Clinical usefulness of bone turnover marker concentrations in osteoporosis

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ARTICLE INFO

Article history:
Received 17 February 2016
Received in revised form 27 June 2016
Accepted 28 June 2016
Available online xxxx

Keywords:
Bone turnover markers
Osteoporosis
Fracture risk
Reference intervals
Monitoring efficacy for treatment of osteoporosis
CTX
PINP

ABSTRACT

Current evidence continues to support the potential for bone turnover markers (BTM) to provide clinically useful information particularly for monitoring the efficacy of osteoporosis treatment. Many of the limitations identified earlier remain, principally in regard to the relationship between BTM and incident fractures. Important data are now available on reference interval values for CTX and PINP across a range of geographic regions and for individual clinical assays. An apparent lack of comparability between current clinical assays for CTX has become evident indicating the possible limitations of combining such data for meta-analyses. Harmonization of units for reporting serum/plasma CTX (ng/L) and PINP (μg/L) is recommended. The development of international collaborations continues with an important initiative to combine BTM results from clinical trials in osteoporosis in a meta-analysis and an assay harmonization program are likely to be beneficial. It is possible that knowledge derived from clinical studies can further enhance fracture risk estimation tools with inclusion of BTM together with other independent risk factors. Further data of the relationships between the clinical assays for CTX and PINP as well as physiological and pre-analytical factors contributing to variability in BTM concentrations are required.

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1. Introduction

Osteoporosis is the most prevalent metabolic bone disease and with an aging population its impact is expected to rise throughout the world. It is defined as a disease characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and consequent increase in risk of fracture [1]. Low bone mass, measured as bone mineral density (BMD), is asymptomatic and its important outcome is fracture, a cause of morbidity and mortality [2]. Therefore, the clinical management focus in osteoporosis is to prevent or reduce the risk of fracture and follow the response to therapy. Its total cost burden, including pharmacological prevention, in the European Union was recently estimated to correspond to approximately 3.5% of the total spending on health care at €37 billion [3]. Similar relative cost burdens are experienced in other parts of the world with the steepest rises in number of fractures in the coming years expected to be reported from the high population countries of Asia, all largely dependent on the aging of the population [4].

The first line of medical testing for diagnosis of osteoporosis and estimation of risk of fracture whether at clinical presentation or following initiation of treatment is measurement of BMD, most commonly using dual-energy X-ray absorptiometry (DXA) [5]. Algorithms to estimate fracture risk based on BMD and other clinical features such as FRAX® are commonly used in clinical practice to guide the treatment of individual patients [6]. Bone turnover markers (BTM) are not included in such algorithms.

BTM have a long history in research on metabolic bone diseases including osteoporosis and assays for a wide range have been developed. A review of this complete range is beyond the scope of this manuscript although others are available [7,8]. BTM largely represent products of bone proteins, particularly type I collagen which undergoes considerable post-translational modification during synthesis of new bone and
within the bone environment such that particular modifications increase the specificity for assessing bone formation or bone resorption. Other BTMs are products of bone cells, reflecting the number of particular cells within the bone environment at any time.

In 2010 the International Osteoporosis Foundation (IOF)-International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Joint Working Group on Bone Marker Standards (WG-BMS) published an extensive review concluding that there were insufficient data to include bone turnover markers values in current clinical practice [9]. The Working Group recommended one bone formation marker (serum-procollagen type I N-propeptide (PINP)) and one bone resorption marker (serum C-terminal telopeptide of type I collagen, (CTX)) be used as reference markers, to be measured by standardized assays in observational and intervention studies in order to assess their clinical performance as well as provide data by which alternatives could be assessed thus enlarging the international experience of the application of these markers to clinical medicine. In 2012 the National Bone Health Alliance extended the literature review on this subject arriving at similar recommendations [10].

The IFCC-IOF Working Group for the Standardization of Bone Marker Assays was established in 2012 to standardize or harmonize serum/plasma CTX and PINP assays depending on feasibility. After initial discussions with representatives of clinicians, clinical laboriandors and the In Vitro Diagnostic industry, it was agreed that a strategy of harmonization of assays was preferable because of the current lack of data indicating their clinical usefulness. A project is underway to describe the relationship for CTX and PINP values generated by the various assays used by clinical laboratories for patients presenting to an osteoporosis clinic. In the first instance a statistical method will be used to harmonize values where the assays provide significantly different concentrations.

