Study on critical illness and bone turnover

Gemma Marcucci[1], Morena Cozzolino[2], Mirko Duradoni[3], Simone Parri[1], Caterina Fossi[1], Carla Signorini[1], Manuela Bonizzoli[2], Laura Masi[4], Adriano Peris[2], Maria Luisa Brandi[5]

[1] Bone Metabolic Diseases Unit, Department of Biomedical, Experimental and Clinical Sciences, University of Florence, Italy; [2] Intensive Care Unit and Regional ECMO Referral Centre, Azienda Ospedaliero-Universitaria Careggi, Florence, Italy; [3] Department of Information Engineering, University of Florence, Florence, Italy; [4] Bone Metabolic Diseases Unit, University Hospital Careggi, Florence, Italy; [5] F.I.R.M.O., Italian Foundation for the Research on Bone Disease, Florence, Italy

ABSTRACT

Purpose: Critical illness has been recognized to acutely influence bone metabolism and, consequently, bone mineral density. The main purpose of this study was to describe bone metabolism changes in adult survivors of critical illness in an attempt to correlate them with severity scores.

Methods: An open, prospective, observational, monocentric study in patients admitted to the intensive care unit (ICU) was conducted, evaluating bone metabolism at baseline (within 72 hours of ICU admission), 6 months, and 12 months.

Results: Fifty-nine patients admitted to the ICU (63% males), mean age 58 ± 16 years, were enrolled. Of these, 20 patients (34%) completed the one-year follow up. At baseline, bone resorption showed an increase, which was maintained at 6 months, and followed by normalization at 12 months. Patients showed, in the majority of cases, hypovitaminosis D with hyperparathyroidism at baseline with subsequent normalization. A trend towards a correlation was described between severity scores and serum 25(OH) vitamin D and bone turnover marker levels.

Conclusions: These results help to confirm a positive association between critical illness requiring ICU admission and bone metabolism changes. This study lays the foundations for further studies evaluating bone health in ICU patients.

KEYWORDS

Critical illness, osteoporosis, bone turnover, bone metabolism, treatment.

Introduction

Intensive care patients face several problems after their critical illness, such as increased mortality, physical and cognitive impairment, and reduced quality of life compared with pre-illness [1-12]. Among the long-term sequelae of critical illness, few published data are available on its short- and long-term effects on bone metabolism and bone mass, and their consequences, such as osteoporosis [1,9]. Osteoporosis is characterized by low bone mineral density (BMD), micro-architectural bone alterations, and high risk of fragility fractures, with a significant associated health burden of mortality, morbidity, and costs [9,13-15].

Osteoporosis is a chronic progressive disorder with a multifactorial etiology. It is a major public health problem, even though it remains an under-diagnosed and under-treated disease [1,16-18]. Several known diseases cause secondary osteoporosis [1,16,19]. Among these, critical illness is a risk factor for osteoporosis in adult intensive care unit (ICU) survivors [1, 20, 21]. Indeed, immobilization, inflammation, treatments, and endocrine dysfunctions related to critical illness may cause accelerated bone turnover, leading to bone fragility and worsening the burden of morbidity in survivors of intensive care [1,20,21]. Altogether, these findings highlight ICU patients as potential targets for pharmacological intervention [1,9,22].

The aim of this study was to describe bone metabolism changes over a 12-month period in adult survivors of critical illness compared with an age- and sex-matched control population, and BMD changes after 12 months. The bone metabolism changes were also correlated with severity scores, i.e., the Simplified Acute Physiology Score (SAPS II) and the Sequential Organ Failure Assessment (SOFA) score, and with specific ICU interventions.

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Contact
Gemma Marcucci; gemma.marcucci@unifi.it
Bone Metabolic Diseases Unit, Department of Biomedical, Experimental and Clinical Sciences, University of Florence. Largo Piero Palagi 1 50139, Florence, Italy - Phone: +39 055 7948087

Methods

Study design, ethics and outcomes

A prospective, observational, open, monocentric study in patients admitted to the ICU was conducted between February 2017 and February 2020 to evaluate bone metabolism parameters and osteoporosis risk factors at baseline (ICU admission), and at 6 months and 12 months, and BMD at 12 months.

