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Modern Spectrum of Bone Turnover Markers: Are They Clinically Useful?

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Abstract

BTM are useful in clinical practice as they are inexpensive and they have proven useful for treatment monitoring and identification of poor adherence. BTM cannot be used in individual patients for identifying accelerated bone loss or an increase in fracture risk or in

deciding on the optimal therapy. They are useful for monitoring both anti-resorptive and anabolic treatment. Response can be defined as a result that exceeds an absolute target, or by a change greater than the least significant change; if such a response is not present, then poor compliance or secondary osteoporosis are likely causes. A baseline BTM measurement is not always made; in that case, a value of BTM on anti-resorptive treatment that is low or low normal or above the reference interval for anabolic therapy may be taken to indicate satisfactory response. We provide an approach to using these bone turnover markers in clinical practice by describing algorithms for anti-resorptive and anabolic therapy and describing the changes we observe in the clinical practice setting.

Introduction

The fractures that result from osteoporosis are a major public health problem (Eastell 2016 [1]). Osteoporosis is characterised by reduced bone mass and microarchitectural deterioration leading to increased bone fragility and may be diagnosed by measurement of bone mineral density (BMD) using dual-energy x-ray absorptiometry. A BMD value at the spine or hip that is 2.5 standard deviations or more below the average value for healthy young women is considered to represent osteoporosis, according to the WHO Working Group. Several treatments have been licensed for use in osteoporosis that are effective in reducing the risk of fracture..

This article focuses on the use of bone turnover markers (BTM) in osteoporosis. BTM can be measured in serum, plasma and urine and their levels relate to the activity of osteoblasts (bone formation markers) and osteoclasts (bone resorption markers). Bone formation markers include proteins that are specific to bone (osteocalcin), or not so specific to bone such as fragments of type I procollagen released during formation of type I collagen (N-propeptide of type I collagen, PINP) and the bone isoform of alkaline phosphatase (bone ALP). Bone resorption markers include fragments released from the telopeptide (end) region of type I collagen following its enzymatic degradation, including the N-telopeptide of type I collagen (NTX) and the C-telopeptide of type I collagen (CTX), deoxypyridinoline and the enzyme tartrate resistant acid phosphatase (Table 1).

In women, the BTMs increase after the menopause and in other situations of accelerated bone loss. In men, there is little increase with age. In cohort studies of women (but not of men), the higher the BTM, the more rapid the bone loss and the greater the risk of fracture. Thus, the measurement of BTM may have clinical relevance to the individual. Currently, the main clinical use for BTM is for the monitoring of response to therapy. A typical goal of therapy might be to lower BTM to values found in women before the menopause.

History, assays and validation

Bone histomorphometry is the gold standard for assessment of bone turnover, but it is invasive, cannot be repeated many times in an individual and requires specialist laboratory interpretation. Bone turnover can also be quantified with calcium balance and kinetic studies, but they are time-consuming, use radio-isotopes and again need specialist interpretation.

Therefore, for clinical use in large numbers of patients there is a need for measures that can be made on easily accessible samples (single measurements of blood or urine), inexpensively, don't require time-consuming specialist processing, and give results that can be interpreted by non-specialist health care practitioners.

The BTM that were developed initially were not bone-specific (for example, hydroxyproline and total alkaline phosphatase), the assays were technically challenging (HPLC for total deoxypyridinoline) and therefore costly and difficult to implement widely. The newer BTM are more bone-specific and the use of enzyme-linked immunosorbent assays (ELISA) and autoanalyser techniques have made them widely available and more affordable (Table 1).

Although assays for total alkaline phosphatase (ALP) were available in the 1920s, only about half of the total ALP is from bone. Hydroxyproline assays were developed in the 1950s, but again were not specific for bone, and were laborious and dangerous (they

resulted in explosions). There were significant developments in the 1980s and 1990s with assays for pyridinium crosslinks (deoxypyridinoline and pyridinolone), bone ALP, PINP and osteocalcin and progression from HPLC to immunoassays.

The introduction of automated immunoassay analysers in 2000 was a major technical advance. These are widely used in clinical practice for measuring many analytes, including hormones, as well as BTM and they do so with high precision (CV less than 5%) and reliability.

BTM have been validated against gold standard methods for studying bone turnover such as a comparison with tracer kinetics and bone histomorphometry, both in health (Eastell 1988 [2]) and in response to osteoporosis treatments (Eastell 1997 [3]). Currently-used BTM were evaluated in a study of 370 women with osteoporosis (Chavassieux 2015 [4]). BTM were assessed against dynamic histomorphometry of iliac crest biopsies. There were weak to moderate correlations (highest r-value was 0.41) between the bone formation markers PINP or bone ALP and bone formation estimates, and between CTX and bone resorption estimates.

Practical aspects

There are different requirements for the use of BTM in clinical practice compared to the research setting. In clinical practice, patients may attend appointments at any time of day and there may be a delay before samples are transported to the laboratory. Patients often have complex medical problems and take multiple medications. In this context, some properties of BTM present challenges to their clinical use.

BTM need to be measured reliably and easily, be locally accessible, inexpensive and be unaffected by the time of day the samples are obtained.

