The predictive value of bone turnover markers during discontinuation with alendronate: a cohort study

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Introduction

Osteoporosis increases the risk of fractures. A number of treatments are available that decreases the fracture risk. Ultimately the treatment must prevent hip fractures as these fractures dramatically increase mortality to 30% [1]. At present three treatments with evidence for prevention of hip fractures: alendronate (an oral bisphosphonate) [2], zoledronic acid (an intravenous bisphosphonate) [3], and denosumab (a subcutaneously administered RANKL-antibody) [4] are available. First line treatment is alendronate that by far is the cheapest. At present, denosumab can only be reimbursed if the patient cannot take alendronate due to side effects, treatment failure or contra indications why denosumab is second line therapy. Zoledronic acid is administered intravenously and therefore in clinical practice almost exclusively administered in hospitals, which limits its use.

Alendronate adheres to the bone, induces apoptosis of the osteoclast and prevents bone resorption [5]. The adhesion is very strong meaning that the half-time of alendronate is years and that the effect on bone remains long after administration has stopped [6].

Alendronate reduces the risk of both vertebral- and hip fractures by approximately 50% [7], [8]. It has, however, become evident that long-term anti-resorptive therapy (bisphosphonates and denosumab) may lead to serious side effects such as atypical femoral fractures [9] or osteonecrosis of the jaw [10]. This has obviated the need for an evidence-based assessment of long-term benefits and risks at the patient level. The alendronate extension study showed that despite stopping treatment after five years the anti-fracture efficacy regarding non-vertebral and radiological vertebral fractures persists for an additional five years in patients with bone mineral density (BMD) T-score > -2.5 at the femoral neck, no fractures during treatment, and no previous vertebral fracture [11], [12]. It is therefore now clinical practice, that treatment is discontinued after five years in patients that fulfil these criteria. Based on the alendronate extension study it was assumed, that bone turnover monitored by biochemical markers would stay suppressed for years after stopping treatment, however, other studies have demonstrated that there is a great variability in the change in bone turnover markers seen after stopping treatment with alendronate in a real-life setting [13]. It is currently unknown which factors predict the outcome after stopping treatment. The alendronate extension study also demonstrated that women stopping treatment with alendronate will lose BMD at the hip. In fact the average BMD at the hip was back to baseline levels after 5 years off treatment [11]. Zoledronic acid adheres even stronger to bone than alendronate and zoledronic acid treatment can be discontinued after three years based on the same criteria as alendronate [14].

Therefore, this treatment break algorithm has raised a number of concerns: Currently patients off treatment are being monitored with yearly Dual-energy X-ray Absorptiometry (DXA) scan, but is DXA the optimal method for monitoring? Could other more easily assessable tools, such as biochemical markers of bone turnover be superior? When and if should treatment be re-initiated? Finally, is it possible to predict who will fracture during discontinuation of therapy and therefore should not stop treatment? We want to investigate if biochemical markers of bone turnover predict bone loss after stopping alendronate and therefore potentially can replace the yearly DXA.

Aims

To investigate the predictive value of markers of bone turnover on bone loss 12 months after stopping alendronate therapy.

Study population and methods

Design

The study is a cohort study comprising 140 patients with osteoporosis stopping treatment with alendronate.

Primary endpoint

- To investigate to what extent changes in carboxy-terminal collagen crosslinks (CTX)[1] three and six months after stopping alendronate treatment predict changes in total hip BMD after one year.

Secondary endpoints

- To investigate to what extent baseline CTX when stopping alendronate treatment predicts changes in total hip BMD after one year.
- To investigate to what extent changes in procollagen type I N-terminal propeptide (PINP)[2] three and six months after stopping alendronate treatment predict changes in total hip BMD after one year.
- To investigate to what extent changes in the ratio CTX/PINP 3 and 6 months after stopping alendronate treatment predict changes in total hip BMD after one year.
- The proportion of the study population in which bone turnover increases to above premenopausal/young adult reference levels[3] after 3, 6, and 12 months.
- The proportion of the study population who loses BMD beyond least significant change in lumbar spine and total hip.

Study population

Inclusion criteria:

- Postmenopausal women (postmenopausal for at least two years)
- Men above 50 years
- Treatment for at least five years with alendronat
- BMD T-score total hip > -2.5
- BMD T-score lumbar spine (L1-L4) > -4

Exclusion criteria:

- Any low-energy fracture within the previous five years during alendronat treatment (not including fingers, toes, or skull)
- Low-energy vertebral fracture at any time
- Low-energy hip fracture at any time
- Ongoing treatment with glucocorticoids

[1] Electrochemiluminescence immunoassay (ECLIA) will be used.

