Bone Turnover Markers

Educational Course

IOF International Osteoporosis Foundation

Cooper C
Ebeling PR
Eastell R
Silverman S
Vasikaran S

Supported by an unrestricted educational grant from Roche Diagnostics Ltd
www.iofbonehealth.org
Clinical Needs In Osteoporosis

- Identification of individuals who would best benefit from intervention
- For those on treatment, the optimal manner in which response to treatment should be monitored

What is the role of bone turnover markers?
Bone Remodelling Cycle

Quiescence

Formation Osteoblasts

Microdamage, Other stimulus

Activation

Resorption Osteoclasts
Age Dependency Patterns In Women

- Pubertal Girls
- Premenopausal Women
- Postmenopausal Women

Δ change, % per year

- Formation
- Resorption
- Net balance
# Classification of BTMs

<table>
<thead>
<tr>
<th>Resorption</th>
<th>Formation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DPD</strong>&lt;sup&gt;*&lt;/sup&gt;</td>
<td><strong>OC</strong>&lt;sup&gt;#&lt;/sup&gt;</td>
</tr>
<tr>
<td>Deoxypyridinoline</td>
<td>Osteocalcin</td>
</tr>
<tr>
<td><strong>PYD</strong>&lt;sup&gt;*&lt;/sup&gt;</td>
<td><strong>ALP</strong>&lt;sup&gt;&amp;&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pyridinoline</td>
<td>Alkaline phosphatase (Total)</td>
</tr>
<tr>
<td><strong>NTX</strong>&lt;sup&gt;*&lt;/sup&gt;</td>
<td><strong>BALP</strong>&lt;sup&gt;&amp;&lt;/sup&gt;</td>
</tr>
<tr>
<td>Amino-terminal crosslinking telopeptide of type I collagen</td>
<td>Bone-specific alkaline phosphatase</td>
</tr>
<tr>
<td><strong>CTX</strong>&lt;sup&gt;*&lt;/sup&gt;</td>
<td><strong>P1CP</strong>&lt;sup&gt;#&lt;/sup&gt;</td>
</tr>
<tr>
<td>Carboxy-terminal crosslinking telopeptide of type I collagen</td>
<td>Procollagen type I C propeptide</td>
</tr>
<tr>
<td><strong>TRACP</strong>&lt;sup&gt;&amp;&lt;/sup&gt;</td>
<td><strong>P1NP</strong>&lt;sup&gt;#&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tartrate-resistant acid phosphatase</td>
<td>Procollagen type I N propeptide</td>
</tr>
</tbody>
</table>

*collagen degradation product; # matrix protein; & enzyme
Attractiveness of BTMs

- Samples of blood and urine easily collected
- Relative specificity for bone resorption or bone formation
- Variety of assays available
- Complementary information to BMD
- Changes in bone turnover markers occur earlier than changes in BMD
Limitations of BTMs

- Reflect total skeletal turnover
- Not always specific to bone metabolism
- Substantial biological variability (controllable and uncontrollable)
- Multiple assays for same analyte
- Do not assess osteocyte activity
Uncontrollable Sources of Pre-analytical Variability

- Age
- Menopausal status
- Gender
- Fractures
- Pregnancy and lactation
- Drugs
- Disease
- Bed rest/immobility
- Geography
- Ethnicity
- Oral contraception

Controllable Sources of Pre-analytical Variability

- Circadian
- Fasting status
- Exercise
- Menstrual
- Seasonal
- Diet

Seasonal Variability

Diurnal Variation in Urine Dpd

Time (hours)

Mean /SEM

DPD/Creat

1 7 2 0 2 3 2 5 8 1 1 1 4 1 7
- 2 0
- 1 5
- 1 0
- 5
0
5
1 0
1 5
2 0
2 5

Pre Menopause
Early Menopause
Late Menopause

Diurnal Variation in Serum CTX

Individual profile of 6 healthy male volunteers

BTMs Predict Fracture Independently of BMD

EVIDOS prospective cohort study of 7598 healthy women; age 75+ yrs

- Low Hip BMD: 2.7
- High CTX: 2.2
- Low Hip BMD & High CTX: 4.8

The Additive Effects of BTMs and BMD on Fracture Risk

Case-cohort control study of 151 older men from the Dubbo Study followed prospectively over 6.3 years.

Patients with raised bone resorption markers have an increased risk of future fractures.

This is independent of the current BMD.

