Bone Markers and Their Use: 2012

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on behalf of
IFCC-IOF Joint Working Group – Standardization of Bone Marker Assays
Bone turnover markers assess the average activity of the bone remodelling cycle.
Bone turnover markers (BTM)

**Bone formation markers** (7 assays available)

Products of active osteoblasts expressed during different phases of osteoblast development or type 1 pro-collagen products

**Bone resorption markers** (8 assays available)

Degradation products of type 1 collagen (i.e., mature collagen) or osteoclast (bone resorbing cell) markers

Levels of BTM in blood/urine reflect bone turnover rate
Bone turnover markers (BTM)
Limiting and potential clinical applications

- Cannot identify if this patient has low bone mass or osteoporosis? Bone densitometry is required.

  But possibly can indicate

- Is this patient at risk of further significant bone loss?
- Is this patient at a higher risk of fracture?
- What is the best treatment for this patient?
- Is this patient responding to treatment
Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: a need for international reference standards

S. Vasikaran · R. Eastell · O. Bruyère · A. J. Foldes · P. Garnero · A. Griesmacher · M. McClung · H. A. Morris · S. Silverman · T. Trenti · D. A. Wahl · C. Cooper · J. A. Kanis · for the IOF-IFCC Bone Marker Standards Working Group

Review of published literature 2000 to 2010 recommending the use of serum CTx and serum PINP be used in all clinical trials
Evidence for the utility of BTMs in fracture risk prediction

• 22 studies examining the relationship between BTMs and subsequent fractures were identified

• 18 studies showed that one or more marker of bone formation or resorption was significantly associated with fracture risk

• Several studies reported that for women with a low BMD, the presence of increased BTMs has an additive effect on fracture risk
Impact of urine-CTx, BMD & prior fracture on 10 year hip fracture probability

Johnell O et al. Osteoporos Int 13: 523-526

An example of the contribution of u-CTX to hip # probability and its independence from BMD based on the EPIDOS data, applied to women from Sweden
Limitations in studies of fracture risk prediction

- 15 different BTMs are available for use
  - up to 10 BTMs have been used in one study
    - Increased possibility of false positive results.
- Heterogeneity in the fracture outcomes at different anatomical sites e.g. spine, hip, non-spine and all fractures
- Multiple statistical approaches have been used with few studies using the most appropriate evaluation; the risk for the individual compared with the risk in the normal population.
Limitations in fracture risk prediction - 2

- Inconsistency in predictive value of specific markers

- The association between bone formation markers and fracture risk was often but not always not significant

- The association of bone resorption markers and fracture risk was somewhat more consistent
The lack of consistency of relationships to fracture risk was also related to the BTM analytic method.

Variable times of specimen collection amongst studies although this factor is critical for some BTMs.
What is required?

• Enlarge the experience of BTMs for fracture risk assessment in population-based studies around the world

• There is no perfect BTM, designate reference bone markers to be incorporated in all future clinical trials generate large data bases suitable for meta-analyses to determine
  • the quantum of their predictive value for different fracture outcomes
  • the dependence of BTMs on the other clinical risk factors used in FRAX
The rationale for use of BTM in monitoring osteoporosis treatment

• Following initiation of treatment the rise in BMD takes 12 to 24 months to be detectable. The reduction in fracture risk occurs much earlier than the BMD changes.

• BTMs often show large and rapid responses to treatments which provides a rationale for their use to monitor treatment in a clinical setting.

• The change in bone turnover has been shown to predict fracture risk independently of and better than change in BMD

• The increase in bone strength following anti-resorptive treatment may be partly explained by a reduction in trabecular perforations (i.e. improved bone microarchitecture) that might be captured by BTMs, but not by BMD
Changes in s-CTX following treatment with oral and iv bisphosphonates

Saag K et al, *Bone* 2007; 40: 1238-43

![Graph showing changes in s-CTX following treatment with oral and iv bisphosphonates.](image)

- Alendronate given orally, weekly
- Zoledronic acid given as a single IV dose
BTM in monitoring osteoporosis treatment - Limitations

• BTMs analysed in only 5 of the many clinical trials of recent years.
• Only a subset of patients had the BTM measured in these trials, so that the number of fractures considered was small
• The BTMs were not always collected in the correct way
  – the s-CTX samples from FIT were mostly non-fasting
  – the u-CTX samples from MORE were on first morning void (not the usual second morning void)
• Statistical pitfalls - changes in BTMs have a skewed distribution and data were not consistently normalised
Criteria for the selection of reference bone turnover marker standards

- Adequately characterised
- Bone specificity
- High performance in fracture risk prediction and in monitoring osteoporosis treatments among women and men
- Widely available on automated platforms and not the monopoly of a single supplier
- Suitable biological and analytical variability, sample handling, stability, ease of analysis
- The ideal medium is blood
Similar conclusions were reached with further analyses of data from 2010 and 2011 with recommendation of serum CTx and serum PINP in future clinical trials.
Aims:

- To standardize or harmonize (as technically feasible or appropriate at this time) clinical assays available for routine and research use for the following two bone turnover markers;
  1) the serum assay for C-telopeptide fragments of collagen type I α1 chains containing the following 8-amino acid peptide epitope Glu-Lys-Ala-His-Asp-β-Gly-Gly-Arg in an isomerised form (also known as serum Crosslaps (βCTx)) and
  2) the serum assay for N-terminal Propeptide of Type I Procollagen (P1NP).
Relationship between serum βCTx levels as assayed by the two major automated instruments –

Data courtesy of UK NEQAS (March 2010-Feb 2011)

Mean bias between methods 0.15μg/L (95%CI 0.07 - 0.23).

\[ y = 0.73x - 0.01 \]

\[ R^2 = 0.96 \]
Schematic presentation of type I collagen showing the amino acid sequence of the human C-terminal telopeptide α1 chain.

