ERRATUM

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Correction to "Denosumab for Prevention of Acute Onset Immobilization-Induced Alterations of Bone Turnover: A Randomized Controlled Trial"

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In the originally published version of the article, the funding information was missing from the Acknowledgments section. The corrected text appears below.

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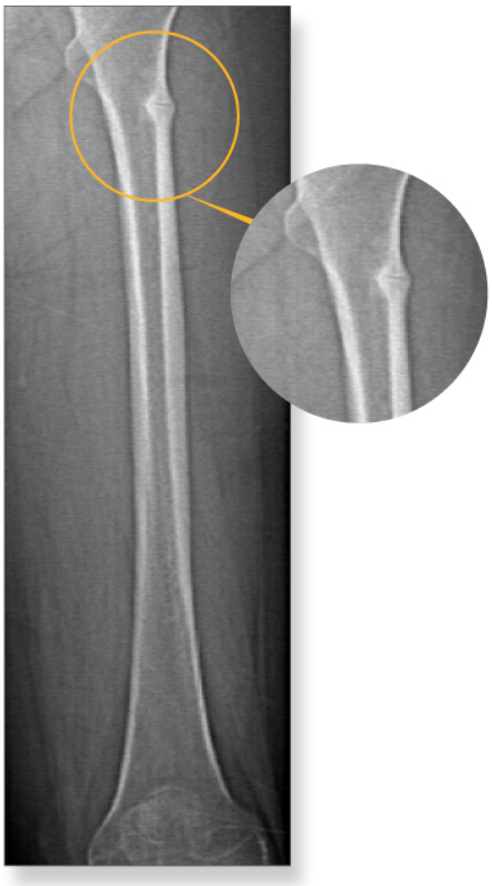

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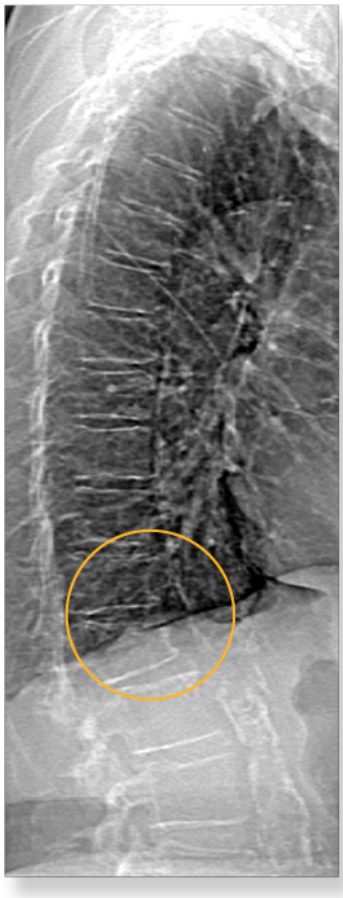

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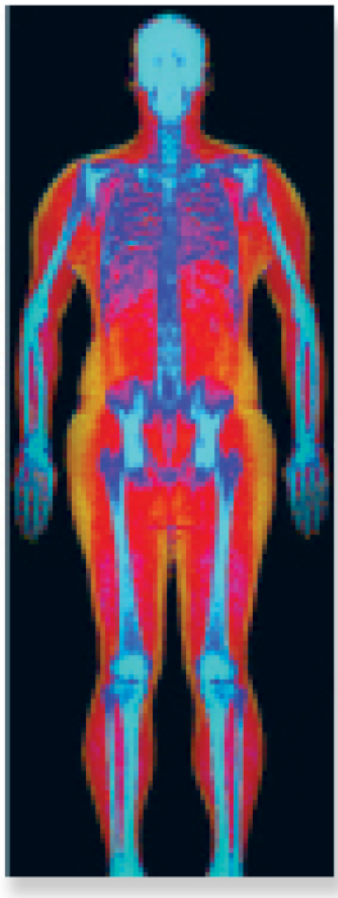

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RESEARCH ARTICLE

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Denosumab for Prevention of Acute Onset Immobilization-Induced Alterations of Bone Turnover: A Randomized Controlled Trial

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ABSTRACT

Metabolic bone disease is a devastating condition in critically ill patients admitted to an intensive care unit (ICU). We investigated the effects of early administration of the antiresorptive drug denosumab on bone metabolism in previously healthy patients. Fourteen patients with severe intracerebral or subarachnoid hemorrhage were included in a phase 2 trial. Within 72 hours after ICU admission, they were randomized in a 1:1 ratio to receive denosumab 60 mg or placebo subcutaneously. The primary endpoint was group differences in the percentage change of C-terminal telopeptide of type 1 collagen (CTX-1) levels in serum from denosumab/placebo application to 4 weeks thereafter. Changes in serum levels of bone formation markers and urinary calcium excretion were secondary outcome parameters. Regarding serum levels of CTX-1, changes over time averaged −0.45 ng/mL (95% confidence interval [CI] −0.72, −0.18) for the denosumab group and 0.29 ng/mL (95% CI −0.01, 0.58) for the placebo group. The primary endpoint, the group difference in changes between baseline and secondary measurement, adjusted for baseline serum levels and baseline neurological status, averaged −0.74 ng/mL (95% CI −1.14, −0.34; $p = 0.002$). The group difference in changes between baseline and secondary osteocalcin measurement averaged −5.60 ng/mL (95% CI −11.2, −0.04; $p = 0.049$). The group difference in averaged change between baseline and secondary measurement of 24-hour urine calcium excretion was significant (−1.77 mmol/L [95% CI −3.48, −0.06; $p = 0.044$]). No adverse events could be attributed to the study medication. The investigation proved that a single application of denosumab early after admission to an ICU prevents acute immobilization-associated increase in bone resorption among previously healthy individuals. © 2022 The Authors. *Journal of Bone and Mineral Research* published by Wiley Periodicals LLC on behalf of American Society for Bone and Mineral Research (ASBMR).

