

Research Paper

Bone Turnover Status: Classification Model and Clinical Implications

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Abstract

Aim: To develop a practical model for classification bone turnover status and evaluate its clinical usefulness.

Methods: Our classification of bone turnover status is based on internationally recommended biomarkers of both bone formation (N-terminal propeptide of type I procollagen, PINP) and bone resorption (beta C-terminal cross-linked telopeptide of type I collagen, bCTX), using the cutoffs proposed as therapeutic targets. The relationships between turnover subtypes and clinical characteristic were assessed in 1223 hospitalised orthogeriatric patients (846 women, 377 men; mean age 78.1±9.50 years): 451 (36.9%) subjects with hip fracture (HF), 396 (32.4%) with other non-vertebral (non-HF) fractures (HF) and 376 (30.7%) patients without fractures.

Results: Six subtypes of bone turnover status were identified: 1 - normal turnover (PINP>32 µg/L, bCTX≤0.250 µg/L and PINP/bCTX>100.0[median value]); 2- low bone formation (PINP ≤32 µg/L), normal bone resorption (bCTX≤0.250 µg/L) and PINP/bCTX>100.0 (subtype 2A) or PINP/bCTX<100.0 (subtype 2B); 3- low bone formation, high bone resorption (bCTX>0.250 µg/L) and PINP/bCTX<100.0; 4- high bone turnover (both markers elevated) and PINP/bCTX>100.0 (subtype 4A) or PINP/bCTX<100.0 (subtype 4B). Compared to subtypes 1 and 2A, subtype 2B was strongly associated with nonvertebral fractures (odds ratio [OR] 2.0), especially HF (OR 3.2), age>75 years and hyperparathyroidism. Hypoalbuminaemia and not using osteoporotic therapy were two independent indicators common for subtypes 3, 4A and 4B; these three subtypes were associated with in-hospital mortality. Subtype 3 was associated with fractures (OR 1.7, for HF OR 2.4), age>75 years, chronic heart failure (CHF), anaemia, and history of malignancy, and predicted post-operative myocardial injury, high inflammatory response and length of hospital stay (LOS) above 10 days. Subtype 4A was associated with chronic kidney disease (CKD), anaemia, history of malignancy and walking aids use and predicted LOS>20 days, but was not discriminative for fractures. Subtype 4B was associated with fractures (OR 2.1, for HF OR 2.5), age>75 years, CKD and indicated risks of myocardial injury, high inflammatory response and LOS>10 days.

Conclusions: We proposed a classification model of bone turnover status and demonstrated that in orthogeriatric patients altered subtypes are closely related to presence of nonvertebral fractures, comorbidities and poorer in-hospital outcomes. However, further research is needed to establish optimal cut points of various biomarkers and improve the classification model.

Key words: bone turnover markers; classification; nonvertebral fracture; prediction

Introduction

As the world's population ages, the prevalence of osteoporotic fractures is increasing, but the existing

prevention strategies are only partially effective. Although altered bone and mineral metabolism is

considered as one of the most important and modifiable risk factors for osteoporotic fractures, the diagnostic and prognostic value of bone turnover markers (BTMs) is still disputed. Currently BTMs, which reflect the status of total bone metabolism, are recommended only for the monitoring the efficacy of osteoporosis treatment and compliance [1-7]. The reasons for scepticism about the practical value of BTMs include their significant analytical and biological variability [8-11], parallel dynamics (due to coupling bone formation and resorption), and, more importantly, large overlap in BTMs values between those with and without fractures [2, 4, 7, 12]. Moreover, both increased and low bone turnover have been shown to be associated with bone gain or loss as well as with increased risk of fracture [13-20]. Despite accumulating evidence suggesting heterogeneity of the osteoporotic processes as a reflection of sophisticated and multifactorial regulation of bone metabolism, osteoporosis is still often considered as a single entity. One possible way to deal with this complex disorder is to identify clinical subtypes based on selected variables. However, there is currently no international consensus regarding characteristics (absolute values) of normal, high or low bone turnover, and the balance between bone formation and resorption is mostly neglected, although after midlife bone is lost because remodelling, despite of coupling, becomes unbalanced [13, 20, 21].

In light of paucity of studies investigating the phenomenon of variants of BTMs we attempted to develop and introduce a practical classification model based on both bone formation and resorption biomarkers and their ratio. We aimed to identify distinct subtypes of bone metabolism and analysed in a cohort of hospitalised orthogeriatric patients the relationships between these subtypes and (1) presence and type of a non-vertebral fracture, (2) clinical and laboratory characteristics (2) and (3) in-hospital outcomes.

Patients and Methods

Patients

This was an observational study using prospectively collected data on 1899 consecutive older (>60 years) patients admitted to the Department of Orthopaedic Surgery at the Canberra hospital (a university-affiliated tertiary care centre, Australian Capital Territory, Australia) between 1 January 2012 and 31 December 2014. After excluding patients with high-trauma fracture, primary hyperparathyroidism, Paget's disease, metastatic cancer to bone, or who lacked adequate laboratory data, 1223 patients (846

women, 377 men) were evaluated for the study. Of these 1223 hospitalized orthogeriatric patients 847 (69.3%) had a non-vertebral bone fracture. Patients with hip fracture (HF, n=451) constituted 53.2% among all fracture patients, and 36.9% of the total cohort. There were 396 (32.4%) patients with other non-vertebral (non-HF) fractures (humerus -79, femur -74, ankle -68, tibia or/and fibula -27, knee -16, wrist -16, forearm -15, other -101) and 376 (30.7%) patients without fractures (elective hip or knee replacement -340, suspected surgical site infections not confirmed by further investigation -12, and 24 patients with a prosthetic joint infection following total hip [n=17] or knee [n=7] arthroplasty).

Data on demographics, orthopaedic and medical diagnoses, chronic comorbid conditions, residential and smoking status, alcohol consumption, laboratory characteristics, procedures performed, medication used, and short-term (in-hospital) outcomes were analysed.

The study was conducted according to the ethical guidelines of the current Declaration of Helsinki and was approved by the local Health Human Research Ethical Committee. Informed consent from each patient or carer was obtained.

Laboratory measurements

In each patient fasting venous blood samples were collected in the morning, usually within 24h after arrival. The following serum indicators of bone and mineral metabolism were measured: two bone formation markers (N-terminal propeptide of type 1 procollagen, P1NP, and osteocalcin, OC), bone resorption marker (beta C-terminal cross-linked telopeptide of type I collagen, bCTX), parathyroid hormone (PTH), 25 hydroxyvitamin D [25(OH)D], calcium, phosphate and magnesium concentrations. The serum concentrations of P1NP, OC and bCTX were measured using an electrochemiluminescent immunoassay (Elecsys 2010 analyser, Roche Diagnostics, Ltd Corp., IN, USA). Intra- and inter-assay coefficients of variation (CV) for P1NP were 2.6% and 4.1 %, respectively; for OC 3.6% and 6.6%, respectively, and for bCTX 3.2% and 6.5%, respectively. Serum 25(OH)D level was measured by a radioimmunoassay (Dia Sorin, Stillwater, MN, USA) and intact PTH was determined by a two-site chemiluminescent enzyme-linked immunoassay on DPC Immulite 2000 (Diagnostic Products Corp., Los Angeles, CA, USA); the intra- and inter-assay CV ranged from 2.1% to 12.7%. Calcium concentrations were corrected for serum albumin. The ratio of P1NP to bCTX was calculated by dividing the P1NP by bCTX. Vitamin D status was defined as deficient for circulating 25(OH)D concentration <25nmol/L, and

as insufficient for 25–50nmol/L. Secondary hyperparathyroidism (SHPT) was defined as elevated serum PTH (>6.8pmol/L, the upper limit of the laboratory reference range). Chronic kidney disease (CKD) was defined as glomerular filtration rate (GFR)<60 ml/min/1.73m² (CKD stage ≥3), anaemia as haemoglobin<120g/L and hypoalbuminaemia as albumin<33g/L.

