Importance of bone markers in the diagnosis and treatment monitoring of osteoporosis

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Osteoporosis

Systemic and progressive disease of the skeleton, characterised by the decrease of bone mineral content, damaged bone microarchitecture and increased bone fragility
Expected fracture incidence by WHO
(proximal femur)
(thousands)

<table>
<thead>
<tr>
<th>Year</th>
<th>2000</th>
<th>2010</th>
<th>2030</th>
<th>2050</th>
</tr>
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<tbody>
<tr>
<td>EU</td>
<td>414</td>
<td>495</td>
<td>719</td>
<td>972</td>
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<tr>
<td>USA</td>
<td>337</td>
<td>418</td>
<td>560</td>
<td>650</td>
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</table>
schedule of the lecture

Bone remodeling
Bone markers
Reflection of bone remodeling
Why to measure
When to measure
What to measure
Perspectives
schedule of the lecture

Bone remodeling

Bone markers

Reflection of bone remodeling

Why to measure

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What to measure

Perspectives
Bone Remodeling

Continuous Process
Childhood – Modeling
Adulthood – Remodeling
Balance Between Resorption and Formation
Dysbalance – Metabolic Bone Disease
Bone Remodeling Sequence in Healthy Subjects

- Oc Precursor
- Osteoclast
- Mononuclear Cells
- Ob Precursors
- Osteoblast
- Resting Bone Surface
- “Activation”
- Resorption
- Reversal
- Bone Formation
- Mineralization

LC = Lining Cells  CL = Cement Line  OS = Osteoid  BRU = Bone Remodeling Unit

~3 WEEKS  ~3 MONTHS
schedule of the lecture

Bone remodeling

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Bone markers

cellular and extracellular components of the skeleton and skeletal matrix released into the bloodstream (and urine) during the bone formation and/or resorption enzymatic activity associated with the osteoblast or the osteoclast function
Markers of bone formation

alkaline phosphatase (bone isoform)

osteocalcin

type 1 collagen extension propeptides (P1NP, P1CP)
Markers of bone resorption

pyridinium crosslinks
(PYD, DPD)
crosslinking telopeptides of type 1 collagen
(CTx, NTx)
tartarate resistant acid phosphatase
(TRAP)
galactosyl-hydroxylysine, hydroxyproline,
urinary OC-fragments,...
Bone Markers

Most Used
Hy-Pro, DPD, ALP, OC

Actual
CTx, NTx, B-ALP, OC, PINP
Type I collagen molecules within bone matrix

N-Telopeptides

Pyr or D-Pyr

Osteoclastic bone resorption

C-Telopeptides

N-Telopeptides

Serum and urine

C-Telopeptides

Pyr or D-Pyr

CTX

NTX

Cross-linked C-and N-Telopeptides

Free Pyr and D-Pyr
Bone Markers

Most Used
Hy-Pro, DPD, ALP, OC

Actual
CTx, NTx, B-ALP, OC, PINP

Perspectives
RANKL, RANK, OPG, Caspase, Aromatase, Cathepsin ...
schedule of the lecture

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Reflection of Remodeling

Measurement of Bone Mineral Density (BMD)
Reflection of Remodeling

Measurement of Bone Mineral Density (BMD)

Histomorphometry
Cells involved in bone remodeling

- Osteoblasts
- Osteocytes
- Osteoclasts
- Bone matrix
Reflection of Remodeling

Measurement of Bone Mineral Density (BMD)

Histomorphometry

Special Imaging Methods
(biopsy, double-labelling, MR, μCT,...)
Clinical Trial Milestones

hPTH(1-34) Markedly Increases New Bone Formation

Before hPTH(1-34) Treatment  After hPTH(1-34) Treatment

(slides taken from patients enrolled in the study)
Reflection of Remodeling

Measurement of Bone Mineral Density (BMD)

Histomorphometry

Special Imaging Methods
(biopsy, double-labelling, MR, μCT,…)

Bone Markers
schedule of the lecture

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Bone Markers - Advantages

quick response
Influence of calcitonin on bone resorption

Before exposition 60 min after

Bone Markers - Advantages

quick response
easy to get
cheap
broad spectrum
Bone Markers - Disadvantages

Analytical
No Tracebility
No Standardization
High Analytical Variabiliy
Uncertainty of Measured Substance

Biological
High Biological Variability
Non-Specificity
Critical Difference of Consequent Measurements

$$CD\% = 2.77 \times (CV_a^2 + CV_i^2)^{\frac{1}{2}}$$

$CV_a$ - analytical CV
$CV_i$ – intraindividual CV
# Values of CD of repeated measurements

