Biochemical markers in the follow-up of the osteoporotic patients

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Summary

Osteoporosis, a disease characterised by low bone mass and micro-architectural deterioration of bone tissue, is viewed as an emerging medical condition. Bone mineral density (BMD) is considered the gold standard of bone status assessment, however it does not offer the timely response desirable for monitoring. Biological markers of bone turnover (BTMs) are claimed to be suitable for that purpose. There is not generalised agreement on which marker could be used in routine. The present paper reviews pros and cons of currently used BTMs and relative analytical methods. Several analytical issues, such as biological variability, molecules stability, lack of reference materials jeopardize the field and, consequently, recommendations are difficult to be drawn. Reference range can’t be used to support clinical judgement and, in this view, Least Significant Change (LSC) is regarded as a way to improve the interpretation of analytical results.

Bone formation markers

Bone Alkaline phosphatase (bALP) is still a marker of interest and its use is widespread in clinical laboratories; Tartrate Resistant Acid Phosphatase band Sb (TRAP Sb) appears to be a promising marker. N-terminal propeptides of type I collagen (s-PINP) and beta-collagen 1 C-terminal cross linked telopeptides (s-CTX), given low biological variability and assay availability for automatised instruments, should be the marker of choice in future clinical trials, to overcome the paucity of uniform data and should be used in clinical routine, to monitor osteoporosis treatment. Finally, the lack of standardisation of currently available diagnostic methods, could be overcome by harmonisation.

KEY WORDS: Bone Turnover Markers; osteoporosis.

Introduction

Osteoporosis is defined as a disease characterised by low bone mass and micro-architectural deterioration of bone tissue, leading to enhanced bone fragility and consequent increase in fracture risk.

(1) Osteoporosis is identified as one of the emerging medical conditions requiring attention during the 21st century, due to its clinical consequences, such as hip fractures and related human and economical costs. Bone mineral density (BMD) is the gold standard of bone status assessment in osteoporosis, leading to internationally applied diagnostic criteria. However, BMD does not offer the timely response desirable for monitoring therapeutic response (2). Biological markers of bone metabolism offer the potential for screening bone turnover conditions, as well as for monitoring early response to therapy, providing a rationale for their use to monitor treatment in a clinical setting (3). The importance of markers in osteoporosis was recently reviewed by Bouxsein and Delmas (4).

An ideal marker must have specific characteristics: biological plausibility (i.e., relation between biomarkers and pathogenetic mechanisms, leading to increased skeletal fragility), association between biomarker and fracture in the target population; consistent change in response to therapy. This latter, possibly, in a predictable and dose-dependent fashion that underlies known mechanisms of action of therapeutic intervention. Eventually changes in biomarkers with treatment must account for a substantial proportion of the antifracture efficacy.

Bone turnover markers (BTMs) are biochemical products, measured usually in blood or urine, that reflect the metabolic activity of bone. It needs to be stressed that markers themselves have no function in controlling skeletal metabolism. They are traditionally categorised as markers of bone formation or bone resorption (3). Resorption markers are either osteoclastic enzymes or products of collagen degradation, released into the circulation. Formation markers are either osteoblastic enzymes or breakdown products of collagen synthesis or matrix proteins.

The present review focuses on the most common bone turnover markers used in clinical practice and, based on the best available evidences, suggests how to use them in the management of osteoporotic patients.

Bone Formation Markers

Bone Alkaline phosphatase

Bone Alkaline phosphatase (bALP), introduced into clinical use in 1929, was the first biochemical marker of bone turnover and is still the one most widely used. Bone alkaline phosphatase has a molecular weight of approximately 140000 Da and is found in the membrane of osteoblasts. It is released into the circulation during bone formation.

