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Guideline

Title	**Harmonised Australian Reference Intervals for Serum PINP and CTX in Adults**
Document Number:	2014 GL01
Publication date:	2104
Next review date:	2021
Owner:	Australasian Association of Clinical Biochemists Harmonisation Committee

The following publication in Clin Biochem Rev 35 (4) 2014 recommends harmonised reference intervals for bone turnover biomarkers.

Mini-Review

Harmonised Australian Reference Intervals for Serum PINP and CTX in Adults

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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-

Table 1. Nomenclature for BTM. Based on recommendations of the Committee of Scientific Advisors of the International Osteoporosis Foundation.[2]

BTM	Abbreviation	Comment
Bone formation markers		
Osteocalcin	OC	
Undercarboxylated osteocalcin	ucOC	
Total osteocalcin	Total OC	Intact [OC (1–49)] + N-mid fragment [OC (1–43)]
Alkaline phosphatase		
Total alkaline phosphatase	Total ALP	Bone + liver + other sources
Bone alkaline phosphatase	Bone ALP	
Procollagen type I N propeptide	PINP	Also called N-terminal extension peptide of type I collagen
Intact procollagen type I N propeptide	Intact PINP	Refers to the trimer of two pro-α1 chains and one pro-α2 chain
Monomer of procollagen type I N propeptide	mon PINP	Thought to be a single pro-α1 chain
Total procollagen type I N propeptide	Total PINP	Monomer + trimer
Bone resorption markers		
Deoxypyridinoline	DPD	Total or free
N-terminal cross-linking telopeptide of type I collagen	NTX-I	In publications concerning bone, the 'I' can be omitted
C-terminal cross-linking telopeptide of type I collagen	CTX-I	β (beta) isomerised unless otherwise specified In publications concerning bone, the 'I' can be omitted
Tartrate-resistant acid phosphatase	TRACP5b	Bone specific isoform of acid phosphatase secreted by osteoclast

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.[18,19] 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

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

BTM	Analytical and Pre-analytical Details
s-PINP	Specificity: Mostly derived from bone collagen type I
	Assay: May recognise trimer alone (intact PINP) or trimer and monomer (total PINP)
	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
	Automated and manual total and intact immunoassays available
	Sample: serum or lithium heparin/EDTA plasma
s-CTX	The measurand is a well characterised 8-amino acid peptide,
	s-CTX is always isomerised to the β-form of the aspartyl residue
	Specificity: collagen type I, with highest contribution probably from bone
	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)
	Automated and manual immunoassays available
	Sample: plasma or serum (EDTA plasma preferred)

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/1.73m^2. 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 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.
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Table 4. Provisional reference intervals for total s-PINP based on limited data.*

Gender	Age group (years)	Reference intervals (µg/L)
Males	20–24	15–115
Premenopausal females	20–24	15–90
Menopausal females	>70	15–115

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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/1.73m^2.

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/1.73m^2.

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 5. Reference intervals for s-CTX in adult males and females.#

Gender	Age group (years)	Reference intervals (ng/L)	Caveats
Males	25–70	100–600	
Males	>70	100–750	
Premenopausal females	20–49	150–800	Higher levels may be seen in women <40 years age*
Menopausal females	50–70	50–800	

*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

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Table 6. Provisional intervals for s-CTX using limited data.*

Gender	Age group (years)	Reference intervals (ng/L)
Males	20–24	400–900
Menopausal females	>70	100–1000

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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.

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