This study involved the Intensive Care Unit, the Regional ECMO Referral Center, and the Bone Metabolic Diseases Unit

at the University Hospital of Careggi in Florence.

The study was approved by the institutional review board (Comitato Etico Area Vasta Centro, Azienda Ospedaliera Universitaria Careggi, Florence, Italy) [number: 10200_oss]. The ethics committee verified the compliance of the study with the Good Clinical Practice standard and the Declaration of Helsinki. Informed consent was collected in accordance with the General Authorization to Process Personal Data for Scientific Research Purposes (Authorization no. 9/2013, The Italian Data Protection Authority). At enrollment, written consent was obtained from the next of kin. Retrospective patient consent was obtained when full mental capacity was regained.

The outcomes were: changes in bone turnover markers (BTMs), bone metabolism analytes compared with the matched-population control subjects, analysis of the factors potentially associated with these parameters, and measurement of BMD 1 year after ICU admission.

Study population, inclusion and exclusion criteria

Adult (>18 years) subjects (both male and female) admitted to the ICU during the study period with presumed prolonged (more than 15 days) immobility (based on medical judgment) were considered eligible for enrollment in the study. Patients with traumatic bone fractures, cancer, metabolic bone disease, use of corticosteroids and/or bisphosphonates, pregnancy, and primary neuromuscular disease were excluded.

The control group was a random population-based sample made up of adult patients of both sexes (as required in the study group), not affected by osteoporosis (BMD was compatible with osteopenia or showed a normal value), attending the Bone Metabolic Diseases Unit at the University Hospital of Careggi in Florence. The exclusion criteria were consistent with those of the study group.

Data collection

At ICU admission, data collection included: age, sex, anthropometric measures, body mass index (BMI), patient's comorbidities (renal, cardiovascular, respiratory, neurological, diabetes mellitus), osteoporosis risk factors (currently smoking and alcohol consumption of three units daily or more), and severity as assessed using SAPS II and the SOFA score. In addition, data were collected on the following aspects: mechanical ventilation (MV) duration, renal replacement therapy (RRT), extra corporeal membrane oxygenation (ECMO), ICU length of stay, hospital length of stay and survival (hospital mortality). The baseline data collection, within 72 hours of ICU admission, also included serum and urinary biochemistry.

After ICU discharge, clinical information and serum and urinary biochemistry were collected at two time points: 6 months and 12 months. At 12 months after ICU admission, measurement of BMD was performed using computerized bone mineralometry with DEXA (dual-energy X-ray absorptiometry), at lumbar (L-BMD) and femoral (F-BMD) sites (Hologic, Discovery A, SN84699, version 13.3.3), and the trabecular bone score (TBS), a new DEXA software (version 3.0.2.0 -DXA, Hologic discovery A # 84699).

Serum and urinary biochemistry, analyzed at baseline, 6 months, and 12 months, included serum and 24-hour urinary calcium and phosphate levels, serum parathyroid hormone (PTH), 25(OH)-vitamin D, glucose, creatinine, albumin, magnesium levels, and concentrations of BTMs [bone alkaline phosphatase (BALP), urinary deoxypyridinoline (DPD), C-terminal telopeptide of type I collagen (beta-CTx), the latter measured only at 6 and 12 months].

Statistical analysis

Analysis of frequencies and descriptive statistics were performed using the IBM Statistical Package for Social Sciences (SPSS 20.0) for Windows (IBM, Armonk, NY, USA). Data are presented as mean ± SD (standard deviation), unless otherwise stated. Repeated measures-related differences were evaluated using Student's t-test for paired samples. P values of less than or equal to 0.05 were considered statistically significant. For all variables that did not meet the assumptions for parametric analysis, the Wilcoxon Signed-Rank Test was employed to assess paired data.

Results

Baseline characteristics

Fifty-nine patients, 37 males (63%) and 22 females, mean age 58 ±16 years (range: 18-87), were enrolled according to the inclusion criteria. Of these, 20 patients (34%) completed the one-year follow up, 23 (39 %) withdrew their consent, and 16 (27 %) died after admission to the ICU.

