Citation: IBMS BoneKEy **9,** Article number: 153 (2012) | doi:10.1038/bonekey.2012.153

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COMMENTARY

Monitoring anti-resorptive treatments for osteoporosis using bone turnover markers

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IBMS BoneKEy **9**, Article number: 153 (2012) | doi:10.1038/bonekey.2012.153; published online 15 August 2012

Commentary on: Bell KJL, Hayen A, Irwig L, Hochberg MC, Ensrud KE, Cummings SR and Bauer DC. The potential value of monitoring bone turnover markers among women on alendronate. J Bone Miner Res 2012; **27**: 195–201.

Bone turnover markers (BTMs) provide useful information about the response of the individual to osteoporosis treatment with anti-resorptive agents, and as a result they are used by some physicians for monitoring the response to treatment. We recently reviewed the recommendations made on this topic by nine national or regional organizations; five of these explicitly recommended the use of BTMs for monitoring the response of the individual to anti-resorptive treatment, but it is recognized that further research is required in this area.[1]

There are a number of factors that render BTMs suitable for use in treatment monitoring. The markers usually show large responses to treatment and the changes are rapid. As an example, the mean change in the bone resorption marker serum C-telopeptide of type I collagen (CTX) to alendronate as compared with baseline is a decrease of 75%,[1] and this level is reached after 8 weeks of treatment. Bone mineral density (BMD) is more commonly used in clinical practice for monitoring treatment response, but the changes are much smaller and slower to evolve (4% at the lumbar spine after 12 months of treatment with alendronate).[2] In studies of raloxifene, risedronate, alendronate and zoledronic acid, BTMs have been shown to explain between 28 and 77% of the fracture risk reduction with these agents; in contrast, change in BMD has only explained 0–28% of the fracture risk reduction with the same agents.[1] The association of BTMs and fracture risk reduction might be higher for raloxifene and alendronate than reported, as the studies made use of manual assays for BTMs, and introduce more variability (see below). The magnitude of the changes in bone turnover assessed by BTMs is similar (if a little smaller than) to that shown by bone histomorphometry.[2]

The approach to monitoring treatment with BTMs is similar to the approach used with BMD. We establish the within-subject variability of each BTM by calculating the coefficient of variation (CV), and if we want to be 95% certain about whether the BTM has increased or decreased, we multiply the CV by 2.77. For example, we recently reported a within-subject CV for CTX of 10%,[3] and so the least significant change (LSC) would be about 28%. We have also observed that the lowest risk of fracture is associated with a BTM measurement that is within the lower half of the premenopausal reference interval.[4] For example, we would aim to decrease serum CTX to a level below $0.3\,ng\,ml^{-1}$.

The identification of non-response is clinically useful: if we identify poor response, we can enquire about compliance with the medication, perform investigations to seek causes of secondary osteoporosis or change medication. Good compliance is associated with a greater response in BMTs,[5] but there is no evidence that feeding back the results of BMTs to a patient improves persistence.[6] However, positive feedback about the change in BTMs increases persistence with medication; negative feedback reduces persistence with medication.[7,8]

There are a number of issues that limit the use of BTMs for monitoring treatment. When the first assays for the measurement of pyridinium crosslinks were introduced, they required specialist equipment, such as high-performance liquid chromatography, and so they were only available in a few laboratories. Furthermore, quality assurance (QA) for the assays used to be relatively poor, with wide variability in results obtained from different laboratories. However, with the advent of immunoassay, and in particular automated immunoassay analyzers, bone turnover measurements are now widely available and QA issues have been resolved.[9] It is important to have expertise in the interpretation of results with appropriate reference ranges and understanding of confounding factors and sources of variability.

It has been proposed that if we want to know about an individual patient's response, we merely need to question the patient about their compliance with the medication. In a recent article in the *Journal of Bone and Mineral Research*, Bell *et al.*[10] provide strong evidence that even among compliant individuals taking alendronate, the BTM responses to treatment are heterogeneous. Bell *et al.*[10] measured a bone resorption marker, urinary N-telopeptide of type I collagen (NTX) expressed as a ratio to urinary creatinine (Cr; NTX/Cr), and a bone formation marker, the bone isoform of alkaline phosphatase (bone ALP) in 1304 postmenopausal women from the Fracture Intervention Trial. They conducted a mixed-models analysis of variance to identify whether all patients treated with alendronate had responses

of similar magnitude, and identified heterogeneity in the treatment response. These results contrast with evidence from their previous study of hip BMD, which did not show such heterogeneity. The authors interpret their results as an indication that BTMs have the potential to provide useful information about individual response to bisphosphonates. This is an important addition to the case for using BTMs for monitoring antiresorptive therapy.

The concept that the response to therapy differs among compliant patients is consistent with other observations. We reported that the non-vertebral fracture risk reduction with risedronate was related to BTMs' response (using either serum CTX or urinary NTX/Cr) even when the analysis was limited to patients with good compliance (>80%), estimated carefully with electronic caps.[5]

The issue that has limited our use of BMTs in clinical practice is the within-subject variability of marker measurements. The study by Bell *et al.*[10] indicates that the within-subject CVs for NTX/Cr and bone ALP were 43% and 26%, respectively. These CV values are very large and would result in estimates of the LSC of 119% and 72%, respectively. No individual would exceed such limits and be considered a responder. Are these estimates valid?

The authors evaluated the within-subject variability by studying the group treated with placebo. However, the 82% of the subjects in the placebo group also received calcium (500 mg per day) and vitamin D (250 IU per day). This supplementation resulted in mean decrease from baseline in NTX/Cr and bone ALP of 26% and 14%, respectively. Thus, the placebo group was not untreated, and therefore is an inappropriate population in which to estimate within-subject variability and the LSC. The reason why treatment with calcium and vitamin D would increase variability is that the baseline and on-treatment samples are included in the calculation of within-subject CV.

