# Denosumab for prevention of immobilization-induced alterations of bone turnover in patients admitted to a neurosurgical intensive care unit: a randomized controlled trial

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# Denosumab for prevention of immobilization-induced alterations of bone turnover in patients admitted to a neurosurgical intensive care unit: a randomized controlled trial

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**Abstract**

**Background:** Metabolic bone disease is a devastating condition in critically ill patients admitted to an intensive care unit (ICU). We investigated the effects of the antiresorptive drug denosumab on bone metabolism in previously healthy patients.

**Methods:** 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 four weeks thereafter. Changes in serum levels of bone formation markers and urinary calcium excretion were secondary outcome parameters.

**Results:** Regarding serum levels of CTX-1, changes over time averaged -0.45 ng/ml (95%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.

**Conclusion:** The investigation proved that a single application of denosumab early after admission to an ICU prevents any immobilization-associated increase in bone resorption among previously healthy individuals.

**Key words:** intensive care unit, denosumab, subarachnoid hemorrhage, intracerebral hemorrhage, CTX-1

## 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 are approved for the prevention of osteoporotic fractures in postmenopausal women and men with a high risk of fractures [3–5]. However, no antiresorptive drugs have been approved yet for immobilized patients. Compared with postmenopausal osteoporosis, immobilization induces specific structural alterations, such as greater cortical porosity, enormous quantities of osteocyte death, and lacunar mineralization [6].

Several months after spinal cord injury, the human monoclonal antibody denosumab, which binds with high specificity to receptor activator nuclear factor κB ligand (RANKL), was shown to reduce osteoclast numbers and activity [7–9]. An improvement of bone metabolism and preservation of bone mineral density (BMD) were observed in these patients [7–9]. 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 [10,11]. We report the results of a phase 2 study comparing the effects of a single application of denosumab versus placebo on bone resorption in persons with acute onset immobility due to severe ICH or aSAH.

## Material 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 [10]. 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 four 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 six months, non-osteoporotic bone disease, severe renal insufficiency, malignant disease in the preceding five years, pregnancy, diabetes mellitus, intake of antiangiogenic agents, ill-fitting dentures, and maxillary or mandibular surgery in the preceding three 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, one month after a single application of denosumab, serum levels of CTX-1 decreased by more than 80% (standard deviation: 13.3%-points) in postmenopausal women [12]. The sample size was calculated using a two-sided t-test with a significance level of 5% for comparing denosumab with placebo in respect of the change from baseline to four weeks thereafter. A total sample size of 10 patients (five denosumab and five 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 one month. Considering a dropout rate of 20% (7% dropouts within one 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 2014to 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 [12,13].

**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 denosumab 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. Due to parenteral feeding, all patients received sufficient calcium daily without supplementation, such as Fresubin® original fibre (Fresenius Kabi; 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/day). Vitamin D supplementation consisted of 4000 IU cholecalciferol (Oleovit D3, Fresenius Kabi, Austria) 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 mg/day).

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 [14]. 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 in order 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 four weeks later, in each case in the morning after an overnight fast. At both time points, biochemical measurements including serum calcium, phosphate, creatinine, 25-0H-vitamin D, and parathyroid hormone were performed the same day. Other serum samples were centrifuged for 10 minutes at 3,000 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, Switzerland, detection limit 0.5 ng/mL, intra-assay coefficient of variation 1.2–4.7 %, inter-assay coefficient of variation 1.5–5.7 %), the bone formation markers osteocalcin (Oc; Cobas 8000 Analyzer, Roche Diagnostics, Switzerland, detection limit: 0.01 ng/mL; intra-assay coefficient of variation: 0.9–1.3 %, inter-assay coefficient of variation: 1.2–2.3 %), bone-specific alkaline phosphatase (BAP; Liaison Analyzer, DiaSorin Inc., USA, detection limit: 0.1 µg/L; intra-assay coefficient of variation: 3.3–4.3 %, inter-assay coefficient of variation: 6.1–8.1 %) and procollagen type 1 amino-terminal propeptide (P1NP; Cobas 8000 Roche Analyzer, Roche Diagnostics, Switzerland, detection limit 5 ng/mL, intra-assay coefficient of variation 1.6–3.5 %, inter-assay 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 %, inter-assay coefficient of variation: ≤10%) and dickkopf 1 (DKK 1; BI-20412, colorimetric sandwich immunoassays, Biomedica, Vienna,