Table I summarizes the baseline findings of the total population enrolled (no. 59 patients), including mean BMI; osteoporosis risk factors, such as smoking (current smoker), alcohol consumption (alcohol > 3 U/day); presence of co-morbidities; and admission categories and severity scoring (SAPII and SOFA).

Table II shows the biochemistry and biomarkers at ICU admission (baseline).

Baseline mean values show elevated PTH levels associated with hypocalcemia and low 25(OH) vitamin D levels, with DPD above the reference range and BALP within the normal reference range. The control group showed significantly higher serum calcium and 25(OH) vitamin D levels compared with the study group, while DPD values tended to be lower (respectively, serum calcium: U = 136; Z = -8.44; p. = .001; 25 (OH) vitamin D: U = 521.50; Z = - 5.64; p. = .001); no statistically significant differences were described for BALP levels.

A negative correlation was described between SAPS II severity scores and BALP levels (r = -0.37; p= 0.20), with higher SAPS II scores tending to be associated with lower baseline BALP values, and a positive correlation with DPD levels (r = -0.30; p= 0.18). No correlation was found between SOFA, MV, RRT, ECMO, ICU length of stay, and BMT changes.

Vitamin D deficiency tended to be negatively associated with SOFA score and ICU length of stay (r = -0.10; p= 0.10), albeit not in a statistically significant way.

Results of follow up at 6 and 12 months

The 20 patients who completed the 12-month follow up all underwent assessment of bone metabolism parameters at 6 and

Table I Baseline features of total population enrolled (no. 59).

PARAMETERS	NUMBER	FREQUENCY (%)
BMI (mean BMI: 28.5 ± 6.9)		
BMI <18.5	1	1.7%
BMI 18.5-24.9	16	27.1%
BMI 25-29.9	25	42.4%
BMI > or = 30	16	27.1%
Osteoporosis risk factors		
Smoker (current)	12	20.3%
Alcohol > 3 U/day	10	16.9%
Co-morbidities		
Renal	1	1.6%
Cardiovascular	23	39.9%
Respiratory	10	16.9%
Neurological	6	10.1%
Diabetes mellitus	9	15.2%
ICU admission category		
Medical disease:	52	88.1%
Neurological failure	13	25%
Respiratory failure	28	53.8%
Septic shock	7	13.4%
Other	4	7.6%
Surgical disease:	7	11.8%
Score related to critical illness		
RRT	7	11.86%
ECMO	14	23.73%
ICU mortality	11	18.64%
H mortality	13	22.03%
	mean ± SD	**median**
MV duration, days	12.65 ± 17.38	5
ICU LOS, days	16.25 ± 15.17	12
H LOS, days	25.28 ± 18.81	19
Severity score	**mean value**	**±SD**
SAPS II	37.59	±14.54
SOFA	7.34	±3.65

BMI, body mass index; ICU, intensive care unit; **RRT**, renal replacement therapy; **ECMO**, extra corporeal membrane oxygenation; **H**, hospital; **H** mortality, survival; **MV**, mechanical ventilation; **LOS**, length of stay; **SAPS II**, Simplified Acute Physiology Score; **SOFA**, Sequential Organ Failure Assessment.

12 months and measurement of DEXA BMD and TBS at 12 months. At baseline, DEXA measurement of BMD was not possible due to the logistical difficulties of performing this in the ICU. Table 3 shows the general characteristics and biochemistry of the study group at baseline and at 12 months, and Table III shows the results of the BMD DEXA performed at 12 months.

Table II Biochemistry and biomarkers at ICU admission (baseline).