The bone resorption markers show a strong circadian rhythm and decrease shortly after feeding. Thus, it is recommended that blood samples for CTX be drawn from the patient

following an overnight fast between 0730 and 1000 (Szulc 2017 [5]). The sample can be collected as EDTA plasma or serum; EDTA is preferable if the sample cannot be processed within 2 hours. The sample can be stored frozen until measured; if the storage is likely to be more than 12 weeks, it is recommended that this is at -70 to -80° C (Okabe 2001 [6]). It is recommended for urinary NTX that the sample is taken as the second morning void, that excessive fluid consumption is avoided and preservative is not added. Variability can be further reduced by obtaining urine samples on three consecutive days, pooling the samples and just making one measurement. As well as measuring the bone resorption marker (NTX, CTX, DPD), it is usual to measure urinary creatinine and to express the result as the BTM to creatinine ratio to correct for urinary dilution.

For bone formation markers, there is a weaker circadian rhythm and so the sample can be drawn at any time of the day (Szulc 2017 [5]). Serum or plasma should be measured the same day or stored in the freezer until measured (Szulc 2017 [5]). EDTA plasma should not be used for bone ALP measurement (Szulc 2017 [5]). Osteocalcin is affected by haemolysis which can lead to a falsely low result. BTM, especially bone formation markers, are increased following fracture and are affected by some medical conditions and treatments, as discussed below (Szulc 2017 [5]).

Choice of BTM

It is logical to include a bone resorption and a bone formation assay when evaluating bone turnover. The choice of BTM will be determined by local availability and cost. It will also be determined by the clinical picture. Thus, in chronic kidney disease, the markers that are usually excreted by the kidney circulate at very high levels and so markers that are not excreted by the kidney are best used, e.g. Bone ALP and intact PINP. In the evaluation of glucocorticoid treatment on bone, markers that are sensitive to the bone effects of these drugs may be most useful, e.g. osteocalcin and PINP which are affected in a dose-dependent manner. These markers are not, however, useful to evaluate the effect of anti-resorptive therapy in these patients.

The IOF has proposed serum CTX and PINP as the two reference markers; they propose that all research studies should include these two at a bare minimum (Vasikaran 2011 [7]), but for clinical practice it may suffice to have one marker only.

Potential Clinical Utility

BTM have proven useful in evaluating the relationship between bone turnover and rates of bone loss, fracture risk and treatment effect in groups of patients using statistical approaches including linear and logistic regression or repeated measures analysis of variance. However, different statistical approaches are needed to evaluate the utility of BTM in the individual. These include tests of diagnostic accuracy (sensitivity, specificity) and assessment of the least significant change to identify response. The least significant change is a change (expressed in absolute units or percentage) that is beyond the day to day changes observed in untreated individuals. It is a statistical approach and is often defined and allows for change up or down and beyond the change expected 95% of the time. It is widely used in clinical chemistry where it is also referred to in some texts as the 'Reference Critical Difference' (Fraser [8]). It is calculated as 2.77 times the coefficient of variation; the latter includes both assay and within-subject variability.

Prediction of bone loss

High bone turnover is associated with more rapid bone loss in postmenopausal women (Shieh 2016 [9]) and BTM have been studied in evaluation this relationship. Higher BTM are associated with bone loss from both trabecular and cortical bone at the hip; and also relate to greater periosteal expansion in the femoral neck (Marques 2016 [10]). The assessment is improved by making more than one BTM measurement (Ivaska 2008 [11]). Estimation of the rate of bone loss in a postmenopausal woman when deciding about her need for anti-resorptive treatment would potentially be useful. Unfortunately, the association between BTM and bone loss is not sufficient to classify individuals reliably by their BTM level (Rogers 2000 [12]).

Prediction of fracture

It would also be of interest to estimate the risk of fracture in the individual postmenopausal woman when deciding about the need for anti-resorptive treatment and high bone turnover is associated with increased risk of several types of fracture in both men and women (Vilaca 2017 [13], Vasikaran 2011 [7]). In a recent meta-analysis of 6 studies that

had measurements of bone resorption (CTX) and bone formation (PINP), the hazard ratio per SD increase was similar for CTX (1,18, 95% CI 1.05 to 1.34) and PINP (1.23, 95% CI 1.09-1.39)(Johansson 2014 [14]). These results were not adjusted for BMD. However, not all studies find an association between BTM and fracture risk (Marques 2016 [10]) and the FRAX Position Development Conference members were unable to find sufficient evidence for inclusion of BTM into the FRAX fracture risk prediction algorithm (McCloskey 2011 [15]).

Selection of therapy

Intuitively, we would like to choose our therapies based on the mechanism of bone loss underlying the osteoporosis. Thus, we might use anti-resorptive therapies (bisphosphonates, raloxifene, denosumab) in patients with high BTM and anabolic therapies (teriparatide, abaloparatide) in patients with low BTM. Unfortunately, this approach is not supported by the results of clinical trials. In the Fracture Intervention Trial, treatment with alendronate was more effective at reducing non-vertebral fracture in those women with higher PINP but this was not true for other BTM or other fracture types (Bauer 2006 [16]). Similarly, the baseline BTM did not predict the fracture benefit with teriparatide (Delmas 2006 [17]). In general, a low PINP is associated with lower rates of bone loss and lower response to zoledronic acid. (Eastell 2015 [18]) Further research is needed.