KEY WORDS: BIOCHEMICAL MARKERS OF BONE TURNOVER; DISEASES AND DISORDERS OF/RELATED TO BONE; ANTIRESORPTIVES

Introduction

In critically ill patients admitted to an intensive care unit (ICU), immobilization alters bone metabolism and reduces bone strength.[1,2] Antiresorptive agents such as bisphosphonates and denosumab (DMab) are approved for the prevention of osteoporotic fractures in postmenopausal women and men with a high risk of fractures.[3-5] Uncoupling of bone metabolism with elevated bone resorption[1] is the main contributing factor to acute disuse-associated bone loss. No antiresorptive drugs are specifically approved for prevention of bone loss in such patients. Compared with postmenopausal osteoporosis, immobilization induces specific structural alterations, such as greater cortical porosity, enormous quantities of osteocyte death, and lacunar mineralization.[6] In detail, apoptosis of osteocytes, the main mechanosensors, triggers the production of receptor activator nuclear factor κB ligand (RANKL) and, thus, bone resorption.[7] Additionally, osteocytes express sclerostin, an inhibitor

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of the Wnt signaling pathway, to an increased extent.[8] Changes in ion channels as well as alterations in the production of myokines, especially irisin, have also been detected as factors with a negative impact on bone integrity during immobilization.[9]

Several months after spinal cord injury, the human monoclonal antibody DMab, which binds with high specificity to RANKL, was shown to reduce osteoclast numbers and activity.[10-12] Improvements of bone metabolism and preservation of bone mineral density (BMD) were observed in these patients.[10-12] However, bone resorption starts immediately after patients become immobilized due to a sudden and severe medical condition.[1]

We analyzed the potential effect of antiresorptive therapy on immobilization-induced bone loss in previously healthy subjects. We included only those patients who had experienced severe intracerebral hemorrhage (ICH) or aneurysmal subarachnoid hemorrhage (aSAH) Hunt and Hess grade IV/V (HH IV/V) and were supposed to remain immobile for weeks or months after the incident.[13,14] We report the results of a phase 2 study comparing the effects of a single application of DMab versus placebo on bone resorption in persons with acute-onset immobility due to severe ICH or aSAH.

Materials and Methods

Study design and participants

Persons eligible for this single-center, randomized, double-blind, placebo-controlled, non-inferiority study were previously mobile and healthy patients admitted to the ICU at the department of neurosurgery, Medical University of Vienna (MUV). We included only those patients who were admitted because of an acute aSAH HH IV/V or ICH (spontaneous or due to arteriovenous malformation bleeding), with severe neurological deficits and a reduced state of consciousness (equivalent to HH IV/V). Severe neurological deficits were defined as stupor or deep coma, moderate to severe hemiparesis, early decerebrate rigidity to decerebrate rigidity, vegetative disturbances, and moribund appearance.[13] Furthermore, the inclusion criteria required that all patients needed ventilation at the time of admission and, in the estimation of the treating physician, were expected to remain more or less immobile during the following 4 weeks. Patients had to be between 30 and 80 years of age. Key exclusion criteria were the intake of drugs with potential effects on BMD, fragility fracture within the previous 6 months, non-osteoporotic bone disease, severe renal insufficiency, malignant disease in the preceding 5 years, pregnancy, diabetes mellitus, intake of anti-angiogenic agents, ill-fitting dentures, and maxillary or mandibular surgery in the preceding 3 months.

The study protocol was approved by the local ethics committee of the MUV (approval number 1155/2018) and performed in accordance with the ethical standards of the 1964 Declaration of Helsinki and its subsequent amendments. The ethics committee waived the need for informed consent before admission. As soon as a study participant's health status ameliorated and he/she was able to understand the possible consequences of the study, we explained the procedures and he/she signed the patient information sheet. This trial was registered at https://eudract.ema.europe.eu/, number 2018-000552-18.

Sample size considerations

Sample size was based on previous reports stating that, 1 month after a single application of DMab, serum levels of CTX-1 decreased by more than 80% (standard deviation: 13.3% points) in postmenopausal women.[15] The sample size was calculated using a two-sided *t* test with a significance level of 5% for comparing DMab with placebo in respect of the change from baseline to 4 weeks thereafter. A total sample size of 10 patients (5 DMab and 5 placebo; nQuery Advanced 8.0) provided a power of 80% to detect a difference of 30% points of the change in serum levels of CTX-1, which was considered the minimum clinically relevant group difference with respect to changes in 1 month. Considering a dropout rate of 20% (7% dropouts within 1 month; 18% in-hospital mortality; 12% mortality of all aSAH HH IV/V patients admitted to the ICU at the department of neurosurgery, MUV, from March 2014 to September 2017 during their stay at the ICU [unpublished data]), a total number of 14 patients was deemed necessary for the primary outcome of CTX-1.[15,16]

Randomization

Randomization was prepared by the statistician and performed online by the pharmacist after the patients had been stabilized. The Randomizer.at software was used with the minimization method; patients were stratified according to the severity of their neurological status.