BIOCHEMISTRY EXAMINATIONS, UNITS, NORMAL RANGE	MEAN	± SD	RANGE (MIN-MAX)
Albumin-corrected calcium, mg/dl [8.5-10.1]	**7.99**	±1.84	6.09-9.30
Magnesium, mg/dl [1.5-2.6]	2.22	±0.41	1.40-3.60
Phosphate, mg/dl [2.5-4.9]	2.95	±1.26	1.30-5.60
Urinary calcium, mg/24h [100-300]	172.03	±133.87	2.00-440.00
PTH, pMol/l [1.3-7.6]	**10.11**	±6.65	1.00-33.90
BALP, µg/L [men: 7-20.1]	16.20	±12.73	5.90-72.00
BALP, µg/L [pre-menopause women: 4-14.3]	22.90	±7.36	18.60-31.40
BALP, µg/L [post-menopause women: 6-22.5]	19.73	±10.97	6.20-52.80
DPD, nmol/mmol c [3-7.4]	**11.63**	±6.38	4.00-32.60
25(OH)-vitamin D, pg/ml [30-100]	**16.13**	±17.77	4.20-96.10
Creatinine, mg/dl [0.64-1.20]	**1.35**	±1.07	0.38-5.87

BMI, **PTH**, parathyrpid hormone; **BALP**, bone alkaline phosphatase; **DPD**, urinary deoxypyridinoline. *Legend*: in blood values out of range.

Table III BMD DEXA performed at 12 months.

DEXA PARAMETERS	STUDY GROUP (N:20)	
	Mean ± SD	Range
Lumbar (L1-L4) BMD	1.011 ± 0.140	0.800 / 1.260
L1-L4 T- score	-1.09 ± 0.98	-2.50 / 0.40
L1-L4 Z-score	0.38 ± 1.20	-1.30 / 2.30
Total femur BMD	0.936 ± 0.129	0.705 / 1.127
Total femur T-score	- 0.92 ± 0.84	-2.50 / 0.10
Total femur Z-score	0.28 ± 0.640	-0.50 / 1.50
Femoral neck BMD	0.730 ± 0.156	0.500 / 1.193
Femoral neck T-score	-1.91 ± 0.960	-4.00 / -0.10
Femoral neck Z-score	-0.32 ± 0.644	-1.20 / 1.00

Bone metabolism parameters

Assessment of bone turnover showed mean levels of the bone resorption marker DPD decreasing from above-reference-range values at baseline to normal levels at 12 months in both sexes (baseline: 10.16 nmol/mmol c ± 4.03; 12 months: 5.02 nmol/mmol c ± 3.86; Wilcoxon Z -3.42; p= 0.001), reaching a statistically significant difference 1 year after ICU hospitalization (Table 3). Mean DPD at 6 months in the study group showed a value still above the reference range (17.8 ± 12.09 nmol/mmol c). The mean DPD levels at baseline and in the year after ICU tended to be higher, albeit not significantly so, in the study population when stratified by sex and compared with population reference (control group) values. In the study group, measurement of beta-CTx, as a marker of bone resorption, showed values still above the reference range at 6 months (1.01 ± 0.09 ng/ml) and within the reference range at 12 months (0.9 ± 0.06 ng/ml; normal range: 0.1-1).

Mean levels of bone BALP, a bone formation marker, tended to increase at 12 months from ICU hospitalization compared with baseline (baseline: 15.78 mcg/L ± 7.86; 6 months: 13.5 ± 5.03 mcg/L; 12 months: 17.23 ± 5.41 mcg/L), albeit always re-

Table IV General characteristics and biochemistry of the study group at baseline and at 12 months.