Treatment used for osteoporosis

Despite having several treatments that reduce the risk of fracture in osteoporosis it is well established that adherence to these treatments can be poor, especially in the case of oral bisphosphonates for which the dosing instructions are complex. There is therefore a need to identify optimal treatment response in individual patients. It has been proposed that treatment failure may be considered if two or more fractures occur on treatment (Diez Perez 2012 [19]) based on evidence from clinical trials of drugs for osteoporosis in which there is a large reduction in risk for spine and hip fracture, although the reduction in risk of other fractures is lower. In practice, the occurrence of two or more fractures during treatment is a very rare event. Bone mineral density is commonly used as a tool to monitor treatment in

the individual and an increase that exceeds the least significant change, for example an increase in lumbar spine or total hip BMD more than 4% (Diez Perez 2012 [19]) may be considered a response. However, such changes occur over many months and persistence with medication declines very early in treatment (less than 50% after 12 months, Netelenbos 2011 [20]) so an earlier response marker would be preferred. The International Osteoporosis Foundation has proposed that a BTM such as PINP or CTX measured within 3 months of starting therapy would help identify poor adherence with the commonest osteoporosis therapy, oral bisphosphonates (Diez Perez 2017 [21]). Another advantage to using BTM rather than bone mineral density is that measurements are less expensive. In our hospital setting, a PINP measurement costs less than 20% that of a bone mineral density measurement. Finally, BTM may be a better surrogate for fracture risk reduction than BMD. The proportion of treatment effect explained by BTM has usually been higher than for BMD (Vasikaran 2011 [7]).

Bisphosphonate

Bisphosphonates are the most commonly-used drugs for osteoporosis. There are three oral bisphosphonates that are licensed in most countries, namely alendronate, risedronate and ibandronate. The absorption of the oral bisphosphonates is very poor and as the dosing regimen is complex, many patients do not comply fully with the instructions so do not achieve an optimal response even though they may take their medication regularly. The oral bisphosphonates have been compared in the TRIO study (Clinical Trial Number: NCT00666627)(Naylor 2015 [22]) to evaluate the clinical utility of BTM to assess response. Alendronate and ibandronate decreased BTM (CTX, NTX) more than risedronate. In this study, more than 80% of patients responded to treatment as defined by a decrease more than the LSC for CTX (56%) and PINP (38%) after 3 months of treatment. Response can also be defined as a reduction to a level below the mean found in healthy young women (Naylor 2015 [22]). In one study, the mean values were given as 217 to 317 ng/L for CTX and 32 to 38 μg/L for PINP (Morris 2017[23]).. In the assessment of treatment response in the individual, the magnitude of the decrease has also been found to be important; for example, with alendronate (Bauer 2004 [24]) and risedronate (Eastell 2003 [25]) the greater the reduction in BTM, the greater the reduction in vertebral fracture risk.

Zoledronic acid is given by annual intravenous infusion, thus avoiding concerns about poor absorption. It results in a reduction in CTX by 2 weeks and when it is given for 6 years as in the Horizon Study, the suppression of CTX and PINP is maintained (Black 2015 [26]). PINP was found to be even better than CTX and BMD in the Horizon study at identifying clinical (fracture) efficacy and responders (Bell 2016 [27]). As with the oral treatments, the greater the reduction in PINP with zoledronic acid, the greater the reduction in the risk of vertebral fractures (Jacques 2012 [28]).

Denosumab

Denosumab inhibits bone resorption, leading to an early and large decrease in bone resorption markers followed by a later and smaller decrease in bone formation markers. Bone resorption markers (such as CTX) decrease within 24 hours of treatment. In the FREEDOM Study, there was no overlap in CTX levels between treated and control subjects at one month indicating that everyone appears to respond (Eastell 2011 [29]). Denosumab results in a greater inhibition of bone resorption than zoledronic acid (Miller 2016 [30]). PINP decreases over several months to a lesser extent than the bone resorption markers and remains suppressed with continued dosing for up to 8 years (Papapoulos 2015 [31]). Once the drug is stopped, the BTM overshoot so that their levels are increased compared to baseline (Bone 2011 [32]). These high BTM results are associated with accelerated bone loss and there are recent reports of multiple vertebral fractures associated with this high BTM (Lamy 2017 [33]).

SERMs

Selective receptor oestrogen agonists (SERM) such as raloxifene have a weaker effect on bone turnover than bisphosphonates and denosumab. Even so, their effect can be monitored using BTM. In 60 to 65% of women with osteopenia, a significant response could

be demonstrated using the LSC approach with CTX or PINP (Naylor 2016 [34]). The BTM response to raloxifene was greatest in those with greatest adherence (Finigan 2013 [35]) providing further support for use of BTM as a means of identifying poor adherence to therapy (see below).

Teriparatide

Teriparatide is an anabolic agent administered as a daily subcutaneous injection and bone formation markers increase within days of starting treatment (Glover 2009 [36]), peaking by 3 months. PINP has proven to be the most responsive BTM to this treatment. Most patients have a significant response using PINP and an increase greater than the LSC (of more than 10 μg/L, Krege 2014 [37]) may be used to identify responders. The change in BTM relates to the later change in BMD (Niimi 2014 [38]). Poor BMD response is associated with low BTM at baseline (PINP, NTX) or smaller increase in BTM after 4 months on treatment (Niimi 2016 [39]).