Study procedure

Eligible patients were enrolled by the first author and randomized on a 1:1 basis to receive a single dose of DMab 60 mg (Prolia, Amgen, Inc., Thousand Oaks, CA, USA) or placebo subcutaneously within 72 hours after admission to the ICU. The blinded medication was prepared by the hospital pharmacy. Because of parenteral feeding, all patients received sufficient calcium daily without supplementation, such as Fresubin original fiber (Fresenius Kabi, Graz, Austria; 1500 kcal, 1 kcal per mL and 80 mg calcium in 100 mL). After the period of parenteral feeding, the patients received calcium supplementation (depending on their diet, up to 1000 mg/d). Vitamin D supplementation consisted of 4000 IU cholecalciferol (Oleovit D3, Fresenius Kabi) every 48 hours during their stay at the ICU. After this time, the patients were prescribed calcium (depending on diet) and vitamin D supplementation (depending on serum levels of 25OH vitamin D, up to 1000 IU/d).

According to the published literature, nimodipine, a dihydropyridine that blocks calcium influx through the L-type calcium channels, is standard treatment for the prevention of vasospasm after aSAH.[17] Independent of study participation, all patients after aSAH are given nimodipine at a dose of 2 mg/h by continuous intravenous perfusion for 21 days to treat or prevent vasospasm. After cardiorespiratory stabilization, all patients also received standard physiotherapy (approximately 30 minutes a day) during their stay at the ICU.

Blood samples were collected at baseline (before application of the study medication) and 4 weeks later, in each case in the morning after an overnight fast. At both time points, biochemical measurements including serum calcium, phosphate, creatinine, 25-OH-vitamin D, and parathyroid hormone were performed the same day. Other serum samples were centrifuged for 10 minutes at 3000*g*, frozen, and kept at −70°C until analysis of bone turnover markers in a single batch run. Levels of the bone resorption marker C-terminal telopeptide of type 1 collagen (CTX-1; Cobas 8000 Roche Analyzer, Roche Diagnostics, Rotkreuz, Switzerland; detection limit 0.5 ng/mL, intra-assay coefficient of variation 1.2%–4.7%, interassay coefficient of variation 1.5%–

5.7%), the bone formation markers osteocalcin (Oc; Cobas 8000 Analyzer, Roche Diagnostics; detection limit 0.01 ng/mL, intra-assay coefficient of variation 0.9%–1.3%, interassay coefficient of variation 1.2%–2.3%), bone-specific alkaline phosphatase (BAP; Liaison Analyzer, DiaSorin Inc., Stillwater, MN, USA; detection limit 0.1 μg/L, intra-assay coefficient of variation 3.3%–4.3%, interassay coefficient of variation 6.1%–8.1%), and procollagen type 1 amino-terminal propeptide (P1NP; Cobas 8000 Roche Analyzer, Roche Diagnostics; detection limit 5 ng/mL, intra-assay coefficient of variation 1.6%–3.5%, interassay coefficient of variation 2.0%–3.8%) as well as sclerostin (SOST; BI-20492, colorimetric sandwich immunoassays, Biomedica, Vienna, Austria; detection limit 3.2 pmol/L, intra-assay coefficient of variation ≤7%, interassay coefficient of variation ≤10%) and dickkopf 1 (DKK 1; BI-20412, colorimetric sandwich immunoassays, Biomedica; detection limit 0.38 pmol/L (0 pmol/L + 3 SD), intra-assay coefficient of variation ≤8.0%, interassay coefficient of variation ≤12.0%) were evaluated. Twenty-four-hour urine was collected for assessment of calcium excretion.

Using a handheld pulse-echo ultrasound device (Bindex BI-100, Software v.2.0, Bone Index, Finland Ltd., Kuopio, Finland), we performed a baseline measurement of cortical thickness at the proximal tibia. Combining this measure with patient characteristics (age, weight, height) yields the density index, which served as an estimate of proximal femur BMD.[18] The device consists of a focused ultrasound probe (3.0 MHz nominal center frequency) and a pulser unit plugged into a laptop's USB port. Measurements were performed at 1/3 of the length of the tibia from the proximal and distal heads, respectively. The length of the tibia was measured as the distance between the medial malleolus and the knee joint space (top of the medial condyle). Each measurement was performed five times by an experienced physiotherapist.

According to the study protocol, the study participants were required to visit the outpatient clinic of the department of neurosurgery at MUV at least 6 months after inclusion in the study. However, all non-essential control visits were prohibited because of the COVID-19 pandemic. Thus, in most cases, a follow-up inquiry was performed on the phone. The patients or caregivers were asked about potential adverse events and actual physical activity levels, which were then recorded on the modified Rankin scale.[19] Only a few patients who were scheduled to visit the outpatient clinic because of their primary disease and not for study purposes were physically present at the follow-up investigation.

Study outcomes

The prespecified primary endpoint was the percentage change in serum levels of CTX-1 from the time of DMab/placebo application to 4 weeks thereafter. Changes in serum levels of Oc, BAP, P1NP, SOST, DKK1, and urinary calcium excretion were secondary outcome parameters.

Statistical analysis

Raw data are presented as median and quartiles due to non-normal distributions. The single primary outcome and each secondary outcome were investigated in a separate ANCOVA model to adjust the group comparison for the respective baseline values and the stratification factor. Within- and between-group differences were estimated as least-squares means from these models (with 95% confidence intervals).

