Mini-Review

Harmonised Australian Reference Intervals for Serum PINP and CTX in Adults

*Samuel D Vasikaran,1,2 SA Paul Chubb,1,2,3 Peter R Ebeling,4 Nicole Jenkins,5 Graham RD Jones,6,7 Mark A Kotowicz,8,9,10 Howard A Morris,11,12 Hans-Gerhard Schneider,5,13 Markus J Seibel,14,15,16 Greg Ward17

1Department of Clinical Biochemistry, PathWest Laboratory Medicine, Royal Perth and Fremantle Hospitals, Perth, WA, Australia; 2School of Pathology and Laboratory Medicine, University of Western Australia, Nedlands, WA, Australia; 3School of Medicine and Pharmacology, University of Western Australia, Nedlands, WA, Australia; 4Department of Medicine, School for Clinical Sciences, Faculty Medicine, Dentistry and Health Sciences, Monash University, Clayton, Vic., Australia; 5Clinical Biochemistry Unit, Alfred Pathology Service, Melbourne, Australia; 6Department of Medical Pathology, St Vincent’s Hospital, Darlinghurst, NSW, Australia; 7University of NSW, Kensington, NSW, Australia; 8School of Medicine, Deakin University, Geelong, Vic., Australia; 9NorthWest Academic Centre, The University of Melbourne, Sunshine Hospital, St Albans, Vic., Australia; 10Department of Endocrinology & Diabetes, Barwon Health, Geelong, Vic., Australia; 11School of Pharmacy and Medical Sciences, University of South Australia, SA, Australia; 12Chemical Pathology Directorate and Hanson Institute, SA Pathology, Adelaide, SA, Australia; 13Monash University, Melbourne, Vic., Australia; 14Bone Research Program, ANZAC Research Institute, Sydney, NSW, Australia; 15Department of Endocrinology & Metabolism, Concord Hospital, Sydney, NSW, Australia; 16The University of Sydney, Sydney, NSW, Australia; 17Biochemistry & Endocrinology, Sullivan & Nicolaides Pathology, Brisbane, Qld, Australia

*For correspondence: Clin Prof Sam Vasikaran, Samuel.Vasikaran@health.wa.gov.au

Abstract
Bone turnover markers (BTMs) are classified as either formation or resorption markers. Their concentrations in blood or urine of adults are considered to reflect the rate of bone remodelling and may be of use in the management of patients with bone disease. Major inter-method differences exist for BTMs, and harmonisation of methods is currently being pursued at an international level. Based on published data, this article describes age- and sex-specific Australian consensus reference intervals for adults for serum procollagen type I amino-terminal propeptide (s-PINP) and serum β-isomerised carboxy-terminal cross-linking telopeptide of type I collagen (s-CTX).

Introduction
Bone turnover markers (BTMs) are classified as either bone formation markers (i.e. peptides or enzymes secreted by osteoblasts during bone formation), or bone resorption markers (i.e. degradation products of bone collagen or enzymes secreted by osteoclasts, Table 1).1 Their concentrations in blood or urine are considered to reflect bone formation and resorption rates, respectively, depending to varying degrees on their tissue specificity and influenced by physiological and pathological factors.2,3 The changes in BTMs also reflect the fact that bone formation and resorption are generally ‘coupled’.

BTMs Currently Offered by Diagnostic Laboratories in Australia
Bone formation markers available for routine use include osteoblast-derived products such as PINP, bone-specific (bone ALP) and total alkaline phosphatase (ALP) and osteocalcin.4,9 All bone formation markers are measured in blood.

Bone resorption markers available for routine use include the collagen breakdown products amino-terminal cross-linking telopeptides of type I collagen (NTX), the low molecular weight form of the carboxy-terminal cross-linking telopeptide of type I collagen (CTX) and deoxypyridinoline (DPD). Commercial immunoassays measure free DPD, which forms about 40% of the total DPD in urine. Furthermore, tartrate-
resistant acid phosphatase band 5b (TRACP5b), an enzyme secreted by osteoclasts, and which reflects osteoclast numbers, has been used to assess bone resorption.10-17 Of note, NTX and DPD are measured in urine while CTX and TRACP5b are measured in blood.

