Osteoporosis International (2019) 30:917–922
https://doi.org/10.1007/s00198-018-04823-5

SHORT SCIENTIFIC COMMUNICATION

Clinical utility of bone turnover markers in monitoring the withdrawal of treatment with oral bisphosphonates in postmenopausal osteoporosis

K.E. Naylor[1] · E.V. McCloskey[1,2] · R.M. Jacques[3] · N.F.A. Peel[4] · M.A. Paggiosi[1] · F. Gossiel[1] · J.S. Walsh[1] · R. Eastell[1]

Received: 14 August 2018 / Accepted: 18 December 2018 / Published online: 6 January 2019

Abstract

Summary Bone markers may be useful to monitor response to treatment withdrawal in osteoporosis. We used two criteria for investigating the change in BTMs after withdrawal of bisphosphonate treatment. A larger increase in BTMs was associated with greater bone loss. Bone markers may be useful in monitoring of patients taking a pause from treatment.

Introduction Measurement of bone turnover markers (BTMs) may be useful to monitor offset of treatment with bisphosphonates (BP) in osteoporosis. We assessed the effect of withdrawal of BP treatment by comparing the changes in BTMs and total hip (TH) bone density (BMD).

Methods We studied postmenopausal osteoporotic women who had completed a randomised study of three oral BPs. After 2 years of treatment, participants with BMD T-score > − 2.5 and in whom it was considered clinically appropriate to discontinue treatment, were invited to participate in a further 2-year observational study. Biochemical response was assessed using BTMs (CTX and PINP) with offset being defined by two criteria: (1) an increase greater than the least significant change (LSC) and (2) an increase above the reference mean value.

Results Fifty women completed the study. At 48 weeks after stopping BPs, CTX was greater than the LSC for 66% of women and PINP 72%; CTX was above the reference mean for 64% of women and PINP 42%. The decrease in THBMD was greater for women with the largest increase in BTM compared to those with continued suppression (mean difference for CTX was − 2.98%, 95%CI − 4.75 to − 1.22, $P < 0.001$, PINP − 2.25%, 95% CI − 4.46 to − 0.032, $P = 0.046$).

Conclusion The measurement of BTM after withdrawal of BPs is potentially useful to evaluate patients that are taking a pause from treatment. An increase in BTMs more than the LSC and/or reference mean reflects loss of treatment effect and identifies patients that are likely to have a decrease in BMD. Such changes could provide an indication for reintroduction of treatment.

Keywords Bisphosphonate · Bone density · Bone markers · Osteoporosis

Introduction

Oral bisphosphonates (BPs) are widely used for the treatment of postmenopausal osteoporosis [1]. The beneficial effects may persist after treatment is withdrawn due to the retention of BPs in bone [2–4], and it has been suggested that patients who are not at high risk of fracture may be considered for a pause in BP treatment [5]. When bisphosphonate treatment is interrupted, it is important to periodically review the patient to monitor for indications for recommending treatment. Clinical assessment usually includes measurements of BMD, but BTMs may be a useful adjunct for management [6]. Indeed, the International Osteoporosis Foundation (IOF) and International Federation of Clinical Chemistry (IFCC) have recommended the use of reference standard BTMs [7, 8], which have been shown to decrease significantly after the

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00198-018-04823-5) contains supplementary material, which is available to authorized users.

✉ K.E. Naylor
k.e.naylor@sheffield.ac.uk

[1] Academic Unit of Bone Metabolism, The Mellanby Centre for Bone Research, University of Sheffield, Sheffield, UK

[2] Centre for Integrated Research into Musculoskeletal Ageing, Liverpool, UK

[3] School of Health and Related Research, University of Sheffield, Sheffield, UK

[4] Metabolic Bone Centre, Sheffield Teaching Hospitals NHS Foundation Trust, Northern General Hospital Sheffield, Sheffield, UK

commencement of anti-resorptive therapies as a means to identify a response to treatment; the corollary suggests that it would be reasonable to consider that an increase in BTMs after treatment has been stopped may be useful to monitor the offset of effect in patients [9]. Certainly, when BP treatment is withdrawn there is an increase in the bone turnover markers (BTMs) and a decrease in the bone mineral density (BMD) [10, 11], but little is known about their utility in clinical management.