Classification criteria for bone turnover status

In line with the recommendations of the International Osteoporosis Foundation and the International Federation of Clinical Chemistry and Laboratory Medicine on BTMs [7], in our classification we used P1NP as a formation marker and bCTX as a resorption marker. There is to date no consensus on normal reference intervals for BTMs. Because the reports on thresholds of optimal bone metabolism, particularly in the older age, are controversial, to classify bone turnover status we used the cutoffs proposed as therapeutic (fracture-protective) targets, though some researchers concluded “that absolute values for BTMs are not suited as treatment targets” [12]. Two approaches were recommended to choose treatment targets for osteoporotic therapy: (1) provisional threshold values derived from community-dwelling observations [22–24] and (2) the mean/median of premenopausal reference intervals [7, 25, 26]. As a provisional treatment target /threshold for optimal anti-resorptive response values of bCTX ≤0.230 µg/L (Chubb S 2016; 2017) and ≤0.250 µg/L (the equivalent of urinary NTX <21 nmol BCE/mmol [22]) were recommended. In 17 studies, the mean/median reference intervals for bCTX in premenopausal women ranged between 0.217 µg/L and 0.484 µg/L [27–41] being ≤0.260 µg/L in seven reports. In 8 studies, the mean/median reference intervals for bCTX in adult men ranged between 0.260 µg/L and 0.490 µg/L [34–36, 42–45] being ≤0.270 in two studies. Even more controversy exists in relation to the target/desired level of P1NP during osteoporosis treatment because of the direction of changes associated with different classes of drugs: P1NP increases greatly with teriparatide administration (Sugimoto T 2014) and decreases (but less than bCTX) with antiresorptive therapy [3, 46–48]. In 15 studies, the mean/median reference intervals for P1NP in premenopausal women ranged between 33.0 µg/L and 47.7 µg/L [27, 29–31, 33–41, 49, 50]; similarly, in 8 studies, the mean/median reference intervals for P1NP in adult men ranged between 32.7 µg/L and 64.9 µg/L [34–36, 38, 42–45]. Based on data from a cohort of community-dwelling older men receiving antiresorptive therapy, serum P1NP concentrations of <32 µg/L (equivalent to the

provisional βCTX threshold of <0.230 µg/L) has recently been recommended as an indicator of optimal therapeutic response to bisphosphonate treatment [23].

In the present study, to classify the bone turnover status we have chosen as the cut points for serum P1NP 32 µg/L and for bCTX 0.250 µg/L; these arbitrary levels are relatively close to those recommended by the majority of experts and based on data reported by both abovementioned approaches.

Our classification of bone turnover status combines analysis of P1NP, bCTX and their ratio, assuming that the circulating concentrations of these markers are related to and reflect the integrated formation and resorption processes of the skeleton, while the ratio P1NP/bCTX<100 (median value) indicates a shift towards accelerated bone resorption.

Outcomes

The following short-term outcomes have been analysed: in-hospital death, myocardial injury (as reflected by cardiac troponin I rise), high postoperative (>3 days) inflammatory responses (CRP>100 mg/L and CRP>150 mg/L), length of hospital stay (LOS >10 days and >20 days), and new discharges to a permanent residential care facility (RCF).

Statistical analyses

Data analyses were performed using Stata software version10 (StataCorp., College Station, TX, USA). The patient characteristics were summarised using descriptive statistics; data presented as mean ± standard deviation (SD) for continuous variables and as numbers (and percentages) for categorical variables. Associations between bone turnover subtypes and fracture prevalence as well as comorbid conditions and outcomes were assessed using multiple linear regression models with a backward stepwise approach adjusting for age and gender. For multivariate logistic regression models all variables with $p \leq 0.100$ at univariate analysis were selected. The discriminative accuracy of each bone turnover subtype was expressed with two descriptors: (1) the area under the receiver operating characteristic curve (ROC), and (2) the percentage of correctly classified patients. Two tailed tests were used and results were considered statistically significant if $p < 0.05$.

Results

Patient characteristics

In the total cohort of orthogeriatric patients the mean age was 78.1±9.50 years, 846(69.2%) were women, and 190(15.4%) were living in a RCF. Patients

averaged 2.7 chronic diseases per person. Four or more chronic conditions were identified in 28.5% of patients with the greatest burden among individuals with HF (36.0% vs. 29.4% in the non-fracture group, $p=0.040$). The most common comorbidities were hypertension requiring medications (60.0%), osteoarthritis (42.5%), abnormal gait with use of an assistive device (42.0%), diabetes mellitus type 2 (DM, 22.0%), CKD (21.3%), coronary artery disease (CAD, 17.1%), chronic obstructive airway disease (COPD, 15.4%), atrial fibrillation (AF, 14.8%), dementia (14.4%), cerebrovascular disease (12.2%), malignancy (10.4%) and chronic/congestive heart failure (CHF, 7.8%).

On admission, vitamin D insufficiency exhibited 295(24.1%) patients, vitamin D deficiency 95(7.8%), hyperparathyroidism 468(38.3%), anaemia 872(71.3%) and hypoalbuminaemia 680(55.6%) subjects. There were 17.3% ex-smokers and 8.0% current smokers, and 31.8% of patients consumed alcohol on average ≥ 3 times per week. At the time of admission antiresorptive treatment (bisphosphonates or denosumab) received 182(14.9%) patients (26.4% with HF, 18.2% with a non-HF and 11.2% without fractures). Compared with patients without a fracture, subjects with a nonvertebral fracture were significantly older (for HF 83.0 ± 8.48 years, for non-HF 76.6 ± 9.49 years vs. 73.9 ± 8.06), much more frequent female (73.2%, 72.8 vs. 60.6%, respectively), more often living in a RCF (27.7%, 10.5% vs. 5.7%, respectively). The proportion of patients with hypertension, CHF, DM, COPD, CKD, history of malignancy, as well as current smokers and anticoagulation medication (mainly warfarin) users were similar in the three groups. Patients with fracture had significantly higher mean values of serum bCTX (+20.9%, $p=0.000$) and PTH (+11.8%, $p=0.021$), lower P1NP/bCTX ratio (-22.1%, $p=0.000$), haemoglobin ($p=0.001$) and albumin ($p=0.000$) levels. The mean serum levels of P1NP, OC, P1NP/OC ratio, 25(OH)D, creatinine, alkaline phosphatase (ALP), thyroid-stimulating hormone (TSH), free thyroxine (fT4) on admission did not differ between the three groups.

Classification of bone turnover status and fracture prevalence by subtypes

To classify bone turnover status we integrated the evidence available in the literature and used the cutoffs proposed as fracture-protective targets for osteoporotic therapy (see Methods). We used three criteria: 1) serum P1NP concentrations of $32 \mu\text{g/L}$, 2) serum bCTX of $0.250 \mu\text{g/L}$, and (3) P1NP/bCTX ratio of 100.0 (the median value in our cohort). In this study, serum bCTX $< 0.250 \mu\text{g/L}$ is referred as "normal", and the serum P1NP $< 32 \mu\text{g/L}$ is referred as

low. Subjects were initially divided into 4 groups according to bone turnover marker levels: 1) normal bone turnover- both markers (P1NP and bCTX) are normal; 2) low bone formation (P1NP $\leq 32 \mu\text{g/L}$) and normal bone resorption (bCTX $\leq 0.250 \mu\text{g/L}$); 3) low bone formation (P1NP $\leq 32 \mu\text{g/L}$) and high bone resorption (bCTX $> 0.250 \mu\text{g/L}$); 4) high bone turnover- both markers are high (P1NP $> 32 \mu\text{g/L}$ and bCTX $> 0.250 \mu\text{g/L}$). All subjects in group 1, as would be expected, had P1NP/bCTX > 100.0 , indicating that bone formation was equal or exceeded bone resorption; the absolute majority of patients in group 3 had P1NP/bCTX < 100.0 (97.6% among patients with fractures). Groups 2 and 4 were further divided into two subtypes (A and B) on the basis of the ratio P1NP/bCTX (≥ 100.0 or < 100.0). In this paper, for simplicity, we are referring to six subtypes (avoiding terms "variant" or "group"). Figure 1 illustrates the principles of classification and the prevalence of each subtype among patients admitted with and without fracture. In our cohort in total, the prevalence of elevated bCTX was 78.1%, and the prevalence of low P1NP was 38.7%; ratio P1NP/bCTX < 100.0 (bone resorption predominates bone formation) was observed in 300 (66.5%) patients with HF, but only in 116(30.9%) individuals without a fracture.

Subtype1 (normal bone turnover) was found in 67 (5.5% of the total cohort) subjects: in 39 patients with fractures (4.6% among all fractures), including 11 with HF (2.4% of all HFs), and in 28 patients without fractures (7.4% among the non-fractured). In subjects with subtype 1, compared to the rest of the cohort, risk of HF (but not other nonvertebral fractures) was 2.3 times lower (inverse association: OR 0.43, Table 1), and receiver operating characteristic (ROC) curve analysis showed the area under the curve (AUC) value of 0.7837 (75% sensitivity, 67.6% specificity and 71.6% accuracy). Interestingly, P1NP $> 62 \mu\text{g/L}$ (treatment target for anabolic therapy/ teriparatide) and normal serum bCTX ($< 0.250 \mu\text{g/L}$) was observed in total only in 9 (0.74%) patients, including 5(1.3%) without fracture, 3(0.76%) with non-HF and 1(0.22%) subject with a HF; 8 of these 9 patients (including all 4 with fractures) have been receiving antiresorptive medications.