Patients with low BMD and high resorption markers have a 4-5-fold higher risk of future fractures.

BTMs Predict Fracture Independently of Prior Fracture

Markers of Bone Formation and Prediction of Fracture

Bone formation markers have yielded contradictory results in the assessment of fracture risk.

No association

Elderly

- Any fracture (bALP, OC)\(^1\)
- Hip fracture (bALP, OC)\(^2\)
- Hip & non-vertebral fracture (OC)\(^3\)

Postmenopausal women

- Any fracture (bALP, OC)\(^4\)

1. Ivaska, JBMR 2010; 2. Garnero, JBMR 1996;

Sornay-Rendu, 2005 JBMR
Limitations

- Altogether 17 different BTMs available (various methods)

- In a given study, there have been up to 10 different BTMs measured. The large number of predictions published raises the possibility of false positive results.

- Heterogeneity in the fracture outcomes reported. Up to four different fracture classifications, such as spine, hip, non-spine and all fractures

- Multiple statistical approaches, e.g.,
  - bone turnover considered as odds ratio per standard deviation increase in BTM;
  - a BTM lying within the top three quartiles (compared to the lowest quartile); or
  - value more than 2 standard deviations above the premenopausal reference interval
Limitations (2)

- For any given analyte, there is some inconsistency in the predictive value of specific markers. For example, s-OC is variously a strong, moderate, borderline or non-significant predictor of fracture risk.

- The association with bone formation markers and fracture risk was usually, though not invariably, not statistically significant.

- The association of bone resorption markers and fracture risk was somewhat more consistent.

- The time of day is critical to the concentration of some BTMs.

- There is a negative correlation between BMD and BTMs, which becomes stronger with advancing age. The prediction of BTMs for fracture was independent of BMD in some studies, but not in all.
Predictive Capacity of BTMs Attenuates with Time

BTMs in Fracture Risk Algorithms

- BTMs are currently not included in the FRAX algorithms because
  1. of the scarcity of population based prospective studies with any single analyte
  2. the applicability of the research database in an international setting is also insecure
     • e.g. > 1/3 studies are from France
     • none from Asia

- Garvan model uses age, BMD, previous fractures and falls
## sCTX and Fracture Prediction in Postmenopausal Women

<table>
<thead>
<tr>
<th>Prospective Studies</th>
<th>Age (years)</th>
<th>Study f-u (years)</th>
<th>Fx type</th>
<th>Marker</th>
<th>Outcome risk (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPIDOS</td>
<td>&gt;74</td>
<td>1.8</td>
<td>hip</td>
<td>u-CTX</td>
<td>OR 2.2 (1.3, 3.6)</td>
</tr>
<tr>
<td></td>
<td>&gt;74</td>
<td>3.3</td>
<td>hip</td>
<td>s-CTX</td>
<td>HR 1.9 (1.0, 3.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>u-CTX</td>
<td>HR 1.7 (1.2, 2.3)</td>
</tr>
<tr>
<td>OFELY</td>
<td>50-89</td>
<td>5.0</td>
<td>all</td>
<td>u-CTX</td>
<td>RR 2.3 (1.3, 4.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>s-CTX</td>
<td>RR 1.9 (1.0, 3.6)</td>
</tr>
<tr>
<td>HOS</td>
<td>43-80</td>
<td>2.7</td>
<td>all</td>
<td>u-CTX</td>
<td>OR 1.5 (1.2, 2.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>OR 1.4 (1.1-1.8)</td>
</tr>
<tr>
<td>Malmö</td>
<td>75</td>
<td>3-6.5</td>
<td>spine</td>
<td>s-CTX</td>
<td>OR 1.9 (1.0, 3.6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>all</td>
<td>S-CTX</td>
<td>OR 1.5 (0.8–3.0)</td>
</tr>
<tr>
<td>MrOs (men)</td>
<td>&gt;65</td>
<td>5</td>
<td>hip</td>
<td>s-CTX</td>
<td>HR 1.2 (0.6-2.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>non-spine</td>
<td>s-CTX</td>
<td>HR 1.3 (0.98-1.7)</td>
</tr>
</tbody>
</table>