N-terminal telopeptide

C-terminal telopeptide

CrossLaps® peptide

β isomerization

Intermolecular Pyridinolone Crosslink


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Specificity of Serum CrossLaps® (β-CTx) Assays

Size of fragments in serum range between 1000 to 10,000 Da with ~50% <3000 Da

The analytes in serum are polypeptides containing this sequence.

The measurand is this specific crosslinked peptide

MATERIAL

CALIBRATION

Value assignment

PROCEDURE

IMPLEMENTATION

uc(y)

A) definition of SI unit by CGPM

B) primary reference measurement procedure

Bipm, nmi, arml

Bipm, nmi

C) primary calibrator

D) secondary reference measurement procedure

Nmi, arml

Nmi, arml, ml

E) secondary calibrator

F) manufacturer’s standing measurement procedure

MI

G) manufacturer’s product calibrator

F) manufacturer’s standing measurement procedure

MI

Routine Serum Specimen

H) end-user’s routine measurement procedure

MI

Patient CTx Result

End-user

End-user

Extensive calibration hierarchy and metrological traceability to SI
Current Practice for $\beta$-CTx Assays

Extensive calibration hierarchy and metrological traceability to SI
Strategy for Standardization of Serum βCTx Assay

A strategy similar to that used for HbA1c assay is likely to be suitable
Traceability by identifying the biologically active peptide – the example of HbA$_{1c}$ (1)

- HbA$_{1c}$ and HbA$_0$ were defined as the globin β-chain amino-terminal hexapeptide with HbA$_{1c}$ containing an extra 1-deoxyfructose at the amino-terminal NH$_2$ group.
- Gravimetry is the primary reference method for the preparation of primary reference materials for each peptide.
- Secondary reference measurement procedures utilise tryptic digestion of haemoglobin and quantification by HPLC/MS and CE/MS.
Specificity of Serum CrossLaps® (β-CTX) Assays

- Cathepsin-K sensitive peptide bond
- Monoclonal Antibody
- C-terminal amino acid
- -crosslinked-
- E-K-A-H-βD-G-G-R-A

Strategy for Standardization of Serum $\beta$CTx Assay

A strategy similar to that used for HbA1c assay is likely to be suitable.

Definition of the analyte—a precise, unambiguous chemical name we will be derived in consultation with the IFCC-IUPAC – Committee on Nomenclature, Properties and Units (C-NPU). Common usage will continue to utilise CrossLaps® and CTx. This similar to the strategy for HbA$_1$c.
A strategy similar to that used for HbA1c assay is likely to be suitable – procedure as defined by ISO15193

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Source sufficient CrossLaps® 8-amino acid peptide of appropriate quality according to ISO15194 suitable for preparation of a primary calibrator utilising gravimetry as the primary reference method
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**Source sufficient CrossLaps of appropriate quality** according to ISO15194 suitable for preparation of a primary calibrator utilising gravimetry as the primary reference method

A higher level analytical procedure utilising, for example LC-MS/MS following appropriate specific peptide digestion, and calibrated with the primary calibrator will be assessed for suitability as a secondary reference method
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The most likely strategy for preparation of secondary calibrators will be to utilise a panel of human serum pools calibrated for CrossLaps® utilising the secondary reference procedure. Samples of these pools can be used to calibrate the IVD manufacturer’s assays.

This strategy will be assessed for standardization and commutability of the manufacturer’s assays using panels of patient sera.
Strategy for Standardization of Serum βCTx Assay

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Alternate Strategy

A) definition of SI unit by CGPM
B) primary reference measurement procedure
C) primary calibrator
D) extensive calibration hierarchy and metrological traceability to SI
E) manufacturer’s standing measurement procedure
F) manufacturer’s product calibrator
G) routine serum specimen
H) end-user’s routine measurement procedure
I) patient CTx result
J) end-user

MATERIAL
CALIBRATION
PROCEDURE
IMPLEMENTATION

Value assignment
A) definition of SI unit by CGPM
B) primary reference measurement procedure
C) primary calibrator

Routine Serum Specimen
Patient CTx Result

End-user

Extensive calibration hierarchy and metrological traceability to SI
Type I Pro-collagen comprising two molecules of A1 and one molecule of A2

Aminoterminal propeptide (PINP) comprises 3 domains:  
NH$_2$-terminal globular domain (most immunogenic domain)  
Helical domain (H) and COOH-terminal domain

The bone remodelling cycle

PINP released by mature osteoblasts
Characterization of PINP antigens in human sera by Gel-exclusion Chromatography and 3 different immunoassays measuring Total PINP and Intact PINP

Relationship between serum $\beta$CTx levels as assayed by the two major automated instruments

Data courtesy of UK NEQAS (March 2010-Feb 2011)

Measuring Intact PINP
Strategy for Standardization of Serum PINP Assay

This likely to be significantly more complex than for \( \beta \text{CTx} \)
Will it be possible to define the measurand for PINP?
If not then the assay may require harmonization rather than standardization
This project will be considered by the WG following achieving significant progress with the \( \beta \text{CTx} \)
Conclusions:

• Current assays for serum βCTx apparently provide disparate results, this issue is under investigation.
• The βCTx measurand has been characterised at the molecular level and therefore the assay is likely to be amenable to a strategy for standardization.
• Current assays for serum PINP provide apparently complementary results despite measuring different forms of PINP ie Intact plus ‘Monomer’ (Total) or Intact. More data are required in this area.
• Standardization of assays for serum PINP is likely to be significantly more complex and possibly harmonization may be need to be considered.