Austria; detection limit: 0.38 pmol/l (0 pmol/l + 3 SD); intra-assay coefficient of variation: ≤8.0 %, inter-assay 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, Bone Index, Finland Ltd., Kuopio, Finland Software v.2.0), 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 [15]. 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 six months after inclusion in the study. However, all non-essential control visits were prohibited due to 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 [16]. 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 pre-specified primary endpoint was the percentage change in serum levels of C-terminal telopeptide of type 1 collagen (CTX-1) from the time of denosumab/placebo application to four weeks thereafter. Changes in serum levels of osteocalcin (Oc), bone-specific alkaline phosphatase (BAP), procollagen type 1 amino-terminal propeptide (P1NP), sclerostin (SOST), dickkopf (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 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 (Figure 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 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 seen 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 denosumab group. Mean 24-hour urine calcium excretion increased in the placebo group, whereas no change was observed in the denosumab group. Concerning the WNT signaling pathway inhibitors, average serum levels of DKK1 increased in the denosumab group (Table 3). The other parameters revealed no relevant intergroup differences (data not 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%CI -0.72, -0.18) for the denosumab 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 -0.74ng/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). Figure 2 shows changes in these biochemical 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 four weeks after application as well as during the follow-up period. Serum calcium levels remained within the normal range. One patient died within four weeks after aSAH due to fatal general brain edema based on previous vasospasm and cerebral infarction. The patient never received denosumab 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**

At 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.

**Discussion**

The present investigation demonstrated the effectiveness of a single shot of denosumab as a prophylactic regimen for immobilization-related bone loss in previously healthy, mobile patients admitted to an ICU because of severe intracerebral hemorrhage. At month one, the median reduction in CTX-1 reached nearly 80% in the denosumab 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.

Uncoupling of osteoclast and osteoblast regulation is known to occur in critical illness. Bone resorption starts immediately; CTX-1 values peak two weeks after baseline and return to initial values by four weeks [17]. 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 [18]. 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 four weeks later in the placebo group. The decrease in serum levels of Oc in the denosumab group concurs with the antiresorptive effect of the drug. The mean group-specific difference in change between the first and second measurement of Oc was significant. Less sensitive assays of the other bone formation markers 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 [19,20]. No such change was observed in the denosumab group, which led to a significant group-specific difference und underlined the efficacy of a single shot of denosumab in preventing immobilization-induced bone loss in previously healthy and mobile persons.

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 and the diameter of the trepanation was kept as small as possible, thus reducing its impact on bone metabolism. Immobilization is evidently the main factor contributing to changes in bone turnover in ICU patients. Facilitation of physical activity and early mobilization are recommended [21]. 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 which showed an increase in serum levels of SOST and DKK1 among young healthy males in bedrest [22]. Belavy et al. 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 denosumab group. This is in line with a previous study evaluating treatment-naïve persons, which reported no changes in serum

levels of sclerostin three months after the initiation of denosumab [23]. Serum levels of DKK1 increased in the denosumab group. This concurs with the reduction in the bone formation marker Oc.

BMD loss has been reported to continue, and the 10-year probability of a fragility fracture increase, within one year after ICU discharge [24]. Retrospective data from pre-admission bisphosphonate users as well as prospective observational data concerning diverse anti-fracture therapy regimens revealed positive effects on BMD loss [25,26]. For several reasons, we decided to investigate the effect of denosumab 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 [27]. 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 T & Amling M [6]). An advantage of denosumab 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 genesis, but plays a role in muscle strength as well. In contrast to bisphosphonate therapy, denosumab was shown to improve muscle mass and muscle strength in postmenopausal women [28]. An equivalent effect of denosumab 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 denosumab is the short-term effect of the latter treatment. Discontinuation of denosumab 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 denosumab raised concerns about a rebound phenomenon with an increase in bone turnover markers [29,30]. According to a post hoc analysis of the Freedom Trial and its extension, the rate of vertebral fractures increases after discontinuation of denosumab, but no higher incidence was observed after discontinuation of placebo [31]. 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 denosumab [30,32]; no such cases have been reported after a single dose [33].

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 [34]. Furthermore, we designed the study to evaluate the effect of a single application of denosumab 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 four 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 denosumab [5], which also reported changes in bone turnover markers by month one. Therefore, we considered four weeks a good timespan. 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. Lastly, due to 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 of potential adverse events and actual physical activity levels.