We have previously reported the changes in BTM and BMD in a randomised trial of three oral BPs during and after withdrawal of treatment [12–14]. The criteria to define a clinically meaningful change or response in BTMs are not standardised, but two approaches can be used; firstly, a response can be assumed if the BTM changes from baseline by an amount that is greater than the least significant change (LSC), or the value on treatment can be compared to the reference interval, with response constituting a value that lies below the reference mean (for healthy young women) [7]. Similar approaches might be taken to define offset of the effect of treatment. In this analysis, we have investigated the relationship between changes in BTMs and BMD following the withdrawal of BP treatment to see if this could provide clinically applicable information for clinicians managing individual patients.

Methods

Study design

The study comprised a 2-year, open-label, parallel, randomised controlled intervention trial of three oral BPs (TRIO study) [12, 13]. At the end of the treatment study, eligible participants were invited to participate in an observational extension study for a further 2 years with no treatment (TRIO offset study) [14].

Study population

The TRIO study recruited women with postmenopausal osteoporosis defined by dual-energy X-ray absorptiometry (DXA) at the lumbar spine or proximal femur as (i) a BMD T-score ≤ -2.5 or (ii) a BMD T-score ≤ -1.0 plus a prevalent non-traumatic fracture. The recruitment details have been described previously [12]. Participants were randomised to receive one of the three oral BPs at the licensed dose for 2 years: (i) ibandronate (Bonviva, Roche, 150 mg), (ii) alendronate (Fosamax, Merck, 70 mg) or (iii) risedronate (Actonel, Warner-Chilcott, 35 mg) [12] and also received calcium carbonate 3 g (1200 mg elemental calcium) and cholecalciferol 20 μg (800 IU) per day (Adcal D3, ProStrakan), initiated 1 week before the commencement of treatment. The study, approved by the Sheffield Research Ethics Committee and the Medicines and Healthcare Products Regulatory Agency (MHRA), was carried out in accordance with the Declaration of Helsinki and the International Conference on Harmonisation Good Clinical Practice (ICH GCP) guidelines. Written informed consent was obtained for all participants. At the end of the 2-year treatment study, women with hip BMD T-score > -2.5 and in whom it was considered clinically appropriate to pause treatment ($n = 57$) were invited to participate in a further 2-year observational study. The criteria for the invitation and subsequent inclusion in this TRIO Offset study have been published [14].

Study outcomes and assessments

The total study was conducted over 192 weeks with visits at baseline one (week − 1), baseline two (week 0, randomisation and commencement of BP), then at 12, 48 and 96 weeks (TRIO study), then after the withdrawal of BP at 24, 48, 72 and 96 weeks (TRIO offset study). Fasting blood was collected (left to clot for 30 min, centrifuged at 2500*g* for 10 min) and stored at − 80 °C until analysis. The C-telopeptide of type I collagen (CTX) and intact pro-collagen I N-propeptide (PINP) were measured using the IDS-iSYS (Immunodiagnostic Systems, Boldon, UK), inter-assay CV 4.4 and 4.5% respectively.

BMD (g/cm^2) of the total hip (TH) was measured by DXA (Discovery A densitometer, Hologic Inc., Bedford, MA) at baseline and weeks 12 and 96 on treatment then at week 48 and 96 off treatment (visit window $\pm$2 weeks).

Definition of BTM offset

The sample size for the TRIO offset study was constrained by the proportion of eligible patients from the original TRIO study [14]. Change in BTMs after withdrawal of treatment was classified according to two criteria, namely increases greater than the LSC and values rising to above the reference mean.

LSC

The least significant change (LSC) was calculated using measurements from the 12- and 13-week visits of the treatment group ($n = 147$) as follows:

$$\mathrm{LSC} = Z' \times \sqrt{2} \times \mathrm{SD_{RMS}}$$

Where $\mathrm{SD_{RMS}}$ is the root-mean-square standard deviation calculated from the data, and Z' is equal to 1.96 for 95% confidence level. Participants in whom the 48-week BTM measurement in the TRIO offset study showed increases greater than

the LSC from the baseline value at an entry to the TRIO offset study was defined as having treatment offset. The LSC for PINP was 10 μg/L, while for CTX the LSC was 160 ng/L.

Reference mean

The reference mean value was calculated from 20 premenopausal women (geometric mean, 95% CI) who had participated as controls in the TRIO study. Participants in whom the BTM measurement at 48 weeks in the TRIO offset study lay above the premenopausal mean were defined as having treatment offset. The premenopausal means for PINP and CTX were 35 μg/L and 240 ng/L respectively.