[2] Electrochemiluminescence immunoassay (ECLIA) will be used.

[3] s-CTX reference range: Men < 30 years: 0,16 - 0,87 µg/l. Men 30 - 50 years: 0,09 - 0,63 µg/l. Men 50 – 70 years: 0,04 - 0,84 µg/l. Women < 30 years: 0,04 - 0,87 µg/l. Women 30 - 50 years: 0,04 - 0,59 µg/l.
s- PINP reference range: Men > 18 years: 21 - 110 µg/l. Women 18 - 50 years: 17 – 124µg/l.

- Metabolic bone disease
- Hormone replacement therapy
- Cancer
- Other conditions affecting bone metabolism

Duration of study

Included patients will participate in the study for 12 months. The participants will receive a letter from the investigator after the termination of the study with information about the study results.

Study plan

	Baseline	M1	M3	M6	M12
Informed consent	X				
Medical history	X				
Bone markers	X	X	X	X	X
Biochemistry	X				
DXA	X			X	X
Fracture history					X

Investigations

Bone turnover markers: We will collect peripheral blood samples in the morning (7.30 – 10.45 a.m.) after a minimum of eight hours of fasting. CTX, PINP, osteocalcin and BSAP will be measured at every clinical visit. The blood samples will be centrifuged at 400 rpm at 5 degrees Celsius for 10 minutes and stored for maximum 12 months at -80 degrees Celsius until batch analysis. All the samples will be analyzed as a single batch at the Department of Biochemistry, Aarhus University Hospital analysis to reduce the analytical variation. BSAP will be measured on serum, whereas the rest of the BTMs will be analyzed on EDTA plasma. PINP, CTX and osteocalcin will be determined by Electrochemiluminescence immunoassay (ECLIA) (Cobas 8000 immunoassay analyzer, Roche Diagnostics) with a precision of ±7.4% at 32 µg/l (95% CI), ±10% at 0.30 µg/l, and ±6% at 18 µg/l, receptively. BASP will be analyzed by immunoassay (ISYS Ostase, IDS) with a precision of ±20% at 6.5 µg/l. If the results of any of the blood samples were below or above the detection range, we used the lowest or highest value, respective.

Biochemistry: We will collect peripheral blood samples in the morning (7.30 – 10.45 a.m.) after a minimum of eight hours of fasting. We will measure markers of diseases affecting bone metabolism: ionized calcium, parathyroid hormone (PTH), 25-hydroxyvitamin D, magnesium, creatinine, estimated glomerular filtration rate (eGFR), phosphate, thyroid-stimulating hormone (TSH) at baseline. The samples will be analyzed at by the Department of Biochemistry, Aarhus University Hospital.

Bone mass: DXA scans measuring the areal bone mineral density (aBMD) (g/cm^2) of the lumbar spine (L1-L4) and hip (left) using a Hologic Discovery scanner (Hologic, Inc., Waltham, MA, USA) will be performed at baseline and after 6

and 12 months. The right hip will be scanned in case of prosthesis. We will cross-calibrate the DXA scanner with a phantom to read aBMD. All participants will be scanned on the same scanner throughout the trial. We consider the least significant change (LSC) ≥ 5% at the total hip and femoral neck and ≥ 3% at the lumbar spine. We will estimate incident fractures using the vertebral fracture assessment (VFA) tool on the scanner. Additionally, we will obtain lumbar spine trabecular bone score (TBS) using the TBS iNsight software, version 2.1.0.0 (Medimaps, Merignac, France).

Fracture history: After 12 months participants will be questioned about incident fractures during the discontinuation period. Information will be confirmed using discharge notes from hospitals.

Data management

Source data identification and verification

Source data will be entered into the patient file and/or the CRF. Most data will be found both in the patient file and the CRF, but some source data, for example body weight and height will be entered directly into the CRF. This will be specified in the source data file.

Information about incident fractures during the discontinuation period will be passed on from discharge notes from hospitals, and these data will be entered into the patient file and the CRF.

