Markers of Bone Resorption

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Biochemical Markers of Bone Turnover

Bone Formation
- Products of active OB:
  - Alkaline phosphatase (TAP, BAP)
  - Osteocalcin (OC)
  - Procollagen type I propeptides (PINP, PICP)

Bone Resorption
- Degradation products of bone collagen:
  - Hydroxyproline (OHP)
  - Pyridinium crosslinks (PYD, DPD)
  - Crosslinked telopeptides of type I collagen (NTX, CTX, ICTP)

- Non-collagenous proteins of bone matrix:
  - Bone sialoprotein
  - Osteopontin
  - Osteocalcin fragments (urine)

- Osteoclast enzymes:
  - Tartrate-resistant acid phosphatase (TRACP 5b)
  - Cathepsin K

Collagen Related Markers of Bone Resorption

<table>
<thead>
<tr>
<th>Marker</th>
<th>Tissue of origin</th>
<th>Analytical specimen</th>
<th>Analytical method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxyproline (Hyp)</td>
<td>Bone, cartilage, skin, soft tissue</td>
<td>Urine</td>
<td>Colorimetry HPLC</td>
</tr>
<tr>
<td>Pyridinoline (PYD)</td>
<td>Bone, cartilage, tendon, blood vessels</td>
<td>Serum</td>
<td>RIA, ELISA</td>
</tr>
<tr>
<td>Deoxypyridinoline (DPD)</td>
<td>Bone, dermis</td>
<td>Urine</td>
<td>HPLC ELISA</td>
</tr>
<tr>
<td>Carboxy-terminal crosslinked telopeptide of type I collagen (ICTP, CTX-MMP)</td>
<td>Bone, skin</td>
<td>Serum</td>
<td>RIA</td>
</tr>
<tr>
<td>Carboxy-terminal crosslinked telopeptide of type I collagen (CTX-I)</td>
<td>All tissues containing type I collagen</td>
<td>Urine (β only)</td>
<td>ELISA RIA</td>
</tr>
<tr>
<td>Amino-terminal crosslinked telopeptide of type I collagen (NTX-I)</td>
<td>All tissues containing type I collagen</td>
<td>Urine</td>
<td>ELISA RIA</td>
</tr>
</tbody>
</table>

Molecular Origin of Markers of Collagen Degradation

Response in Collagen-related Markers in Different Clinical Conditions

- Serum and urine CTX or NTX levels are markedly increased in postmenopausal osteoporosis, and their values decrease rapidly with antiresorptive treatment (in contrast to ICTP)
- Serum ICTP is a sensitive marker in other pathological conditions (metastatic bone disease, myeloma)
- Differences in marker responses may result from differences in the enzymatic pathways leading to the release of CTX/NTX and ICTP from collagen type I.

Caveats in the Value of Bone Markers

- Collagen-related markers are based primarily on type I collagen, which is not bone specific and is widely distributed in several tissues
- Changes in bone markers are not disease specific, but reflect alterations in skeletal metabolism independent of the underlying cause
- Systemic levels of biochemical markers reflect global skeletal turnover, i.e. no distinct information on the remodeling of trabecular and cortical bone
- Some markers are characterized by significant intra-individual variability
Biochemical Markers in the Assessment and Monitoring of Osteoporosis

- Evaluation of bone turnover and bone loss
- Fracture risk assessment
- Short-term evaluation of treatment effect