GENERAL CHARACTERISTICS				
Age (mean ± SD)	58.2 ± 17.3			
BMI (mean ± SD)	31.5 ± 9			
Smoker (current)	N:3		15%	
Alcohol > 3 U/day	N:1		5%	
Co-morbidities				
Renal	N:0		0%	
Cardiovascular	N:8		40%	
Respiratory	N:5		25%	
Neurological	N:2		10%	
Diabetes mellitus	N:2		10%	
ICU admission category				
Medical disease:				
Neurologic failure	N:2		10%	
Respiratory failure	N:8		40%	
Septic shock	N:1		5%	
Other	N:9		45%	
Surgical disease:	N:0		0%	
Score related to critical illness (ICU admission)				
RRT	N:2		10%	
ECMO	N:4		20%	
MV duration, days (mean ± SD)	7.1 ± 8.7			
ICU LOS, days (mean ± SD)	13.1 ± 10.9			
H LOS, days (mean ± SD)	23.6 ± 13.9			
Severity score (ICU admission)				
SOFA	6 ± 3		±14.54	
SAPS II	32.3 ± 10.3		±3.65	
BIOCHEMICAL EXAMINATIONS, UNITS, NORMAL RANGE	**BASELINE**		**12 MONTHS**	
	Mean ± SD	**Range**	**Mean ± SD**	**Range**
Albumin-corrected calcium, mg/dl [8.5-10.1]	7.78 ± 0.52*	6.90-8.90	9.50 ± 0.54	8.50-10.50
Phosphate, mg/dl [2.5-4.9]	2.73 ± 0.98	1.40-5.60	3.58 ± 0.70	2.50-5.20
Urinary calcium, mg/24h [100-300]	209.70 ± 130.56	10.00-416.50	132.56 ± 54.90	24.00-200.00
PTH, pMol/l [1.3-7.6]	9.14 ± 5.24*	2.30-23.80	5.00 ± 2.81	1.30-9.60
BALP, mcg/L [men: 7-20.1]	18.77 ± 9.96	7.00-31.40	17.23 ± 5.41	10.00-28.00
BALP, mcg/L [post-menopause women: 6-22.5]	14.04 ± 6.42	6.10-26.20	16.42 ± 6.11	7.00-27.30
DPD, nmol/mmol c [3-7.4]	10.16 ± 4.03*	6.00-20.10	5.02 ± 3.86	2.50-16.00
25(OH)-vitaminD, pg/ml [30-100]	11.60 ± 10.02*	4.20-44.00	23.96 ± 11.19	10.00-52.30

Note: the values outside the reference range are indicated in bold. *: P value <0.05.
BMI, body mass index; **ICU**, intensive care unit; **RRT**, renal replacement therapy; **ECMO**, extra corporeal membrane oxygenation; **MV**, mechanical ventilation; **LOS**, length of stay; H, hospital; **SOFA**, Sequential Organ Failure Assessment; **SAPS II**, Simplified Acute Physiology Score; **PTH**, parathyroid hormone; **BALP**, bone alkaline phosphatase; **DPD**, urinary deoxypyridinoline

maining within the reference range in both females and males, and without showing any statistically significant difference compared with population reference levels (Table IV).

At baseline, mean serum concentrations of 25(OH) vitamin D were below the reference range, and statistically significantly lower than the control group values in both females and males (Table 3). The mean levels of 25(OH) vitamin D increased significantly from baseline to 6-month and 12-month follow up ($p = 0.03$).

The mean PTH concentrations were above the reference range at baseline and decreased at 12 months, albeit without showing a significant change (baseline: 9.14 pmol/L ± 5.24; 12 months: 5.00 pmol/L ± 2.81) (Table 3). At baseline, PTH values were statistically increased compared with the control group ones (study group: 9.14 pmol/L ± 5.24 *versus* 4.76 pmol/L ± 2.07 p= 0.001).

The mean albumin-corrected serum calcium levels were below the reference range at baseline, before normalizing at 6 months and during the year after ICU (baseline: 7.78 ± 0.52 mg/dl; 6 months: 9.50 ± 0.39 mg/dl; 12 months: 9.50 ± 0.54 mg/dl). At baseline, the mean albumin-corrected serum calcium value was statistically lower than the control group value (baseline: 7.78 mg/dl ± 0.52 *versus* 9.41 mg/dl ± 0.49; p= 0.001).

Bone mineral density

Seventy percent (14/20) of the follow-up patients, with no previous known history of osteoporosis/osteopenia or fragility fractures, showed T-scores compatible with osteopenia/osteoporosis (osteoporosis, 7 patients; osteopenia, 7 patients) at 12 months. Two patients had T-scores <-2.5 at the lumbar level and five at the femoral neck level. The mean total lumbar BMD TBS at 12 months was 0.900 ± 84.78, and the mean T-score was -2.5. Analyzing the scores indicating the severity of the health status of ICU patients, the study showed only a negative correlation between the SOFA score and lumbar BMD (r = -0.51; p= 0.065): when SOFA scores increased, lumbar BMD levels tended to be lower. Only 1 fragility fracture at the dorsal vertebra was reported at the 12-month follow up.