**Conclusion**

This study proved that a single application of denosumab 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 denosumab on bone density and bone structure.

**Abbreviations**

aSAH: aneurysmal subarachnoid hemorrhage; BAP: bone-specific alkaline phosphatase; BMD: bone mineral density; CTX-1: C-terminal telopeptide of type 1 collagen; dickkopf; ICH: intracerebral hemorrhage; HH IV/V: Hunt and Hess grade IV/V; ICU: intensive care unit; Oc: osteocalcin; P1NP: procollagen type 1 amino-terminal propeptide; SOST: sclerostin; RANKL: receptor activator nuclear factor κB ligand

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**Author contributions**

LW, AR, MBA, PP, and KKS designed the research. LW, MB, and KKS performed the research and collected the data. AR performed the sample size calculation and analyzed the data. LW and KKS drafted the manuscript. All authors read and approved the final manuscript.

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**Availability of data and material**

Datasets can be provided upon request by the corresponding author.

**Declarations**

**Ethics approval and consent to participate**

This study was performed in accordance with the principles of the Declaration of Helsinki. Approval was granted by the ethics committee of the Medical University of Vienna (approval number 1155/2018). 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.

**Consent for publication**

Not applicable

**Compeding interests**

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## References


1. Baecker N, Tomic A, Mika C, Gotzmann A, Platen P, Gerzer R, et al. Bone resorption is induced on the second day of bed rest: results of a controlled crossover trial. J Appl Physiol [Internet]. 2003;95:977–82. Available from: https://www.physiology.org/doi/10.1152/japplphysiol.00264.2003

2. Shirazi-Fard Y, Kupke JS, Bloomfield SA, Hogan HA. Discordant recovery of bone mass and mechanical properties during prolonged recovery from disuse. Bone [Internet]. 2013;52:433–43. Available from: https://linkinghub.elsevier.com/retrieve/pii/S8756328212012501

3. Black DM, Thompson DE, Bauer DC, Ensrud K, Musliner T, Hochberg MC, et al. Fracture Risk Reduction with Alendronate in Women with Osteoporosis: The Fracture Intervention Trial. J Clin Endocrinol Metab [Internet]. 2000;85:4118–24. Available from: https://academic.oup.com/jcem/article-lookup/doi/10.1210/jcem.85.11.6953

4. Black DM, Delmas PD, Eastell R, Reid IR, Boonen S, Cauley JA, et al. Once-Yearly Zoledronic Acid for Treatment of Postmenopausal Osteoporosis. N Engl J Med [Internet]. 2007;356:1809–22. Available from: http://www.nejm.org/doi/abs/10.1056/NEJMoa067312

5. Cummings SR, Martin JS, McClung MR, Siris ES, Eastell R, Reid IR, et al. Denosumab for Prevention of Fractures in Postmenopausal Women With Osteoporosis. Obstet Gynecol Surv [Internet]. 2009;64:805–7. Available from: https://journals.lww.com/00006254-200912000-00019

6. Rolvien T, Milovanovic P, Schmidt FN, Kroge S, Wölfel EM, Krause M, et al. Long-Term Immobilization in Elderly Females Causes a Specific Pattern of Cortical Bone and Osteocyte Deterioration Different From Postmenopausal Osteoporosis. J Bone Miner Res [Internet]. 2020;35:1343–51. Available from: https://onlinelibrary.wiley.com/doi/10.1002/jbmr.3970

7. Gifre L, Ruiz-Gaspà S, Carrasco JL, Portell E, Vidal J, Muxi A, et al. Effect of recent spinal cord injury on the OPG/RANKL system and its relationship with bone loss and the response to denosumab therapy. Osteoporos Int [Internet]. 2017;28:2707–15. Available from: http://link.springer.com/10.1007/s00198-017-4090-4

8. Gifre L, Vidal J, Carrasco JL, Muxi A, Portell E, Monegal A, et al. Denosumab increases sublesional bone mass in osteoporotic individuals with recent spinal cord injury. Osteoporos Int [Internet]. 2016;27:405–10. Available from: http://link.springer.com/10.1007/s00198-015-3333-5

9. Cirnigliaro CM, La Fountaine MF, Parrott JS, Kirshblum SC, McKenna C, Sauer SJ, et al. Administration of Denosumab Preserves Bone Mineral Density at the Knee in Persons With Subacute Spinal Cord Injury: Findings From a Randomized Clinical Trial. JBMR Plus [Internet]. 2020;4. Available from:

https://onlinelibrary.wiley.com/doi/10.1002/jbm4.10375

10. Hunt WE, Hess RM. Surgical risk as related to time of intervention in the repair of intracranial aneurysms. J Neurosurg. 1968;28:14–20.