Changes in Bone Resorption with Menopause

Bone Resorption and Fracture Prediction in Postmenopausal Women

<table>
<thead>
<tr>
<th>Prospective Studies</th>
<th>Age (yrs)</th>
<th>Study follow-up (yrs)</th>
<th>Fx type</th>
<th>Marker</th>
<th>RR (95%CI) for levels &gt;+2SD premp</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPIDOS</td>
<td>&gt;75</td>
<td>1.8</td>
<td>Np</td>
<td>u-CTX/</td>
<td>2.2 (1.3, 3.6)</td>
</tr>
<tr>
<td></td>
<td>&gt;75</td>
<td>3.3</td>
<td>Np</td>
<td>s-CTX</td>
<td>1.9 (1.0, 3.3)</td>
</tr>
<tr>
<td>OFELY</td>
<td>50-89</td>
<td>5.0</td>
<td>all</td>
<td>u-CTX/</td>
<td>2.3 (1.3, 4.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>s-CTX</td>
<td>1.9 (1.0, 3.6)</td>
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<tr>
<td>HOS</td>
<td>43-80</td>
<td>2.7</td>
<td>all</td>
<td>u-CTX</td>
<td>1.5 (1.2, 2.0)</td>
</tr>
<tr>
<td>Rotterdam</td>
<td>&gt;55</td>
<td>4.0</td>
<td>all</td>
<td>u-CTX</td>
<td>1.9 (1.2, 3.8)</td>
</tr>
<tr>
<td>Malmo</td>
<td>75</td>
<td>4.6</td>
<td>al aged 2</td>
<td>TRAP/1a</td>
<td>2.3 (1.3, 4.1)</td>
</tr>
</tbody>
</table>

Changes of Bone Turnover and BMD on Treatment

<table>
<thead>
<tr>
<th>Treatment Duration (Months)</th>
<th>Serum CTX (% of baseline)</th>
<th>BMD Spine (% baseline)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>6</td>
<td>97.5</td>
<td>97.5</td>
</tr>
<tr>
<td>9</td>
<td>95.0</td>
<td>95.0</td>
</tr>
<tr>
<td>12</td>
<td>92.5</td>
<td>92.5</td>
</tr>
<tr>
<td>15</td>
<td>90.0</td>
<td>90.0</td>
</tr>
<tr>
<td>18</td>
<td>87.5</td>
<td>87.5</td>
</tr>
<tr>
<td>21</td>
<td>85.0</td>
<td>85.0</td>
</tr>
<tr>
<td>24</td>
<td>82.5</td>
<td>82.5</td>
</tr>
</tbody>
</table>

Suppression of Bone Markers during Antiresorptive Therapy depends on selected Bone Marker

Biochemical Markers of Bone Turnover

Sources of Preanalytical Variability:

- Controllable factors:
  - Sample storage
  - Diurnal variability
  - Diet
  - Exercise
  - Seasonal rhythms

- Uncontrollable factors:
  - Age
  - Gender
  - Recent fractures
  - Renal function
  - Immobility
  - Non-skeletal diseases
Release of Osteoclast Enzymes and Resorption Markers during Osteoclast Differentiation

- High amounts of tartrate-resistant acid phosphatase are expressed in OC, alveolar macrophages and dendritic cells
- TRACP has two distinct enzymatic activities. It can function as a phosphatase at acid pH, and as a generator of reactive oxygen species at neutral pH
- Two isoforms of type 5 TRACP found in human serum:
  - TRACP 5b: only secreted by osteoclasts (pH 5.8)
  - TRACP 5a: secreted by macrophages, dendritic cells (containing sialic acid residues, pH 5.2)

TRACP and Cathepsin K in Resorbing Osteoclast

- TRACP 5b, cathepsin K
  - proportional to the number of osteoclasts reflecting osteoclast number

Low Diurnal Variability of TRACP 5b

- Low diurnal variability of TRACP 5b
- TRACP 5b and S-βCTX

Clinical Performance of Immunoreactive TRACP 5b

- Analytical Performance
  - Bone marker | Analytical variability (CV, fasting) | Individual variability (CV, fasting) | Bone marker | % Difference (fed-fasting) ± SE
    - TRACP 5b | 3.2 | 6.6 | TRACP 5b | -2.4 ± 0.79
    - S-CTX | 1.1 | 19.1 | S-CTX | -17.8 ± 2.6
    - S-NTX | 0.7 | 12.2 | S-NTX | -8.5 ± 1.7
    - U-CTX | 7.5 | 24.6 | U-CTX | -7.0 ± 2.6
    - U-NTX | 6.9 | 43.7 | U-NTX | -7.9 ± 3.7