The licence for teriparatide is for 2-years as there is a concern about osteosarcoma with long-term use and the effect of the drug wanes after three years of therapy. There is accelerated bone loss after stopping teriparatide, but this can be prevented by administering bisphosphonates, raloxifene or denosumab (Ebina 2016 [40]).

Abaloparatide is a new licensed anabolic therapy for osteoporosis (Shirley 2017 [41]). It is a synthetic peptide analogue of the human parathyroid hormone-related protein and works through the PTH receptor as does teriparatide. However, the increase in PINP is less than with teriparatide (Miller 2016 [42]). The clinical utility of BTMs for monitoring abaloparatide therapy have not yet been fully reported.

Practical approach to monitoring

We have been using BTM to monitor osteoporosis therapy in our secondary care practice for 20 years. We have observed that many patients commencing treatment and having a

poor BTM response are identified as having minor errors in following the dosing instructions that may not be picked up by a brief medication review. This is particularly important as most osteoporosis medication prescribing takes place in general practice by non-specialists who may not appreciate the limited absorption of oral bisphosphonates and the need for complete and consistent adherence to the dose regime. In primary care, time and resource to undertake early assessment of compliance is also limited and so we felt it appropriate to roll out the approach of monitoring osteoporosis therapy using BTM into general practice.

Figure 1 illustrates the algorithm that has been implemented in clinical practice. The physician decides to treat; most commonly, this would be with an oral bisphosphonate such as alendronate. At this point, PINP is measured. Our local laboratory uses the automated immunoassay (Roche Cobas) for this measurement; the results are similar for other automated immunoassays (IDS iSYS)(Morris 2017[23], Table 2); we need more data on the Orion PINP assay in comparison to the other assays. In one study, the results were similar (Eastell 2012 [43]) but in another the Orion assay give results lower than either Roche or IDS (Cavalier, personal communication). A discussion is held with the patient after one month to assess compliance and any problems or concerns with their treatment. This discussion is often held over the phone and may be initiated by their doctor, nurse or a pharmacist. The PINP measurement is repeated after 6 months to assess response. Treatment response is defined as a decrease in PINP that exceeds the least significant change of 10 µg/L or a decrease to below the geometric mean for young women (35 µg/L). As we describe later, the management advice accompanying the algorithm highlights factors such as incident fracture that may affect the interpretation of the PINP result and the actions to take if a clear response is not identified.

CTX can be used in the same way as for PINP for monitoring in practice and it has the advantage of the change being earlier than for PINP (Naylor 2015 [22]). The mean CTX value is 280 ng/L (Morris 2016[23]) and the least significant change value is about 100 ng/L (in Table 2 it is 60 to 80 ng/L depending on the method).

The estimates of least significant change (and the geometric means) are based on the assay and within-subject variability and so are a statistical approach and can be used for the monitoring of any intervention on bone turnover.

The mean values for PINP and CTX for the population were based on values obtained from studies in Italy, UK, France, Belgium, USA, UK, Saudi Arabia and Denmark (Morris 2016

[23]); there don't appear to be important differences between countries (Glover 2009 [44]) and so they should be suitable for international use. The mean values for PINP using Roche and IDS assays is probably similar (Morris 2016 [23], Table 2). However, the mean values for CTX used to be higher for IDS (Table 2, Eastell 2012 [43]) but more recent reports show them to be lower (Morris 2016[23]). There is a clear need for harmonisation of assay results for BTMs (Vasikaran 2011 [7]).

Treatment targets

The rationale for choosing a least significant change of 10 µg/L is that changes up to this level could occur by chance in up to 95% of people whereas a change greater than this is relatively uncommon and it is based on untreated postmenopausal women with low bone mineral density (Eastell 2006 [45]). The least significant change is also similar for both the Roche Cobas and the IDS iSYS assays as too is the mean response to oral bisphosphonates (Table 2).

The rationale for choosing a target PINP value of $\leq$35 µg/L is that in clinical trials of anti-resorptive drugs the lowest fracture risk is found in those women with bone turnover marker levels on treatment below the average value for young women (Eastell 2007 [46], Delmas 2009 [47]). Bone turnover markers have a skewed distribution, so it is best to take the geometric mean or the median. This value is around 35 µg/L for the Roche Cobas assay (Morris 2016[23]). The critical values for PINP (and CTX) are supported by results from those obtained in 50 women from the TRIO study with postmenopausal osteoporosis treated with oral bisphosphonate and compared to 200 healthy young control women. It can be seen from Table 2 that the mean values for young women are similar for PINP and close to 35 µg/L for both assays. It can also be seen that the least significant change is similar and as the baseline PINP was around 46 µg/L, an LSC of 23% equivalent to about 10 µg/L. It is also notable that the mean reduction on treatment after 12 to 13 weeks is very similar. The attraction of using PINP rather than CTX can be seen from this table - the LSC is lower for PINP than CTX and PINP does not need to be taken in the fasting state.

The concepts of least significant change and target for treatment are not unique to the use of bone turnover markers in osteoporosis and so are already familiar to colleagues in primary care. In type 2 diabetes, it is usual to monitor with haemoglobin A_{1c} and consider

the reference change value (the same as least significant change) and the target; the critical values used are 0.5% change and a target of 7.0% (Little 2011 [48]).