2. BTM concentrations for predicting fracture risk

The IOF-IFCC WG-BMS review by Vasikaran et al. described 22 studies, in which the relationship between bone turnover markers and incident fractures was examined [9]. Eighteen of them showed that one or more markers were associated with risk of subsequent fracture with the concentration of bone resorption markers more consistently associated with fracture risk than bone formation markers. This was the case for studies in both men and women. Since that time three more studies have been published including a meta-analysis (Table 1). The meta-analysis examined the performance characteristics of two BTM, PINP and CTX, for fracture risk prediction in untreated individuals. The analysis included 6 prospective, cohort studies with the first incidence as the primary outcome. Only studies in middle-aged or older men and women were included. The expression of risk varied between the original studies, but all results were transformed into hazard ratio (HR) per standard deviation (SD) which is the gradient of risk (GR). The meta-analysis found a modest, but significant association between both PINP and CTX concentrations at baseline and fracture risk (see Table 1) [11]. This analysis combined results for CTX generated by the two clinical laboratory automated assay methods currently available. As presented below (see Section 6) these assays do not appear to provide comparable values for CTX. Similarly the PINP data were generated by different assays and while the lack of comparability of these assays is less certain again the GR would likely be reduced by combining assay data which are not comparable. In the Australian Health In Men Study the association of bone turnover markers with hip fracture incidence in older men was examined. Total osteocalcin (tOC), undercarboxylated osteocalcin (ucOC) and CTX were associated with hip fractures in univariate analyses, but only tOC remained significantly associated with incident hip fractures in multivariate analyses adjusting for age and glucocorticoid use [12]. In contrast to the above, a Japanese study of the Taiji cohort of both men and women failed to demonstrate a significant association between a broad range of markers of bone formation and bone resorption and incident fracture risk. However, the study was insufficiently powered for a fracture endpoint as this cohort included relatively young subjects (mean age approximately 60 years) resulting in a low number of osteoporotic fractures (32) during the 10-year follow-up period [13].

Table 1

<table>
<thead>
<tr>
<th>Study</th>
<th>Population and setting</th>
<th>Age (years)</th>
<th>Expression of risk</th>
<th>Length of follow-up</th>
<th>Fracture type</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Johansson [11]</td>
<td>Meta-analysis, 6 prospective cohort studies, middle-aged or older men (2 studies) and women (4 studies)</td>
<td>&gt;50</td>
<td>HR for fracture per SD in BTM (GR)</td>
<td>From 2 to 6.5 years</td>
<td>Different between studies: hip, non-vertebral, osteoporotic</td>
<td>HR per SD (95% CI). Different settings for adjustment.</td>
</tr>
<tr>
<td></td>
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<td>Fracture combined (hip, non-spine, osteoporotic, any, low-trauma)</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td>PINP HR = 1.23 (1.09–1.39)</td>
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<td></td>
<td></td>
<td>CTX HR = 1.18 (1.08–1.29)</td>
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<td></td>
<td></td>
<td>HR = 1.19 (1.05–1.34) (if women only)</td>
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<td></td>
<td></td>
<td>HR = 1.17 (1.04–1.31) (if adjusted for age)</td>
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<td></td>
<td></td>
<td></td>
<td>HR = 1.12 (0.97–1.29) (if adjusted for BMD)</td>
<td></td>
</tr>
<tr>
<td>Yoshimura [13]</td>
<td>307 middle-aged and elderly Japanese recruited by age- and gender–stratification in the Taiji cohort (147 men and 160 women), 32 with fractures</td>
<td>40–79</td>
<td>HR per SD</td>
<td>10 years</td>
<td>Osteoporotic (spine, pelvis, ribs, distal radius, forearm, humerus and hip)</td>
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<td></td>
<td></td>
<td></td>
<td>Hip fractures</td>
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<td></td>
<td></td>
<td></td>
<td>CTX HR = 1.23 (1.04–1.47)</td>
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<tr>
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<td></td>
<td></td>
<td>HR = 1.17 (0.95–1.44) (if women only)</td>
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<td></td>
<td>HR per SD. However, HR are not shown in article, as no significant associations were found</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>s-OC, s-OCT, s-BAP, s-ICTP, s-IP, s-PINP, s-beta-CTX, s-NTX, u-PP, u-PPD</td>
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<td></td>
<td>OR per SD (95% CI)</td>
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<td></td>
<td></td>
<td>Log10(tOC) 1.20 (1.00–1.42) (after adjustment for age and GC use)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Log10(PINP) and Log10(CTX-I) not significantly associated with incident hip fracture after adjustment for age and GC use (P = 0.17)</td>
<td></td>
</tr>
<tr>
<td>Chubb [12]</td>
<td>6028 community-dwelling older men from Perth, Australia enrolled in the population-based Health In Men Study (HIMS), 114 with hip fractures, 3896 in control group</td>
<td>70–89</td>
<td>OR per SD in BTM</td>
<td>From 8 to 11 years</td>
<td>Hip fractures</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>s-OC, s-OCT, s-BAP, s-ICTP, s-IP, s-PINP, s-beta-CTX, s-NTX, u-PP, u-PPD</td>
<td></td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td>OR per SD (95% CI)</td>
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<td></td>
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<td></td>
<td>Log10(tOC) 1.20 (1.00–1.42) (after adjustment for age and GC use)</td>
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<td></td>
<td></td>
<td></td>
<td>Log10(PINP) and Log10(CTX-I) not significantly associated with incident hip fracture after adjustment for age and GC use (P = 0.17)</td>
<td></td>
</tr>
</tbody>
</table>