Subtypes2A and 2B (low P1NP and normal bCTX) were observed in 201(16.4%) patients: in 64 with HF (14.2% of all HFs), 66 with non-HF (16.6% among the non-HFs) and in 71 without fracture (18.9% of all non-fractured). Among 57 subjects with subtype 2B (an imbalance between bone formation and resorption) 44(77.2%) patients presented with fractures, including 26 with HF. There was no significant difference between subjects with subtype 2A and subtype1 in prevalence of HFs ($p=0.155$) or

non-HFs ($p=0.300$), whereas in patients with subtype 2B the risk of any fracture was 3.3 times higher (OR 3.3, 95% CI 1.45-7.61, $p=0.004$) and risk of HF was 4.3 times higher (OR 4.3, 95% CI 1.73-10.68, $p=0.001$) than in subjects with subtype 1. After adjustment for age and gender, compared to the rest of the cohort, patients with subtype 1 and subtype 2A did not show significant association with presence of nonvertebral fractures, while subjects with subtype 2B, had 2.1-fold increased risk of HF (OR 2.12, 95% CI 1.00-4.53, $p=0.050$; AUC value 0.7821, 76.7% sensitivity, 66.0% specificity and 71.8% accuracy). In other words, despite low/normal levels of both BTMs contrasting association with fracture prevalence were related to the inadequate formation /resorption balance.

Subtype 3 (low P1NP and elevated bCTX) accounted for 272(22.2%) patients in the total cohort, including 137 with HF (30.4% among all HFs), 74 with non-HF (18.7% among the non-HFs) and in 61 patients without fractures (16.2% among the non-fractured). Compared to the rest of the cohort (adjusted for age and gender), patients with subtype 3 had a 1.5-fold

increased risk for any fracture (OR 1.45, 95%CI 1.04-2.01, $p=0.027$) and 1.8-fold increased risk for HF (OR 1.77, 95%CI 1.21- 2.58, $p=0.003$) with AUC values of 0.6920 and 0.7852, respectively.

Subtypes 4A and 4B (high bone turnover) were found in 683 (55.8%) patients, and in 272 (39.8%) of them the P1NP level was $>62\mu\text{g/L}$. The subtype 4B (bone resorption predominating the formation) demonstrated 295 subjects (24.1% of the total cohort), including 137 with HF (30.4% among the HFs), 97 with non-HF (24.5% among the non-HFs) and 61 without fractures (16.2% among the non-fractured). When compared to the rest of the cohort and adjusted for age and gender, subtype 4B was a significant indicator of presence of both HF (OR 1.78, 95%CI 1.121-2.62, $p=0.003$; AUC value 0.7853) or non-HF (OR 1.64, 95%CI 1.14-2.36, $p=0.008$; AUC value 0.6172). Comparison of subtypes 4A and 4B showed that in the latter odds ratio (OR) for presence of HF was 2.4-fold higher (OR 2.41, 95%CI 1.74-3.40, $p=0.000$) and for any fracture 2.6-fold higher (OR 2.55, 95%CI 1.78-3.67, $p=0.000$).

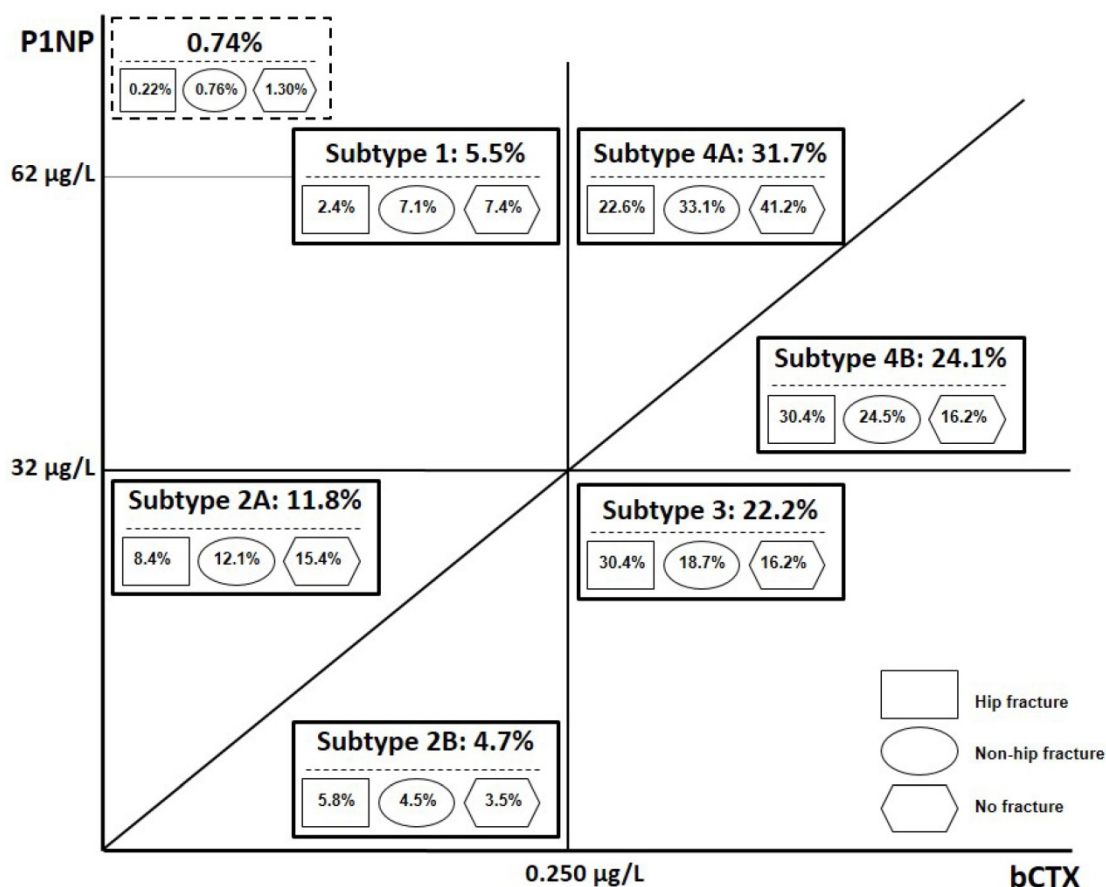


Figure 1. Schematic presentation of principles of classification of bone turnover marker status and the prevalence (%) of each subtype among hospitalized orthogeriatric patients. In subtypes 2A and 4A the ratio P1NP/bCTX >100.0 , while in subtypes 2B and 4B the ratio P1NP/bCTX <100.0 . The proportion (%) of patients with each subtype among all subjects admitted with a hip or non-hip fracture and without a fracture is shown in geometrical figures. Abbreviations: P1NP, N-terminal propeptide of type I procollagen; bCTX, C-terminal β cross-linked telopeptide of type I collagen; HF, hip fracture; non-FH, other non-vertebral fracture.

Table 1. Discriminative value of bone turnover status for non-vertebral fracture presence/prediction

Bone turnover status	Fracture site	OR	95%CI	AUC	Sensitivity, %	Specificity, %	PPV, %	NPV, %	Accuracy, %
1*. P1NP>32 µg/L, bCTX<0.250 µg/L, P1NP/ bCTX >100.0 (n=67)	Hip	0.43	0.19-0.97 (p=0.043)	0.7837	75.0	67.6	73.3	69.4	71.6
	Any fracture	0.77	0.46-1.30 (p=0.324)	0.6916	91.2	18.02	71.3	47.9	68.6
2A*. P1NP<32 µg/L, bCTX <0.250 µg/L, P1NP/ bCTX >100.0 (n=144)	Hip	0.70	0.43-1.14 (p=0.151)	0.7815	76.7	66.0	73.0	70.3	71.8
	Any fracture	0.73	0.50-1.06 (p=0.095)	0.6918	91.3	17.8	71.4	47.5	68.7
2B. P1NP<32 µg/L, bCTX <0.250 µg/L, P1NP/ bCTX <100.0 (n=57)	Hip	3.23	1.37-7.65 (p=0.008)	0.8061	66.7	84.9	76.9	77.1	71.0
	Any fracture	2.04	1.00-4.17 (p=0.051)	0.7220	82.3	39.4	69.9	56.5	66.4
3. P1NP<32 µg/L, bCTX >0.250 µg/L, P1NP/ bCTX >100.0 (n=272)	Hip	2.40	1.42-4.06 (p=0.001)	0.8124	79.0	70.1	77.0	72.5	75.1
	Any fracture	1.74	1.14-2.65 (p=0.010)	0.7194	89.9	27.9	74.0	54.7	71.0
4A. P1NP>32 µg/L, bCTX>0.250 µg/L, P1NP/ bCTX >100.0 (n=388)	Hip	0.94	0.58-1.53 (p=0.815)	0.7597	57.6	84.7	70.2	76.1	74.2
	Any fracture	0.94	0.66-1.34 (p=0.714)	0.6647	77.7	38.2	65.1	53.5	61.8
4B. P1NP>32 µg/L, bCTX >0.250 µg/L, P1NP/ bCTX <100.0 (n=295)	Hip	2.53	1.48-4.33 (p=0.001)	0.8247	81.2	72.8	79.1	75.4	77.5
	Any fracture	2.08	1.37-3.16 (p=0.001)	0.7412	91.4	26.5	75.2	55.7	72.5

The asterisk (*) on the subtypes 1 and 2A indicates comparison with the rest of the cohort. For all other subtypes comparison was made with combined data for subtypes 1 and 2A.