Statistical analysis was performed using SAS 9.4 (SAS Institute, Cary, NC, USA) based on a two-sided significance level of 5%. Statistical significance after correction for multiple secondary outcomes is shown in Table 3.

Results

Patient characteristics

Between May 2020 and April 2021, 24 patients were admitted to the ICU at the department of neurosurgery, MUV, because of aSAB (HH IV/V) or equally severe ICH. Fourteen consecutive patients were included in the study (Fig. 1). Baseline characteristics are given in Table 1. A density index beyond the upper threshold of 0.844 g/cm^2 evaluated by pulse-echo ultrasonometry suggested a normal BMD in most study participants. One patient in each group had a density index between 0.844 g/cm^2 and 0.779 g/cm^2, which would require additional dual-energy X-ray absorptiometry (DXA) measurement for verification of the diagnosis, and one patient was below the lower threshold (0.779 g/cm^2), suggesting osteoporosis. In all but two persons, cortical thickness suggested a normal BMD.

Efficacy

Biochemical parameters evaluated at baseline and follow-up are shown in Table 2. No clinically relevant abnormalities were found in the baseline routine chemistry. Follow-up values of gamma-glutamyl-transpeptidase were above normal in both groups. Concerning vitamin D, most patients had baseline and follow-up values below the normal range.

Mean levels of the bone resorption marker CTX-1 and the bone formation marker Oc decreased in the DMab group. Mean 24-hour urine calcium excretion increased in the placebo group, whereas no change was observed in the DMab group. Concerning the WNT signaling pathway inhibitors, average serum levels of DKK1 increased in the DMab group (Table 3). The other parameters (including SOST) revealed no relevant intergroup differences (not all data shown).

Changes in CTX-1 over time (calculated as the 4-week level minus baseline value), adjusted for baseline serum levels and baseline neurological status, averaged −0.45 ng/mL (95% confidence interval [CI] −0.72, −0.18) for the DMab group and +0.29 ng/mL (95% CI −0.01, +0.58) for the placebo group (Table 3). The primary endpoint, group differences in change between baseline and secondary measurement, adjusted for baseline serum levels and baseline neurological status, averaged −0.74 ng/mL (95% CI −1.14, −0.34) and was statistically significant ($p = 0.002$). Conservatively imputing the 4-week CTX-1 value for the deceased patient in the placebo group by the baseline value resulted in an adjusted mean change of +0.25 ng/mL (95% CI −0.00, +0.51) for the placebo group and a group difference of −0.71 (95% CI −1.08, −0.34, $p = 0.002$). Excluding one patient in the placebo group who had a very high baseline CTX-1 value, a sensitivity analysis showed a mean difference of −0.56 ng/mL (95% CI −0.82, −0.30, $p = 0.001$). Concerning the secondary endpoints, the group difference in change between baseline and the secondary Oc measurement, adjusted for baseline serum levels and baseline neurological status, averaged −5.60 ng/mL (95% CI −11.2, −0.049) and was statistically significant ($p = 0.049$, not adjusted for testing multiple secondary outcomes). Twenty-four-hour urine calcium excretion also revealed a significant group difference in averaged change between baseline and secondary measurement, adjusted for baseline levels and baseline neurological status (−1.77 mmol/L, 95% CI −3.48, −0.06, $p = 0.044$). Fig. 2 shows changes in these biochemical