Discussion

This prospective-observational study investigated the relationship between critical illness, bone turnover markers, and bone metabolism.

The baseline evaluation of BTMs, both in the total enrolled group (no. 59) and the follow-up group (no. 20), showed increased bone resorption with no commensurate response in terms of bone formation. Subsequently, the trend of BMT levels in the follow-up group at 6 and 12 months showed persistence of increased bone resorption at 6 months and normalization at 12 months, without compensatory increased formation activity throughout the period. In physiological conditions, there is a continuous cycle of bone formation and resorption, called bone remodeling, which allows bone tissue to meet the requirements of mineral homeostasis, repair microdamage, and adapt to altered mechanical loading [23,24]. In several pathological conditions, an uncoupling of bone resorption and formation can occur; this can also occur in the case of prolonged immobilization, interfering drugs, and the presence of proinflammatory cytokines associated with various diseases, including critical illness [24,25]. Overall, the increase in resorption at the expense of bone formation can lead to trabecular thinning, loss of connectivity between trabeculae, cortical thinning, and increased cortical porosity, resulting in bone fragility [23,24].

BTM concentrations mirror these processes and are important tools for assessing bone fragility and fracture risk. However, they are usually influenced by several factors, and can therefore be difficult to interpret [26]. In this regard, the chosen exclusion criteria allowed us to eliminate confounding factors in the patient clinical history, such as the use of glucocorticoids, bisphosphonates, and major disorders interfering with bone metabolism.

Thirteen studies (case series or cohort studies) have been published that include short-term evaluations of BTMs as outcome measures in ICU patients [9,25,27-37]. All studies that evaluated BTMs described an initial increase in markers of bone resorption, suggesting increased osteoclastic activity in ICU patients [1,9,25-38]. One study also measured osteoclast precursors and mature osteoclasts in serum and described a significant increase in osteoclast precursors in ICU patients compared with controls [34]. Among these studies, only Orford et al. described BTM changes both during the ICU stay and the subsequent year; they showed an increase in bone resorption during critical illness, and subsequent normal bone resorption, whereas the bone formation marker level was within normal limits during the ICU stay and at 1 year, albeit with an increasing trend at 12 months [9,27]. Therefore, our findings regarding BTM changes over the 12-month period are in line with these previous results [9,27], and confirm that an initial increase in bone resorption may be associated with critical illness, and that over time this process tends to normalize [1,9].

We also described BTM alterations at 6 months, showing that the increase in bone resorption markers persisted at this timepoint, too, but with no increase in the bone formation marker level, as was instead described at 12 months. A previous prospective observational study conducted in 28 adult patients with prolonged critical illness also described BTM levels at 5 weeks, reporting high beta-CTx levels in 45% of subjects at admission, increasing to more than 80% of subjects at weeks 1 and 2, and more than 50% of subjects at week 5. In contrast, concentrations of P1NP (a bone formation marker) were reduced in 55% of subjects at ICU admission, and in 10% of subjects by week 5 [37]. Bone formation marker levels tend not to increase initially, probably because pathological (i.e., inflammation-related) or drug-induced alterations cause prevalent bone resorption at the expense of bone formation. However, this process should be further investigated; in particular, further studies would be needed to investigate the role of sclerostin in these conditions.

Moreover, our study found a correlation trend between SAPS II score and BTM concentrations during the ICU stay; although no significant correlation with SAPS II was achieved, due to the limited sample size, this finding provides sufficient preliminary data to support its use. SAPS II was designed to measure the severity of critical illness in ICU patients aged 18

or over on admission [39].

In the past, a prospective observational study described that levels of sclerostin, a key negative regulator of bone formation measured at admission and at 1 week in 264 critically ill adults admitted to a medical ICU, varied with illness severity measured using the Acute Physiology and Chronic Health Evaluation (APACHE) II score, with significantly higher levels recorded in patients with scores greater than 20 compared with those scoring less than 20 [40]. The APACHE score has been replaced by other illness severity scores, such as SOFA and SAPS II; therefore, in the future, it would be interesting to evaluate correlations in large patient samples between BTMs and these severity scores, since the role of illness severity in bone loss is still unclear. Finally, in previous studies, the relationship between inflammation, sepsis, loss of hypothalamic-pituitary axis pulsatility, and increases in BTMs was investigated [24], but several confounding effects relating to premorbid disease, organ failure, and medications made it impossible to establish clear relationships.