Abbreviations: P1NP, N-terminal propeptide of type I procollagen; β-CTX, C-terminal β-cross-linked telopeptide of type I collagen; HF, hip fracture; OR, odds ratio; AUC, area under the receiver operating characteristic curve; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

As can be seen, the most common subtypes were 4A, 4B and 3, representing 31.7%, 24.1% and 22.2%, respectively, of patients in the total cohort and 27.5%, 27.6% and 24.9%, respectively, among patients with fractures. Among individuals with P1NP/bCTX<100, patients with fractures comprised 79.2%. In patients with nonvertebral fractures, subtypes 2B, 3 and 4B were found in 5.2%, 24.9% and 27.6%, respectively, compared to 3.5%, 16.2% and 16.2% among subjects without a fracture. Conversely, among patients with subtypes 2B, 3 and 4B nonvertebral fractures had 77.2%, 77.6% and 79.3% (including a HF - 45.6%, 50.4% and 46.4%, respectively).

Because both subtypes 1 and 2A, compared to the rest of the cohort, were not significantly associated with presence of nonvertebral fractures (except an inverse association of subtype 1 with HF presence) and there were no major differences between subtype 1 and 2A in regard to fracture prevalence, we further evaluated the relationship between subtypes 2B, 3, 4A and 4B and fracture presence in comparison with combined data for subtypes 1 and 2A (Table 1). These analyses revealed that subtype 2B increases the risk of HF by 3.2-fold and the risk of any non-vertebral fracture by 2.0-fold, subtype 3 by 2.4- and 1.7-fold, respectively, and subtype 4B by 2.5- and 2.1-fold, respectively, whereas subtype 4A does not show such discriminative value (Table 1). Receiver operating characteristic (ROC) curve analyses for distinguishing HF and non-fracture patients showed the highest area under the curve (AUC) values for subtype 1 when compared to the rest of the cohort (0.7837), and for subtypes 2B (0.8061), 3 (0.8124,) and 4B (0.8247) when

compared to subtypes 1 and 2 combined. For distinguishing any non-vertebral fracture the AUC values were lower (0.7220, 0.7194, and 0.7412 for subtypes 2B, 3 and 4B, respectively). For HF, subtypes 2B, 3 and 4B had, respectively, an accuracy of 71.0%, 75.1% and 77.5%, sensitivity of 66.7%, 79.0% and 81.2%, specificity of 84.9%, 70.1% and 72.8%; for any non-vertebral fracture the corresponding values for sensitivity were 82.3%, 89.9% and 91.4%, and for specificity 39.4%, 27.9% and 26.5%, respectively.

On the other hand, subtypes 2A and 4A, both with P1NP/bCTX>100.0, were not discriminative for fracture presence, although in 37.7% of patients with fractures these subtypes of bone turnover were observed. These findings suggest that in subjects with subtypes 2A and 4A metabolic factors other than reflected by serum P1NP and bCTX may be more relevant for assessing bone quality and fracture development.

Bone turnover status and other parameters related to bone and mineral metabolism

The profiles of bone-mineral metabolism in subjects with different subtypes of bone turnover demonstrated, as would be expected, significant differences in a number of parameters in addition to the variables used for classification (Table 2). Subtype 1, compared to subtype 2A, showed higher mean levels of bone formation markers (P1NP, OC, alkaline phosphatase [ALP]), bone resorption (bCTX), as well as P1NP/bCTX and P1NP/OC ratios. Comparison with combined data from subtypes 1 and 2A revealed the following statistically significant

differences in the mean values. For subtype 2B: lower P1NP, OC, ALP, phosphate, albumin and haemoglobin concentrations, P1NP/bCTX and P1NP/OC ratios, and higher bCTX and PTH levels. For subtype3: higher bCTX (2.7-fold) and lower P1NP (2-fold), ALP, magnesium, albumin, haemoglobin, transferrin saturation and GFR levels, P1NP/bCTX and P1NP/OC ratios. For subtype 4A: higher concentrations of P1NP (3.7-fold), bCTX (3.3-fold) OC, ALP (about 2-fold each), phosphate, calcium (corrected for albumin) and GGT, significantly elevated P1NP/OC ratio, but lower magnesium, albumin and haemoglobin levels. For subtype 4B: a 4.3-fold higher bCTX concentration, higher P1NP, OC (both about 2-fold), ALP, PTH, phosphate, and lower magnesium, albumin, haemoglobin and GFR levels, as well as P1NP/bCTX ratio. Subtypes 4A and 4B, despite similarities in the direction of changes in P1NP, OC, ALP, bCTX, phosphate, magnesium, albumin, haemoglobin and GFR, demonstrated significant differences. Patients with subtype 4A comparing to those with subtype 4B exhibited higher mean values for P1NP, calcium (in absence of overt

hypercalcaemia), P1NP/OC ratio and lower values for bCTX and PTH ($p < 0.001$ for all variables), indicating a higher bone formation, lower bone resorption as well as a strong coupling of bone formation and resorption.

Bone turnover status and clinical characteristics

We analysed the associations of bone turnover subtypes with the following chronic comorbidities: dementia, hypertension, coronary artery disease (CAD), atrial fibrillation (AF), chronic heart failure (CHF), history of myocardial infarction, stroke, transitional ischaemic attack, malignancy, peripheral vascular disease (PVD), diabetes (DM), chronic kidney disease (CKD), chronic obstructive pulmonary disease (COPD), anaemia, Parkinson's disease, osteoarthritis, and use of osteoporotic medications prior to admission. The analysis also included relation to smoking (current or ex-smoker), alcohol consumption (more than 3 times a week), use of a walking device and residential status (living in a long-term RCF).

Table 2. Parameters of mineral-bone metabolism and related variables in orthogeriatric patients by bone turnover status

Variable	Bone turnover status						
	Subtype 1	Subtype 2A	Subtypes 1+2A	Subtype 2B	Subtype 3	Subtype 4A	Subtype 4B
P1NP, µg/L	46.20±18.71	22.85±5.85***	30.26±15.88	14.83±3.98***	23.02±5.84***	112.02±142.45***	50.45±19.57***
bCTX, µg/L	0.195±0.038	0.160±0.049***	0.171±0.048	0.190±0.036**	0.458±0.210***	0.563±0.405***	0.740±0.317***
P1NP/bCTX	247.62±112.04	151.88±52.31***	182.28±88.34***	78.30±15.52***	57.17±22.82***	187.12±113.49	71.96±17.98***
OC, pg/ml	5.91±2.35	4.02±1.86***	4.62±2.21	3.86±2.65*	4.60±2.30	8.48±5.32***	8.60±5.13***
P1NP/OC	9.36±6.58	6.82±3.51***	7.63±4.83	4.75±2.04***	6.15±3.30***	15.48±17.21***	7.28±4.16
PTH, pmol/L	6.88±6.33	6.80±4.99	6.82±5.44	8.49±5.69*	7.67±5.21	6.30±4.52	8.65±6.09***
25(OH)D, mmol/L	62.55±21.54	63.22±23.67	63.00±22.97	62.23±23.67	62.78±26.67	64.53±25.85	61.04±29.37
Ca (corrected), mmol/L	2.41±0.14	2.38±0.11	2.39±0.12	2.36±0.14	2.38±0.14	2.44±0.13***	2.41±0.14
PO ₄ , mmol/L	0.87±0.22	0.83±0.24	0.84±0.23	0.77±0.24*	0.84±0.23	0.98±0.23***	0.97±0.25***
Mg, mmol/L	0.79±0.08	0.77±0.09	0.78±0.09	0.77±0.10	0.76±0.11*	0.76±0.10*	0.76±0.09*
ALP, IU	78.73±27.66	66.85±26.47*	72.00±27.18	59.32±17.62***	73.48±38.20	112.28±104.33***	91.43±72.98***
GGT, IU	44.30±33.46	47.02±72.78	46.15±62.88	40.32±51.53	49.41±64.69	62.88±82.82*	51.16±84.28
Albumin, g/L	34.49±3.87	33.90±4.06	34.09±4.00	32.82±3.6*	31.03±3.85***	32.34±4.72***	31.07±4.40***
TSAT, %	12.52±7.68	11.51±7.63	11.82±7.64	9.68±6.38	9.65±7.00***	12.63±8.38	12.04±9.54
Hb, g/L	117.10±17.90	115.01±17.56	115.7±17.65	107.37±16.66**	107.71±18.03***	109.00±16.93***	106.89±18.02***
GFR, ml/min/1.73m ²	79.43±10.50	78.47±14.11	78.77±13.05	74.96±14.75	74.24±16.77***	71.58±19.51***	67.39±22.45***
Age, years	74.4±8.63	75.5±8.66	75.1±8.65	77.6±8.60	80.4±9.17***	76.6±9.25	80.3.5±9.92***