11. Wadiura LI, Herta J, Mischkulnig M, Hirschmann D, Borkovec M, Hosmann A, et al. The Evoked Potential Score for SSEP and BAEP—A Prognostic Marker for the Long-Term Neurological Outcome in Patients after Poor-Grade Subarachnoid Hemorrhage. Diagnostics [Internet]. 2021;11:1075. Available from: https://www.mdpi.com/2075-4418/11/6/1075

12. McClung MR, Lewiecki EM, Cohen SB, Bolognese MA, Woodson GC, Moffett AH, et al. Denosumab in Postmenopausal Women With Low Bone Mineral Density. Obstet Gynecol Surv [Internet]. 2006;61:384–6. Available from: http://journals.lww.com/00006254-200606000-00017

13. Zafar SF, Postma EN, Biswal S, Fleuren L, Boyle EJ, Bechek S, et al. Electronic Health Data Predict Outcomes After Aneurysmal Subarachnoid Hemorrhage. Neurocrit Care [Internet]. 2018;28:184–93. Available from: http://link.springer.com/10.1007/s12028-017-0466-8

14. Dorhout Mees S, Rinkel GJE, Feigin VL, Algra A, van den Bergh WM, Vermeulen M, et al. Calcium antagonists for aneurysmal subarachnoid haemorrhage. Cochrane Database Syst Rev [Internet]. 2007; Available from: https://doi.wiley.com/10.1002/14651858.CD000277.pub3

15. Karjalainen JP, Riekkinen O, Töyräs J, Jurvelin JS, Kröger H. New method for point-of-care osteoporosis screening and diagnostics. Osteoporos Int [Internet]. 2016;27:971–7. Available from: http://link.springer.com/10.1007/s00198-015-3387-4

16. Wilson DA, Nakaji P, Albuquerque FC, McDougall CG, Zabramski JM, Spetzler RF. Time course of recovery following poor-grade SAH: the incidence of delayed improvement and implications for SAH outcome study design. J Neurosurg [Internet]. 2013;119:606–12. Available from: https://thejns.org/view/journals/j-neurosurg/119/3/article-p606.xml

17. Gavala A, Makris K, Korompeli A, Myrianthefs P. Evaluation of Bone Metabolism in Critically Ill Patients Using CTx and PINP. Biomed Res Int [Internet]. 2016;2016:1–9. Available from: https://www.hindawi.com/journals/bmri/2016/1951707/

18. Orford N, Cattigan C, Brennan SL, Kotowicz M, Pasco J, Cooper DJ. The association between critical illness and changes in bone turnover in adults: a systematic review. Osteoporos Int [Internet]. 2014;25:2335–46. Available from: http://link.springer.com/10.1007/s00198-014-2734-1