Subject data protection

Access to CRFs will be limited to investigators and other healthcare professionals involved in this study. The patient files are electronic and kept on the hospital electronic patient file system. Any printouts of the patient files and the CRFs will be kept behind locked doors after working hours.

Data handling

When data analyses is finalized, the data will be converted to an anonymous form using participation numbers only, and names and social security numbers will be removed.

Statistical analysis and power calculation

Changes over time will be investigated using paired t-tests or repeated measurements statistics. The relations between baseline levels and changes in the levels of the biochemical markers and changes in BMD will be investigated using correlation and regression analyses.

The primary endpoint is if changes in CTX after three and six months predict changes in lumbar spine BMD. It has previously been demonstrated that the change and the variability of the change in CTX three and six months after stopping alendronate is large: +115±25% and +140±30%, respectively. The change in total hip BMD was in the same study -1.4±0.4% after 12 months [13]. It can be estimated that 140 patients would be needed to have 80% statistical power to demonstrate a significant correlation with a $r^2 = 0.25$.

Recruitment of participants

The patients will be recruited from the outpatient clinic and the DXA unit to which patients are referred from their general practitioner or from other departments. Potential participants will be approached during visits or by letter (appendix

1). Patients with osteoporosis living in the Central Region of Denmark, who have redeemed 22 prescriptions for alendronate within the last five years[4], will be approached by letter (appendix 1a).
All staff at the outpatient clinics will be informed about the study including inclusion and exclusion criteria. If the patient is interested in receiving further information he or she will receive written information about the project (appendix 2). Moreover, we will advertise in daily newspapers and on the webpages www.forsøgsperson.dk and www.sundhed.dk (appendix 3). Participants who respond to advertisements will be sent written information (appendix 2). Subsequently, all potential participants will be invited to a consultation to obtain oral information from one of the investigators. The consultation will take place at the Department of Endocrinology and Internal Medicine, Aarhus University Hospital, Tage-Hansens Gade 2, 8000 Aarhus C where research facilities including offices are available. Family members or friends are welcome to participate in the meeting as well. Afterwards the potential participant will be given 14 days to consider whether they wish to participate in the study or not, before they sign the consent form. The participants will not take part in the study before the consent form has been signed.

Bio-banks

A research bio-bank will be made during the study. 20 ml of blood (ten glasses of two ml each) will be sampled. The blood samples will be analysed in batches for changes in bone markers. After the trial has ended, the remaining blood samples will be transferred to another bio-bank at Aarhus University Hospital and stored for a maximum of ten years before destruction. Participants will be made aware that the bio-bank is for future research and that blood samples can be destroyed at all times on request of the patient. The participants will be asked to sign a separate consent form before the remaining blood samples are transferred to the research bio-banks.

Assessment of safety

Definition of adverse events (AE)

Any untoward medical occurrence in the patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment. An adverse event can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a product, whether or not considered related to the product.
The severity is assessed as follows:

- Mild: Awareness of sign or symptom, but easily tolerated.
- Moderate: Discomfort enough to cause interference with usual activity.
- Severe: Incapacitating with inability to work or do usual activity.

Outcomes are defined as “recovered”, “recovering”, “recovered with sequelae”, “not recovered”, “fatal”, or “unknown”.

Assessment of AEs

At baseline a thoroughly physical examination of the participants and questioning concerning any conditions or diseases will take place. This way the investigators will be able to evaluate possible changes though out the study. Patients will be interviewed about the occurrence of AEs at each visit from the first trial related activity after the subject has signed

[4] Information on redeemed prescriptions for alendronate is obtained though Register of Medicinal Products Statistics.

the informed consent. Subjects that experience adverse events or develop a disease during the trial period will be managed until the condition is cured or stationary. If this is not the case at the end of the study, subjects will be referred to a relevant physician, e.g. the general practitioner or a specialist, to be followed up. All queries related to these AEs will be resolved. Cases of chronic conditions, cancer or AEs ongoing at time of death (where death is due to another AE) will be closed with the outcome "recovering/resolving" or "not recovered/not resolved". Cases can be closed with the outcome of "recovering/resolving" when the subject has completed the follow up period and is expected by the investigator to recover.

