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Original research manuscripts

"A prospective study on critical illness and changes in bone turnover in adult patients"

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Abstract 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 the attempt to correlate changes with severity scores.

It is an open, prospective, observational, monocentric study on patients admitted to the ICU was conducted, evaluating bone metabolism at baseline (within 72 hours of ICU admission), 6 months, and 12 months. 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, with normalization at 12 months. Patients showed, in a 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. These results contribute to the confirmation of a positive association between critical illness requiring ICU and bone metabolism changes. This study poses the bases for further studies to evaluate bone health in ICU patients.

Keywords: Critical illness, Osteoporosis, Bone turnover, Bone metabolism, Treatment.

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1. 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 to pre-illness [1-12]. Among the long-term sequelae of critical illness, little data has been published on 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 mass density, micro-architectural bone alterations, and high risk of fragility fractures, with a significant associated health burden of mortality, morbidity, and costs [9, 13-15]. It is well known that osteoporosis is a chronic progressive disorder with multifactorial etiology and represents 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 also represents a risk factor for osteoporosis in adult survivors of the intensive care unit (ICU) [1, 20, 21]. Indeed, immobilization, inflammation, treatments, and endocrine dysfunctions related to critical illness may cause an accelerated bone turnover, determining bone fragility and worsening the burden of morbidity in survivors of intensive care [1, 20, 21]. Altogether, these findings indicate ICU patients as potential targets for pharmacological intervention [1, 9, 22].

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

2. Results

2.1 Baseline characteristics

Fifty-nine patients, 37 males (63%) and 22 females, mean age of 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.

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Table 1 summarizes baseline findings of total population enrolled (n: 59), including mean body mass index (BMI); osteoporosis risk factors, such as smoke (current smoker), alcohol (Alcohol > 3 U/day); presence of co-morbidities; and admission categories and severity scoring (SAPII and SOFA).

Table 1 Baseline features of total population enrolled (n: 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%
Osteoporotic Factors Risks		
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%
Neurologic 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		
CRRT	7	11.86%
ECMO	14	23.73%
ICU mortality	11	18.64%
H mortality	13	22.03%
	mean ± SD	**median**
VM 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

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Legends: Body mass index (BMI), intensive care unit (ICU), Mechanical Ventilation duration (MV), Renal Replacement Therapy (RRT), and Extra Corporeal Membrane Oxygenation (ECMO), ICU length of stay, hospital length of stay (H LOS) and survival (H mortality), Simplified Acute Physiology Score (SAPS II) and Sequential Organ Failure Assessment (SOFA).

Table 2 shows biochemistry and biomarkers at ICU admission (baseline).

Table 2 Biochemical exams and biomarkers levels at ICU admission (baseline).

Biochemical exams, 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)-vitaminD, 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

Legend: in blod values out of range; bone alkaline phosphatase (BALP), urinary deoxypyridinoline (DPD)

Baseline mean values show elevated PTH levels associated with hypocalcemia and low 25(OH) vitamin D levels, with deossypyridinoline (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 to study group, and 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 between severity score SAPS II and BALP levels (r = -0.37; p= 0.20), as SAPS II score increased, BALP values tended to be lower at baseline, and a positive correlation with DPD levels (r = -0.30; p = 0.18), were described. 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.

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2.2 Results of Follow-up at 6 and 12 months

The 20 patients who completed the 12-month follow-up were all assessed with measurement of bone metabolism exams at 6 and 12 months and measurement of DEXA BMD and TBS at 12 months. At baseline, DEXA measurement of BMD was not possible due to logistic difficulties of execution in the ICU. Table 3 shows the general characteristics and biochemistry of the study group at baseline and at 12 months, and Table 4 shows the results related to the BMD DEXA performed at 12 months.

Table 3 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)				
CRRT	N:2	10%		
ECMO	N:4	20%		
VM 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			
SAPS II	32.3 ± 10.3			
Biochemical exams, 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.

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Table 4 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
Femur neck BMD	0.730 ± 0.156	0.500 / 1.193
Femur neck T-score	-1.91 ± 0.960	-4.00 / -0.10
Femur neck Z-score	-0.32 ± 0.644	-1.20 / 1.00

2.3 Bone metabolism parameters

Regarding bone turnover, the mean levels of DPD bone resorption marker decreased with statistically significant difference 1 year after ICU hospitalization, varying from values above the reference range at baseline to values within normal levels for men and women at 12 months (baseline: 10.16 nmol/mmol c ± 4.03; 12 months: 5.02 nmol/mmol c ± 3.86; Wilcoxon Z -3.42; *p* value: 0.001) (Table 3). The mean level of DPD at 6 months in the study group showed a persistent value 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 with statistically significant difference, in the study population when stratified by sex and compared with population reference levels (control group). The dosage of beta-CTx in the study group, as a marker of bone resorption, confirmed values 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).