Sources of variability in BTMs

Many clinical factors influence BTM measurements but we pay particular attention to the occurrence of a fracture or to treatment with glucocorticoids as these are common confounders and have a clinically important impact. There is a large increase in PINP after a fracture, with a mean increase of 55% six weeks after wrist fracture (Ingle 1999 [49]), 96% six weeks after ankle fracture (Ingle 1999 [50]) and 100% 12 weeks after tibial shaft fracture (Veitch 2006 [51]). BTMs have also been report to be increased after vertebral fracture (Hashidate 2011 [52]). The magnitude of the increase appears to relate to the bone size at the site of fracture and may be greater if the fracture is managed surgically. Many patients initiate osteoporosis therapy in the weeks following a fracture and in the GLOW study, 7% of patients taking osteoporosis medication for 3 years sustained an incident fracture, illustrating the importance of this effect (Diez-Perez 2014 [53]).

Glucocorticoid therapy reduces the level of PINP in a dose-responsive manner and this also makes the interpretation of PINP difficult - is any reduction due to a beneficial effect of bisphosphonate treatment or due to the harmful effect of the glucocorticoid? For example, a daily dose of 10 mg prednisone resulted in a 20% reduction in PINP over a week. Thus, PINP is only helpful in monitoring treatment of glucocorticoid-induced osteoporosis if the glucocorticoid dose is established and remains stable. If accessible, it may be preferable to use another BTM in this clinical situation, such as bone ALP, CTX or NTX.

Evaluation of use in clinical practice

Antiresorptive Therapy

We introduced the monitoring algorithm (Figure 1) into primary care in Sheffield in September 2011 and conducted an audit on all patients being evaluated for osteoporosis at the Metabolic Bone Centre in Sheffield in July 2012. New treatment was recommended to the general practitioner in 108 cases (mean age 65 years, 86% female) and baseline PINP

was obtained by the GP in 76 of these. Follow-up measurement was made in 34 of these. We found that at follow-up, 27 (79%) met the criteria for treatment response (Figure 2). Among the 7 people with poor response, we found reasons for this in 3 cases (poor compliance, intercurrent surgery and the sample measured too early). We were encouraged by this early uptake of PINP monitoring and are working further to develop awareness among general practitioners and to develop confidence in its use.

We acknowledge that monitoring using PINP in clinical practice is by necessity pragmatic, needing to minimise cost and patient inconvenience and differs considerably from the research approach. Nonetheless, the approach has been welcomed by GP colleagues and we believe is preferable to no monitoring or reassessment of BMD after 2 years, by which time a high proportion of patients have stopped treatment.

The interpretation of PINP results and the need to change treatment needs to be considered on an individual basis using clinical judgement and considering factors such as the severity of the osteoporosis, likelihood of poor compliance (e.g. presence of dementia) and presence of known confounders.

In all patients with suboptimal response, especially if repeat PINP remains increased, change in treatment needs to be considered. We would often move onto parenteral treatment at this point to eliminate problems due to poor compliance and/or poor absorption.

Our initial evaluation of the use of PINP for monitoring anti-resorptive therapy in clinical practice (Figure 2) drew our attention to several types of responses.

Black – a significant decrease to below 35µg/L

This is consistent with a good response to a level that is associated with low fracture risk and so is the optimal response. We would recommend the physician confirms compliance, enquires about any drug side-effects and encourages the patient to continue therapy and report any new issues. Medications should be reviewed at least annually and risk assessment including DXA planned at 5 years. Further PINP measurement is not considered necessary unless the clinical situation alters.

Red – a significant decrease but not to below 35µg/L

Many patients like this will have high baseline BTM. The high PINP on treatment may indicate that fracture risk is still somewhat elevated (Eastell 2006 [46], Delmas 2012 [47]) and the patient may benefit from treatment with a parenteral drug such as zoledronic acid or denosumab.

- A high baseline PINP may be due to fracture within the preceding 6-12 months, especially if very recent. In that case, any subsequent decrease may be due to the healing fracture and so does not indicate good response. Thus, it is important to check compliance and if this is adequate then continue with treatment and repeat PINP in another 3 months. If PINP is still above 35 then consider investigation for secondary osteoporosis.
- A high baseline PINP may occur in a patient with another disease affecting PINP e.g. skin disease, liver dysfunction. Consider if an additional confounder has developed and if investigations are indicated; check compliance and repeat in another 3 months. If uncertainty remains at that point, consider change in treatment.

Yellow – no significant change and remains under 35µg/L

- Low PINP values are associated with a lower risk of fracture. It is not certain whether patients with low BTM have fracture benefit from treatment – one study with alendronate would indicate there is no benefit (Bauer 2006 [16]) whereas a study with risedronate indicates there may be some benefit (Siebel [54]). Certainly, patients with low PINP tend to have low rates of bone loss when left untreated and low BMD increases in response to anti-resorptive treatment (Eastell 2015 [18]). Nonetheless, continued treatment would generally be considered appropriate and the patient may be advised that the BTM result indicates a low risk of fracture.

