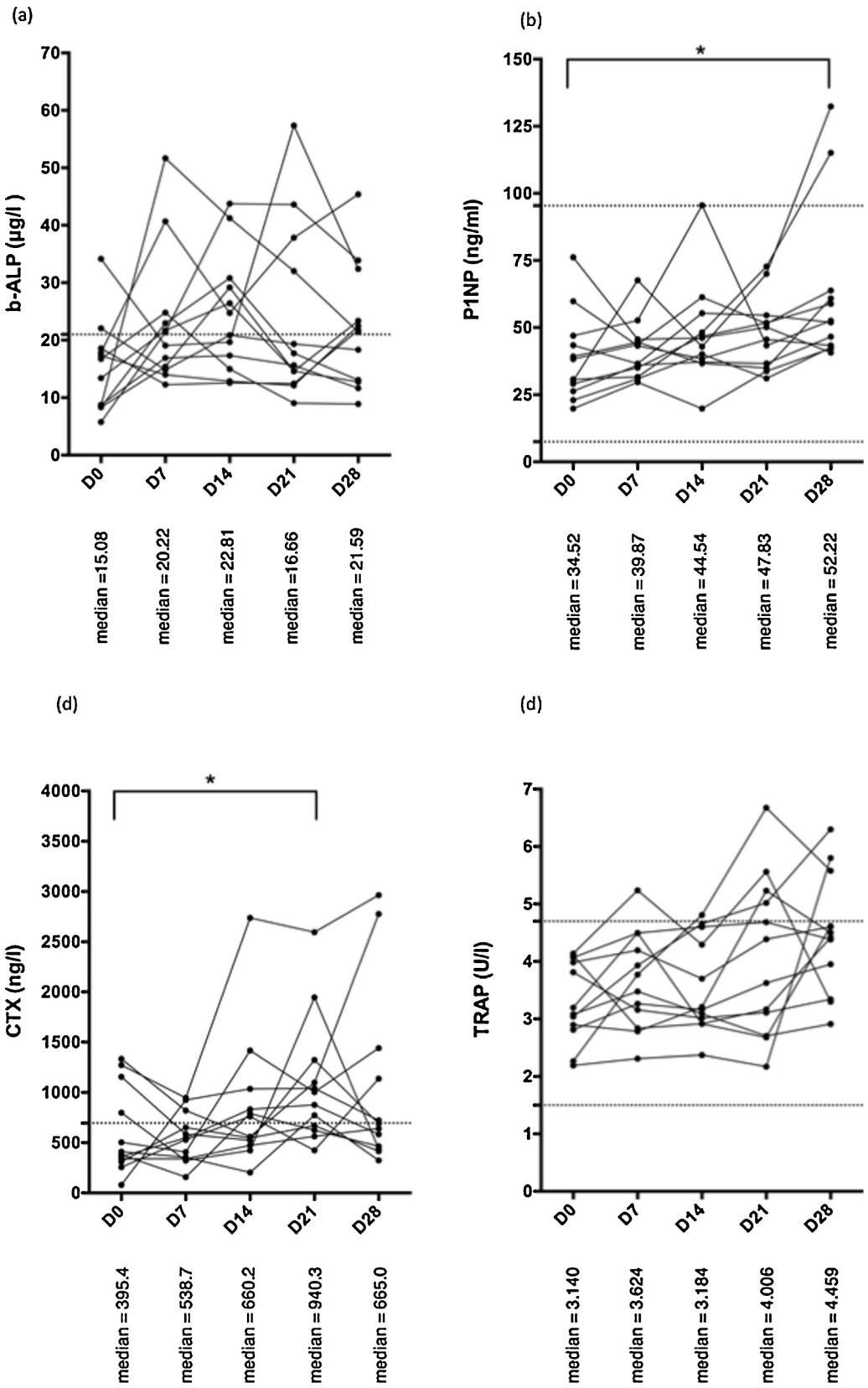

Fig. 2 – "Before–after" graphs represent paired data of b-ALP (a), P1NP (b), CTX (c) and TRAP (d) at admission (D0), day 7 (D7), day 14 (D14), day 21 (D21) and day 28 (D28). Median values of the 12 patients who completed the study are noted below x axis. Dotted lines represent normal range. b-ALP concentration <21 µg/l and CTX concentration <695 ng/l were considered normal. Normal range for P1NP was 7.5–95.4 ng/ml and for TRAP was 1.5–4.7 U/l.
Paired data were analyzed using Friedman test with multiple comparisons (*$p < 0.05$).

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Table 1 – Proportions of clinically significant changes between two consecutive measurements.