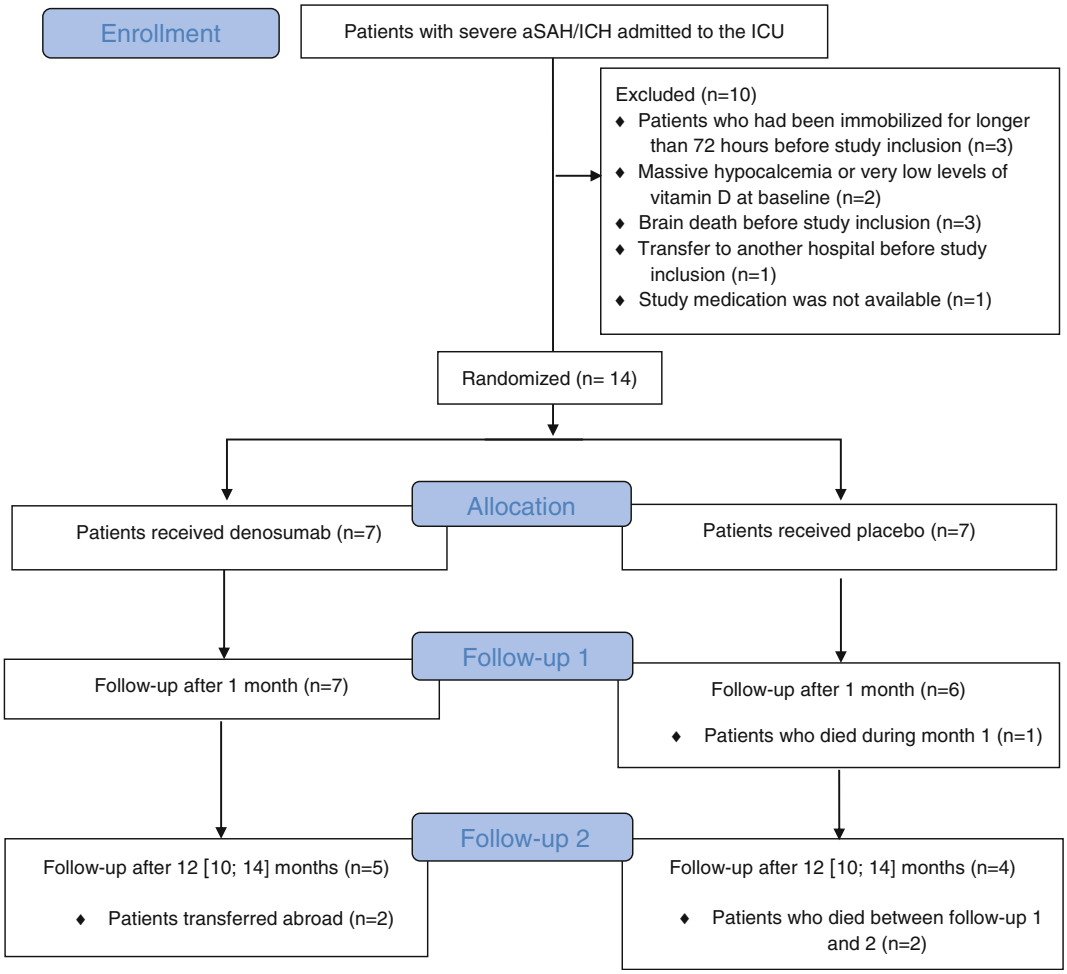

Fig. 1. Flow diagram of participants.

markers, which reflected bone resorption as well as bone formation.

Adverse events

No adverse events related to the study medication were observed during the first 4 weeks after application as well as during the follow-up period. Serum calcium levels remained within the normal range. One patient died within 4 weeks after aSAH due to fatal general brain edema based on previous vasospasm and cerebral infarction. The patient never received DMab because she was in the placebo group. None of the patients nor their caregivers, who were available for follow-up (12 [10; 14] months after baseline), reported any bone fractures or symptoms such as back pain.

Follow-up physical activity

In our study, nine patients were available for long-term follow up. Three patients had a score of 5 on the modified Rankin scale, three patients a score of 4, one patient a score of 2, and two patients a score of 1. In total, five of the patients were able to go back home. In detail, three patients had received DMab and two patients had received placebo. In comparison, four patients (two of each group) were transferred to a nursing home based on their low level of neurological status.

Table 1. Demographics and Characteristics of Study Participants

	DSMB group	Placebo group
n (male/female)	7 (3/4)	7 (4/3)
Age (years)	51 (44; 56)	59 (59; 65)
BMI (kg/m$^{2)}$	24.2 (21.6; 26.1)	25.7 (24.7; 27.8)
aSAB, *n*	4	5
ICH, *n*	3	2
Embolization, *n*	3	3
Clipping, *n*	2	1
Trepanation, *n*	5	4
External ventricular drainage, *n*	7	7
Density index (g/cm^2)	0.93 (0.90; 1.05)	0.90 (0.81; 0.98)
Cortical thickness (mm)	3.1 (2.8; 3.8)	2.9 (2.6; 3.4)

aSAB = aneurysmal subarachnoid hemorrhage; BMI = body mass index; DSMB = denosumab; ICH = intracerebral hemorrhage; *n* = number of subjects.

Data shown as median (quartiles).

Table 2. Biochemical Parameters at Baseline and Follow-Up

	DSMB group		Placebo group	
	Baseline ($n = 7$)	4-week follow-up ($n = 7$)	Baseline ($n = 7$)	4-week follow-up ($n = 6$)
Albumin-corrected Ca (mmol/L)	2.37 (2.31; 2.38)	2.26 (2.21; 2.33)	2.43 (2.32; 2.44)	2.37 (2.15; 2.58)
Phosphate (mmol/L)	1.1 (0.8; 1.2)	0.9 (0.8; 1.0)	0.7 (0.6; 1.3)	1.1 (0.8; 1.2)
GOT (U/L)	21 (14; 53)	27 (18; 47)	18 (16; 36)	28 (14; 42)
GPT (U/L)	23 (13; 25)	45 (29; 143)	16 (12; 37)	44 (25; 61)
GGT (U/L)	21 (10; 38)	134 (67; 187)	35 (16; 59)	271 (128; 433)
Creatinine (mg/dL)	0.54 (0.38, 0.83)	0.31 (0.27; 0.72)	0.93 (0.51; 1.42)	0.69 (0.55; 0.84)
BUN (mg/dL)	10.9 (5.9; 12.6)	11.8 (8.8; 19.8)	16.0 (12.5; 21.9)	22.6 (14.5; 28.6)
PTH (pg/mL)	31 (16; 45)	45 (27; 101)	43 (36; 88)	40 (40; 48)
25OHD (nmol/L)	58 (19; 80)	70 (32; 111)	47 (18; 79)	49 (41; 54)
CTX-1 (ng/mL)	0.57 (0.53; 0.79)	0.12 (0.10; 0.15)	0.58 (0.34; 0.71)	0.72 (0.60; 1.13)
Osteocalcin (ng/mL)	12.30 (11.90; 23.40)	8.6 (7.8; 10.5)	12.7 (9.9; 17.3)	14.35 (7.00; 15.90)
BAP (ng/mL)	8.2 (5.3; 12.0)	12.0 (9.3; 20.0)	11.0 (8.1; 16.0)	12.9 (7.3; 25.0)
P1NP (ng/mL)	40.0 (32.0; 62.0)	56.0 (41.0; 83.0)	45.0 (28.0; 50.0)	61.0 (33.0; 77.0)
SOST (pmol/L)	10.7 (9.5; 18.0)	17.1 (14.6; 22.8)	27.6 (8.9; 33.5)	27.0 (22.3; 29.8)
DKK1 (pg/mL)	19.5 (11.0; 24.0)	47.9 (13.3; 58.4)	17.6 (4.9; 35.0)	31.0 (9.4; 36.0)
24-hour urine: Ca excretion (mmol/L)	1.60 (1.20; 2.30)	1.45 (1.10; 2.00)	1.40 (0.20; 2.80)	3.70 (2.20; 4.20)