19. Spatz JM, Fields EE, Yu EW, Pajevic PD, Bouxsein ML, Sibonga JD, et al. Serum Sclerostin Increases in

391 Healthy Adult Men during Bed Rest. J Clin Endocrinol Metab [Internet]. 2012;97:E1736–40. Available from:
392 https://academic.oup.com/jcem/article/97/9/E1736/2536590
393 20. Zerwekh JE, Ruml LA, Gottschalk F, Pak CYC. The Effects of Twelve Weeks of Bed Rest on Bone
394 Histology, Biochemical Markers of Bone Turnover, and Calcium Homeostasis in Eleven Normal Subjects. J
395 Bone Miner Res [Internet]. 2009;13:1594–601. Available from:
396 http://doi.wiley.com/10.1359/jbmr.1998.13.10.1594
397 21. Rousseau A-F, Kerschan-Schindl K, Scherkl M, Amrein K. Bone metabolism and fracture risk during and
398 after critical illness. Curr Opin Crit Care [Internet]. 2020;Publish Ah. Available from:
399 https://journals.lww.com/10.1097/MCC.0000000000000734
400 22. Belavý DL, Baecker N, Armbrecht G, Beller G, Buehlmeier J, Frings-Meuthen P, et al. Serum sclerostin and
401 DKK1 in relation to exercise against bone loss in experimental bed rest. J Bone Miner Metab [Internet].
402 2016;34:354–65. Available from: http://link.springer.com/10.1007/s00774-015-0681-3
403 23. Tsourdi E, Makras P, Rachner TD, Polyzos S, Rauner M, Mandanas S, et al. Denosumab effects on bone
404 density and turnover in postmenopausal women with low bone mass with or without previous treatment. Bone
405 [Internet]. 2019;120:44–9. Available from: https://linkinghub.elsevier.com/retrieve/pii/S875632821830365X
406 24. Orford NR, Lane SE, Bailey M, Pasco JA, Cattigan C, Elderkin T, et al. Changes in Bone Mineral Density in
407 the Year after Critical Illness. Am J Respir Crit Care Med [Internet]. 2016;193:736–44. Available from:
408 http://www.atsjournals.org/doi/10.1164/rccm.201508-1514OC
409 25. Lee P, Ng C, Slattery A, Nair P, Eisman JA, Center JR. Preadmission Bisphosphonate and Mortality in
410 Critically Ill Patients. J Clin Endocrinol Metab [Internet]. 2016;101:1945–53. Available from:
411 https://academic.oup.com/jcem/article-lookup/doi/10.1210/jc.2015-3467
412 26. Orford NR, Bailey M, Bellomo R, Pasco JA, Cattigan C, Elderkin T, et al. The association of time and
413 medications with changes in bone mineral density in the 2 years after critical illness. Crit Care [Internet].
414 2017;21:69. Available from: http://ccforum.biomedcentral.com/articles/10.1186/s13054-017-1657-6
415 27. Sapir-Koren R, Livshits G. Osteocyte control of bone remodeling: is sclerostin a key molecular coordinator
416 of the balanced bone resorption–formation cycles? Osteoporos Int [Internet]. 2014;25:2685–700. Available from:
417 http://link.springer.com/10.1007/s00198-014-2808-0
418 28. Bonnet N, Bourgoin L, Biver E, Douni E, Ferrari S. RANKL inhibition improves muscle strength and insulin
419 sensitivity and restores bone mass. J Clin Invest [Internet]. 2019;129:3214–23. Available from:
420 https://www.jci.org/articles/view/125915

421 29. Bone HG, Bolognese MA, Yuen CK, Kendler DL, Miller PD, Yang Y-C, et al. Effects of Denosumab
422 Treatment and Discontinuation on Bone Mineral Density and Bone Turnover Markers in Postmenopausal
423 Women with Low Bone Mass. J Clin Endocrinol Metab [Internet]. 2011;96:972–80. Available from:
424 https://academic.oup.com/jcem/article-lookup/doi/10.1210/jc.2010-1502
425 30. Tsourdi E, Zillikens MC, Meier C, Body J-J, Gonzalez Rodriguez E, Anastasilakis AD, et al. Fracture Risk
426 and Management of Discontinuation of Denosumab Therapy: A Systematic Review and Position Statement by
427 ECTS. J Clin Endocrinol Metab [Internet]. 2021;106:264–81. Available from:
428 https://academic.oup.com/jcem/article/106/1/264/5939974
429 31. Cummings SR, Ferrari S, Eastell R, Gilchrist N, Jensen J-EB, McClung M, et al. Vertebral Fractures After
430 Discontinuation of Denosumab: A Post Hoc Analysis of the Randomized Placebo-Controlled FREEDOM Trial
431 and Its Extension. J Bone Miner Res [Internet]. 2018;33:190–8. Available from:
432 https://onlinelibrary.wiley.com/doi/10.1002/jbmr.3337
433 32. Lamy O, Stoll D, Aubry-Rozier B, Rodriguez EG. Stopping Denosumab. Curr Osteoporos Rep [Internet].
434 2019;17:8–15. Available from: http://link.springer.com/10.1007/s11914-019-00502-4
435 33. Anastasilakis AD, Polyzos SA, Makras P, Aubry-Rozier B, Kaouri S, Lamy O. Clinical Features of 24
436 Patients With Rebound-Associated Vertebral Fractures After Denosumab Discontinuation: Systematic Review
437 and Additional Cases. J Bone Miner Res [Internet]. 2017;32:1291–6. Available from:
438 https://onlinelibrary.wiley.com/doi/10.1002/jbmr.3110
439 34. Schousboe JT, Riekkinen O, Karjalainen J. Prediction of hip osteoporosis by DXA using a novel pulse-echo
440 ultrasound device. Osteoporos Int [Internet]. 2017;28:85–93. Available from:
441 http://link.springer.com/10.1007/s00198-016-3722-4
442
443 **Figure 1 Flow diagram of participants**
444 **Figure 2 Changes in biochemical markers of bone turnover**