Subtype 2A is compared with subtype 1, while subtypes 2B, 3, 4A and 4B are compared with combined data for subtypes 1 and 2A; *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$.

Abbreviations: P1NP, N-terminal propeptide of type I procollagen; bCTX, C-terminal β-cross-linked telopeptide of type I collagen; OC, osteocalcin; PO₄, phosphate; Ca, calcium corrected for albumin; Mg, magnesium; ALP, alkaline phosphatase; GGT, gamma-glutamyltransferase; 25(OH)D, 25hydroxyvitamin D; PTH, parathyroid hormone; TSAT, transferrin saturation; Hb, haemoglobin; GFR, glomerular filtration rate.

There was no significant difference between patients with subtypes 1 and 2A in regard to sociodemographic parameters, prevalence of fractures (including HF) and comorbid conditions, as well as in mean values of most laboratory variables (except P1NP, OC, ALP, bCTX, P1NP/bCTX and P1NP/OC ratios) and short-term outcomes. Therefore, data for types 1 and 2A were combined, and other subtypes were compared with the combined data. Patients with 2B, 3, 4A and 4B subtypes showed remarkable differences in regard to clinical characteristics. Compared to subtypes 1 and 2A, individuals with 3, 4A and 4B subtypes were more likely to have CKD (18.0%, 23.5% and 30.2% vs. 10.4%, respectively), anaemia (76.5%, 72.7% and 75.3% vs. 56.9%), history of malignancy (12.1%, 12.1% and 11.9% vs. 6.2%), to use a walking device (42.9%, 45.4% and 48.1% vs. 26.1%), and least likely to receive anti-osteoporotic treatment (13.6%, 11.9% and 10.5% vs. 23.2%). Patients with subtypes 3 and 4B were significantly older (+ 5 years on average) and demonstrated a significantly higher prevalence of dementia (20.2% and 17.3% vs. 9.0%, respectively), CHF (9.2% and 11.5% vs. 2.8%) and hyperparathyroidism (43.8% and 49.7% vs. 32.2%). Subtype 2B was also associated with hyperparathyroidism (49.1% vs. 32.2%). Subjects with subtype 3 were more likely to be residents of RCF (21.7% vs. 12.8%). Subtype 4B demonstrated a lower prevalence of diabetes mellitus (DM, 18.6% vs. 26.5%) and alcohol over-users (26.8% vs. 31.8%).

Independent clinical indicators/predictors of bone turnover status

We further performed multivariate logistic regression analyses with a backward stepwise approach for presence of bone turnover subtypes 2B, 3, 4A and 4B, including in the models the following variables: dementia, CHF, anaemia, CKD, history of malignancy, DM, vitamin D status, hyperparathyroidism, hypoalbuminaemia, use of walking aids, RCF residence, alcohol overuse, smoking (current and previous), use of anti-resorptive medications (>3 months), gender and age; age was evaluated as a continuous and as a categorical (>75 years) variable in separate models. As can be seen in Table 3, following these analyses, subtype 2B was independently predicted by 2 variables, subtype 3 by 6 variables, subtype 4A by 6, and subtype 4B by 4 variables. For every year increase in age there was a 6% increase in probability of subtype 3 and a 5% increase in probability of subtype 4B. Compared to subjects with subtypes 1 and 2A, among aged >75 years the presence of subtype 2B was 1.9-fold higher and presence of subtypes 3 and 4B was 2.5-fold higher. Hyperparathyroidism was the only other

independent predictor for subtype 2B. For subtypes 3, 4A and 4B hypoalbuminaemia on admission was a significant independent positive indicator while use of osteoporotic treatment was an independent negative predictor. Anaemia and history of malignancy were independent predictors of subtypes 3 and 4A, presence of CHF strongly indicated subtype 3, and CKD correlated independently with subtypes 4A and 4B.

Taken together, these results suggest that different bone turnover subtypes are linked to specific clinical characteristics (constellation of specific clinical variables) which can be used as indicators/predictors of altered bone turnover status. In other words, the clinical profile may serve as an early warning sign indicative of a possibly abnormal bone turnover status, and, *vice versa*, the bone turnover subtype may suggest the need of further evaluation for extraskeletal diseases. For example, subtype 3 is associated with and can be predicted by a clinical profile encompassing advanced age, CHF, anaemia, hypoalbuminaemia and history of malignancy. Presence of any of these conditions should raise the alarm regarding bone status and associated high risk for nonvertebral fracture, especially HF; conversely, in a patient with subtype 3 presence of previously non-diagnosed chronic conditions (e.g., CHF, anaemia, hypoalbuminaemia) as well as lack of osteoporotic treatment should be considered.

Bone turnover status and short-term outcomes

The association between bone turnover subtypes and comorbidities led us to investigate whether bone status can predict adverse in-hospital outcomes. In total, there were 32 deaths corresponding to in-hospital mortality of 2.6%: 25 (5.5%) deaths occurred among patients admitted with HF, and 7 (1.8%) among subjects with non-HF. Among patients with subtype 3 there were 11 (4.0%) non-survivors, among subjects with subtype 4A - 11 (2.8%), among patients with subtype 4B - 9 (3.0%) and among patients with subtype 2B - 1 (1.8%). None of the patients with subtypes 1 or 2A died.

Post-operative myocardial injury with cardiac troponin I rise was observed in 444 (36.4%) patients including 16 (24.2%) with subtype 1, 36 (25.0%) with subtype 2A, 16 (28.1%) with subtype 2B, 125 (46.0%) with subtype 3, 112 (28.9%) with subtype 4A and 139 (47.3%) patients with subtype 4B. Comparing to subjects with subtypes 1 and 2A, the OR for this complication obtained in patients with subtype 3 was 2.6 (95% CI 1.7-3.9, $p=0.000$) and in subjects with subtype 4B 2.7 (95% CI 1.8-4.1, $p=0.000$); after adjustment for age and gender the ORs were 2.1 and

2.2, respectively (Table 4); however, these associations become non-significant in fully adjusted models.

A high and persistent (≥ 3 days) post-operative inflammatory response was mostly related to urinary tract, respiratory or skin infections; elevated CRP of $>100\text{mg/L}$ and $>150\text{mg/L}$ demonstrated 553(45.3%) and 348(28.5%) patients, respectively. In models adjusted for age and gender, subtype 3 was a significant predictor of both CRP $>100\text{mg/L}$ (OR 2.4, $p<0.001$) and CRP $>150\text{mg/L}$ (OR 1.7, $p=0.006$), subtype 4B predicted CRP $>150\text{mg/L}$ (OR1.5, $p=0.038$), while subtypes 2B and 4A were not predictive for inflammatory marker raise. In fully adjusted models, only subtype 3 showed a significant link with CRP $>100\text{mg/L}$ (OR1.8, $p=0.013$).

The length of hospital stay (LOS) was ≥ 10 days in 530(43.3%) patients and ≥ 20 days in 256(20.9%). Compared to patients with subtypes1 and 2, in

subjects with subtypes 3, 4A and 4B the corresponding ORs for LOS ≥ 10 days were 1.8 (95%CI 1.2-2.7, $p=0.004$), 2.3 (95%CI 1.6-3.1, $p=0.000$) and 2.3 (95%CI 1.6-3.4, $p=0.000$), and for LOS ≥ 20 days 1.8 (95%CI 1.1-3.1, $p=0.026$), 2.6 (95%CI 1.6-4.2, $p=0.000$), and 1.7 (95%CI 1.1-2.9, $p=0.044$), respectively. After adjusting for age and gender the ORs did not change significantly, although subtypes 3 and 4B showed borderline significance for LOS ≥ 20 days. In fully adjusted models, a strong association remained only for subtype 4A.