**Pre-analytical Issues**
Significant intra-individual variations are seen in most BTMs. These fluctuations are due to a multitude of factors, including diurnal variation and fasting status. Therefore, BTMs should be collected in the fasting state in the morning within a standardised time period (ideally between 8.00 and 10.00 am). This is of particular relevance to s-CTX, which displays a high degree of variability depending on fasting status. Other markers, such as s-PINP, s-TRACP or urine DPD (u-DPD) or u-NTX are much less affected by food intake but still show diurnal variation. Analytical and pre-analytical details are included in Table 2 for the BTMs for which consensus reference intervals are presented here. Further details for all BTMs have been published elsewhere.1,3

Urine measurements can be performed in a spot sample (second void) but require correction for urinary creatinine. Attention to details regarding sample type and storage are important to minimise degradation, and have been addressed elsewhere.20-25 In brief, both serum and EDTA or heparin plasma are acceptable for PINP measurement; once separated, PINP is stable in serum/plasma for at least five days at room temperature and for at least four weeks at 4 °C. EDTA plasma is preferred for CTX, which is stable at room temperature.
Bone Marker Reference Intervals

in whole blood collected into EDTA for 24 hours; and after separation, for 48 hours at room temperature or 7 days at 4 °C. If a clotted sample is collected it should be centrifuged immediately and serum analysed or frozen within 4 hours. Once frozen, CTX is stable for long-term storage. There are no significant differences in measured values between plasma and serum for either marker.26

Renal failure may lead to the accumulation of some BTMs or their fragments in blood, and therefore can lead to an increase in measured concentrations, in addition to it causing metabolic bone disease. The blood BTMs mostly affected are total PINP (due to accumulation of the monomeric form) and s-CTX, which should be used with caution when eGFR <30 mL/min/1.73m². As would be expected, levels of BTM measured in urine are always affected by renal failure and should not be used in such circumstances. In contrast, the intact form of PINP, ALP and TRACP5b are least affected by renal impairment.27,28

There are method-specific differences between commercial assays for each BTM due to assay specificity, fragment recognition as well as differences in standardisation.3,29 Until harmonisation of methods for each is achieved, results and reference intervals from different methods cannot be used interchangeably for clinical care or in research studies, and patients should be monitored by the use of the same method over time.

Table 2. Analytical and pre-analytical details for s-PINP and s-CTX.

<table>
<thead>
<tr>
<th>BTM</th>
<th>Analytical and Pre-analytical Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>s-PINP</td>
<td>Specificity: Mostly derived from bone collagen type I</td>
</tr>
<tr>
<td></td>
<td>Assay: May recognise trimer alone (intact PINP) or trimer and monomer (total PINP)</td>
</tr>
<tr>
<td></td>
<td>Sources of variability: small circadian rhythm; bone acting agents, sex hormone and glucocorticoid therapy; not significantly affected by food. Total PINP assay, but not intact PINP, influenced by renal function</td>
</tr>
<tr>
<td></td>
<td>Automated and manual total and intact immunoassays available</td>
</tr>
<tr>
<td></td>
<td>Sample: serum or lithium heparin/EDTA plasma</td>
</tr>
<tr>
<td>s-CTX</td>
<td>The measurand is a well characterised 8-amino acid peptide, s-CTX is always isomerised to the β-form of the aspartyl residue</td>
</tr>
<tr>
<td></td>
<td>Specificity: collagen type I, with highest contribution probably from bone</td>
</tr>
<tr>
<td></td>
<td>Sources of variability: Very dependent on time of day and food (must be collected after an overnight fast); bone acting agents, sex hormone and glucocorticoid therapy; influenced by renal function, liver function and circadian rhythm (large effect)</td>
</tr>
<tr>
<td></td>
<td>Automated and manual immunoassays available</td>
</tr>
<tr>
<td></td>
<td>Sample: plasma or serum (EDTA plasma preferred)</td>
</tr>
</tbody>
</table>

| Table 3. Reference intervals for total s-PINP in adult males and females.# |
|-------------------------|-----------------|-----------------|-----------------|
| Gender                  | Age group (years) | Reference intervals (µg/L) | Caveats                      |
| Males                   | 25–70            | 15–80            | -                           |
| Males                   | >70              | 15–115           | -                           |
| Premenopausal females   | 25–49            | 15–70            | higher levels may be seen in women <35 years* |
| Menopausal females      | 50–70            | 15–90            | -                           |

*The reference intervals for premenopausal females between 25 and 34 years is 15–90 µg/L.  
#Reproduced with permission from Vasikaran SD et al. Towards optimising the provision of laboratory services for bone turnover markers. Pathology 2014;46(4):267-73.
Working Group

Consensus reference intervals in adults for s-PINP and s-CTX were developed under the umbrella of the Australasian Association of Clinical Biochemists (AACB) Reference Intervals Harmonisation Project. The working-group was composed of Clinical Biochemists and Endocrinologists with experience in the area; this activity was endorsed by the Royal College of Pathologists of Australasia (RCPA).