Green – a significant increase in PINP, to above 35µg/L

- This is likely to reflect either an intercurrent event leading to an increase in bone turnover or presence of a disease that provides a non-bone source of PINP

- A common cause of increased PINP is a new fracture (magnitude of increase related to size of bone affected) with an additional impact from surgical intervention. If a fracture is confirmed, then check the compliance, reassure the patient that a fracture occurring very early in treatment is not due to treatment failure and reassess after another 3-6 months
- Other causes include a reduction in glucocorticoid dose since treatment initiation, ie with greater suppression of PINP at baseline when on a higher dose – if the 6 month result remains above 35µg/L this still suggests poor treatment response. In practice, we would measure another marker unaffected by glucocorticoid
- at this point eg NTX and if result is low then presume response
- May be due to new secondary osteoporosis eg development of thyrotoxicosis – clinical evaluation and relevant investigation should be undertaken and any underlying cause treated. Compliance with the osteoporosis treatment should also be checked and encouraged.
- We may observe fluctuations in PINP (and other BTM) in patients with co-morbidities especially those affecting liver, kidney and skin. For example, increases in PINP are observed after non-bone surgical intervention reflecting PINP from type I collagen in skin. In these patients, check compliance and consider using another marker

If there remains a suboptimal response then a change to an alternative agent such a parenteral drug (zoledronic acid or denosumab) may be considered. If there is a good BTM response, but the patient has one or more new vertebral fractures, then a change to an anabolic drug (teriparatide) may be considered.

Monitoring offset of effect in the individual

It is often recommended that oral bisphosphonate therapy is stopped after 5 years in milder forms of osteoporosis (Adler 2016 [55]). The rationale for this is that longer-term therapy may

increase the risk of atypical femur fractures and once treatment is stopped there is continued benefit with little bone loss from the spine and continued (if mild) suppression of bone turnover (Black 2006 [56]). Attempts have been made to monitor the offset of effect with BMD, but the changes at the hip are quite small relative to the least significant change and so only a small proportion of patients are identified as having offset of effect, with just 29% having more than 5% bone loss from the total hip 5 years after stopping alendronate (McNabb 2013 [57]). BTM could be used for this purpose, perhaps using the LSC approach to examine for an increase or the threshold approach to identify a value that is above the mean for young women and so merits re-starting therapy. There has been little published on this topic and there appears to be little association between change in BMD or BTM and fracture risk off treatment with alendronate (Bauer 2014 [58]). We await further research before making any recommendations.

Anabolic Therapy

We also use PINP to assess response to teriparatide treatment. Teriparatide is used in patients with severe and complicated osteoporosis so it is important to consider if response is optimal as early as possible, particularly as treatment is limited to 24 months. Suboptimal response may be due to issues with compliance, drug storage or injection technique.

We evaluated 91 patients monitored using PINP. All had previously been treated with anti-resorptive therapy, mean age 71 years (89% female). The baseline PINP was 35 µg/L using the Roche Cobas automated immunoassay analyser, reflecting the effect of the prior anti-resorptive treatment. We took our treatment targets as an increase of more than the least significant change in PINP at months one and three (10 µg/L)(Eastell 2006 [45]) and an increase to above the reference interval of 69 µg/L (Glover 2008 [59]) on at least one occasion. We found that by 3 months of treatment 93% exceeded the least significant change and 66% exceeded the upper limit of the reference interval (Figure 4). This responder rate of 93% was similar to that found in clinical trials of teriparatide of 87, 77, and 87% (McClung 2005 [60], Neer 2001 [61], Tsujimoto 2011 [62]). There was no significant correlation between PINP results and change in lumbar spine BMD at two years but this was difficult to evaluate as only 29% of our patients had reliable spine scans due to very high prevalence of vertebral fracture and degenerative change. The baseline PINP in this evaluation were low as all patients had previously been treated with anti-resorptive drugs and so these findings not relevant to patients starting teriparatide with no such prior therapy.

In our experience, treatment is often commenced without measurement of a baseline PINP, especially in primary care. In this situation, it is particularly important to undertake a thorough evaluation of adherence to treatment and we find it remains valuable to make the 6 month measurement. A PINP value on treatment that is low or low normal for anti-resorptive treatment (ie <35 µg/L) or above the reference interval for anabolic therapy (ie >69 µg/L) may be presumed to indicate adequate response. However, this approach is less well documented than the least significant change approach.

Current recommendations: Examples

The IOF and IFCC made recommendations concerning BTM and reviewed national guidelines; five out of nine national societies or organisations recommended the use of BTM for treatment monitoring, although recognising that further research and evaluation remains necessary (Vasikaran 2011 [7]).

The IOF proposed that a PINP or CTX value at 12 weeks on treatment with oral bisphosphonate can identify poor response and be used to identify patients who are unlikely to be adhering to therapy (Diez-Perez 2017 [21]) or who have failed therapy (Diez-Perez 2013). The IOF proposed using the BTMs at 12 weeks rather than 3 months on treatment and the responder rate in the TRIO study was similar for 12 and 48 weeks (Naylor 2015 [22]) and so this approach is appropriate. National guidelines supporting the use of bone turnover markers are also available, such as those from and Austrian Group and from the Japanese Osteoporosis Society (Bieglmayer 2012 [63], Nishizawa 2013 [64]).

Conflicts of Interest:

Dr. Eastell reports grants and personal fees from Amgen, grants from AstraZeneca, grants and personal fees from Alexion, grants, personal fees and non-financial support from Immunodiagnostic Systems, grants, personal fees and non-financial support from Roche, personal fees from Lilly, personal fees from D3 Biomedical Science Institutes, personal fees from GSK Nutrition, within 3 years of writing this review. Dr Naylor, Dr Pigott, and Miss Gossiel have no disclosures. Dr Walsh has received speaker's honoraria from Lilly and the donation of drug and placebo from Prostrakan. Dr N Peel has received speaker's honoraria and funding to attend educational events from Warner-Chilcott, Lilly, Amgen, GSK and Prostrakan and consultancy fees from Internis Pharma and Lilly.