New discharges to a RCF required 45(5.7%) patients: 1.9% of subjects with subtype1, 5.1% with subtype 2A and 6.8%, 6.8%, 5.2% and 6.8% of patients with subtypes 2B, 3, 4A and 4B, respectively; the differences between subtypes in the percentage of patients being discharged to RCFs did not reach statistical significance (Table 4).

Table 3. Independent and significant clinical and biochemical correlates/predictors of bone turnover status in orthogeriatric patients

Variables	Bone turnover status											
	Subtype 2B			Subtype 3			Subtype 4A			Subtype 4B		
	OR	95%CI	P Value	OR	95%CI	P Value	OR	95%CI	P Value	OR	95%CI	P Value
Age				1.06	1.03-1.08	<0.001				1.05	1.03-1.07	<0.001
Age>75yrs*	1.87	1.01-3.47	0.048	2.49	1.64-3.79	<0.001				2.51	1.65-3.82	<0.001
Anaemia				1.82	1.15-2.87	0.010	1.57	1.00-2.47	0.048			
CKD							2.04	1.16-3.58	0.013	3.08	1.78-5.32	<0.001
CHF				3.01	1.13-7.97	0.027						
History of malignancy				2.16	1.04-4.48	0.039	2.17	1.02-4.60	0.045			
Hyperparathyroidism	1.87	1.01-3.46	0.046									
Hypoalbuminaemia				2.48	1.62-3.80	<0.001	1.6	1.03-2.49	0.036	2.14	1.44-3.19	<0.001
OPT				0.32	0.19-0.56	<0.001	0.29	0.17-0.49	<0.001	0.25	0.15-0.44	<0.001
Walking aids use							2.3	1.32-3.12	0.001			

Only statistically significant associations (compared to subjects with subtypes 1 and 2A) are shown. The backward stepwise regression models included dementia, CHF, anaemia ($<120\text{g/L}$), CKD (GFR $<60\text{ ml/min/1.73m}^2$), history of malignancy, diabetes mellitus, vitamin D insufficiency (25(OH) D $<50\text{ mmol/L}$) or deficiency (25(OH) D $<25\text{ mmol/L}$), hyperparathyroidism (PTH $>6.8\text{pmol/L}$), hypoalbuminaemia ($<33\text{g/L}$), use of walking aids, nursing home residence, alcohol use (> 3 times/week), smoking (current and previous), use of anti-osteoporotic medications (>3 months) and adjusted for age and gender. * evaluated in separate models.

Abbreviations: OR, odds ratio; CI, confidence interval; CKD, chronic kidney disease; CHF, chronic heart failure; OPT, osteoporotic therapy; GFR, estimated glomerular filtration rate.

Table 4. Bone turnover status and in-hospital outcomes

Outcomes	Bone turnover status											
	Subtype 2B			Subtype 3			Subtype 4A			Subtype 4B		
	OR	95%CI	P Value	OR	95%CI	P Value	OR	95%CI	P Value	OR	95%CI	P Value
cTnl rise	0.97	0.49-1.92	0.934	2.05	1.36-3.09	0.001	1.13	0.77-1.68	0.535	2.17	1.44-3.26	<0.001
	0.80	0.38-1.68	0.561	1.40	0.90-2.19	0.139	0.84	0.55-1.30	0.440	1.38	0.88-2.17	0.162
LOS>10days	1.08	0.57-2.04	0.891	1.54	1.03-2.28	0.034	2.20	1.54-3.15	<0.001	2.08	1.41-3.06	<0.001
	0.95	0.49-1.84	0.876	1.16	0.76-1.76	0.496	2.18	1.48-3.20	<0.001	1.74	1.17-2.61	0.007
LOS>20days	0.88	0.36-2.17	0.783	1.65	0.99-2.75	0.058	2.49	1.57-3.96	<0.001	1.54	0.92-2.56	0.098
	0.80	0.32-2.01	0.640	1.30	0.76-2.23	0.333	2.61	1.60-4.26	<0.001	1.40	0.82-2.36	0.209
CRP>100mg/L	1.74	0.92-3.29	0.088	2.40	1.59-3.63	<0.001	1.23	0.87-1.74	0.238	1.30	0.88-1.91	0.184
	1.67	0.81-3.43	0.165	1.75	1.12-2.72	0.013	0.97	0.66-1.42	0.883	0.94	0.61-1.44	0.778
CRP>150mg/L	1.24	0.68-2.26	0.483	1.70	1.16-2.49	0.006	1.28	0.91- 1.81	0.156	1.49	1.02-2.17	0.038
	1.18	0.62-2.24	0.622	1.44	0.96-2.15	0.078	1.04	0.72-1.52	0.821	1.20	0.80-1.78	0.374
New RCF d/c	1.31	0.31-5.50	0.709	1.26	0.47-3.42	0.649	1.09	0.42-2.83	0.858	1.36	0.50-3.68	0.858
	0.85	0.17-4.30	0.847	1.18	0.38-3.67	0.781	1.09	0.41-2.89	0.857	1.48	0.47-4.66	0.507

Multivariate regression comparisons with subtypes1and 2A combined.

Model 1 (1st line): adjustment for age and gender. Model 2 (2nd line): included chronic heart failure, dementia, chronic kidney disease (GFR $<60\text{ml/min/1.73m}^2$), history of malignancy, PTH $>6.8\text{pmol/L}$, albumin $<33\text{ g/L}$, anaemia (haemoglobin $<120\text{g/L}$), hip or any non-vertebral fracture, use of osteoporotic treatment, age and gender.

Abbreviations: OR, odds ratio; CI, confidence interval; cTnl, cardiac troponin I; LOS, length of hospital stay; CRP, C-reactive protein; RCF d/c, new discharges to a permanent residential care facility.

Discussion

Main findings

In the current study, we proposed a model for classification bone turnover status and evaluated the clinical usefulness (advantages and limitations) of such approach. The classification scheme is based on optimal treatment targets and captures three significant and widely accepted factors of bone metabolism - bone formation, bone resorption and their ratio, indices that reflect bone remodelling in the entire skeleton. We defined six subtypes of bone turnover and showed that among hospitalized orthogeriatric patients these subtypes differed substantially in terms of clinical characteristics, including prevalence of nonvertebral fractures, especially HF, chronic comorbid conditions and in-hospital outcomes. Subtypes suggestive an imbalance in bone turnover favouring an increase in bone resorption demonstrated a good/moderate discriminative ability in regard to non-vertebral fracture presence. The study highlights the similarities and differences between subtypes and indicates that the future classification should also include other indices of bone metabolism which may better reflect bone health and fracture risk.

In osteoporosis, a multifactorial heterogeneous disease, bone formation and bone resorption, though mutually dependent through crosstalk between osteoblasts and osteoclasts, may be affected differently, and, not surprisingly, various patterns of bone metabolism (determined by specific genetic, metabolic and clinical factors) occur. The present study, to our knowledge, is the first of its kind, evaluating the clinical significance of different subtypes of bone turnover markers in the elderly. Identifying the bone turnover status in the elderly is an important key to better understand underlying pathophysiological mechanisms and may have an advantage in at least three areas: individualized management, prediction of nonvertebral fractures, and prognosis of in-hospital outcomes. Table 5 presents an overview of our findings.

Classification of bone turnover status

Our classification is three-fold: it takes into account bone formation, bone resorption and the balance between these processes. Due to existing controversy concerning reference intervals of BTMs, for classification we used as cutoffs values recently proposed optimal treatment targets for anti-resorptive therapy. It worthy of mention in this connection that many, but not all [1, 12], studies suggested that P1NP and bCTX may provide information about both response to treatment and reduction of fracture risk

following osteoporotic therapy with antiresorptive [7, 23, 24, 26, 48, 51, 52] or anabolic [6, 53-59] agents. Almost all published studies demonstrated reduction in serum bCTX during antiresorptive therapy and rise in serum P1NP during therapy with teriparatide; these changes have been associated with an improvement in BMD and reduced fracture risk. The importance to examine the balance between formation and resorption when evaluating bone turnover has also been recognized [60-64].