**Bold** – adjusted for BMD
sP1NP and Fracture Prediction in Postmenopausal Women

<table>
<thead>
<tr>
<th>Prospective Studies</th>
<th>Age (years)</th>
<th>Study f-u (years)</th>
<th>Fx type</th>
<th>Expression of risk</th>
<th>Relative risk (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OFELY</td>
<td>50-89</td>
<td>5.0</td>
<td>all</td>
<td>Highest vs. lowest quartile</td>
<td>1.3 (0.7, 2.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Concentrations &gt;2SD of prem. women</td>
<td>1.6 (0.8, 3.4)</td>
</tr>
<tr>
<td>DOES</td>
<td>&gt;70</td>
<td>6.3</td>
<td>all</td>
<td>Highest quartile of the distribution compared to lowest</td>
<td>1.4 (0.8-1.6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Univariate analysis, RR for 1SD change (+15 g/L)</td>
<td>1.1 (0.9-1.4)</td>
</tr>
<tr>
<td>MrOS (men)</td>
<td>&gt;65</td>
<td>5</td>
<td>hip non-spine</td>
<td>Highest quartile versus three lower quartiles</td>
<td>2.1 (1.2-3.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.6 (1.2- 2.0)</td>
<td></td>
</tr>
</tbody>
</table>

Relationship Between Bone Turnover and Bone Loss

Typical BTM Changes After Anti-Resorptive vs Anabolic Therapy

Response of BTMs To Anabolic Therapy (hPTH1-34)

Response of BTMs to Strontium Ranelate

2 g/d oral strontium ranelate

Dose Response of Serum CTX With Ibandronate

MOBILE study: 1609 women with postmenopausal osteoporosis

- 2.5 mg daily
- 50/50 mg monthly (single dose, consecutive days)
- 100 mg monthly
- 150 mg monthly

% change s-CTX

Miller et al (2005) JBMR 20:1315-1322
Frequency of Administration of an Anti-Resorptive Therapy

DAILY VS. MONTHLY RISEDRONATE

- 5 mg daily
- 150 mg monthly

Urine NTX/Cr
Serum CTX
Serum BAP

Mean Percent Change in Baseline

Months

Route of Administration Determines BTM Response

Changes in s-CTX following treatment with oral alendronate given weekly and zoledronic acid given as a single annual IV dose.

## Wide Variability of Changes in BTM After Therapy

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Author</th>
<th>Dose</th>
<th>P\textsuperscript{1}NP</th>
<th>OC</th>
<th>BALP</th>
<th>s-CTX</th>
<th>u-NTX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strontium ranelate</td>
<td>Meunier</td>
<td>2 g/d</td>
<td></td>
<td>+8</td>
<td>-12</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bruyère</td>
<td>2 g/d</td>
<td></td>
<td></td>
<td>-11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raloxifene</td>
<td>Naylor</td>
<td>60 mg/d</td>
<td>-34</td>
<td>-21</td>
<td>-21</td>
<td>-25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chesnut</td>
<td>60 mg/d</td>
<td></td>
<td>-20</td>
<td>-28</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Meunier</td>
<td>60 mg/d</td>
<td></td>
<td>-20</td>
<td>-28</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ettinger</td>
<td>60 mg/d</td>
<td></td>
<td>-18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Risedronate</td>
<td>Harris</td>
<td>5 mg/d</td>
<td></td>
<td>-23</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rosen</td>
<td>35 mg/w</td>
<td>-48 *</td>
<td>-28 *</td>
<td>-55 *</td>
<td>-40 *</td>
<td></td>
</tr>
<tr>
<td>Alendronate</td>
<td>Naylor</td>
<td>10 mg/d</td>
<td>-28</td>
<td>-31</td>
<td>-49</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hannon</td>
<td>10 mg/d</td>
<td></td>
<td></td>
<td>-71</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rosen</td>
<td>70 mg/w</td>
<td>-64 *</td>
<td>-41 *</td>
<td>-74 *</td>
<td>-53 *</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Emkey</td>
<td>70 mg/w</td>
<td>-68 *</td>
<td></td>
<td>-81</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Arlot</td>
<td>70 mg/w</td>
<td>-70 *</td>
<td></td>
<td></td>
<td>-70 *</td>
<td></td>
</tr>
<tr>
<td>Zoledronate</td>
<td>Black</td>
<td>5 mg/y i.v.</td>
<td>-59</td>
<td>-30</td>
<td>-58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Denosumab</td>
<td>Cummings</td>
<td>60 mg/6/12 sc</td>
<td>-50</td>
<td></td>
<td>-72</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bone</td>
<td>60 mg/6/12 sc</td>
<td>-60</td>
<td></td>
<td>75</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lewiecki</td>
<td>60 mg/6/12 sc</td>
<td>-60</td>
<td>-60</td>
<td>-70</td>
<td>-60</td>
<td></td>
</tr>
<tr>
<td>Teriparatide</td>
<td>Glover</td>
<td>20 µg/d</td>
<td>+111 *\textsuperscript{a}</td>
<td>+76 *\textsuperscript{a}</td>
<td>+18 *\textsuperscript{a}</td>
<td>+5 *\textsuperscript{a}</td>
<td>+8 *\textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td>Arlot</td>
<td>20 µg/d</td>
<td>+135 *</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} Indicates significant change compared to baseline.
Effects of Discontinuing Alendronate on sCTX and BSAP