# Figures

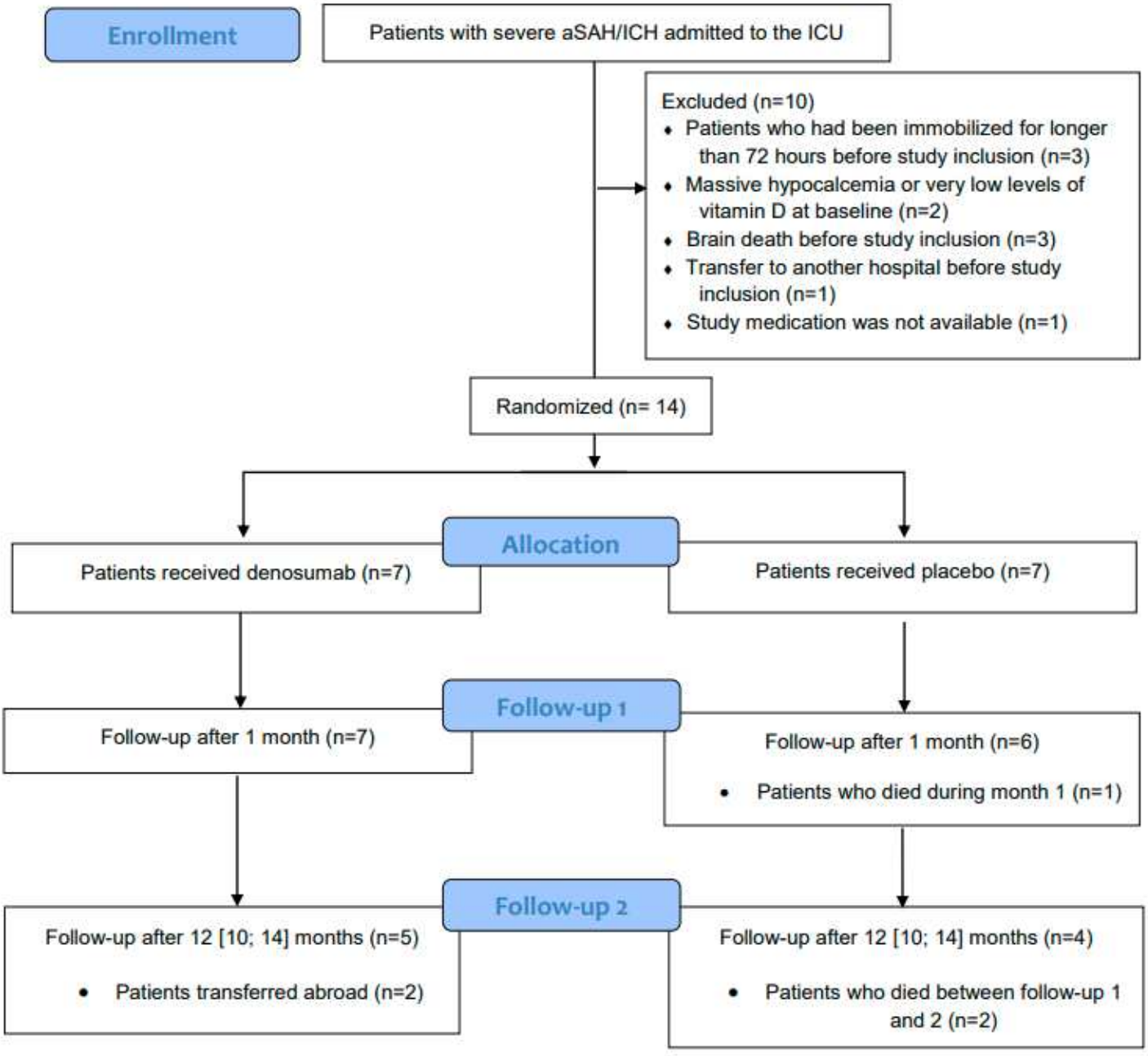


**Figure 1**

Flow diagram of participants

**Figure 2**

Changes in biochemical markers of bone turnover

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