Definition of serious adverse events (SAE)

Any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening ("life threatening" in the definition of SAE refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it was more severe)
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Is an important medical event that may not result in death, be life-threatening, or require hospitalization but may be considered a serious adverse event when, based upon appropriate medical judgment, it may jeopardise the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition

Definition of serious adverse reactions (SAR)

A SAR is an adverse event, which fulfil both the criteria for a SAE and the criteria for an adverse reaction. An adverse reaction is a response to a medicinal product which is noxious and unintended, and for which the causal relationship between the product and the adverse event is suspected (judged possible or probable by the sponsor or the investigator).

Definition of suspected unexpected serious adverse reactions (SUSAR)

A suspected unexpected serious adverse reaction is an SAE, which is unexpected and regarded as possibly or probably related to the study product by the Investigator.

Reporting of SAEs, SARs, and SUSARs

SAEs and SARs will be reported to the sponsor no later than 24 hours after the investigator has become aware of them. SUSARs that have been deadly or life threatening will be reported to the Danish Health and Medicines Authority no later than seven days after sponsor is notified. No later than eight days after this notification the Danish Health and Medicines Authority will be notified about follow-up procedures and information. Other SUSARs will be reported after no more than 15 days. In addition, a yearly report on SAEs and SARs will be sent to the Danish Health and Medicines Authority and The Central Denmark Region Committees on Health Research Ethics.

All SAEs will be managed until the outcome of the event is "recovered", "recovered with sequelae" or "fatal" and until all queries have been resolved. The follow up information on SAEs will only include new (corrections or new or addi-

tional) information and will be reported within 24 hours of obtaining knowledge of the information. This will also be the case with previously non-serious AEs, which subsequently become SAEs.

Safety reporting requirements

When reporting events the following parameters will be recorded:

- Study name
- Event start/stop date
- Severity
- Seriousness
- Patient identification (e.g. subject number, initials, sex, age)
- Event (preferably a diagnosis)
- Drug
- Reporter identification (e.g. name, or initials)
- Causality
- Outcome

Reporting to Health Authorities

The investigator is responsible for all required periodic updates to health authorities and expedited reporting of Adverse Events occurring during the performance of the study, in accordance with local regulations and the agreed protocol. The approving Health Authority may have special requests beyond SUSAR reporting. A full report on all events during the study will be made to The Central Denmark Region Committees on Health Research Ethics and the Danish Health and Medicines Authority after the study has ended.

Patients are covered by a publicly funded compensation scheme. As usual participants are covered by the blue European Health Insurance Card when travelling in Europe.

Termination of the study

The study will be terminated for the individual participant if the investigator suspects that the participant will be at risk of serious, life-threatening events if he or she continues as part of the study. If the DXA scans show a rapidly declining BMD > 8% during the first six months, the study will be terminated for this participant and treatment for osteoporosis will be re-initiated.

The patients will be referred to the outpatient clinic at Aarhus University Hospital or their GP after termination of the study.

Perspectives

The study will contribute with new knowledge about biochemical markers of bone turnover as predictors of bone loss after stopping treatment with alendronate. It will thus be possible to identify patients who will experience a decrease in BMD during treatment break, and for this particular group of patients treatment can be re-initiated earlier so further loss of bone will be avoided. On the other hand, the biochemical markers of bone turnover could also shed light on who can

tolerate treatment break, thereby avoid long-term treatment with alendronate, which may be associated with serious side effects (atypical femur fractures and osteonecrosis of the jaw).
Finally, the use of blood samples rather than DXA will reduce the use of X-rays.

Safety and ethical considerations

Puncture of veins for blood sampling can result in a bruise and very rarely in infection. The DXA examinations results in radiation doses of 60μSv, which increases the risk of cancer by 0,003% and the lifetime risk of cancer from 25% to 25,003%. If a patient has been examined by DXA within two months of inclusion in the study, this scan will be used as the baseline scan. In this case the DXA examinations results in radiation doses of 40μSv, which increases the risk of cancer by 0,002% and the lifetime risk of cancer from 25% to 25,002%. The risks associated with participating in this study are limited and we believe the potential gains from this study outweigh the risks.

Economy

The study is initiated by the investigators. Aarhus University (550,000kr), The Svend Fælding Foundation (20.000kr), The Foundation of Aase and Ejnar Danielsen (100.000kr) and The Carl and Ellen Hertz' Foundation (10.000kr) have granted financial support. Additional support will be applied for at other foundations as well. The investigators have connection no to these foundations. Participants will have expenses for transport reimbursed but will not otherwise be paid to participate.