Statistical analysis

Changes in TH BMD, from the start of the TRIO Offset study, are presented as least square mean percentage changes with two-sided 95% CI. The change in TH BMD after withdrawal of BP was compared by analysis of covariance (ANCOVA) with Bonferroni correction, adjusted for THBMD at baseline (end of treatment), in three groups for each of the BTMs; those showing offset by both a greater than LSC increase and a value lying above the threshold mean offset, those showing partial offset by only one of these criteria, and finally those showing neither of these criteria (no offset). The change in the TH BMD was compared between those above or below the mean and greater than or less than LSC for each BTM (ANCOVA with Bonferroni correction). Cohen's kappa coefficient was used to test for concordance between offset category as defined by LSC and reference mean criteria within and between the BTMs. The relationship between the change in BTMs at 48 weeks off treatment and percentage change in THBMD was investigated using Pearson's correlation. Baseline BTMs were compared between the three responder groups by Kruskal–Wallis analysis. Statistical analyses were conducted using Stata statistical software (StataCorp. 2015 Stata Statistical Software: Release 14. College Station, TX: StataCorp LP.) and MedCalc Statistical Software version 14.10.2 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2014).

Results

Baseline characteristics

From the original TRIO study, 94 participants were assessed for eligibility and 59 consented to continue into the TRIO offset study, with two subsequently withdrawing before the start of the study. The baseline characteristics of those that entered the TRIO offset study compared to those who did not participate have been published previously [14]. In summary, at the TRIO baseline, the participants in the TRIO offset study ($n = 57$) were similar to all the participants in the TRIO study in terms of mean age (66.6 vs. 65.9 years, $P = 0.67$) and mean BMI (26.1 vs 25.1 kg/m^2, $P = 0.97$) but, given the selection criteria for the Offset study, had higher TH BMD at both TRIO baseline (T-score − 1.14 vs − 1.70, $P = 0.006$) and at the end of TRIO (T-score − 0.9 vs − 1.6, $P = 0.006$). Of the 57 women recruited from the TRIO study, 18 had been allocated ibandronate, 21 alendronate and 18 risedronate [14].

Bone turnover markers

Of the 57 women entering TRIO Offset, BTM data were available for 50 of them at 48 weeks after stopping BP. The increase in CTX was by more than the LSC for 33 women (66%) (Fig. 1a) and was above the reference mean for 32 women (64%) (Fig. 1b), with 29 women (58%) showing changes in both measurements, seven women (14%) showing

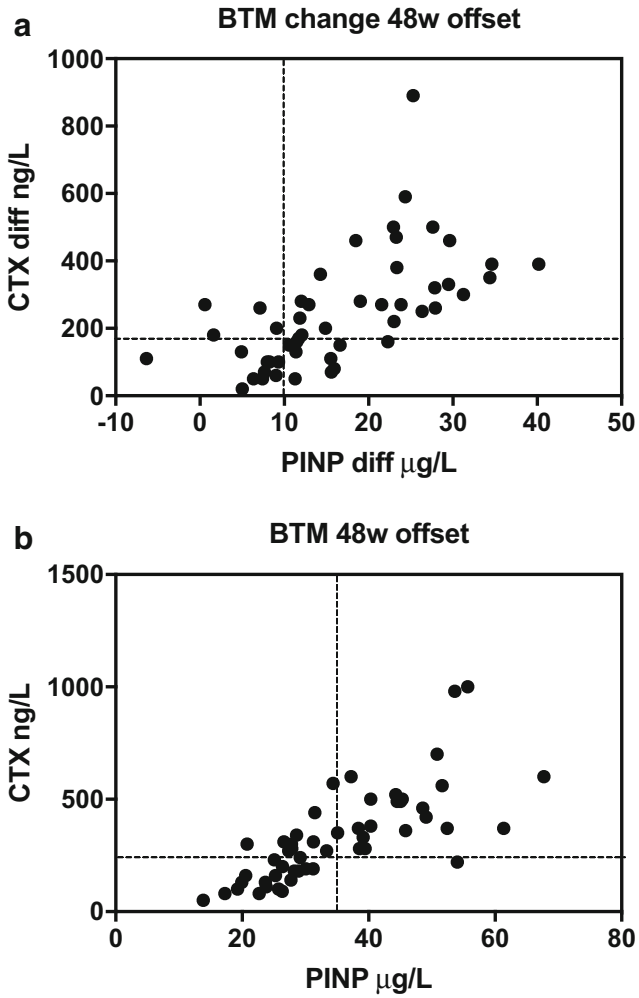

Fig. 1 **a** Change in BTM with LSC and **b** absolute value with reference mean for BTM at 48 weeks after stopping BP treatment. The LSC and reference mean are shown as dashed lines