BAP = bone-specific alkaline phosphatase; BUN = blood urea nitrogen; CTX = cross-linked-C-telopeptide of type I collagen; DKK1 = dickkopf 1; DSMB = denosumab; we = weeks; GGT = gamma-glutamyltransferase; GOT = glutamic-oxaloacetic transaminase; GPT = gamma-glutamyl-transpeptidase; P1NP = N-terminal propeptide of type I collagen; PTH = parathyroid hormone; SOST = sclerostin; 25OHD = 25-OH-Vitamin D.

Data shown as median (quartiles). Number of measured baseline values of all parameters: 7; number of measured follow-up values: 5–7.

Discussion

The present investigation demonstrated the effectiveness of a single injection of DMab as a prophylactic regimen for immobilization-related bone loss in previously healthy, mobile patients admitted to an ICU because of severe intracerebral hemorrhage. DMab has been previously investigated in osteoporotic patients and patients who had been immobilized for several months due to spinal cord injury, but this is the first study evaluating acute-onset immobilization-induced alterations of bone turnover. At month one, the median reduction in CTX-1 reached nearly 80% in the DMab group, whereas it increased by 56% in the placebo group. This large intergroup difference of 136% points is even higher than the 86% points difference reported in the pivotal FREEDOM trial,[5] which included non-immobilized postmenopausal osteoporotic women. Thus, DMab may be more effective in acute-onset immobilization than in postmenopausal osteoporosis.

Uncoupling of osteoclast and osteoblast regulation is known to occur in critical illness. Bone resorption accelerates immediately; CTX-1 values peak 2 weeks after baseline and return to initial values by 4 weeks.[20] A systematic review revealed increased bone resorption but yielded inconsistent data regarding bone formation markers in persons with prolonged critical illness admitted to an ICU.[21] In the present study, which included persons with sudden-onset critical illness and immobilization, serum levels of bone resorption and bone formation markers did not change significantly from baseline to 4 weeks later in the placebo group. The decrease in serum levels of Oc in the DMab group concurs with the antiresorptive effect of the drug. The mean group-specific difference in change between the first and second measurement of this marker of osteoid mineralization was significant. Paradoxically, the bone formation markers representing earlier stages of bone formation P1NP and BAP[22] did not show significant changes between the first and second measurement. We speculate that less-sensitive assays of P1NP and BAP may fail to disclose other differences as well. The increase in 24-hour urine calcium excretion in the placebo group is in line with previous experimental studies,[23,24] No such change was observed in the DMab group, which led to a significant group-specific difference and underlined the efficacy of a single injection of DMab in preventing immobilization-induced bone loss in previously healthy and mobile persons.

Table 3. Biochemical Parameters: Group Difference in Changes Over Time as Least Squares Means (Adjusted for Respective Baseline Values and Baseline Neurological Status)

Outcome	DSMB mean change (95% CI)	Placebo mean change (95% CI)	Group difference of mean changes (95% CI)	*p* Value[a] for group difference
CTX-1 (ng/mL)	−0.45 (−0.72; −0.18)	0.29 (−0.01; 0.58)	−0.74 (−1.14; −0.34)	0.002
Oc (ng/mL)	−5.93 (−9.71; −2.16)	−0.34 (−4.40; 3.73)	−5.60 (−11.2; −0.04)	0.049
BAP (ng/mL)	11.2 (−5.0; 27.4)	2.5 (−14.9; 20.0)	8.6 (−16.2; −33.4)	0.451
P1NP (ng/mL)	56.7 (−39.8; 153.1)	21.9 (−82.6; 126.4)	34.8 (−109.3; 178.9)	0.598
SOST (pmol/L)	4.4 (−0.9; 9.8)	2.6 (−3.3; 8.4)	1.9 (−6.7; 10.5)	0.636
DKK1 (pg/mL)	25.1 (11.9; 38.3)	9.4 (−4.7; 23.5)	15.7 (−3.9; 35.3)	0.104
24-hour urine Ca (mmol/L)	0.02 (−1.09; 1.14)	1.79 (0.60; 2.99)	−1.77 (−3.48; −0.06)	0.044

BAP = bone-specific alkaline phosphatase; CI = confidence interval; CTX = cross-linked-C-telopeptide of type I collagen; DKK1 = dickkopf 1; DSMB = denosumab; P1NP = N-terminal propeptide of type I collagen; SOST = sclerostin.

[a]None of the secondary outcomes showed a statistically significant group difference after correction for multiple testing.