Bone marker	CV_a	CV_i	CD	D7-D0 (n = 17)	D14-D7 (n = 15)	D21-D14 (n = 14)	D28-D21 (n = 12)
b-ALP	4.9%	6.2%	18%	82%	67%	57%	67%
P1NP	4.7%	7.4%	22%	58%	60%	50%	50%
CTX	3.5%	10.85%	32%	76%	67%	43%	67%
TRAP	2.6%	8.3%	24%	41%	13%	29%	42%

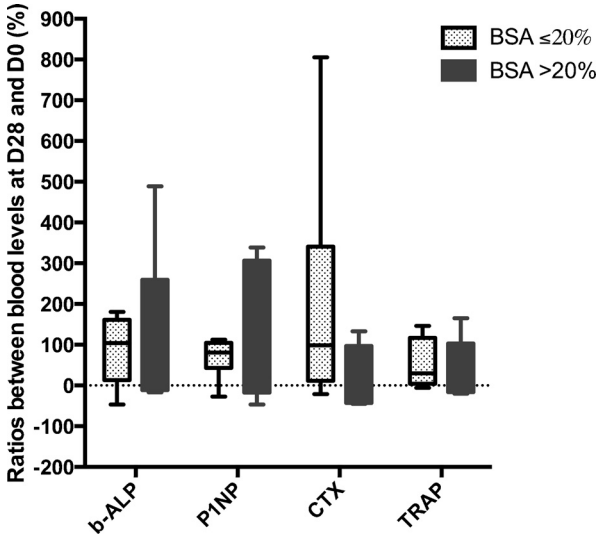

Fig. 3 – Box and whisker plots representing ratios between values of bone markers at D28 and D0 in patients with BSA ≤20% (dotted boxes) and in patients with BSA >20% (grey boxes). Ratios of lab values are expressed as percentages.

of bone markers were clinically insignificant in 18% to 87% of the cases, depending on the biomarker. TRAP changes were the less significant. Its meaning in terms of bone resorption extent is uncertain. The concept of CD should be more widely used in clinical studies, especially if comparing biological changes to clinical outcomes. This would allow specifying utility of bone markers in patients' follow-up and optimal timing for measurements. Indeed, in hemodialysis patients, it has been suggested that variation of bone markers need repeated measures on a sustained period, knowing the slow process of bone turnover [17].

Of note, interpretation of the bone markers during acute phase of burn may be hazardous. First, hemodilution following fluid resuscitation may have negatively influenced D0 results. However, according to the moderate severity of burn, fluid shifts or fluid resuscitation are supposed to be limited. Second, immunoassays classically used to measure bone markers may not be suitable in case of proteins changes in relation to burn and resuscitation. In a similar way, vitamin D immunoassays have been challenged in a hemodilution model: they have been demonstrated to be less accurate when comparing to the reference method (mass spectrometry), due to matrix effects [18]. Third, regarding the panel of available bone markers, it is important to keep in mind some specific aspects related to burn [19]. Markers derived from type 1 collagen (such as P1NP) may not be sensitive in burn patients because of a production in regenerating skin. Osteocalcin levels may be lowered by vitamin K deficiency, a frequent condition encountered by acute burn patients, and critically ill patients as well. Finally, CTX measurements require specific pre-analytical conditions that may be met with difficulty during critical care conditions (such as long fasting in case of continuous enteral nutrition). Considering this information, BALP and TRAP may be the most suitable bone markers in burn patients. The present study is actually the first report of TRAP evolution in burn patients. However, the aim of the study was not to validate the superiority of TRAP as marker of bone resorption.

To conclude, considering available data, role and interest of bone markers in management of burn related bone disease remain unclear. Further large and integrative studies are urgently needed. They should be designed with the aim of determining the clinical value of bone markers in mild to moderate burns but also in severely burned patients in whom bone loss is thought to be more striking. In that perspective, it would be important to consider the limitations to the use of CTX or P1NP. Ideally, bone markers should be used as serial measurements to overcome pre-analytical pitfalls and values should be apprehended through the critical difference concept. Coupling bone markers measurements to histologic data or bone mass evaluation, even if quite difficult in daily clinical settings, would unquestionably be of added value in order to establish the relevance of biological results.

Table 2 – Phospho-calcic metabolism.

Parameters	Normal ranges	D0 (n = 12)	D28 (n = 12)	p value
25(OH)-D	30–100 ng/ml	13 [8–23]	12.5 [8–23]	0.78
$1,25(OH)_2$-D	<85 pg/ml	41 [22–78]	34.5 [19–63]	0.12
PTH	4–26 pg/ml	42 [10–105]	19.5 [9–128]	0.15
Albumin	36–49 g/l	31 [19–38]	25.5 [18–35]	0.08
Corr Ca Tot	2.15–2.6 mmol/l	2.22 [2.05–2.52]	2.46 [2.17–2.53]	0.02*
P	0.74–1.51 mmol/l	1.16 [0.5–1.63]	1.04 [0.48–1.64]	0.9

* Statistically significant.

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Author's contributions

AFR, EC designed research; AFR conducted research; AFR, PDel and EC analyzed data; AFR wrote paper; PDa, PDel and EC critically reviewed paper. All authors approved the final manuscript.

Conflict of interest

Pierre Delanaye and Etienne Cavalier received honoria from IDS. Anne-Françoise Rousseau and Pierre Damas declare that they have no conflict of interest.

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