changes in only one and 14 women (28%) in neither. The increase in PINP was by more than the LSC for 36 women (72%) (Fig. 1a) and was above the mean for 21 (42%) (Fig. 1b), with 18 women (36%) having both changes, 21 women (42%) showing changes in only one parameter and 12 women (24%) neither parameter. Within the markers, there was a good concordance between the classification of offset by the LSC and mean parameters for CTX (Kappa 0.69) but not with PINP (Kappa 0.22). Between markers, there was a good concordance between CTX and PINP classification using the LSC method (Kappa 0.49) and the mean threshold approach (Kappa 0.58).

The time course of changes in BTM over the study period for those classified as showing offset, partial offset or no offset is shown in Supplemental Fig. 2. For both PINP and CTX, the women with offset (i.e. fulfilling both LSC and mean parameters) had significantly higher BTMs prior to bisphosphonate treatment at an entry to the TRIO study than those showing offset by only one criterion or not showing offset. For example, the median (IQR) value of serum PINP in those showing offset was 63.6 μg/L (53.4 to 81.1, $n = 18$) compared to 49.2 μg/L (95% CI 40.6 to 55.9, $n = 21$) and 51.6 μg/L (37.2 to 57.1, $n = 11$) in those with partial or no offset respectively ($p < 0.05$). For CTX, the baseline median value was 580 ng/L (405 to 742, $n = 29$) in those showing offset compared to 310 ng/L (230 to 430, $n = 14$) in those without offset, while women showing a partial offset had intermediate values (430 ng/L, 300 to 528, $n = 7$) ($P < 0.05$ between the groups).

Bone mineral density

The changes in BMD during treatment have been previously published [12]. We compared the change in TH BMD over 96 weeks off treatment in the three groups classified by BTM change after the withdrawal of treatment and at 48 weeks after stopping treatment (data were available for 49 women). At the end of the 2-year discontinuation period, only one participant had a BMD T-score of < -2.5. Total hip BMD loss was significantly greater in those women showing BTM offset (a change in BTMs greater than the LSC and above the mean value at week 48 after stopping treatment) compared to those who only met one threshold (partial offset) or neither (Table 1). There was a significant difference in the TH BMD percentage change for those with offset by both criteria (LSC and reference mean) for CTX or PINP compared to those with neither criteria met (− 2.98%, $P < 0.001$, − 2.25%, $P = 0.046$, respectively) but no significant difference to those with neither criteria met, (either greater than LSC or the mean).

The participants with the greatest increase in BTMs at 48 weeks after stopping treatment had the largest decrease in TH BMD 96 weeks after stopping treatment CTX $r = -0.58$ (95% CI − 0.74 to − 0.36) $P < 0.001$, PINP $r = -0.41$ (95% CI − 0.62 to − 0.14) $P < 0.005$.

Discussion

BTMs have been used in clinical research for many years but their uptake in clinical practice varies widely. One of the limitations to more widespread use is the relatively poor reproducibility of BTM, which can be addressed by adopting standardised sample handling and patient preparation procedures [15]. We assessed the change in BTM after the withdrawal of BP treatment using two criteria as suggested by the IOF and IFCC to monitor treatment of osteoporosis in postmenopausal women [7]. The values for CTX and PINP LSC and reference mean were comparable to those reported by Morris et al. [16]. Importantly, the offset of treatment effect using criteria for either the resorption marker CTX or the bone formation marker PINP was associated with more rapid bone loss at the hip over a 2-year period. Specifically, those women showing offset of the bone turnover suppression by changes in either marker at 48 weeks off treatment, particularly, when those changes were greater than the LSC, demonstrated greater decreases in hip BMD. These observations suggest that the monitoring of BTM at the earlier time point could prove useful in influencing follow-up and decisions to reintroduce treatment.