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Figure legends

Figure 1. Sheffield PINP monitoring algorithm for anti-resorptive treatment. Optimal treatment response with PINP is a decrease of 10 μg/L to below 35 μg/L. Optimal treatment response with CTX is a decrease of 100 ng/L to below 280 ng/L.

Figure 2. Serum PINP (μg/L) measured using Roche Cobas in patients starting anti-resorptive therapy in 34 patients based in general practice setting. Solid lines indicate

response with a significant change in PINP to a level below 35 μg/L, and dotted lines no response. The lines are coloured red to indicate those patients who had a significant change in PINP but didn't reach the target, yellow to indicate those who reached the target but PINP didn't change, and green to show an increase in PINP. The second PINP value should have been taken after 6 months of treatment and the broken horizontal line shows the critical value of 35 μg/L (the mean value of PINP in healthy young women).

Figure 3. Sheffield PINP monitoring algorithm for anabolic treatment. An optimal response would be an increase in PINP of more than 10 ug/L to above 69 μg/L.

Figure 4. The absolute value of PINP (μg/L) measured using Roche Cobas at baseline, one and three months after starting teriparatide in 91 people for osteoporosis. The blue dashed horizontal line represents the upper limit of the reference interval for healthy young women (69 μg/L). Overall, 95% responded with an increase of more than 10 μg/L above baseline at both 1 and 3 months and 66% exceeded the upper limit of the reference interval at at least one timepoint. Patients meeting both response criteria, i.e. demonstrating an optimal response, are shown in black; those who had a significant increase in PINP but not exceeding the reference interval are shown in red. Patients in whom no BTM response was demonstrated are shown in green. The extremely high value of PINP of more than 500 μg/L was observed in a patient with auto-immune hepatitis.

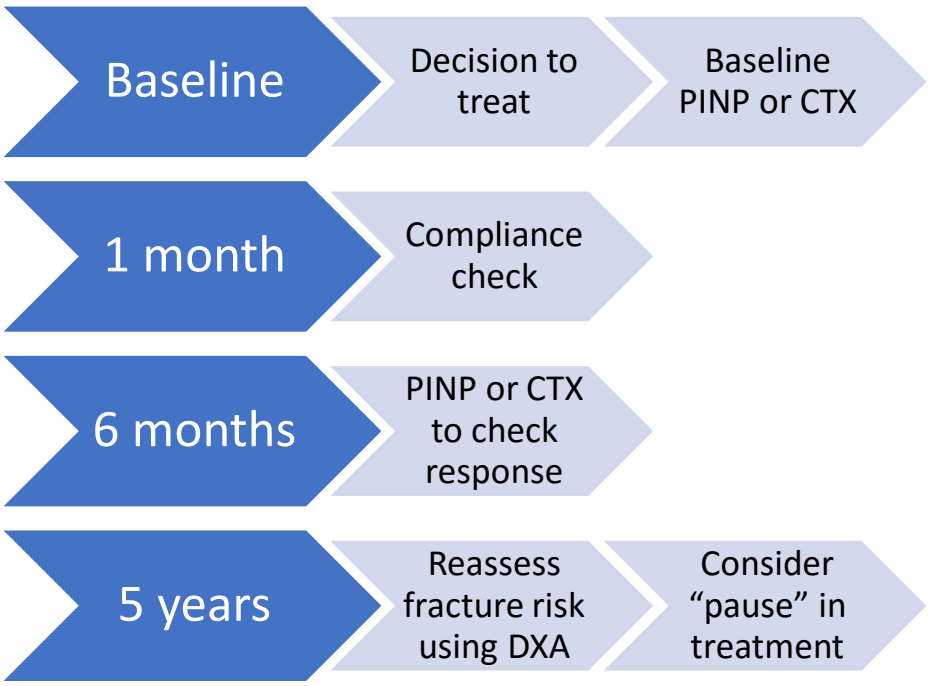
Table 1. Bone turnover markers commonly used in clinical practice.

Bone formation	N-propeptide of type I collagen, PINP*	Serum or plasma	Radioimmunoassay, automated assay (Roche Cobas, IDS iSYS)
	Osteocalcin (OC)	Serum or plasma	ELISA, automated assay (Roche Cobas, IDS iSYS)
	Bone alkaline phosphatase (bone ALP)	Serum	IRMA, ELISA, automated assay (IDS iSYS)
Bone resorption	C-terminal telopeptide of type I collagen (CTX)*	Serum, plasma or urine	ELISA, automated assay (Roche Cobas, IDS iSYS)
	N-terminal telopeptide of type I collagen (NTX)	Serum, plasma or urine	ELISA, automated assay (Ortho Clinical Diagnostics)
	Deoxypyridinoline (DPD)	Urine	HPLC for total, ELISA for free
	Tartrate-resistant acid phosphatase, 5b	Serum or plasma	ELISA, automated assay (IDS iSYS)

Table 2. The critical values for PINP and CTX are supported by results in 50 women from the TRIO study with postmenopausal osteoporosis and treated with oral bisphosphonate and 200 healthy young control women. The means in the control group are geometric means; LSC estimates were based on the CV of samples taken at 12 and 13 weeks on treatment and multiplied by 2.77; effects are change from baseline to the average of the 12 and 13 week values. The full study has been described before (Naylor 2015 [20]).