The mean bone BALP levels, a bone formation marker, tended to increase at 12 months from ICU hospitalization compared to 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 within the reference range for both females and males, and without statistically significant difference when compared with population reference levels (Table 3).

At baseline, the mean serum concentrations of 25(OH) vitamin D were below the reference range, and showed statistically significant lower values, when compared to the control group for both females and males

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(Table 3). The mean levels of 25(OH) vitamin D showed a significant increase from baseline to 6-month and 12-month follow-up (p value: 0.03).

The mean PTH concentrations were above the reference range at baseline and decreased to 12 months without 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 to the control group (study group: 9.14 pmol/L ± 5.24 *versus* 4.76 pmol/L ± 2.07 *p* value 0.001).

The mean albumin-corrected serum calcium levels were below the reference range at baseline, and normalized 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 value of albumin-corrected serum calcium was statistically lower when compared to the control group (baseline: 7.78 mg/dl ± 0.52 *versus* 9.41 mg/dl ± 0.49; *p* value: 0.001).

2.4 Bone mineral density

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

3. Discussion

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

The baseline evaluation of BTMs, both in total enrolled group (n:59) and follow-up group (n:20), showed increased bone resorption with no commensurate response in 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 a 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, such as in the case of prolonged immobilization, interfering drugs, and in presence of proinflammatory cytokines associated to 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 to assess fragility bone and fracture risk. However, they are usually influenced by several factors, requiring a difficult interpretation (26). In this regard, the chosen exclusion criteria allowed us to eliminate confounding factors, such as the use of glucocorticoids, bisphosphonates, and major disorders interfering with bone metabolism in the patient's previous clinical history.

Thirteen studies (case series or cohort studies) have been published that include short-term evaluations of BTMs as outcome in ICU patients [9, 25, 27-37]. All studies that evaluated BTMs described an initial increase of markers of bone reabsorption, suggesting an increased osteoclastic activity in ICU patients [1, 9, 25-40]. One study also measured osteoclast precursors and mature osteoclasts in serum and described a significant increase in osteoclast precursors in ICU patients compared to controls [40]. Among these studies, only Orford et al. described BTM changes both during ICU admission and the subsequent year, showing an increase of bone resorption during critical illness, and a subsequent normal bone resorption, whereas bone formation marker was within normal limits during ICU admission and at 1 year [9, 27]. Therefore, our findings regarding BTM changes over the 12-month period are in line with these previous results [9, 27], confirming that an initial increase in bone resorption may be associated with critical illness, and that over time this process tends to normalize [1, 9]. Our investigation also described BTM alterations at 6 months, showing that the increase of bone resorption markers persisted even at 6 months, with no increase in bone formation marker level, as instead described at 12 months. A previous prospective observational study conducted on 28 adult patients with prolonged critical illness also described BTM levels at 5 weeks, reporting high beta-CTx levels in 45% of

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subjects at admission, increasing to more than 80% of subjects week 1 and 2, and more than 50% of subjects at week 5. In contrast, P1NP (bone formation marker) concentrations were reduced in 55% of subjects at admission to ICU and 10% of subjects by week 5 [37].

Moreover, our study found a correlation trend between SAPS II and BTM concentrations during ICU stay; although a statistical significance of correlation with SAPS II was not achieved, due to limited sample size, this correlation provides sufficient preliminary data to support its conduct. SAPS II was designed to measure the severity of critical illness for patients admitted to ICU aged 18 or more [41]. In the past, a prospective observational study described that sclerostin levels, 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 a severity score of illness, Acute Physiology and Chronic Health Evaluation (APACHE) II score, with significantly higher levels in patients with scores greater than 20 compared with less than 20 [42]. 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 increase in BTMs has been investigated [24], but there were several confounding effects regarding premorbid disease, organ failure, and medications, making it impossible to determine clear relationships.

The deficit of vitamin D 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 [43-49]. ICU patients are at high risk for 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 [50]. Our study shows a trend of negative correlation between vitamin D deficit at ICU admission and ICU length of stay, as described in two previous cohort studies [46, 51]. 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 ICU stay. SOFA score is a severity score used in ICU to determine the extent of a person's organ function or rate of failure, including cardiovascular, hepatic, coagulation, renal, and neurological systems,

previously known as the sepsis-related organ failure assessment score [52]. A correlation between sepsis and vitamin D deficit has already been described [45]; therefore, 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 has suggested that vitamin D deficiency is associated with high mortality in critically ill patients [49]. 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 vitamin D deficit (vitamin D level ≤ 12 ng/mL) [45]. In conclusion, serum 25(OH) vitamin D levels at ICU admission may identify patients at high risk for 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 [53].