Another clinically important observation from our analysis is that women with higher BTM at baseline (prior to treatment) had a greater increase and return towards higher turnover during the offset period. Furthermore, the mean percentage decrease in TH BMD during the 2 years off treatment was greater in these women. These findings suggest that those women with high baseline BTM may require earlier assessment of offset to determine if there is an indication to restart treatment. The relationship between high baseline turnover and a subsequent return to relatively high turnover has been observed during denosumab treatment [17]. However, after the withdrawal of denosumab, there is a return to baseline followed by a transient increase in BTM above baseline associated with accelerated bone loss, whereas after BP withdrawal, BTM returned to baseline and bone loss is slower [18].

It is important to note that the LSC method used absolute changes as used in clinical practice, rather than percentage changes, as the CTX value can be suppressed to zero with BP treatment so it would not be a suitable baseline for the calculation of percentage change during offset. Where a pretreatment baseline BTM measurement may not be available in clinical practice, a measurement at the end of treatment appears to be an appropriate baseline by which to assess the offset of the treatment effect. In the absence of a sample at the end of treatment, thus excluding the use of the LSC response, then values that lie above the premenopausal mean at 48 weeks can also identify apparent offset of effect, particularly when using CTX.

This study has a number of limitations. Firstly, the assay used for measurement of BTMs should be taken into account,

Table 1 The mean percentage change in total hip BMD during the 2 years after withdrawal of BP treatment for study participants with BTM values greater than or less than the LSC or reference mean at 48 weeks offset. Comparison of groups by ANCOVA, week 48 offset change in BTM [1]both >LSC and >mean, [2]change in BTM <LSC and <mean, [3]either >LSC or >mean ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$)

	Criteria	*N* (%)	Mean TH BMD change (95% CI) over 2 years	Mean difference (95% CI)
CTX	> Mean	32 (65)	− 2.35 (− 3.16 to − 1.55)	2.09 (0.71 to 3.47) **
	< Mean	17 (35)	− 0.26 (− 1.37 to 0.85)	
	> LSC	32 (65)	− 2.65 (− 3.39 to − 1.91)	2.94 (1.65 to 4.23) ***
	< LSC	17 (35)	0.30 (− 0.73 to 1.32)	
	[1]Both	29 (59)	− 2.58 (− 3.38 to − 1.78)	− 2.98 (− 4.75 to − 1.22)***
	[2]None	14 (29)	0.41 (− 0.75 to 1.57)	
	[3]Either	6 (12)	− 1.78 (− 3.52 to − 0.05)	− 0.79 (− 3.15 to 1.57)[1,3]
				2.19 (− 0.38 to 4.76)[2,3]
PINP	> Mean	21 (43)	− 2.35 (− 3.39 to − 1.30)	1.26 (− 0.12 to 2.64)
	< Mean	28 (57)	− 1.09 (− 1.99. to − 0.18)	
	> LSC	35 (71)	− 2.10 (− 2.90 to − 1.31)	1.66 (0.17 to 3.16) *
	< LSC	14 (29)	− 0.44 (− 1.70 to 0.82)	
	[1]Both	18 (37)	− 2.63 (− 3.74 to − 1.53)	− 2.25 (− 4.46 to − 0.03)*
	[2]None	11 (22)	− 0.39 (− 1.80 to 1.02)	
	[3]Either	20 (41)	− 1.40 (− 2.45 to − 0.36)	− 1.23 (− 3.11 to 0.65)[1,3]
				1.02 (− 1.15 to 3.19)[2,3]

as there are discrepancies between results from different manufacturers [15, 19]. Jorgensen et al. found significant disagreement between the IDS-iSYS and Roche Cobas assay for PINP and CTX. This will influence the reference intervals and it is recommended that patients should be monitored with the same assay [19]. The National Bone Health Alliance has recommended that standardised sample handling and patient preparation procedures are adopted to minimise variability [15]. It is important that the laboratory adheres to robust internal and external quality assurance programmes.

Furthermore, we acknowledge that the 2-year duration of BP treatment is not representative of the usual recommended clinical practice in postmenopausal osteoporosis, but many patients with poor compliance or persistence will withdraw from treatment in relatively short timeframes [20]. The effect of treatment may diminish at a different rate after withdrawal of treatment if the women were treated for a more standard 5 years with oral BP, but data from the oral risedronate study showed parallel rates of offset at 2 and 7 years [11]. We were not able to consider fracture rates, as the number of subjects is small, but the data on total hip BMD suggest that the observed offset is associated with potential clinical consequences. It is not clear that the use of BTMs in the setting of postmenopausal osteoporosis is translatable to offset in other forms of osteoporosis, particularly the causes of secondary osteoporosis such as glucocorticoid use. Finally, though allocation to BP was randomised, the criteria for the discontinuation of the treatment may have resulted in a non-random comparison amongst those who participated in the TRIO Offset study and does not include the women with more severe osteoporosis.