Table 5. Overview of the relationships between altered bone turnover status and presence of non-vertebral fracture, clinical characteristics and in-hospital outcomes

	Bone turnover status			
	Subtype 2B	Subtype 3	Subtype 4A	Subtype 4B
Fracture risk (compared to subtypes 1 and 2):				
HF, (OR)	↑,3.2	↑,2.4		↑,2.5
Any fracture, (OR)	↑,2.0	↑,1.7		↑,2.1
Independent clinical indicators/predictors:				
Age>75years, (OR)	↑,1.9	↑,2.5		↑,2.5
Hypoalbuminaemia,(OR)		↑,2.5	↑,1.6	↑,2.1
Anaemia, (OR)		↑,1.8	↑,1.6	
CKD, (OR)			↑,2.0	↑,3.1
CHF, (OR)		↑,3.0		
Hyperparathyroidism, (OR)	↑,1.9			
History of malignancy, (OR)		↑,2.1	↑,2.2	
Walking aids use, (OR)			↑,2.3	
OPT, (OR)		↓,0.32	↓,0.29	↓,0.25
In-hospital outcomes:				
Myocardial injury with cTnI rise, (OR)		↑, 2.1*		↑,2.2*
LOS>10 days, (OR)		↑,1.5*	↑,2.2	↑,1.7
LOS>20 days, (OR)			↑,2.6	
CRP>100mg/L, (OR)		↑,1.8		
CRP>150mg/L, (OR)		↑,1.7*		↑,1.5*
In-hospital death, (%)	1.8	4	2.8	3

Data reflect only statistically significant results compared to subtypes 1 and 2A (combined) in multivariate adjusted regression models; the asterisk (*) indicates statistical significance in models adjusted only for age and gender.

Abbreviations: OR, odds ratio; CHF, chronic heart failure; CKD, chronic kidney disease; cTnI, cardiac troponin I; LOS, length of hospital stay; CRP, C-reactive protein (marker of systemic inflammatory response).

Bone turnover status and fractures

Despite the wide heterogeneity of bone turnover markers, from low to significantly elevated, even within the same fracture type, specific subtypes of turnover status demonstrate different impact on fracture development, and may, therefore, provide a rough estimate of individual risk. Elevated BTMs, a sign of an increased turnover rate, is commonly reported as a factor which adversely influences BMD and increases fracture risk. Consistent with this data, in our study, high levels of both biomarkers were found in 55.1% of all patients with fractures. In this context, the low prevalence of fractures among subjects with subtype1 (normal bCTX and P1NP>32

$\mu\text{g/L}$ and, especially, with $\text{P1NP} > 62 \mu\text{g/L}$), appears to contradict previous studies, who found a protective effect of low P1NP. In the present study, among patients with $\text{bCTX} < 0.250 \mu\text{g/L}$, higher levels of P1NP were associated with lower prevalence of fractures: with $\text{P1NP} > 32 \mu\text{g/L}$ there were in total 67(5.5%) patients [11(2.4%) among HFs], including with P1NP levels between $32 \mu\text{g/L}$ and $62 \mu\text{g/L}$ – 35(4.1%) patients [10(2.2%) among HFs] and with $\text{P1NP} > 62 \mu\text{g/L}$ only 4(0.47%) patients [including 1(0.22%) among HFs], whereas with $\text{P1NP} < 32 \mu\text{g/L}$ there were 130(15.3%) patients [64(14.2%) among HFs]. On the other hand, bCTX was elevated in 683(91.1%) of 750 subjects with $\text{P1NP} > 32 \mu\text{g/L}$ and in 272(96.8%) of 281 patients with $\text{P1NP} > 62 \mu\text{g/L}$. These data strongly suggest that higher P1NP becomes a risk factor for fracture only in the presence of elevated bCTX and lower P1NP/bCTX ratio (subtype 4B), showing that the ratio is of major importance in assessing bone status rather than each of the markers taken alone; it appears that well-balanced formation/resorption processes are protective. These observations indicate that an increase or a decrease in serum P1NP can be related to positive or negative effects, depending on the bCTX level and P1NP/bCTX ratio, and should not be interpreted in isolation. These data may explain the conflicting results in the literature on the associations between P1NP, BMD and fractures [42, 65-67].

Obviously, bone turnover status as defined by subtypes is more closely related to presence of osteoporotic fractures compared with separate indicators of bone metabolism. Patients with subtypes 2B, 3 and 4B, compared to those with subtypes 1 and 2A, have a 3.2-, 2.4- and 2.5-fold increase in risk for HF, respectively, and a 2.0-, 1.7- and 2.1-fold increase in risk for any nonvertebral fracture, respectively. Subtypes 2B, 3 and 4B showed good AUC values (0.8061- 0.8247) for HF, and fair AUC values (0.7194 - 0.7412) for any nonvertebral fracture, and had an acceptable sensitivity for estimating risk of any fracture of 82.3%, 89.9% and 91.4%, respectively, but a low specificity (39.4%, 27.9% and 26.5%, respectively). These data shows that dysregulation in skeletal metabolism causing bone frailty is a necessary but not a sufficient condition for fracture development. A nonvertebral bone fracture usually occurs as a result of a fall in a person with frail bones. The deterioration of main components of bone strength - bone mass, microarchitecture and remodelling/metabolism - not always remains in parallel; bone quality is only partially reflected by BTMs. Most patients with osteoporosis (as defined by BMD) do not have a fracture and at least half of fractures occur in patients without osteoporosis, while both high and low BTMs

are associated with increased fracture risk. Subtypes 2B, 3 and 4B because of their relatively high sensitivity can be useful as screening tools to identify patients at fracture risk. However, subtype 4A (both markers elevated and the ratio $\text{P1NP/bCTX} > 100.0$) which constituted 27.5% of all patients with fractures (22.6% among all HF patients), does not discriminate presence of nonvertebral fractures against subtypes 1 and 2A (seen in 14.8% of all patients with fractures and in 10.9% of HF patients). The fact that fractures in subjects with subtype 4A are not entirely explained by elevated P1NP and bCTX may suggest that high bone turnover with preserved P1NP/bCTX ratio occur rather as a compensatory/adaptive response to bone loss but it is not sufficient enough to provide adequate bone quality and prevent fracture. Apparently, the described classification model does not completely represent the (patho)physiology of bone metabolism; P1NP, bCTX and their ratio reflect important but not all factors contributing to bone health and fragility fractures, and additional biomarkers are required to characterise more accurately bone quality and predict fractures, especially in subjects with subtype 4A.

Nevertheless, the results of the study raise the possibility that identifying subtypes of bone turnover may provide the basis for pharmaceutical treatment decisions: (a) focus on those at greatest risk and avoid complications of therapy (although current osteoporotic drugs are relatively benign) in individuals with low risk, and (b) choose between therapeutics which mainly suppress bone resorption or stimulate bone formation.

Bone turnover status and other indices of bone and mineral metabolism

Compared to subjects with subtypes 1 and 2A, patients with subtypes 2B-4B demonstrated significant differences in calcium (higher in subtype 4A), phosphate (lower in subtype 2B and higher in 4A and 4B), magnesium (lower in subtypes 3, 4A and 4B) and PTH concentrations (elevated in subtypes 2B and 4B), as well as in P1NP/OC ratio (decreased in subtypes 2B and 3 but elevated in subtype 4A). These heterogeneous disturbances of parameters of mineral-bone metabolism reflect the complexities underneath the bone turnover subtypes indicating, among other mechanisms, the important role of fibroblast growth factor 23 (FGF23), a hormone secreted mainly by osteocytes that modulates serum phosphate balance, PTH and 1,25(OH) vitamin D synthesis [68-71] and sclerostin, a glycoprotein also produced by mature osteocytes that influences differentiation and survival of osteoblasts [72-74]. It could be speculated that these and other hormones, not evaluated in this study, may be the modifying

factors contributing to bone fragility and fractures, especially in subjects with subtype 4A.

Our findings add to previous studies linking separate BTMs with fracture risk evidence of higher discriminative value of specific bone turnover subtypes, and also suggest that other biomarkers of bone metabolism need to be included in the classification to improve fracture prediction and identify novel therapeutic targets in the geriatric population.