The secondary hyperparathyroidism reported in most of our ICU patients confirms what is described in previously performed studies [9, 43, 44, 54-62]. Secondary hyperparathyroidism causes increased bone resorption, although it is not the only cause in these patients [50]. 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, 43, 44, 50, 54- 62]. Indeed, the only correction of vitamin D deficit in these patients does not seem to correlate with a reduction of BTMs [39, 50]. A cohort study, conducted on 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 [50]. 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 starting 1 month after the loading dose five monthly maintenance doses of 90,000 IU) compared to placebo on 6-month serum OC, sclerostin, or beta-CTx levels in 289 adult critically ill patients [63]. Moreover, regarding treatment with high-dose vitamin D, it is important to remember that high-doses of vitamin D are associated with potential safety issues, such as increase in falls and fractures, as observed in community-dwelling older women at risk of fractures [64].

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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, a lack of muscle activity, inflammation, neuroendocrine stress reaction, malnutrition, gut microbiota dysregulation, and drugs [1, 65, 66]. Loss of bone mass appears to persist for up to 2 years [27] and, despite gradual improvement in bone formation, bone mass density may not completely recover for several years following acute illness.

Our study showed that more than half of 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, 63]. Baseline DEXA data are not available, since DEXA testing during ICU stay 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, 63]. 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 to age-matched and sex-matched population controls [9]. At ICU discharge, 45% of all subjects were osteopenic or osteoporotic, increasing to 55% at 1 year [9]. The factors that influence the trajectory of bone mass density 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. This method showed worse values of lumbar BMD and T-score compared to results obtained with DEXA, albeit evaluated in a small study group. 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 [67], also considering the significant reduction of lumbar BMD values described in a recent 2-year prospective observational study [27]. In addition to typical risk factors for osteoporosis, in this particular

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patient population, it would be interesting to further investigate the negative correlation between the severity score of SOFA score and lumbar BMD reduction, given the trend 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 [68].

4. Methods

4.1 Study design, Ethics and Outcomes

A prospective, observational, open, monocentric study on patients admitted to the ICU was conducted between February 2017 and February 2020 to evaluate bone metabolism exams and osteoporosis risk factors at baseline (ICU admission), and at 6 months and 12 months, and bone mineral density (BMD) at 12 months. This study involved the Intensive Care Unit, the Regional ECMO Referral Centre, 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 conformity of the study to the Good Clinical Practice Standard and the Declaration of Helsinki. Informed consent was collected in accordance with 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, with retrospective patient consent 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.

4.2 Study population, inclusion and exclusion criteria

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Adult (>18 years) subjects (both male and female) admitted to the ICU during the study period with presumed prolonged immobility (more than 15 days) 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, characterized by adult patients of both sexes, as required in the study group, not affected by osteoporosis (BMD was compatible with osteopenia or normal value), afferent to the Bone Metabolic Diseases Unit at the University Hospital of Careggi in Florence. The exclusion criteria were consistent with those of the study group.

4.3 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 greater), and severity scoring per SAPS II and SOFA score. In addition, information regarding Mechanical Ventilation duration (MV), Renal Replacement Therapy (RRT), and Extra Corporeal Membrane Oxygenation (ECMO), ICU length of stay, hospital length of stay (H LOS) and survival (H mortality) were collected. 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 using computerized bone mineralometry with a DEXA (Dual-energy X-ray absorptiometry), at lumbar (L-BMD) and femoral (F-BMD) sites (Hologic, Discovery A, SN84699, version 13.3.3), and with trabecular bone score (TBS), a new DEXA software (version 3.0.2.0 -DXA, Hologic discovery A # 84699), was performed.

Serum and urinary biochemistry, analyzed at baseline, 6 months, and 12 months, included serum and 24-hour urine calcium and phosphate levels, serum PTH, 25(OH)-vitamin D, glucose, creatinine, albumin, magnesium levels, and BTM concentrations [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].

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4.5 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 by using the Student's t-test for paired sample. P value of less than or equal to 0.05 was 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.

5. Conclusions

Clinical research on bone metabolism alterations both in the course of multiorgan failure and in the study of the sequelae of intensive care still represents 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 with a larger sample, 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 up to 6 months from the acute condition of the disease, with an increase in the level of the bone formation marker 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.

Finally, in the future, additional imaging exams such as TBS could be used in clinical research to monitor the BMD trend in these patients. Further studies are needed to investigate the long-term health impact of altered bone metabolism after critical illness and risk fragility fractures. Currently, few data are published on anti-fracture treatment use and change in BMD following critical illness [69-71]. However, the positive association described between anti-fracture therapy use and BMD provides support for future studies in this specific population.

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Informed Consent Statement: Informed consent was collected in accordance with 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, with retrospective patient consent obtained when full mental capacity was regained.

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