FLEX trial: the effects of continuing alendronate (ALN) for 5 years compared with cessation of treatment for the next 5 years

People discontinuing Aln experience a gradual rise in markers over 5 years

Effects of Discontinuing Denosumab on Serum CTX and BSAP

Effect of Denosumab Re-treatment on Serum CTX and BSAP

Percent Change (Median [Q1, Q3])

Serum CTX

- On treatment
- Off treatment

BSAP

Placebo 30 mg Q3M
Baseline sCTX and P1NP Predict Change in BMD with Alendronate or Denosumab Therapy

1 yr treatment with alendronate and denosumab

- Alendronate 70 mg QW
- Denosumab 60 mg Q6M

![Graphs showing percent change from baseline in BMD least squares mean (95% CI) for different baseline levels of sCTX1 and P1NP.](image)

*P ≤ 0.014

Changes in BTMs After Treatment Correlate With Fracture Risk Reduction

--- placebo
__risedronate 5 mg

Percent of Treatment Effect Explained by Bone Turnover Markers

- In the VERT study, the change in u-CTX and u-NTX at 3 to 6 months explained between 54 to 77% of the fracture risk reduction with risedronate, depending on the marker, the method of analysis and the fracture type (Harris et al, 1999, Reginster et al, 2000, Watts et al, 2003)

- In the MORE study, the change in PINP and OC explained 28% and 34%, respectively, of the vertebral fracture risk reduction with raloxifene (Ettinger et al, 1999, Delmas et al, 2002)
BTM vs BMD Monitoring

- The decrease in marker values, particularly the indices of bone resorption occurs within days or weeks of starting treatment with anti-resorptive agents.

- In contrast, the change in BMD occurs over months or years so that BTMs may give earlier information on the response to treatment than BMD.

- Moreover, the decrement in marker values is large in the case of bisphosphonates (e.g. by 50% or more), whereas the increment in BMD is modest (e.g. 5%).
Evidence for the Utility of Bone Turnover Markers In Monitoring Osteoporosis Treatment

- Changes in BTMs with treatment are associated with changes in BMD, both for anti-resorptive therapy and for anabolic therapy.

- However, the changes in BMD with therapy are not closely related to the fracture risk reduction, particularly with anti-resorptive therapy.

- For example, the change in spine BMD over three years explained:
  - only 11% of the reduction in spine fracture risk with alendronate
  - 18% for risedronate and
  - close to zero for raloxifene.
# BMD vs BTMs for Monitoring

<table>
<thead>
<tr>
<th></th>
<th>BMD</th>
<th>BTMs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expected change (&quot;signal&quot;)</td>
<td>~6-8%</td>
<td>~20-300%</td>
</tr>
<tr>
<td>LSC (&quot;noise&quot;)</td>
<td>~3%</td>
<td>~14-133%</td>
</tr>
<tr>
<td>Signal:Noise Ratio</td>
<td>~2</td>
<td>~2 or better</td>
</tr>
<tr>
<td>Time for significant change</td>
<td>1-3 years</td>
<td>~days to 3 months</td>
</tr>
<tr>
<td>Standardised methodology</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Clinical practice guidelines</td>
<td>+++</td>
<td>+</td>
</tr>
</tbody>
</table>

*Adapted from Miller et al. (1999) J Clin Densitom. 2:323-342.*
BTMs Vary With Age In Premenopausal Women

Reference Intervals For sCTX

Adapted from slide provided by Eastell R.
Reference Intervals For sP1NP

Adapted from slide provided by Eastell R
« Drug Holidays »