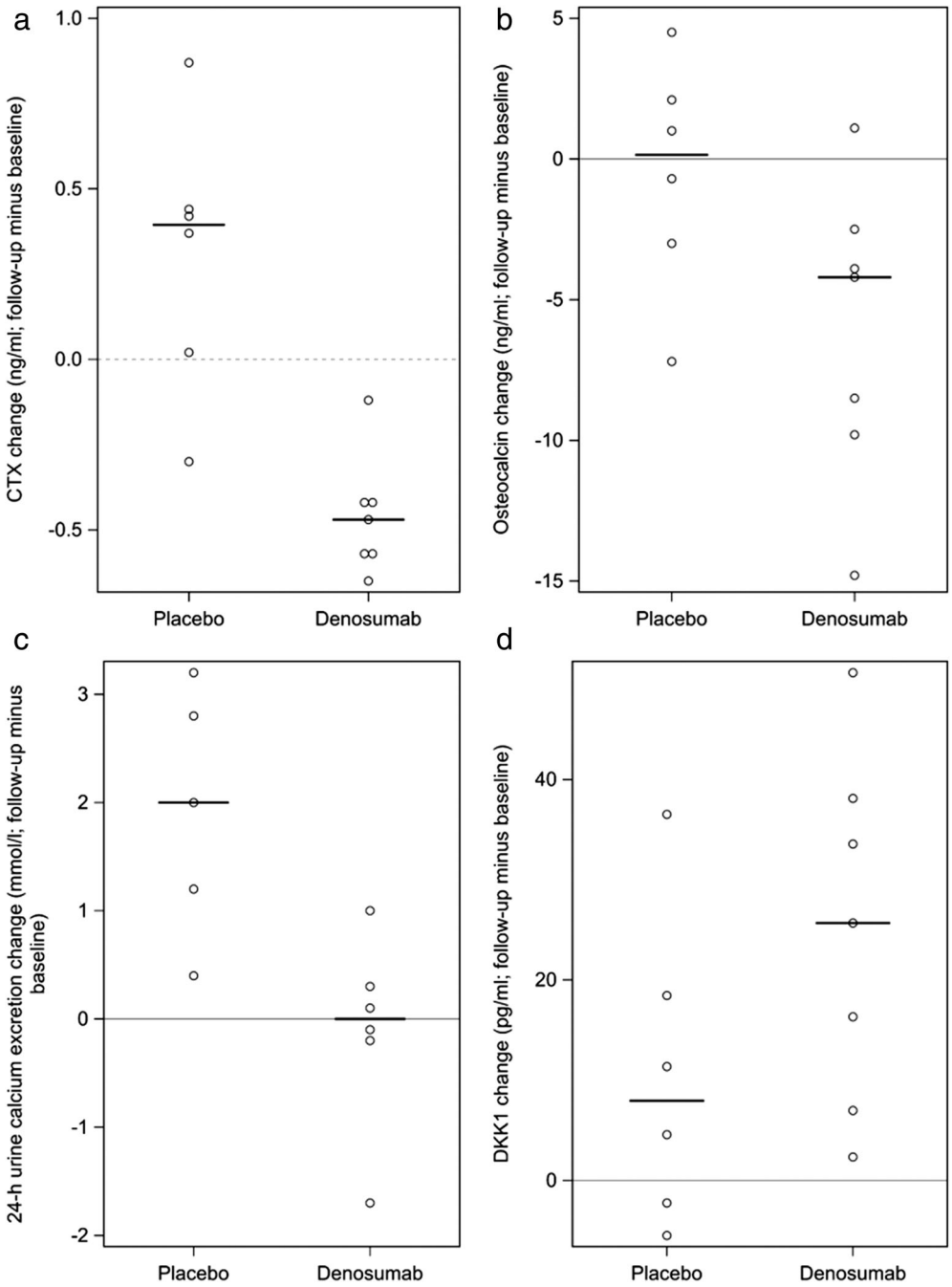

Fig. 2. Changes in biochemical markers of bone turnover.

In critically ill patients admitted to an ICU, bone turnover is driven by several factors. One factor that may have affected bone turnover in our population is decompressive craniotomy, which was needed in some patients to prevent critical levels of intracranial pressure. The number of persons who underwent decompressive craniotomy was balanced between groups. In case the trepanation has an impact on bone metabolism, we kept the diameter of the trepanation as small as possible.

Immobilization is evidently the main factor contributing to changes in bone turnover in ICU patients. Facilitation of physical activity and early mobilization are recommended.[25] Daily physiotherapy sessions of 30 minutes each may have been important in preserving the integrity of the musculoskeletal system in our patients, and may have been the reason for the absence of a significant change in bone turnover in the placebo group.

Neither of the two investigated WNT signaling pathway inhibitors—SOST or DKK1—was altered in the placebo group. This is in contrast to an experimental study that showed an increase in serum levels of SOST and DKK1 among young healthy males in bedrest.[26] Belavy and colleagues also noted the impact of resistive exercise on SOST and DKK1. Thus, the daily

physiotherapy sessions in our patients may have been the reason for no increase in WNT signaling pathway inhibitors. Serum sclerostin levels did not change in the DMab group. This is in line with a previous study evaluating treatment-naïve persons, which reported no changes in serum levels of sclerostin 3 months after the initiation of DMab.[27] Serum levels of DKK1 increased in the DMab group. This concurs with the reduction in the bone formation marker Oc. In this setting, DKK1 seems to better reflect the effect of DMab than SOST.