The vitamin D deficit at baseline in ICU patients described in our study confirms that this endocrine alteration is very common in these patients, as reported in previous studies [30,41-46]. ICU patients are at high risk of vitamin D deficiency for several reasons, such as: decreased dietary vitamin D or malabsorption, decreased sunlight exposure, impaired hepatic 25-vitamin D formation, and/or impaired renal 1,25-vitamin D formation [47]. Our study showed a trend of negative correlation between vitamin D deficit at ICU admission and ICU length of stay, as described in two previous cohort studies [44,48]. In addition, a trend of negative correlation was also reported between a severity score (SOFA score) and 25(OH) vitamin D deficit in our study group during the ICU stay. Previously known as the sepsis-related organ failure assessment score, the SOFA score is a severity score used in the ICU to determine the extent of a person's organ function or rate of failure, and it considers cardiovascular, hepatic, coagulation, renal, and neurological systems [49]. Since correlation between sepsis and vitamin D deficit has already been described [43], it might be useful to evaluate the correlation between SOFA and vitamin D levels in large multicenter trials to confirm this association in ICU patients. A recent meta-analysis suggested that vitamin D deficiency is associated with high mortality in critically ill patients [30]. The largest randomized clinical trial performed so far on enteral administration of vitamin D3 in a large group of ICU patients with vitamin D deficit showed that vitamin D administration was significantly associated with 18% relative risk reduction of death in a subgroup of patients with severe deficits (vitamin D level ≤ 12 ng/mL) [43].

In conclusion, serum 25(OH) vitamin D levels at ICU admission may identify patients at high risk of prolonged hospitalization and mortality. However, randomized trials are needed to assess whether vitamin D supplementation can improve these clinically relevant outcomes in ICU patients; currently, the existing data are insufficient to make an evidence-based recommendation regarding its use in the ICU [50].

The secondary hyperparathyroidism reported in most of our ICU patients confirms what is described in previously performed studies [9,41,42,47,51-58]. Secondary hyperparathyroidism causes increased bone resorption, although it is not the only cause in these patients [47]. Stress, disease, and/or immobilization with prolonged bedrest stimulates the production of certain cytokines and local bone growth factors, resulting in high bone resorption [9,41,42,47,51-58]. Indeed, correction of vitamin D deficit alone in these patients does not seem to correlate with reduction in BTMs [36,47]. A cohort study conducted in 55 ventilator-dependent chronic critically ill patients showed that treating vitamin D deficiency with calcitriol did not lead to a reduction in bone resorption markers [47]. Finally, a post-hoc analysis of the Correction of Vitamin D Deficiency Ill Patients (VITdAL-ICU) study, a randomized, double-blind, placebo-controlled trial, reported no effect of high-dose vitamin D3 (a loading dose of 540,000 IU and five monthly maintenance doses of 90,000 IU starting 1 month after the loading dose) compared with placebo on 6-month serum osteocalcin (OC), sclerostin, or beta-CTx levels in 289 adult critically ill patients [59].

Moreover, with high-dose vitamin D treatment, it is important to remember that high doses of vitamin D are associated with potential safety issues, such as increases in falls and fractures, as observed in community-dwelling older women at risk of fractures [60].

From studies conducted so far, an evident association between critical illness and high bone resorption, leading to secondary osteopenia and osteoporosis, emerges. In addition to vitamin D deficit (especially in long stayers), several other factors can have a role in bone loss, including immobilization, lack of muscle activity, inflammation, neuroendocrine stress reaction, malnutrition, gut microbiota dysregulation, and drugs [1,61,62]. Loss of bone mass appears to persist for up to 2 years [27] and, despite gradual improvement in bone formation, BMD may not completely recover for several years following acute illness.