ICTP: C-terminal cross-linking telopeptide of type I collagen generated by matrix metalloproteinase; BAP: bone-specific alkaline phosphatase; beta-CTX: beta-isoamidized C-terminal cross-linking telopeptide of type I collagen; BTM: bone turnover marker; CI: confidence interval; CTX: C-terminal cross-linking telopeptide of type I collagen; DPD: deoxypyridinoline cross-links of collagen; GC: glucocorticoid; GR: gradient of risk; HR: hazard ratio; NTX: N-terminal cross-linking telopeptide of type I collagen; OC: intact osteocalcin; OR: odds ratio; PICP: C-terminal propeptide of type I collagen; PINP: N-terminal propeptide of type I collagen; PYR: pyridinoline cross-links of collagen; SD: standard deviation; TOC: total osteocalcin.

Please cite this article as: H.A. Morris, et al., Clinical usefulness of bone turnover marker concentrations in osteoporosis. Clin Chim Acta (2016), http://dx.doi.org/10.1016/j.cca.2016.06.036
These more recent findings support the previous interpretation in the Vasikaran review [9]. There are significant associations between bone turnover markers and incident fracture risk, though the association is modest. Most studies demonstrate a relation between bone turn- over markers and fracture, yet there are limitations to the studies. These include the variable use of markers of bone formation (BAP, PINP, PICP, total osteocalcin, intact osteocalcin) and of bone resorption (ICTP, CTX, NTX-I, PYR, DPD, beta-CTX), differences in analytical assays and platforms, inconsistencies in expression of risk, as well as inconsistent predictive value for a specific marker in the individual studies reported. (See Table 1 for abbreviations of BTMs).

3. BTM concentrations for monitoring treatment

The IOF-IIFC WG-BMS review [9] also reported seven studies concerning the relationship between change in BTM and fracture risk reduction with drugs given for postmenopausal osteoporosis. These drugs included alendronate, risedronate, zoledronic acid, raloxifene, and strontium ranelate. One of the outcomes from such studies is to assess the extent to which a biological marker is a surrogate end-point for a clinical event, which is known as the ‘treatment effect explained’. In the case of clinical trials for osteoporosis treatment the clinical end- point is fracture and the surrogate biological markers are BTM. In these trials the treatment effect explained varied from 27 to 77% indicating that about half of the fracture risk reduction with these drugs, which work through the inhibition of bone turnover, could be associated with the measured change in BTM during the first year of treatment.

There have now been two further studies that examine this question, one a follow-up analysis of zoledronic acid and the other a new analysis with bazedoxifene, a selective estrogen receptor modulator, similar to raloxifene (Table 2). They are both believed to reduce the risk of fracture by the reduction in bone turnover. Jacques and colleagues [14] reported on the relationship of changes in PINP and fracture risk reduction in the HORIZON trial. This was a study of 7736 postmenopausal women with osteoporosis who were randomized to receive zoledronic acid 5 mg intravenously once a year for three years, or placebo. All patients received calcium and vitamin D. A bone marker subset analysis included 1132 women in whom PINP was measured. This marker was chosen as the samples were not taken with the patients in the fasting state and PINP has proven to be informative in other studies, for example with raloxifene where the change in PINP at 12 months was 56% [15]. The change in PINP at one year explained 58% of the treatment effect on new vertebral fracture (statistically significant), and there was a significant association with non-vertebral fracture. This figure was similar to the 54% treatment effect explained change in total hip BMD over three years and vertebral fracture. The effect explained by PINP was independent of that explained by total hip BMD. The change in CTX at one year explained 16% and change in OC 6% of the treatment effect on new vertebral fracture (statistically significant). There was no overall reduction of non-vertebral fractures in this study so any relationship with marker change could not be tested. These figures were similar to the figures of 14% treatment effect explained by the change in total hip BMD and 5% for lumbar spine BMD over three years and vertebral fracture.

Once again the conclusions made in the original report [9] are at least partially supported by these new analyses. The treatment effect explained by BTM is at least as great as BMD. The finding of significant positive associations between the reduction in BTM and the reduction in fracture risk support the use of BTM in monitoring treatment. The limitation noted in the original report that studies were often small subsets of the main trial was true for the zoledronic acid study but not for the bazedoxifene study, which is the largest study to date. The studies were also criticized for not obtaining samples under optimal conditions. This again was not true of these two studies as the patients from the bazedoxifene study were in the fasting state for the blood draw, a critical requirement for serum CTX.