Evaluating vitamin D levels is important because due to different therapeutic interventions its metabolism may be dysregulated, leading to rapid reductions of vitamin D in critically ill patients.[28] We assume that regular supplementation prevented the reduction of vitamin D levels in participants of this study. In osteoporotic patients, a transient increase of serum levels of vitamin D has been observed after DMab application.[29] This fact may be responsible for the increase of the median levels of vitamin D levels by about 20% in our DMab group. We do not suspect an effect of vitamin D supplementation on bone turnover markers (including SOST) because it has been shown that even a loading dose of 540,000 IU did not significantly change the bone turnover markers in critically ill patients reevaluated 28 days after application.[30]

BMD loss has been reported to continue, and the 10-year probability of a fragility fracture increase, within 1 year after ICU discharge.[31] Retrospective data from pre-admission bisphosphonate users as well as prospective observational data concerning diverse antifracture therapy regimens revealed positive effects on BMD loss.[32,33] In general, DMab is not the first choice for treatment of relatively young patients suffering from osteoporosis. However, for several reasons, we decided to investigate the effect of DMab rather than bisphosphonate. One factor is the pathophysiology of immobilization-induced bone loss. In the absence of mechanical loading, osteocytes—which are the most crucial mechanosensors—increase the secretion of sclerostin and RANKL.[34] Thus, the use of an antibody against RANKL is meaningful from the pathophysiologic point of view. Experimental studies on the inhibition of sclerostin as well as RANKL production have shown that skeletal unloading induces less bone loss (for a review, see Rolvien and Amling[6]). An advantage of DMab is that it may also be used in patients with renal dysfunction, which is a frequent problem in critically ill patients. Another point is that the RANK/RANKL system is not only important for osteoclast generation but plays a role in muscle strength as well. In contrast to bisphosphonate therapy, DMab was shown to improve muscle mass and muscle strength in postmenopausal women.[35] An equivalent effect of DMab on immobilization would preserve muscle strength and serve as a very important additive effect of the drug in immobilized patients. A difference between parenteral bisphosphonate and DMab is the short-term effect of the latter treatment. Discontinuation of DMab leads to complete and rapid reversal of its effects on bone turnover markers.

On the one hand, the timed effect probably is an advantage in short-term immobilization. On the other hand, several case reports describing the occurrence of vertebral fractures after the discontinuation of DMab raised concerns about a rebound phenomenon with an increase in bone turnover markers.[36,37] According to a post hoc analysis of the Freedom Trial and its extension, the rate of vertebral fractures increases after discontinuation of DMab, but no higher incidence was observed after discontinuation of placebo.[38] The risk of such rebound-associated vertebral fractures increases with the duration of treatment and does not seem to occur before the second dose of DMab;[37,39] no such cases have been reported after a single dose.[40]

The limitations of the present study are worthy of mention. First, we did not perform areal BMD measurement using dual-energy X-ray absorptiometry. Owing to the patients' critical health status, we decided to use the pulse-echo ultrasound device; the investigation is performed at the bedside and without radiation exposure. The density index identifies hip osteoporosis with 82% specificity and 80% sensitivity.[41] Furthermore, we designed the study to evaluate the effect of a single application of DMab on bone turnover markers and not on BMD. Second, the interval between baseline and the assessment of the primary endpoint was relatively short. Mobility is not expected to improve markedly during 4 weeks after the onset of severe hemorrhage, and the majority of patients are presumed to be hospitalized during this time. Additionally, we were able to compare our data with a previous pivotal trial evaluating DMab,[5] which also reported changes in bone turnover markers by month one. Therefore, we considered 4 weeks a good time span. This investigation is a proof-of-concept study. The optimal duration of DMab treatment and the potential necessity of subsequent bisphosphonate therapy have not been evaluated yet. Regrettably, the two study groups were not of similar age; the difference was incidental. For randomization, patients were stratified by the severity of their neurological status and not by age. However, median baseline values of CTX-1 were similar. As with many subjects in the general population, most study participants had serum levels of vitamin D below the desirable range. Lastly, because of the COVID-19 pandemic, patients were advised to refrain from non-essential control visits to the hospital. Instead of the planned follow-up visit at our outpatient clinic, we called patients who were not scheduled for routine check-ups and interviewed them or their relatives in regard to potential adverse events and actual physical activity levels.

In conclusion, this study proved that a single application of DMab shortly after ICU admission reduced bone turnover in immobilized critically ill patients with severe ICH or aSAH. Extrapolating our findings, we assume that similar effects occur in persons immobilized for other reasons. Long-term studies with larger sample sizes should be performed to investigate the effect of early antiresorptive treatment with DMab on bone density and bone structure in patients with anticipated prolonged immobilization, eg, subarachnoid hemorrhage or severe trauma.

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Author contributions

Lisa Irina Wadiura: Conceptualization; data curation; investigation; resources; writing – original draft. **Maria Butylina:** Investigation; visualization; writing – review and editing. **Andrea Reinprecht:** Conceptualization; methodology; writing – review and editing. **Marie-Bernadette Aretin:** Conceptualization; investigation; writing – review and editing. **Mario Mischkulnig:** Writing – review and editing. **Andreas Gleiss:** Conceptualization; formal analysis; writing – review and editing. **Peter Pietschmann:** Conceptualization; methodology; writing – review and editing. **Katharina Kerschan-Schindl:** Conceptualization; data curation; methodology; project administration; supervision; writing – original draft; writing – review and editing.

Peer review

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Data availability statement

The data that support the findings of this study are available on request by the corresponding author.

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