In our study, more than half of the follow-up patients, without previous history of osteoporosis/osteopenia or fragility fractures, reported osteoporosis/osteopenia at 12 months after ICU discharge, confirming previous findings [9,24,27,59]. Baseline DEXA data are not available, since DEXA testing during ICU stays is not logistically easy to carry out, and also not routinely required. Over the last 5 years, some studies have described a high proportion of osteopenic or osteoporotic patients after ICU, suggesting a disease burden that may contribute to long-term morbidity and mortality [9,24,27,59]. A prospective longitudinal cohort study has described a significantly greater annual decrease in BMD in the year after critical illness in subjects ventilated for more than 24 hours who survived to ICU discharge, compared with age-matched and sex-matched population controls [9]. At ICU discharge, 45% of all subjects were osteopenic or osteoporotic, this rate increasing to 55% at 1 year [9]. The factors that influence the trajectory of BMD before and after ICU are not clear, partly due to difficulty performing long-term research in ICU patients [24].

In our study, an innovative software, TBS, was also used for the evaluation of lumbar BMD at 12 months. Albeit in a small study group, this method showed worse lumbar BMD and T-score values compared with the results obtained with DEXA. TBS is a gray-level textural metric that can be extracted from the two-dimensional lumbar spine DEXA image. It is

related to bone microarchitecture and provides skeletal information that is not captured from the standard BMD measurement. Currently, TBS represents an emerging technology that could become a valuable clinical tool in the diagnosis of osteoporosis and in fracture risk assessment [63], also considering the significant reduction in lumbar BMD values described in a recent 2-year prospective observational study [27]. In addition to typical risk factors for osteoporosis, in this particular patient population, it would be interesting to further investigate the negative correlation between the SOFA severity score and lumbar BMD reduction, given the trend we described in our study as a preliminary finding.

Currently, the evidence regarding fragility fractures after critical illness is scarce and limited. In the future, larger database linkages would be necessary. The only study that has reported the incidence of new fractures following ICU described an increased incident fragility fracture risk in older female ICU survivors compared with age- and gender-matched population controls [64].

Conclusions

Clinical research on bone metabolism alterations both in the course of multiorgan failure and in the study of the sequelae of intensive care is still an underdeveloped field of investigation.

Our study supports the hypothesis that critical illness and associated factors contribute to bone metabolism alterations and an increase in bone loss, as has emerged from recent literature. Furthermore, our study suggests some correlations between ICU severity scores, BTMs, and vitamin D levels, providing preliminary data not only for further studies in larger samples, but also for the study of target subgroups of critically ill patients. Another important aspect that has emerged from our investigation concerns the persistence of an increase in bone resorption markers for up to 6 months after the acute condition, with an increase in the level of the bone formation markers seen only at 12 months; this data could not only have important repercussions in the prediction of recovery times from the critical state, but also provide useful information for physical rehabilitation and recovery of quality of life. In the future, additional imaging examinations such as TBS could be used in clinical research to monitor the BMD trend in these patients.

Currently, few data are published on anti-fracture treatment use and changes in BMD following critical illness [30,65]. However, the positive association described between anti-fracture therapy use and BMD provides support for future studies in this specific population. Considering the increased levels of bone resorption following critical illness, the early use of antiresorptive drugs (i.e., bisphosphonates or denosumab) could be hypothesized, albeit evaluating any contraindications. However, further prospective studies in large populations are needed for further short- and long-term investigation of: bone metabolism, also including the evaluation of specific BTMs such as PINP, beta-CTx, and other molecules like sclerostin; monitoring of BMD, bone quality, and fragility fractures and correlations with critical illness severity scores. These studies should also investigate any differences in outcomes based on the different etiologies of patients admitted to the ICU. In fact, the underlying disease could partly influence the mineral metabolism and bone strength alterations.

Finally, in addition to the evaluation of anti-resorptive therapy, these studies will be needed to evaluate the impact of rehabilitation and mobilization, undertaken as early as possible, on bone strength, since physical rehabilitation and exercise, acting directly and indirectly, could mitigate bone resorption and improve bone strength.

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