Bone turnover status and chronic clinical conditions

Although age-related chronic diseases and frailty [75-81] are among known factors predisposing to bone loss, falls and fractures, no previous studies evaluated the relationship between bone turnover status and clinical characteristics. Our data clearly showed that in the elderly, subtypes of bone metabolism are associated with and can be predicted by specific clinical conditions. On univariate analyses 16 different variables were associated with distinct bone turnover subtypes. Backward stepwise linear regression analyses after adjusting for main confounders revealed several chronic conditions as independent and significant predictors/indicators of specific bone turnover subtypes (Tables 3 and 5). Among patients with subtypes 2B, 3, and 4B the proportion of aged >75 years was 1.9-2.5 times higher than in those with subtypes 1 and 2A. Other independent correlates included the following: for subtype 2B - hyperthyroidism; for subtype 3 - hypoalbuminaemia, anaemia, CHF, and history of malignancy; for subtype 4A - hypoalbuminaemia, anaemia, CKD, history of malignancy and use of walking aids; for subtype 4B - hypoalbuminaemia and CKD. Despite an overlap between the variables, specific conditions characterised the respective subtype. Each of these clinical variables indicated the presence of a specific bone turnover subtype with an OR of 1.6-3.1 (Tables 3 and 5). As would be expected, OPT was inversely associated with subtypes 3, 4A and 4B (risk decreased by 68-75%). The observation that OPT may be highly effective is important because in recent years worldwide the percentage of OPT users has declined [82-84]. In our cohort, approximately 85% of patients did not receive OPT.

Our results are in line with numerous evidences of multidirectional (patho)physiological links between the skeleton as a dynamic, metabolically active organ and the cardiovascular, renal, liver, endocrine, nervous and immune systems. Our observations complement the results from many previous studies showing positive bi- and multi-directional relationships between osteoporosis,

falls and fractures, on one hand, and chronic conditions such as CKD [71, 85-90], CVDs [91-104], anaemia [105-111], hypoalbuminaemia [112-114] and hyperthyroidism [115-119] on the other, supporting the concept that osteoporosis is a systemic disease.

Despite the growing knowledge of bone-extraskeletal interactions, the reasons and implications for this connection(s) remain not fully understood. These associations may be driven by shared risk factors (advanced age, reduced physical activity, malnutrition, disturbed inflammatory and antioxidant responses, etc.), common genetic basis and multiple common biochemical pathways involved in the regulation of both bone and extraskeletal metabolism (PTH, 25(OH) vitamin D, osteoprotegerin/RANK ligand/RANK axis, FGF23, sclerostin-Klotho axis, bone morphogenetic proteins, adipocytokines, autonomic nervous system). Given the wide range of genetic, metabolic and environmental (medications, smoking, alcohol, etc) factors influencing production, release and removal of P1NP and bCTX, it is not surprising that different subtypes of bone turnover status reflecting altered mechanisms of either bone formation or bone resorption or both were found to be associated with different chronic conditions. It can be assumed that common factors contribute to constellation of specific patterns of abnormal metabolism (including bone metabolism) and diseases, predisposing to falls and osteoporotic fractures. The presence of relatively specific clinical profiles for each turnover subtype implies that altered turnover status may serve as a warning sign for both frail bones and extraskeletal diseases. The provided data suggests that in an elderly person presenting with the abovementioned clinical conditions it would be worth considering impaired bone turnover (with increased fracture risk) and the need for special bone examination, and *vice versa*, presence of an unfavourable turnover status may help to identify earlier individuals with serious medical problems requiring intervention (e.g. CKD, CHF, anaemia), despite the absence of clinical signs and symptoms.

Bone turnover status and outcomes

In older orthopaedic patients, hospital complication rates are high (up to 50 % after surgery for hip fracture) indicating a vital need of early identification of individuals prone to postoperative morbidity and mortality. This study demonstrated for the first time that in the orthogeriatric population, altered bone turnover status is associated with poorer in-hospital outcomes, and that specific subtypes independently of established clinical risk factors (age, gender and presence of multi-morbidities) predict

myocardial injury, high inflammatory response, prolonged LOS, and all-cause mortality. Subtypes 3, 4A and 4B accounted for 31 (96.9%) of 32 in-hospital deaths, while no fatal outcomes occurred among 211 subjects with subtypes 1 and 2A. This observation is in line with studies on older adults living in the community or RCFs showing a positive association between all-cause mortality and bCTX levels [120-123] as well as osteoporosis defined by BMD [124-128].

Models adjusted for age and gender demonstrated that, compared to subjects with subtypes 1 and 2A, patients with subtype 3 had a significantly higher risk of developing postoperative myocardial injury (OR 2.1), inflammatory/infective complications with CRP>100 mg/L (OR 2.4) and LOS>10 days (OR 1.5). Similarly, subtype 4A was predictive for prolonged hospitalisation (for LOS>10 days OR 2.2 and for LOS>20 days OR 2.5), and subtype 4B indicated an increased risk of myocardial injury (OR 2.2), high inflammatory response with CRP>150 mg/L (OR 1.5) and LOS>10 days (OR 2.1). After controlling for multiple comorbidities, presence of HF or any non-vertebral fracture, OPT, age and gender (fully adjusted models, Tables 4 and 5), subtype 3 remained a significant independent predictor for CRP>150 mg/L (OR 1.7), subtype 4A for prolonged LOS (OR 2.2 and 2.6 for LOS>10 days and >20 days, respectively), and subtype 4B for LOS>10 days (OR 1.7). Compared to subtypes 1 and 2A, the subtype 2B did not demonstrate significant associations with the outcomes. These analyses show that turnover subtypes independently of a variety of clinical characteristics (on admission) known to adversely affect outcomes can help in individualized risk assessment identifying patients in whom poorer outcomes to be expected and additional interventions planned.

No studies on predictive value of bone turnover status for hospital outcomes in orthogeriatric patients have been reported. Previous studies found in critically ill patients on admission significantly elevated bone resorption markers, including bCTX levels ([129-133]), and low-normal P1NP levels [132, 133]. Lower BMD was shown to predict myocardial infarction in men and women during 5.7 years of follow-up [97]. Other studies identified higher P1NP levels as a risk factor for incident myocardial infarction in older men followed for 7 years [134] and as a biomarker of frailty [77].

Taking together, the study illuminates the close and complex relationship between bone turnover status, nonvertebral fractures, functioning of other systems and in-hospital outcomes.

The proposed classification model of bone turnover status may be of relevance for clinical

management, as well as for research. While requiring further replication, our data highlights the heterogeneity of bone turnover status, identifies distinct subtypes, their association with chronic conditions and usefulness for better patient stratification for more individualised approaches. This pilot classification is a first step towards integrative understanding bone metabolism, further exploration of the underlying pathophysiologic differences between various turnover subtypes is needed. Subtyping could be improved by adding new biomarkers. The combination of bone turnover subtypes with other diagnostic and prognostic tools may improve the preventive and treatment strategies for the elderly.

Limitations and strengths

Several limitations of our study should be considered. Firstly, we recognize the limitations of the classification model: it is based on BTM values proposed as treatment targets, the cutoffs are arbitrarily chosen, the two markers analysed reflect mainly the function of osteoblasts and osteoclasts, but biochemical indicators of the osteocyte activity, important factors in the maintenance of skeletal integrity [135], are not included (currently these markers are used for research purposes). Therefore, the pathogenesis of bone metabolism is only partially represented by various subtypes, and there is no evidence at present that all patients with a particular subtype share the same metabolic defect(s). Although the proposed classification brings a conceptual shift in our understanding of the pathophysiology and clinical applications of bone turnover status, additional biochemical indicators of the complex multilayered regulatory mechanisms need to be found and considered in future classification. The model, despite its limitations, illustrates the clinical opportunities of an integrative approach based on simultaneous use of a formation and resorption marker and their ratio compared to those which relied on analysis of these biomarkers separately. Secondly, the subjects in our study do not represent the general population, they were selected from hospitalised orthogeriatric patients, and a significant proportion of individuals admitted without fractures may have had undiagnosed/undocumented osteoporosis. Thirdly, the study has been done in a single centre, and the patients were mainly Caucasian; that could affect the generalizability of the results. Fourthly, the cross-sectional design of the study does not allow causal conclusions, and, despite multivariate analyses with extensive adjustment for potential confounders, the possibility of residual unmeasured confounders could not be excluded.

Our study has also several strengths. For the first time, a practical method for subtyping bone turnover status using three criteria (both serum bone formation and bone resorption markers and their ratio as a reflection of the bone turnover balance) was provided. Six bone turnover subtypes were identified and in a relatively large cohort of orthogeriatric patients clear relationships between bone turnover status and presence of nonvertebral fracture, chronic comorbid conditions and in-hospital outcomes have been shown.

Conclusions

We proposed a classification model of bone turnover status based on a combination of serum bone formation and resorption markers and demonstrated that in orthogeriatric patients altered subtypes are closely related to presence of nonvertebral fractures, comorbidities and poorer in-hospital outcomes. However, the pathogenesis of bone metabolism is only partially represented by this classification, and the future subtyping model could be improved by adding other biomarkers. Further research is needed to establish optimal cut points of various BTMs, improve the classification and achieve more-individualized prognosis and treatments.

Competing Interests

The authors have declared that no competing interest exists.

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