<table>
<thead>
<tr>
<th>Test</th>
<th>Method</th>
<th>CD</th>
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</thead>
<tbody>
<tr>
<td>t-DPD</td>
<td>HPLC</td>
<td>60%</td>
</tr>
<tr>
<td>f-DPD</td>
<td>EIA</td>
<td>38%</td>
</tr>
<tr>
<td>U-NTx/cr</td>
<td></td>
<td>36%</td>
</tr>
<tr>
<td>U-CTx/cr</td>
<td></td>
<td>51%</td>
</tr>
<tr>
<td>S-β-CTx</td>
<td></td>
<td>54%</td>
</tr>
<tr>
<td>B-ALP</td>
<td></td>
<td>29%</td>
</tr>
<tr>
<td>OC</td>
<td></td>
<td>29%</td>
</tr>
<tr>
<td>PICP</td>
<td>RIA</td>
<td>24%</td>
</tr>
<tr>
<td>PINP</td>
<td>RIA</td>
<td>21%</td>
</tr>
</tbody>
</table>
No standardization

DPD

HPLC – total DPD, immunoassays free DPD

CTx

non-enzymatic posttranslational modifications

four different forms

(native linear peptide, isomerized β-form, racemic linear peptide, isomerized and racemic β-form)

attributed to protein aging (!)
no standardization

pilot study

5 „markers“

PTH, CTx-β, P1NP, OC, 25-OH-Vit D

19 osteocenters and laboratories

no true control (independent) material
no standardization

pilot study

PTH

3 different units

8 methods for measurement

results between 45 – 162 pmol/l
no standardization

pilot study

CTx-β

5 different units

3 methods for measurement

results between 1.82 – 3.10 μg/l
no standardization

pilot study

P1NP

1 unit used

2 methods for measurement

results between 322 – 1020 μg/l
no standardization

pilot study

osteocalcin

2 different units

7 methods for measurement

results between 155 – 517 μg/l
no standardization

pilot study

vitamin D (25-OH)

3 different units

4 methods for measurement

results between 116 – 646 nmol/l
schedule of the lecture

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Bone Markers - Utility

Reason for Measurement:
Not for Diagnosis !!
Differential Diagnostics ?
Detection of Fast Losers
Detection of High Turnover
Predictive Value
(course of the disease, success of therapy)
predictive value

... women who have marker values of bone turnover above the premenopausal range (25-40% of postmenopausal women) have been shown in several (but not in all) studies to have approximately a two-fold increased risk of vertebral and non-vertebral fractures, independently of age and of BMD

European guidance ..2008, Osteoporosis Int. 399
Can we judge treatment efficacy before starting it?

FIT Study

6,186 women, 55-80 y, femoral neck BMD T < -1.6
(3,495 women were osteoporotic)

Randomized to Alendronate or Placebo, 3.2 Years

Markers: B-ALP, PINP, sCTx

Osteoporotic women with lowest tertile of PINP
relative risk for non-spine Fx 0.88 (CI 0.65-1.21)

Osteoporotic women with highest tertile of PINP
relative risk for non-spine Fx 0.54 (CI 0.39-0.74)

Baner DC, JBMR 2006, 21:292
Can we judge treatment efficacy before starting it?

OFELY Study

pre-existing fracture
relative risk for next Fx: 2.7

pre-existing fracture and high B-ALP activity:
relative risk for next Fx: 2.7
Monitoring of Therapy

changes in bone markers are valid intermediate endpoints for efficacy of fracture risk reduction and valuable data on therapeutic success, particularly early in treatment, before any change of BMD become apparent
Risk Stratification

10% decrease of resorption markers
6% decrease of fracture risk

10% decrease of formation markers
13% decrease of fracture risk
schedule of the lecture

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## Response to Estrogen Therapy

<table>
<thead>
<tr>
<th>Marker</th>
<th>Responders (%)</th>
</tr>
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<tbody>
<tr>
<td>S- OC, S- PINP</td>
<td>87</td>
</tr>
<tr>
<td>U- NTx/Cr</td>
<td>27</td>
</tr>
<tr>
<td>U- CTx/Cr</td>
<td>18</td>
</tr>
<tr>
<td>BMD- LP</td>
<td>36</td>
</tr>
<tr>
<td>BMD- Femur</td>
<td>0</td>
</tr>
<tr>
<td>BMD- Total</td>
<td>0</td>
</tr>
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</table>
Response to Alendronate Therapy

<table>
<thead>
<tr>
<th>Marker</th>
<th>Responders (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S- BALP</td>
<td>85</td>
</tr>
<tr>
<td>U- NTx/Cr</td>
<td>66</td>
</tr>
<tr>
<td>U- DPD/Cr</td>
<td>23</td>
</tr>
</tbody>
</table>
Effect of Alendronate, HRT and Combination
After 3 Years of Therapy

Randomized, Double-Blind Study
Premarin (and MPG) 0.625 and 2.5
Alendronate
Calcium and Vitamin D
485 women (65-90 years)
BMD, U-NTx, B-ALP