In conclusion, measurements of CTX and/or PINP appear useful in evaluating the offset of bone turnover suppression in patients that are taking a pause from BP treatment. An increase in BTM by more than LSC and/or change to greater than the reference mean predicts loss of BMD and by inference a possible increase in fracture risk. We propose the LSC as the optimal assessment of BTM offset with the comparison to the reference interval providing additional information to help clinical management of patients, particularly when there is no baseline BTM measurement available.

It would, therefore, be reasonable to use this as an indication to consider reintroduction of treatment if the individual is at high risk of fracture. Those with continued suppression of BTMs are likely to have a stable BMD and may remain off treatment with continued monitoring.

Acknowledgements We are grateful to the data safety monitoring board, the Clinical Trials Research Unit, School of Health and Related Research, for data management and statistical support and the staff of the Academic Unit of Bone Metabolism for conducting the study. We would also like to acknowledge the Lay Advisory Panel for Bone Research and the participants of the study. We acknowledge the support of the NIHR Clinical Research Facility, Northern General Hospital, Sheffield. This work was supported by the South Yorkshire and North Derbyshire Musculoskeletal Biobank, which received ethics approval from NRES REC South Central Oxford C, (REC ref. 15/SC/0132) and is housed in the University of Sheffield Biorepository (HTA Licence no. 12182). The views expressed in this publication are those of the author(s) and not necessarily those of the NIHR.

Funding information The TRIO study was funded by Warner-Chilcott, the bone turnover marker measurements were funded by Immunodiagnostics Systems. Professor Richard Eastell (Academic Unit of Bone Metabolism, The University of Sheffield) is a National Institute for Health Research (NIHR) Senior Investigator (Emeritus). The authors approved the manuscript for publication and vouched for the completeness and accuracy of the data. The funder was involved in the design, but not in the conduct, analysis or reporting of the study.

Compliance with ethical standards

Conflict of interest NP has received speaker's honoraria and funding to attend educational events from Warner-Chilcott, Eli Lilly, Amgen, GSK and Prostrakan and consultancy fees from Internis Pharma and Eli Lilly. JW has received speaker's honoraria from Eli Lilly, grant funding from Alexion and Immunodiagnostic Systems, research drug and kits from Eli Lilly, Prostrakan (Kyowa Kirin), Consilient and Biomedica, consulting fees from Shire and Mereo Biopharma. EM has received speaker's honoraria and/or research funding and/or advisory board funding from Warner-Chilcott, Merck, AgNovos, Amgen, GSK, Bayer, Consilient Healthcare, Hologic, Eli Lilly, Novartis, Pfizer, Radius Pharmaceuticals, Servier, Wyeth, UCB and Roche. RE has received grant funding from Warner-Chilcott and the National Institute for Health Research (NIHR) and consultancy funding from Warner-Chilcott, Roche, Immunodiagnostic Systems and Merck. KN, RJ, MP and FG have nothing to disclose.