4. The effect of renal impairment on BTM concentrations

Bone health is very frequently altered in Chronic Kidney Disease (CKD) and these patients are at increased risk of fractures whether they are dialyzed [18] or not [19]. Indeed, these patients often are characterized by either increased or decreased bone turnover, linked to over- or under-secretion of parathyroid hormone (PTH). The gold standard to evaluate bone turnover is bone biopsy. Unfortunately, use of bone biopsies to determine bone turnover is hampered by the invasive nature of the procedure and the difficulty for correct interpretation of the results, limiting its use to a few specialized centres [20]. In clinical practice repeated bone biopsies are problematic for the follow-up of the patients or to assess effect of a treatment. Hence, BTM are essential in clinical practice to evaluate bone turnover. In 2009 the international recommendations in nephrology, Kidney Disease: Improving Global Outcomes (KDIGO) guidelines [21] recommended the measurement of PTH and the bone turnover marker Bone Specific Alkaline Phosphatase (BAP) in the assessment of metabolic bone disease of CKD (CKD-MBD). BAP was selected because serum concentrations are unaffected by renal function since it is cleared by the liver and with a molecular weight above 50,000 D it is unlikely to be filtered at the kidney. BAP does suffer from some analytical and clinical issues, which have been discussed elsewhere [22].

PINP has been recommended as the bone formation marker by IOF and IIFC for clinical research studies in osteoporosis [9]. It consists of three subunit chains of type 1 procollagen (2 pro-α1 chains and 1 pro-α2 chain) that are non-covalently linked and is produced in equimolar amounts with collagen deposited in bone tissue [23]. Once in the circulation, PINP is rapidly bound and internalized by liver endothelial cells through their scavenger receptors [24]. In human serum, PINP is present in two major forms, an intact trimeric form and a monomeric form. This

Table 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Trial</th>
<th>Author</th>
<th>N</th>
<th>BTM</th>
<th>Months</th>
<th>Change, %</th>
<th>Duration, yr</th>
<th>Fracture</th>
<th>Treatment effect explained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zoledronic acid</td>
<td>HORIZON International</td>
<td>Jacques [14]</td>
<td>1132</td>
<td>PINP</td>
<td>12</td>
<td>56</td>
<td>3</td>
<td>Vertebral</td>
<td>Vertebral (58%) CTX, 18% (3–41) OC, 14% (0–46) CTX, 20% (4–44) OC, 4% (0–21) CTX, 25% (3–68) OC, 29% (0–85)</td>
</tr>
<tr>
<td>Bazedoxifene (all)</td>
<td></td>
<td>Bruyere [16]</td>
<td>5244</td>
<td>CTX, 12</td>
<td>PINP</td>
<td>3</td>
<td>4</td>
<td>OC, 37</td>
<td>OC, 39</td>
</tr>
<tr>
<td>20 mg daily</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>OC, 41</td>
<td>0</td>
<td>3</td>
<td>OC, 37</td>
</tr>
<tr>
<td>40 mg daily</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>OC, 41</td>
<td>0</td>
<td>3</td>
<td>OC, 37</td>
</tr>
</tbody>
</table>

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Fibroblast Growth Factor 23 (FGF23) is produced by osteocytes and is increased in CKD patients. High concentrations of FGF23 are associated with improved indices of skeletal mineralization in dialyzed pediatric patients with high turnover renal osteodystrophy [28]. Thus, FGF-23 measurements may indicate skeletal mineralization status, at least in this population [29]. However, since concentrations of FGF23 are extremely high in CKD patients compared to healthy individuals, it would appear unlikely that subtle changes in FGF23 concentrations will be clinically significant. These high concentrations add to the difficulty of measuring FGF23 with current manual assays. It is unclear whether such highly diluted specimens provide values that reflect the true value in serum or whether matrix effects confound these results. New studies, with better analytical tools, are needed to prove the usefulness of FGF23 to reflect bone mineralization in CKD patients.

Sclerostin, also produced in the osteocytes, is an inhibitor of the Wnt signalling pathway thus decreasing bone formation [30]. Sclerostin has a true protective effect or if these high values arise as a secondary phenomenon [32]. Sclerostin accumulates in CKD which adds further complexity for interpretation of results [33]. Even more problematic is the lack of concordance between the different assay kits confounding the interpretation of serum levels [34]. With a new latter form tends to be elevated in CKD patients. PINP determination can be performed either with automated (Roche Elecsys/Cobas and IDS iSYS) or manual (Orion Diagnostica) methods but the “Total” PINP assay (Roche Elecsys/Cobas) recognizes both the trimeric form and the monomers whereas the “Intact” PINP assays (IDS iSYS and Orion Diagnostica) recognize the trimeric form only. In CKD patients, it has been shown that patients with a glomerular filtration rate (GFR) below 30 mL/min/1.73 m² have PINP concentrations that are significantly correlating with histological indices of osteoclast number, bone formation rate and mineral apposition rate in uremic patients [27]. By the same token, it is not a good marker of change in bone resorption following treatment with cathepsin K inhibitors, which reduce bone resorption without reducing osteoclast numbers. TRAP-5B has recently become available on the automated IDS iSYS platform which may increase its potential as a routine marker for clinical laboratories increasing the data on this marker since such information is scarce [26].

Table 3
Reference intervals for CTX in pre-menopausal women measured by the automated Roche assay.