Greenspan, SL, JCEM 90: 2762
Decrease in NTx at 6 months

BMD change % after 3 years

Greenspan, SL, JCEM 90: 2762
Decrease in B-ALP at 6 months

BMD change % after 3 years

Greenspan, SL, JCEM 90: 2762
# Response to BP or HRT

<table>
<thead>
<tr>
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<th>12 months</th>
<th>CD</th>
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<tbody>
<tr>
<td><strong>BP</strong></td>
<td></td>
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</tr>
<tr>
<td>BMD LS</td>
<td>2.55%</td>
<td>47%</td>
</tr>
<tr>
<td>NTx</td>
<td>28%</td>
<td>78%</td>
</tr>
<tr>
<td>DPD</td>
<td>30%</td>
<td>31%</td>
</tr>
<tr>
<td><strong>HRT</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMD LS</td>
<td>2.55%</td>
<td>44%</td>
</tr>
<tr>
<td>NTx</td>
<td>28%</td>
<td>67%</td>
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Worsfold, M, CC, 50: 2263
Stronciumranelate Markers f Bone Remodelation (SOTI Study)

Teriparatide (rPTH) monitoring

Change in B-ALP at 1 month correlates with:
- 2D trabecular wall thickness at 22 months ($r=0.73, p=0.001$)
- Trabecular bone volume ($r=0.58, p<0.05$)

Change in PICP at 1 month correlates with:
- Trabecular wall thickness ($r=0.60, p=0.001$)
- 2D trabecular bone volume ($r=0.51, p<0.05$)

Changes in markers at 6 or 12 months were not associated with changes in structure and/or dynamic parameters.

Dobnik H, JCEM 90:3970, 2005
correlation between P1NP (1 month) and BMD (18 months)
Predictive Role of BM in Men

203 monzygotic twins (men)
35-69 years
5-year prediction of BMD change

PINP, PICP, ICTP, U-NTx

only U-NTx correlated with BMD in femoral neck (P=0.006)
explained 3.8 % of variance

Donescu, OS, Edmonton, Canada, Vienna 2006
target of therapy

antiresorptive therapy
decrease of bone resorption markers to the premenopausal levels within 3-6 months
decrease of bone formation markers to the premenopausal levels within 6-9 months
target of therapy

osteocanabolic therapy

increase of bone formation marker (P1NP)

2- to 3-fold

within 1-3 months

increase bone formation markers to the 2- to 3-fold

within 3-6 months
schedule of the lecture

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under the BP treatment changes in OPG levels are positively correlated to changes in BMD response. BP therapy modulates serum RANKL concentration.
Aromatase Activity in Men

Important role of circulating estrogens
Threshold level of bioavailable estrogen
15% from testes, 85% peripheral
aromatisation of androgen precursors in tissues
polymorphism of aromatase (CYP19) gene
Cathepsin K

- Expressed in osteoclasts
- Localized in ruffled border of active OC
- Expression is enhanced by RANKL

<table>
<thead>
<tr>
<th></th>
<th>Healthy</th>
<th>Osteopenic</th>
<th>Osteoporotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cathepsin K</td>
<td>$5.4 \pm 2.1$</td>
<td>$6.94 \pm 5.4$</td>
<td>$8.82 \pm 7.1$</td>
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</table>

Correlation between Cathepsin K and Fractures: $p = 0.036$

Genetics

disease susceptibility

pharmacogenetics
## Genetic influences

**Response to therapy – influence of VDR genotypes**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>BB</th>
<th>Bb</th>
<th>bb</th>
<th>P</th>
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<tbody>
<tr>
<td>Alendronate</td>
<td>3.4 ± 1.8</td>
<td>6.1 ± 3.9</td>
<td>7.4 ± 4.3</td>
<td>&lt;.001</td>
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<tr>
<td>Raloxifene</td>
<td>4.3 ± 2.6</td>
<td>2.7 ± 1.5</td>
<td>2.1 ± 0.9</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>HRT</td>
<td>2.4 ± 1.9</td>
<td>4.1 ± 2.7</td>
<td>5.1 ± 2.9</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>ALN + RAL</td>
<td>7.2 ± 2.1</td>
<td>7.2 ± 2.0</td>
<td>7.5 ± 2.1</td>
<td>n.s.</td>
</tr>
<tr>
<td>ALN + HRT</td>
<td>4.5 ± 2.8</td>
<td>6.3 ± 3.3</td>
<td>8.1 ± 3.0</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

*a priori* vs *a posteriori* probability

Palombo S, Osteoporosis Int 2005,16
Conclusions

1. It's very difficult to specify the "best" markers of resorption and formation.

2. There are too many influences on results.
3. Metabolism is different between healthy people and osteoporotic patients

4. Biological variability is very high
   (Critical Differences of Measurements)

5. Interpretation of Results Must Be Done by Specialists
6. Bone Markers Can Bring Very Valuable Information

7. Bone Markers Inform About Metabolic Turnover

8. Bone Markers Are Independent Risk Factor

9. Bone Markers Give Us the Best Information About Compliance As Well As About the Effect of Therapy
6. Bone Markers Can Bring Very Valuable Information

7. Bone Markers Inform About Metabolic Turnover

8. Bone Markers Are Independent Risk Factor

9. Bone Markers Give Us the Best Information About Compliance As Well As About the Effect of Therapy – up to now
Home message

Bone markers are (could be) very useful
We don’t have anything better
Take care with the interpretation
Select the proper marker for proper situation
Follow the same lab
Thinking is better that believing
There is no worse loss than a lost time

Michelangelo Buonarroti (1475-1564)