References

1. Russell RG (2006) Bisphosphonates: from bench to bedside. Ann N Y Acad Sci 1068:367–401
2. Compston J, Cooper A, Cooper C et al (2017) UK clinical guideline for the prevention and treatment of osteoporosis. Arch Osteoporos 12:43
3. Ensrud KE, Barrett-Connor EL, Schwartz A, Santora AC, Bauer DC, Suryawanshi S, Feldstein A, Haskell WL, Hochberg MC, Torner JC, Lombardi A, Black DM, for the Fracture Intervention Trial Long-Term Extension Research Group (2004) Randomized trial of effect of alendronate continuation versus discontinuation in women with low BMD: results from the fracture intervention trial long-term extension. JBone MinerRes 19:1259–1269
4. Papapoulos SE, Cremers SC (2007) Prolonged bisphosphonate release after treatment in children. N Engl J Med 356:1075–1076
5. Adler RA, El-Hajj Fuleihan G, Bauer DC et al (2016) Managing osteoporosis in patients on long-term bisphosphonate treatment: report of a task force of the American Society for Bone and Mineral Research. J Bone Miner Res 31:16–35
6. Chubb SAP, Vasikaran SD (2017) Measurement and clinical utility of betaCTX in serum and plasma. Adv Clin Chem 81:97–134
7. Vasikaran S, Eastell R, Bruyere O et al (2011) Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: a need for international reference standards. Osteoporos Int 22:391–420
8. Vasikaran S, Cooper C, Eastell R, Griesmacher A, Morris HA, Trenti T, Kanis JA (2011) International Osteoporosis Foundation and International Federation of Clinical Chemistry and Laboratory Medicine Position on bone marker standards in osteoporosis. ClinChemLab Med 49:1271–1274
9. Diez-Perez A, Naylor KE, Abrahamsen B et al (2017) International Osteoporosis Foundation and European Calcified Tissue Society Working Group. Recommendations for the screening of adherence to oral bisphosphonates. Osteoporos Int 28:767–774
10. Black DM, Schwartz AV, Ensrud KE, Cauley JA, Levis S, Quandt SA, Satterfield S, Wallace RB, Bauer DC, Palermo L, Wehren LE, Lombardi A, Santora AC, Cummings SR, FLEX Research Group (2006) Effects of continuing or stopping alendronate after 5 years of treatment: the fracture intervention trial long-term extension (FLEX): a randomized trial. JAMA 296:2927–2938
11. Eastell R, Hannon RA, Wenderoth D, Rodriguez-Moreno J, Sawicki A (2011) Effect of stopping risedronate after long-term treatment on bone turnover. J Clin Endocrinol Metab 96:3367–3373
12. Paggiosi MA, Peel N, McCloskey E, Walsh JS, Eastell R (2014) Comparison of the effects of three oral bisphosphonate therapies on the peripheral skeleton in postmenopausal osteoporosis: the TRIO study. Osteoporos Int 25:2729–2741
13. Naylor KE, Jacques RM, Paggiosi M, Gossiel F, Peel NF, McCloskey EV, Walsh JS, Eastell R (2016) Response of bone turnover markers to three oral bisphosphonate therapies in postmenopausal osteoporosis: the TRIO study. Osteoporos Int 27:21–31
14. Naylor KE, Bradburn M, Paggiosi MA, Gossiel F, Peel NFA, McCloskey EV, Walsh JS, Eastell R (2018) Effects of discontinuing oral bisphosphonate treatments for postmenopausal osteoporosis on bone turnover markers and bone density. Osteoporos Int 29:1407–1417
15. Szulc P, Naylor K, Hoyle NR, Eastell R, Leary ET, National Bone Health Alliance Bone Turnover Marker P (2017) Use of CTX-I and PINP as bone turnover markers: National Bone Health Alliance recommendations to standardize sample handling and patient preparation to reduce pre-analytical variability. Osteoporos Int 28:2541–2556
16. Morris HA, Eastell R, Jorgensen NR, Cavalier E, Vasikaran S, Chubb SAP, Kanis JA, Cooper C, Makris K, Assays I-IWGfSoBM (2017) Clinical usefulness of bone turnover marker concentrations in osteoporosis. Clin Chim Acta 467:34–41
17. Eastell R, Christiansen C, Grauer A, Kutilek S, Libanati C, McClung MR, Reid IR, Resch H, Siris E, Uebelhart D, Wang A, Weryha G, Cummings SR (2011) Effects of denosumab on bone turnover markers in postmenopausal osteoporosis. JBone MinerRes 26:530–537
18. Miller PD, Bolognese MA, Lewiecki EM, McClung MR, Ding B, Austin M, Liu Y, San MJ (2008) Effect of denosumab on bone density and turnover in postmenopausal women with low bone mass after long-term continued, discontinued, and restarting of therapy: a randomized blinded phase 2 clinical trial. Bone 43:222–229
19. Jorgensen NR, Mollehave LT, Hansen YBL, Quardon N, Lylloff L, Linneberg A (2017) Comparison of two automated assays of BTM (CTX and P1NP) and reference intervals in a Danish population. Osteoporos Int 28:2103–2113
20. Silverman SL, Schousboe JT, Gold DT (2011) Oral bisphosphonate compliance and persistence: a matter of choice? Osteoporos Int 22:21–26