<table>
<thead>
<tr>
<th>Region</th>
<th>Age range (n)</th>
<th>Reference Interval (ng/L)</th>
<th>Mean/median (μg/L)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA</td>
<td>28–45 (237)</td>
<td>94 to 659</td>
<td>280</td>
<td>De Papp et al. [36]</td>
</tr>
<tr>
<td>Italy</td>
<td>45–50 (82)</td>
<td>70–610</td>
<td>250</td>
<td>Adami et al. [37]</td>
</tr>
<tr>
<td>France</td>
<td>35–45 (157)</td>
<td>105–589</td>
<td>N/A</td>
<td>Claudon et al. [38]</td>
</tr>
<tr>
<td>England</td>
<td>35–45 (153)</td>
<td>100–620</td>
<td>270</td>
<td>Glover et al. [39]</td>
</tr>
<tr>
<td>France, Belgium, US and UK</td>
<td>30–39 (637)</td>
<td>114–628</td>
<td>317</td>
<td>Glover et al. [40]</td>
</tr>
<tr>
<td>Saudi Arabia</td>
<td>35–45 (765)</td>
<td>163–274</td>
<td>217</td>
<td>Ardaei et al. [41]</td>
</tr>
<tr>
<td>France, Denmark</td>
<td>35–39 (188)</td>
<td>111–791</td>
<td>297</td>
<td>Eastell et al. [42]</td>
</tr>
<tr>
<td>Australia</td>
<td>30–39 (215)</td>
<td>100–700</td>
<td>N/A</td>
<td>Jenkins et al. [43]</td>
</tr>
<tr>
<td></td>
<td>40–49 (209)</td>
<td>100–600</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Australia</td>
<td>20–49 (164)</td>
<td>150–800</td>
<td>N/A</td>
<td>Vasikaran et al. [44]</td>
</tr>
<tr>
<td>Spain</td>
<td>35–45 (164)</td>
<td>137–484</td>
<td>255</td>
<td>Guanabens et al. [46]</td>
</tr>
</tbody>
</table>

Table 4
Reference intervals for PINP in pre-menopausal women measured by the automated Roche assay.

<table>
<thead>
<tr>
<th>Region</th>
<th>Age range (n)</th>
<th>Reference Interval (μg/L)</th>
<th>Mean/median (μg/L)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Italy</td>
<td>45–50 (82)</td>
<td>14.6–63.5</td>
<td>34.7</td>
<td>Adami et al. [37]</td>
</tr>
<tr>
<td>France</td>
<td>35–45 (157)</td>
<td>17.9–60.4</td>
<td>N/A</td>
<td>Claudon et al. [38]</td>
</tr>
<tr>
<td>England</td>
<td>35–45 (153)</td>
<td>16.2–60.9</td>
<td>33.1</td>
<td>Glover et al. [39]</td>
</tr>
<tr>
<td>France, Belgium, US and UK</td>
<td>30–39 (637)</td>
<td>16.3–78.2</td>
<td>38.7</td>
<td>Glover et al. [40]</td>
</tr>
<tr>
<td>Saudi Arabia</td>
<td>35–45 (765)</td>
<td>22.3–42.9</td>
<td>32.5</td>
<td>Ardaei et al. [41]</td>
</tr>
<tr>
<td>France, Denmark</td>
<td>35–39 (188)</td>
<td>17.3–83.4</td>
<td>38.0</td>
<td>Eastell et al. [42]</td>
</tr>
<tr>
<td>Australia</td>
<td>30–39 (215)</td>
<td>15–80</td>
<td>N/A</td>
<td>Jenkins et al. [43]</td>
</tr>
<tr>
<td></td>
<td>40–49 (209)</td>
<td>15–60</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Australia</td>
<td>25–49 (164)</td>
<td>15–70</td>
<td>N/A</td>
<td>Vasikaran et al. [44]</td>
</tr>
<tr>
<td>Spain</td>
<td>35–49 (164)</td>
<td>22.7–63.1</td>
<td>N/A</td>
<td>Guanabens et al. [46]</td>
</tr>
</tbody>
</table>

Table 5
Reference intervals for CTX in pre-menopausal women measured by the automated IDS assay.

<table>
<thead>
<tr>
<th>Region</th>
<th>Age range (n)</th>
<th>Reference Interval (ng/L)</th>
<th>Mean/median (μg/L)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spain</td>
<td>35–45 (164)</td>
<td>109–544</td>
<td>249</td>
<td>Guanabens et al. [46]</td>
</tr>
<tr>
<td>Germany</td>
<td>30–54 (382)</td>
<td>50–670</td>
<td>230</td>
<td>Michelsen et al. [47]</td>
</tr>
</tbody>
</table>

Table 6
Reference intervals for PINP in pre-menopausal women measured by the automated IDS assay.

<table>
<thead>
<tr>
<th>Region</th>
<th>Age range (n)</th>
<th>Reference Interval (μg/L)</th>
<th>Mean/median (μg/L)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spain</td>
<td>35–45 (164)</td>
<td>21.8–65.5</td>
<td>36.6</td>
<td>Guanabens et al. [46]</td>
</tr>
<tr>
<td>Belgium and UK</td>
<td>18–50 (180)</td>
<td>13.7–71.1</td>
<td>N/A</td>
<td>Morovat et al. [48]</td>
</tr>
</tbody>
</table>

* Included OCP users.