- Effect of Feeding
  - % Difference (fed-fasting) ± SE
    - TRACP 5b | -2.4 ± 0.79
    - S-CTX | -17.8 ± 2.6
    - S-NTX | -8.5 ± 1.7
    - U-CTX | -7.0 ± 2.6
    - U-NTX | -7.9 ± 3.7

Stability of Serum TRACP 5b

- Stability of Serum TRACP 5b
  - Incubation time (days) Storage time (months)
  - % TRACP 5b activity
    - At 4°C
    - At 25°C
    - At 20°C
    - At -20°C
TRACP 5b in Metabolic Bone Diseases

Halleen et al, Clin Chem 2001, 47: 597

TRACP 5b and Fracture Prediction

Gerdhem et al, J Bone Miner Res 2004, 19: 386

TRACP 5b for Monitoring ALN Treatment

Nenonen et al, J Bone Miner Res 2005, 20: 1804

TRACP 5b for Monitoring ALN Treatment

Pycnodysostosis (Toulouse-Lautrec Disease)

Hannon et al. Bone 2004
ALN±Ca, n=23, evaluation at 24 wks

Signal-to-noise ratio

Bone marker

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>ALN±Ca (RCT), n=148, evaluation at 12 wks</td>
<td></td>
</tr>
<tr>
<td>S-TRACP 5b</td>
<td>5.3</td>
</tr>
<tr>
<td>S-CTX</td>
<td>3.9</td>
</tr>
<tr>
<td>S-PINP</td>
<td>2.9</td>
</tr>
<tr>
<td>S-BALP</td>
<td>2.8</td>
</tr>
<tr>
<td>S-OC</td>
<td>1.8</td>
</tr>
<tr>
<td>U-CTX</td>
<td>1.9</td>
</tr>
<tr>
<td>U-NTX</td>
<td>1.6</td>
</tr>
<tr>
<td>U-DPD</td>
<td>2.3</td>
</tr>
</tbody>
</table>

S-TRACP 5b (U/L)

S-CTX (μg/L)

Serum TRACP 5b is a reliable osteoclast-specific and sensitive marker of bone resorption

TRACP 5b is proportional to the number of osteoclasts, may be used as a marker of osteoclast number (may be of interest in novel treatments inhibiting bone resorption without affecting OC number, i.e. CIC-7 inhibitors)

Serum TRACP 5b activity has low technical and biological variability, does not accumulate in renal and hepatic failure, but has low storage stability above -70°C

Serum TRACP 5b has a favorable signal-to-noise ratio, hence may be a useful marker in monitoring antiresorptive therapy

Pycnodysostosis, autosomal recessive bone sclerosing disorder, is caused by a deficiency in cathepsin K activity characterised by decreased bone turnover and an accumulation of undigested collagen fibrils in OC (osteopetrosis and short-stature)

Cathepsin K null mouse manifest osteopetrosis, characterized by dysfunctional matrix digestion

Fratzl-Zelman et al, J Clin Endocrinol Metab 2004
Gowen et al, J Bone Miner Res 1999

Gelb et al, Science 1996
Saftig et al, Proc Natl Acad Sci USA 1998
Fridal-Odén et al, J Clin Endocrinol Metab 2004

Pycnodysostosis (Toulouse-Lautrec Disease)

Henri Toulouse-Lautrec (1864-1901)
**Clinical Studies Measuring Serum Cathepsin K**

- Cathepsin K decrease with age in women and men (Kershan-Schindl et al, Experimental Gerontology 2005)
- Cathepsin K correlates with BMD and fracture history (Holzer et al, J Lab Clin Med 2005)
- Cathepsin K also expressed in synovial fibroblasts and macrophages. Serum cathepsin K levels are increased patients with rheumatoid arthritis and correlates with radiological destruction in longstanding disease (Skoumal et al, Arthritis Res Ther 2005; Skoumal et al, Rheumatol Int 2008)

**Cathepsin K: Conclusion**

- Serum concentrations of cathepsin K do not appear to reflect the activity of osteoclasts as compared to biochemical markers of bone resorption.
- Cathepsin K may be a better surrogate for osteoclast number than for osteoclast function.
- The clinical utility of cathepsin K measurement as a marker of bone resorption seems to be limited.