Method

A literature search on PubMed was conducted for published reference interval studies for s-PINP and s-CTX. Publications were weighted according to study design, subject numbers, location of study subjects and assay methodology. Consensus reference intervals were calculated for post- and pre-menopausal adult females and adult males subdivided into age groups according to availability of adequate subject numbers. More weighting was given to a single large Australian study.30

Australian Consensus Reference Intervals for s-PINP

Adequate data were available for the Roche (total) s-PINP assay for the following age groups (Table 3).30-38 These serum based reference intervals are also applicable to EDTA or heparin plasma samples.26

Areas of Uncertainty

Further data are awaited for females >70 years and both sexes <25 years age (Table 4).

Notes

S-total PINP may be increased in renal failure; the above reference intervals should be used with caution when eGFR <30 mL/min/1.73m². There appears to be reasonable agreement for s-PINP values reported by intact and total PINP assays in subjects with normal renal function and without metastatic bone disease.28,39

Australian Consensus Reference Intervals for s-CTX

Adequate data were available for the Roche s-CTX assay based on a fasting morning sample for the following age groups (Table 5).30,31,33-35,38,40 These serum based reference intervals are also applicable to EDTA plasma samples.26

Areas of Uncertainty

Further data are awaited for females >70 years and <20 years and males <25 years age (Table 6).

Notes

s-CTX has significant diurnal variation and is lowered by food intake. s-CTX may be increased in renal failure; the above reference intervals should be used with caution when eGFR <30 mL/min/1.73m².

Limitations and Further Directions

More data are needed for intact PINP assays with only two published studies identified.41,42 Data are also awaited for s-CTX values by IDS iSYS with only one published study identified.42 Reference interval data are awaited for other CTX assays. Only two publications have been identified

<table>
<thead>
<tr>
<th>Gender</th>
<th>Age group (years)</th>
<th>Reference intervals (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>20–24</td>
<td>15–115</td>
</tr>
<tr>
<td>Premenopausal females</td>
<td>20–24</td>
<td>15–90</td>
</tr>
<tr>
<td>Menopausal females</td>
<td>&gt;70</td>
<td>15–115</td>
</tr>
</tbody>
</table>

*Reproduced with permission from Vasikaran SD et al. Towards optimising the provision of laboratory services for bone turnover markers. Pathology 2014;46(4):267-73.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Age group (years)</th>
<th>Reference intervals (ng/L)</th>
<th>Caveats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>25–70</td>
<td>100–600</td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>&gt;70</td>
<td>100–750</td>
<td></td>
</tr>
<tr>
<td>Premenopausal females</td>
<td>20–49</td>
<td>150–800</td>
<td>Higher levels may be seen in women &lt;40 years age*</td>
</tr>
<tr>
<td>Menopausal females</td>
<td>50–70</td>
<td>50–800</td>
<td></td>
</tr>
</tbody>
</table>

*The reference intervals for premenopausal females between 30–39 years is 100–700 ng/L and for 20–29 years is 150–800 ng/L

#Reproduced with permission from Vasikaran SD et al. Towards optimising the provision of laboratory services for bone turnover markers. Pathology 2014;46(4):267-73
Table 6. Provisional intervals for s-CTX using limited data.*

<table>
<thead>
<tr>
<th>Gender</th>
<th>Age group (years)</th>
<th>Reference intervals (ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>20–24</td>
<td>400–900</td>
</tr>
<tr>
<td>Menopausal</td>
<td>&gt;70</td>
<td>100–1000</td>
</tr>
<tr>
<td>females</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Reproduced with permission from Vasikaran SD et al. Towards optimising the provision of laboratory services for bone turnover markers. Pathology 2014;46(4):267-73

with reference intervals for total PINP in children and one publication with reference intervals for Roche s-CTX in children.43,44

Conclusion

BTMs are widely used in bone research including therapeutic trials of new medications for osteoporosis and other bone diseases. Whilst their use is well accepted for conditions such as Paget’s disease of bone, and shows promise for malignant bone disease, their utility in the clinical management of the patient with osteoporosis is unclear. Despite this some specialist clinical practices employ BTMs for monitoring treatment. The above consensus reference intervals for adults may help harmonise reporting of s-PINP and s-CTX results by laboratories within Australia using the stated methods.

Competing Interests: None declared (SDV, SAPC, PRE, NJ, MAK, HGS, MJS). GRDJ has previously received travel and research support from Roche Diagnostics Australia, and honoraria from Bio-Rad and Abbott Diagnostics. HAM has received support for travel from Roche Diagnostics Australia, Abbott Diagnostics and Ortho Clinical Diagnostics. GW has received research support from Roche and Ortho Clinical Diagnostics.

References

17. Halleen JM, Karp M, Viloma S, Laaksonen P, Hellman...


44. Bayer M. Reference values of osteocalcin and procollagen type I N-propeptide plasma levels in a healthy Central European population aged 0-18 years. Osteoporos Int 2014;25:729-36.