Please cite this article as: H.A. Morris et al., Clinical usefulness of bone turnover marker concentrations in osteoporosis, Clin Chim Acta (2016), http://dx.doi.org/10.1016/j.cca.2016.06.036
anti-sclerostin agent becoming available, interest in this analyte will likely grow but robust analytical methods are required to provide true measurements suitable for clinical interpretation.

5. Interpretation of bone turnover markers concentrations – The role of reference intervals

BTM reference intervals are useful for interpreting the results from osteoporosis patients but by themselves they are of limited value for fracture prediction in untreated, individual patients. The measurement of very high BTM values (>3 standard deviations above the mean of the reference values) during initial assessment of patients with osteoporosis is suggestive of other metabolic disease including malignancy [9]. The need to establish reference intervals from healthy premenopausal women aged 30–45 years when concentrations are at a nadir has been emphasised [9,35]. Ideally the subjects used for these studies should have normal BMD at the spine [9]. Expert opinion also suggests that the mean of the premenopausal reference interval can be used as a treatment target for anti-resorptive therapy [9,35].

It is considered necessary to establish reference intervals for different geographic areas and ethnicities [9]. Furthermore due to differences that currently exist between results from the different commercial clinical assays, current reference intervals need to be method specific; reference intervals from different methods cannot be used interchangeably. The following data providing reference interval data for CTX and PINP from various countries and assays are summarized in Tables 3–6.

de Papp et al. studied healthy premenopausal women from across the US including users and non-users of the oral contraceptive pill [OCP]. Serum samples were collected in the morning after an overnight fast. CTX values were log transformed to obtain a normal distribution and the geometric mean ± 2 SD was used to determine the overall mid 95% range for CTX (Table 3). Data from Italian healthy premenopausal, non-OCP using women aged 20–49 years were examined for the central 95% distribution for PINP and CTX [37]. Serum samples were collected between 7.30 am and 8.30 am after an overnight fast. BTMs were considerably higher in women aged 20–25 years and decreased progressively until 45–50 years of age. The reference intervals in women aged 45–50 years are presented in Tables 3 and 4. Healthy French premenopausal, non-OCP using women provided serum samples after an overnight fast before 10 am. The 2.5th to 97.5th percentile distribution for CTX and PINP are shown in Tables 3 and 4 [38]. Reference intervals for English premenopausal, non-OCP using women were established from serum samples collected between 8 am and 10 am after an overnight fast. Data for serum CTX and PINP were log transformed and 95% reference interval was calculated as mean ± 1.96 SD (Tables 3 and 4) [39].

French, Belgian, US and UK healthy premenopausal women including OCP non-users and users provided serum samples collected between 8 and 10 am after an overnight fast [40]. CTX and PINP values were log transformed to achieve normal distributions (Tables 3 and 4). Healthy premenopausal Saudi Arabian, non-OCP using women provided serum samples collected between 9:00 and 11:00 am after an overnight fast [41]. The central 95% calculated for each BTM (Tables 3 and 4). A cross-sectional registry study examined premenopausal healthy European Caucasian women not on OCP from France and Denmark [42]. Serum samples were collected after an overnight fast between 08:00 and 09:30 am. BTM data were log transformed to obtain a normal distribution and the reference intervals were determined as mean ± 1.96 SD for normalized values (Tables 3 and 4). An Australian study that included premenopausal women from the Geelong Osteoporosis Study examined reference intervals by decades of age [43]. Serum samples were collected after an overnight fast between 07:30 and 11:45 am and stored at −80 °C for >10 years. Optimal age-related reference intervals were determined for each BTM based on the central 90% of the distribution (Tables 3 and 4). Harmonized reference intervals for use in Australia have been developed for automated Roche assays for CTX and PINP based on published studies listed above with most weighting given for the Australian data [44,45].

Serum samples were collected from healthy premenopausal Spanish, non-OCP using women between 8 and 10 am after an overnight fast [46]. A quantile regression was used to estimate the 5th, 50th and 95th percentiles. The reference intervals are provided in Tables 3 and 4 for the automated Roche assay and Tables 5 and 6 for the automated IDS iSYS assay. The German Study of Health in Pomerania examined healthy premenopausal women after excluding those with any predetermined illness, OCP use or serum 25-hydroxyvitamin D concentration <25 nmol/L. Blood sampling was performed between 8.00 am and 8.00 pm from the mostly non-fasting subjects [47]. Reference intervals were defined as the central 95% range between the 2.5th and 97.5th percentiles (Tables 5 and 6). Note this study included mostly non fasting subjects and sampling was performed throughout the day. Morovat et al. studied apparently healthy premenopausal women as part of a larger study in two centres [48]. No mention is made of OCP use. Serum samples were collected during working hours in Belgium and between 8.30 am and 3.00 pm in UK. PINP was measured by automated IDS iSYS assay. PINP values were log transformed to obtain a normal distribution and the 95% reference interval determined and calculated values were converted back to measured units (Tables 5 and 6).