Publications

The results of the study will be published in an international scientific journal. All results including inconclusive, positive, and negative results will be accessible to the public after the study has ended and will be published in the European Clinical Trials Database. After completion of the study, the participants will receive written information about the results. The participants can seek further information about the project by contacting the investigator.

Monitoring

The studies are conducted according to the final version of the protocol and according to the Helsinki declaration and the Danish Health Law. The Processing of data is carried out in accordance with the Act on Processing of Personal Data and the study will not be initiated until approval has been given from the Danish Data Protection Agency ("Datatilsynet"), The Central Denmark Region Committees on Health Research Ethics, and the Danish Health and Medicines Authority.

Research plan and facilities

October 2016-March 2018: Recruitment of study participants
October 2016-March 2019: Conduction of the study
April 2019-July 2019: Analyses of the results and preparation of a manuscript for publication.

We have all the facilities for the clinical study including offices and DXA machines. We have experienced research nurses and laboratory technicians and we have access to analyses of biochemical markers at the Department of Clinical Biochemistry at Aarhus University Hospital.

Reference list

[1] P. N. Kannegaard *et al.*, 'Excess mortality in men compared with women following a hip fracture. National analysis of comedications, comorbidity and survival', *Age Ageing*, vol. 39, no. 2, pp. 203–9, 2010.

[2] D. M. Black *et al.*, 'Fracture risk reduction with alendronate in women with osteoporosis: the Fracture Intervention Trial. FIT Research Group', *J Clin Endocrinol Metab*, vol. 85, no. 11, pp. 4118–24, 2000.

[3] D. M. Black *et al.*, 'Once-yearly zoledronic acid for treatment of postmenopausal osteoporosis', *N Engl J Med*, vol. 356, no. 18, pp. 1809–22, 2007.

[4] S. R. Cummings *et al.*, 'Denosumab for prevention of fractures in postmenopausal women with osteoporosis', *N Engl J Med*, vol. 361, no. 8, pp. 756–765, 2009.

[5] R. G. Russell *et al.,*, 'Mechanisms of action of bisphosphonates: Similarities and differences and their potential influence on clinical efficacy', *Osteoporos Int*, vol. 19, no. 6, pp. 733–759, 2008.

[6] A. G. Porras *et al.,*, 'Pharmacokinetics of alendronate', *Clin. Pharmacokinet.*, vol. 36, no. 5, pp. 315–28, 1999.

[7] D. Black *et al.*, 'Randomised trial of effect of alendronate on risk of fracture in women with existing vertebral fractures. Fracture Intervention Trial Research Group', *Lancet*, vol. 348, no. 9041, pp. 1535–41, 1996.

[8] U. A. Liberman *et al.*, 'Effect of oral alendronate on bone mineral density and the incidence of fractures in postmenopausal osteoporosis', *N Engl J Med*, vol. 333, no. 22, pp. 1437–1443, 1995.

[9] E. Shane *et al.*, 'Atypical subtrochanteric and diaphyseal femoral fractures: report of a task force of the American Society for Bone and Mineral Research', *J Bone Min. Res*, vol. 25, no. 11, pp. 2267–94, 2010.

[10] S. Khosla *et al.*, 'Bisphosphonate-associated osteonecrosis of the jaw: report of a task force of the American Society for Bone and Mineral Research', *J Bone Min. Res*, vol. 22, no. 10, pp. 1479–91, 2007.

[11] D. M. Black *et al.*, 'Effects of continuing or stopping alendronate after 5 years of treatment: the Fracture Intervention Trial Long-term Extension (FLEX): a randomized trial', *JAMA*, vol. 296, no. 24, pp. 2927–2938, 2006.

[12] A. V. Schwartz *et al.*, 'Efficacy of continued alendronate for fractures in women with and without prevalent vertebral fracture: The FLEX trial', *J Bone Min. Res*, vol. 25, no. 5, pp. 976–982, 2010.

[13] K. Saag *et al.*, 'Resolution of Effects On Bone Turnover Markers and Bone Mineral Density After Discontinuation of Long-Term Bisphosphonate Use', *ACR/ARHP. 2012 Annu. Meet. Abstr. 1971*.

[14] D. M. Black *et al.*, 'The effect of 3 versus 6 years of zoledronic acid treatment of osteoporosis: a randomized extension to the HORIZON-Pivotal Fracture Trial (PFT)', *J Bone Min. Res*, vol. 27, no. 2, pp. 243–54, 2012.