Analyte	PINP		CTX	
Analyser	IDS iSYS	Roche Cobas e411	IDS iSYS	Roche Cobas e411
Controls, mean, µg/L (PINP) and ng/L (CTX)	31	33	327	221
LSC, %	29	23	54	50
LSC, µg/L and ng/L	6.2	5.7	80	60
Effect, %	-51	-54	-75	-74
Effect, µg/L and ng/L	-28	-32	-490	-360

Figure 1. Sheffield PINP monitoring algorithm for anti-resorptive treatment. Optimal treatment response with PINP is a decrease of 10 µg/L to below 35 µg/L. Optimal treatment response with CTX is a decrease of 100 ng/L to below 280 ng/L.



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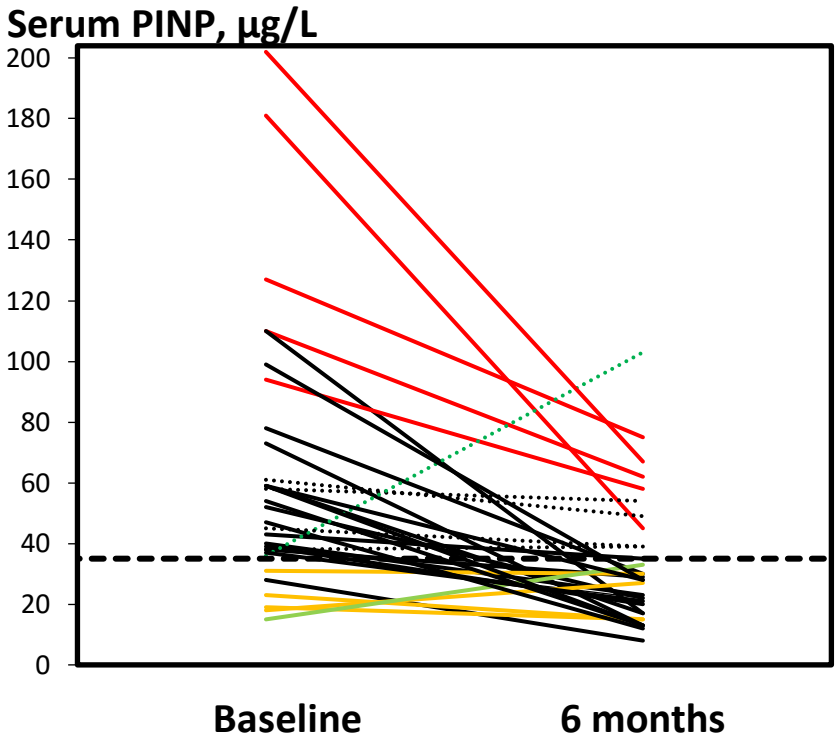

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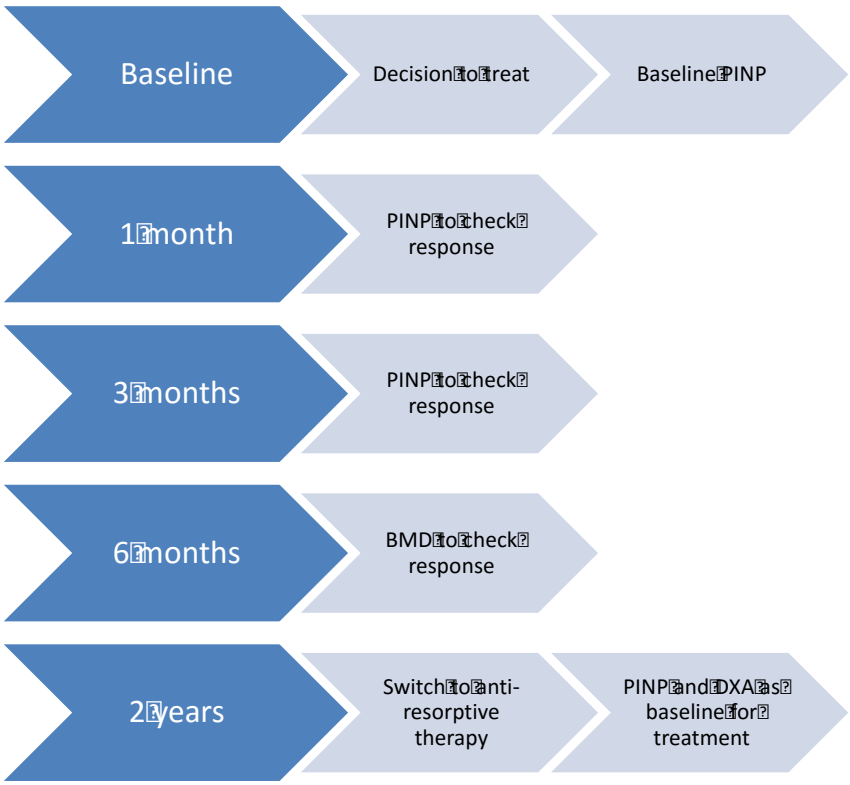

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