A second explanation of the large within-subject variability should be considered. The NTX assay was established on an automated immunoassay analyzer around the year 2000, and we noted that not only was the assay CV improved (from 10% to ~3%), but the non-linear dilution problems that had been noted with this assay were less marked.[11] The first change would improve the assay CV, and the second the within-subject CV. These major technical improvements were not available to Bell *et al.*[10] as their samples were measured in the 1990s, but they are now well established. Automated immunoassay analyzers are reported to perform better than manual assays for the measurement of bone resorption markers.[12] In a recent study, Schafer *et al.*[9] reported a large improvement in short- and long-term assay variability with the automated immunoassay analyzer compared with the manual enzyme-linked immunosorbent assay. The analysis of bone ALP is also now automated.[13]

The study by Bell *et al.*[10] evaluated a further feature of BTMs. They compared the mean response of BTMs with alendronate and expressed this as a ratio to the within-subject variability. This approach is referred to as 'signal-to-noise ratio'. They found that the ratio obtained was larger than in their previous studies of BMD, and again concluded that BTMs showed more potential than BMD for monitoring the response to treatment.

The case for using BTMs for monitoring anti-resorptive treatment response is strengthened by the article by Bell *et al.* The International Osteoporosis Foundation recently considered the evidence for BTMs in monitoring the response to osteoporosis therapies, and concluded that there is still a need for further research in this area. In particular, it would be good practice to ensure that all clinical trials include at least one marker of bone resorption and one marker of bone formation; the Foundation proposed that serum CTX and serum procollagen I N-propeptide (PINP) be used for this purpose.[1] Such an approach will allow us to pool information from all trials. Furthermore, the International Federation of Clinical Chemistry is now working on the standardization of the assays for these two markers to ensure improved consistency among laboratories. Already, automated immunoassay analyzers are available for the measurement of CTX and PINP.[14]

How should we approach the monitoring of anti-resorptive therapy in clinical practice? We should ensure that we reduce sources of variability to a minimum. This can be done by taking samples for the measurement of resorption markers from patients who have been fasting overnight. It is helpful to have more than one sample at baseline; we take samples 2–4 weeks apart and then take their average value as the baseline; this is not always convenient and may be circumvented by the use of serum markers CTX and PINP, as these have lower within-subject variability. This approach does have the limitation that multiple measurements may not be covered by health insurance. The article by Bell *et al.*[10] evaluates the value of a multiple sampling approach; caution needs to be taken in making such calculations, as samples taken a short time apart (consecutive days) will be more closely correlated than samples taken a long time apart (months or years), and so the reduction in variance may not be as large as they propose (serial correlation). There is a need for further studies of long-term CV and its impact on long-term changes in BTMs in the response to treatment.

The laboratory should use a method with good precision, have robust reference intervals and participate in a QA scheme. The physician should be knowledgeable about the expected change with treatment and the factors that can result in changes in bone turnover (such as the increase after fracture). It is common to evaluate the changes in BTMs at baseline and after 3–6 months of treatment. A decrease of more than the LSC to a level that is below the premenopausal mean indicates a good response. The within-subject CVs for serum CTX and PINP are both below 10%,[1] and so the LSC is 30% (or lower).

There are three further practical issues to the application of LSC. These estimates of LSC may be closer to 40% in clinical practice; we have reported before that the CV in an academic center tends to be lower than in a non-academic one.[15] The description of variability as a percentage may have its limits; the variability may be dependent on the level of the analyte. An alternative approach is to use the standard deviation and calculate the LSC in absolute units; for example, for teriparatide monitoring, we described an LSC of 10 ng ml^{-1} (Eastell *et al.*[16]). Finally, the type of treatment needs to be considered when deciding on the timing of sampling and the interpretation of response. Thus, CTX decreases maximally within the first 24 h of administration of denosumab,[17] whereas it decreases maximally by 6 months, and to a lesser extent with raloxifene.[18]

In patients with postmenopausal osteoporosis, about 12% have BTM above the reference interval and about 70% in the upper half of the reference interval.[19] In the 18% of cases in which the starting bone turnover is in the lower half of the reference interval, the target should just be a decrease of more than the LSC.

If there is no response then poor compliance should be suspected, addressed and the test repeated. If there is still no response then check for secondary osteoporosis and consider a change in therapy. BMTs should be complementary to BMD testing. BMD is particularly helpful for assessing current fracture risk and making decisions about continuing anti-resorptive therapy beyond 5 years.

Conflict of Interest

Dr Eastell serves as a consultant, has received honoraria for speaking, and has received grant support from Amgen, AstraZeneca, California Pacific Medical Center, GlaxoSmithKline, Hologic, Kyphon Inc., Lilly Industries, Maxygen, Nastech Pharmaceuticals, Nestle Research Center, New Zealand Milk Limited, Novartis, Novo Nordisk, ONO-Pharma, Organon Laboratories, Osteologix, Pfizer, Procter & Gamble Pharmaceuticals, Roche Diagnostics, Sanofi-Aventis, Servier, Shire, Tethys, TransPharma Medical Limited, Unilever, and Unipath.

ACKNOWLEDGMENTS

The document was edited by Dr Lynne Ferrar on behalf of the Sheffield Biomedical Research Unit in Musculoskeletal Disease.

Author contributions: Dr Eastell wrote the manuscript. Drs Naylor and Jacques commented on it.

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