<table>
<thead>
<tr>
<th>Method 1 (x)</th>
<th>Method 2 (y)</th>
<th>n</th>
<th>Slope (95% CI)</th>
<th>Intercept (95% CI) (μg/L)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orion</td>
<td>Roche</td>
<td>34</td>
<td>0.94 (0.80–1.15)</td>
<td>−3.6 (−18.4–3.6)</td>
<td>Koivula et al. [49]</td>
</tr>
<tr>
<td>Roche</td>
<td>iSYS</td>
<td>127</td>
<td>0.98 (0.94–1.03)</td>
<td>−1.42 (0.00–2.86)</td>
<td>Weather et al. [52]</td>
</tr>
<tr>
<td>Roche</td>
<td>iSYS</td>
<td>820</td>
<td>1.05 (1.04–1.06)</td>
<td>−1.90 (−0.80)</td>
<td>Morovat et al. [50]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Method 1 (x)</th>
<th>Method 2 (y)</th>
<th>n</th>
<th>Slope (95% CI)</th>
<th>Intercept (95% CI) (μg/L)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orion</td>
<td>Roche</td>
<td>39</td>
<td>5.74 (4.56–8.57)</td>
<td>−95.6 (−240.9 to −31.9)</td>
<td>Koivula et al. [49] (Haemodialysis patients)</td>
</tr>
<tr>
<td>Orion</td>
<td>Roche</td>
<td>173</td>
<td>1.57 (1.43–1.73)</td>
<td>−12.0 (−19.0 to −5.7)</td>
<td>Koivula et al. [49] (Elderly bed-bound patients)</td>
</tr>
<tr>
<td>Roche</td>
<td>iSYS</td>
<td>81</td>
<td>0.74 (0.67–0.81)</td>
<td>+3.7 (1.2–5.8)</td>
<td>Cavalier et al. [51] (eGFR 30–60 mL/min/1.73 m²)</td>
</tr>
</tbody>
</table>

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The largest variation between the reference intervals appear to be between the Roche and IDS-iSYS assays for CTX although data for the IDS-iSYS assay are limited. The variation across geographic regions appears to be minor except for those from Saudi Arabia. Possibly data from other regions are largely derived from Caucasian populations and therefore there remain limited data from other ethnic groups as discussed previously [9].

6. Comparability of PINP and CTX values generated by current clinical assays

As discussed previously currently there are three clinical assays available for PINP and for CTX in blood. EDTA plasma has been stated as the preferred specimen type for the assay of CTX and is identified as such when specific reference is made. PINP is less affected by specimen type. The relationships between results produced by these different clinical assays for CTX and PINP have been examined. Note that CTX is variously reported in units of ng/L or ng/ml; in this review all results are converted to ng/L. PINP is reported in μg/L in most studies.

Kolvula et al. examined the relationships between the PINP results produced by two assays, the automated Roche Elecsys 2010 assay which measures total PINP and the radioimmunoassay for intact PINP (Orion Diagnostica UniQ PINP) [49]. The subjects were: 34 apparently healthy blood donors (26 men, 8 women; ages between 19 and 62 years), 39 patients with chronic renal failure and 173 bedridden geriatric (age >65 years) in-patients. The serum samples were kept frozen at –20 °C till analysis. The Passing-Bablok regression data are given in Tables 7 and 8. They concluded that PINP concentrations were similar in healthy blood donors but different in haemodialysis or bedridden geriatric patients with the Roche assay giving significantly higher results. In the most extensive study of PINP methods, Morovat et al. compared automated Roche E170 Total PINP and IDS iSYS Intact PINP in 828 serum specimens from healthy individuals and osteoporotic patients [50]. This study is notable for including a significant number of healthy children (>45% of the whole cohort), which had the effect of extending the range of PINP values in the comparison. The relationship between the two assays was non-linear. Overall the iSYS results were significantly higher than those obtained by the Roche E170 but at total PINP concentrations of <100 μg/L and >670 μg/L, the iSYS assay gave lower values than the E170 assay. Cavalier et al. compared the automated Roche Elecsys Total PINP and IDS iSYS Intact PINP assays in two populations; 157 patients in stage 3–5 CKD and 125 patients in stage 5D patients [51]. They concluded that the two assays produce the most discrepant results when eGFR decreases below 30 ml/min/1.73 m² although discrepancy is apparent even for eGFR values between 30 and 60 ml/min/1.73 m² (Table 8).

Wheater et al. examined the relationships between the results produced by two automated systems, Roche Elecsys 2010 and IDS iSYS, for PINP and CTX in blood from 127 subjects: 72 self-reported healthy volunteers (28 males, 28 females <50 years and 5 males, 11 females >50 years) with no known bone disease and 55 rheumatoid arthritis (RA) patients (1 male, 4 females <50 years and 10 males, 40 females >50 years) [52]. All patients had an estimated glomerular filtration rate (eGFR) > 30 ml/min/1.73 m². Serum samples were stored at −80°C immediately after venepuncture and used for both assays. The Passing-Bablok regression data are shown in Tables 7 and 9. Whereas the PINP assays appeared to give equivalent results, these authors found significant proportional and systematic biases between the CTX assays.