- Concern: long-term suppression of remodelling could result in fragility
- From clinical studies, some biphosphonates (alendronate, zoledronic acid) have a prolonged effect after stopping and others (risedronate) a more rapid offset.
- Potential biophysical explanation: affinity of alendronate and zoledronic acid to hydroxyapatite is greater than risedronate and ibandronate (Nancollas, Bone, 2006).
- A practical approach:
  - If after 5 years FN T-score <-2.5, then continue therapy
  - If FN T-score is >-2.5, take a drug holiday
### Algorithm For Drug Holidays

**TABLE 5. Suggested duration of bisphosphonate treatment and drug holidays**

<table>
<thead>
<tr>
<th>Patient’s fracture risk</th>
<th>Suggested duration of treatment</th>
<th>Suggested duration of drug holiday$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>Treatment rarely indicated</td>
<td>NA</td>
</tr>
<tr>
<td>Mildly increased</td>
<td>Treat for approximately 5 yr</td>
<td>Stay off bisphosphonate until BMD decreases significantly or fracture occurs</td>
</tr>
<tr>
<td>Moderately increased</td>
<td>Treat for 5–10 yr</td>
<td>Stay off bisphosphonate for 2–3 yr (or less if BMD decreases or fracture occurs)</td>
</tr>
<tr>
<td>High</td>
<td>Treat for 10 yr</td>
<td>Stay off bisphosphonate for 1–2 yr (or less if BMD decreases or fracture occurs); alternate medication (e.g. raloxifene, teriparatide) may be given during the holiday from bisphosphonates</td>
</tr>
</tbody>
</table>

Duration is based largely on personal opinion.

$^2$ Longer holidays might be appropriate for patients treated with bisphosphonates that bind most strongly to bone (i.e. zoledronic acid, alendronate), whereas shorter holidays might be considered for patients treated with compounds that bind less strongly (i.e. risedronate, ibandronate).

---

BTMs Could Identify Secondary Osteoporosis

- No systematic study of this study available
- It is possible that BTMs could be used for this purpose
  - ↑BTMs
    - Osteomalacia
    - Paget’s disease
    - Various endocrine disorders
      - Primary hyperparathyroidism
    - Malignant bone diseases
      - Multiple myeloma
  - ↓BTMs
    - Glucocorticoid-induced osteoporosis
BTMs Could Predict the Response to Therapy

- ↑bone resorption in untreated patient → good response to anti-resorptive therapy
- ↓bone formation → favourable response to anabolic therapy
- Do baseline BTMs predict better the reduction in fracture risk?
  - Variable results in the FIT trial
  - Baseline BTMs predict BMD
BTMs Could Improve Adherence to Treatment

- Studies have examined the effect of monitoring treatment on adherence
- Randomised open study of women treated with bisphosphonates
  - Providing BTM results is not a way to enhance compliance and persistence with alendronate therapy *(Silverman, Osteoporosis International, 2012)*
  - IMPACT study was a controlled trial of risedronate
    - No difference in persistence was seen between the 2 groups at one year
An increase in BTM concentration predicts fracture risk independently of BMD and prior fracture

More data needed before routine clinical use can be recommended
- Which marker?
- What threshold?
- How to combine with other risk assessment approaches e.g., FRAX?

BTMs widely adopted in monitoring treatment. Application limited by:
- Inadequate appreciation of sources of variability
- Limited data on comparison of treatments using the same BTM
- Inadequate quality control.
A need for international reference standards

- Reference standard marker for bone resorption
  - serum CTX
- Reference standard marker for bone formation
  - serum P1NP

“Assays based on these standards should be used consistently in future clinical trials and observational studies in order to generate data which will eventually guide clinicians to the appropriate use of BTMs in routine clinical practice.”
sCTX: Reference Standard for Bone Resorption

- well characterised
- most CTX is derived from osteoclastic bone resorption
- has been evaluated both for fracture prediction and monitoring osteoporosis therapies
- assays widely available
- biological and analytical variabilities have been well documented as are requirements of sample handling and stability
- measurable in serum / plasma
sP1NP: Reference Standard For Bone Formation

- less well characterised than CTX
- most PINP is produced during bone formation; reflects synthesis of bone collagen
- found as trimeric (intact) molecule and as monomer
- has been evaluated both for fracture prediction and monitoring osteoporosis therapies
- assays widely available
- biological and analytical variabilities have been well documented as are requirements of sample handling and stability
- measurable in serum or plasma
THANK YOU