Chubb et al. measured plasma CTX by all three commercial assays on 169 adult patients (119 females and 50 males, median age 65 years [inter-quartile range 57–75.75 years] attending hospitals for routine investigation of metabolic bone disease including osteoporosis [53]. EDTA plasma was frozen at −20 °C before analysis after storage at 4 °C for up to 7 days. They also found significant proportional and systematic bias when the IDS iSYS assay was compared to both the IDS ELISA and Roche methods. The Passing Bablok regression parameters are given in Tables 9 and 10. In contrast, in a conference abstract, Cavalier et al. reported no systematic bias and lower proportional bias (the slope of the regression line was 1.12) between the Roche and IDS iSYS automated assays for CTX [54] (Table 9). Huvelle et al. compared CTX results by the IDS iSYS assay and the IDS ELISA on 97 serum samples collected from patients presenting to hospital for bone and mineral metabolism work-up (females 78; males 19; mean age: 67 years) [55]. Their regression data are shown in Table 10. They concluded that their limited study suggested the two assays could be used interchangeably. In summary, the results of two studies suggest that all PINP assays give similar results in healthy subjects with eGFR > 30 ml/min/1.73 m² [49,52]. However, based on the largest comparison study of the IDS iSYS and Roche E170 assays, Morovat et al. have concluded: “although there is a broad, general agreement between the intact and total PINP assays, there are some variations between the two results, and the differences can be large, unpredictable and clinically significant” [50]. Clearly the total PINP assay gives significantly higher values than the intact PINP assays in patients where there is an accumulation of the monomer; e.g. renal failure patients with eGFR < 30 ml/min/1.73 m², and in patients who are bedridden long-term [49,51].

For CTX assays, Wheater et al. and Chubb et al. found significant proportional and systematic inter-method biases [52,53], whereas Cavalier et al. and Huvelle et al. did not [54,55]. Two reference interval studies for CTX, each carried out using more than one assay support the presence of significant inter-assay biases for CTX [42,46]. The basis for these differences in outcomes between studies is unclear although variation between plasma or serum specimens may contribute. Such effects may hamper efforts to achieve harmonization of results between assays.

7. Conclusions

The current status in this field continues to support the potential for BTM to provide clinically useful information although many of the limitations identified earlier remain, particularly in regard to the relationship between BTM and incident fractures. Significant progress has

<table>
<thead>
<tr>
<th>Table 9</th>
<th>Regression equations describing the relationships of CTX values from two automated assays.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method 1 (x)</td>
<td>Method 2 (y)</td>
</tr>
<tr>
<td>Roche</td>
<td>iSYS</td>
</tr>
<tr>
<td>Roche</td>
<td>iSYS</td>
</tr>
<tr>
<td>Roche</td>
<td>iSYS</td>
</tr>
</tbody>
</table>

* Note: EDTA plasma specimens were used for these analyses, N/A not available.

<table>
<thead>
<tr>
<th>Table 10</th>
<th>Regression equations describing the relationships of CTX values from the IDS automated assay and the IDS ELISA.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method 1 (x)</td>
<td>Method 2 (y)</td>
</tr>
<tr>
<td>ELISA</td>
<td>iSYS</td>
</tr>
<tr>
<td>ELISA</td>
<td>iSYS</td>
</tr>
</tbody>
</table>

* Note: EDTA plasma specimens were used for these analyses.
been made on the usefulness of BTM for monitoring the efficacy of osteoporosis treatment. Important data are now available on reference interval values for CTX and PINP across a range of geographical regions and for individual assays. Perhaps most importantly the apparent lack of comparability between current clinical assays for CTX has become evident indicating the possible limitations of combining such data for meta-analyses. In order to overcome the limitations and to gain additional knowledge of the value of bone turnover marker measurements for predicting fracture risk, we reiterate the suggestions of the IOF-IFCC Bone Marker Standards Working Group [9] and NBHA [10] that future clinical studies should focus on using standardized analytical methods of reference analytes. Further study of the relationships between the clinical assays for CTX and PINP as well as factors, including physiological and pre-analytical issue, contributing to variability in BTM concentrations is required.

It is encouraging that the development of international collaborations continues. One is an initiative to bring all data from clinical trials in osteoporosis together in an individual meta-analysis. The Foundation of the National Institutes of Health in the US are obtaining all BTM results from the clinical trials in osteoporosis and planning such an analysis. ([http://www.fnih.org/what-we-do/current-research-programs/biomarkers-consortium-bone-quality-project](http://www.fnih.org/what-we-do/current-research-programs/biomarkers-consortium-bone-quality-project) This should overcome the critical issues of inconsistent statistical methodology and small sample size. It is possible that this knowledge can contribute to further enhance fracture risk estimation tools such as FRAX with inclusion of bone turnover marker over other independent risk factors.

References