This marker is very stable in blood samples and it is not affected by haemolysis. Currently used assays can detect the bone isomert of alkaline phosphatase (5). Since alkaline phosphatase is produced by different cell type, resulting in different carbohydrate content, relatively specific immunoassays for bALP from bone were developed, although cross-reactivity of up to 20% between the bone and liver enzymes (6) is still present in all assays. The long-term intra-individual variability of bone alkaline phosphatase is up to 10%, and such biological variability represents the major component of total variability, since the improvement of analytical methods.
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Osteocalcin
Osteocalcin is a large peptide synthesized by osteoblasts, odonto-
oblasts, and some chondrocytes. It binds to hydroxyapatite and it is
deposited in the bone matrix. As osteocalcin fragments are re-
leased from the bone matrix during resorption, assays for circula-
ting osteocalcin and its fragments reflect both bone formation and
resorption (7). Only a small fraction of osteocalcin is released into
the circulation following a circadian rhythm peaking at 4 a.m. It is
released by the kidney and its levels are affected by renal impair-
ment.
Osteocalcin has a half-life of less than one hour and it is quickly
degraded; the intact molecule and fragments coexist in circula-
tion (8). The presence in variable amount of different fragments
introduces several analytical problems, not to mention the assay’s
lack of standardisation (9). Furthermore, the serum degradation,
even in the absence of haemolysis, causes an important prea-
nalytical problem, making comparison between different assays
even more difficult.
Given all the above mentioned facts (10) osteocalcin cannot be
considered optimal in routine clinical practice.

Procollagen I extension peptides
Type I collagen is synthesized by osteoblasts as the precursor mo-
lecule procollagen, with extension peptides in the carboxy (C) and
amino (N) ends. These extensions are cleaved by proteases du-
ring collagen extra cellular metabolism, producing N-terminal (PINP)
and C-terminal (PICP) propeptides of type I collagen. They are
found in blood as a trimeric form, rapidly converted in a monomeric
form and represent a marker of type I collagen synthesis (11). Dif-
fent assays can measure both monomeric and trimeric forms.
The clearances of the two forms are most probably different, ac-
cording to recent literature. intact PINP is mainly metabolized by
the endothelial cells of the liver whereas clearance of monome-
r PINP depends on kidney function. PINP measurement has the
practical advantage of a low diurnal variability, and its circulating
levels are not significantly influenced by food intake (patient does
not need to be fasting) (12, 13).

Bone Resorption Markers
Hydroxyproline
Hydroxyproline is an amino acid common to and characteristic of
all forms of collagen. Urinary hydroxyproline excretion is the ol-
dest test of bone resorption. However, its lack of specificity is well
recognized: excreted hydroxyproline may originate from skin col-
lagen (which can turn over rapidly in certain disorders), from newly
synthesized collagen not incorporated into tissue, and even from
dietary collagen and gelatin. Hydroxyproline measurement is no
longer recommended (14).

Pyridinium cross-links
Pyridinolines are cross-linking amino acids that strengthen collagen
fibrils in the extracellular matrix. They are found in the main fibril-
forming collagen types I, II, and III of many tissues. Pyridino-
line is the major chemical form, but deoxypyridinoline is also abun-
dant in bone collagen and it is considered to be a relatively se-
lcative bone marker. Assays are currently available for serum and
urine samples. These markers follow a circadian rhythm and are
higher early in the morning and scarcely influenced by diet. To date
immunoassays are widely used as alternative to high performance
liquid chromatography (HPLC) (15, 16).

Telopeptides of type I collagen
These peptides are the non-helical region of type 1 collagen whe-
where the crosslinks attach. The measured molecules are either a tri-
meric carboxy-terminal telopeptide (ICTP), which is measured in se-
rum by radioimmunoassay (10) or the carboxy-terminal region (NTX)
or the carboxy-terminal region (CTX). They are produced by osteo-
clasts during bone resorption. NTX and CTX can be measured in
either serum or urine. The serum levels are influenced by circadian
rhythm and food intake, therefore samples must be collected at a
given time of the day (preferably in the morning) and fasting (17).
The 24-hour urine collection has the advantage of overcoming cir-
cadian changes and is less sensitive to dietary interferences, althou-
gear proper urine collection may be troublesome for the patients.
A further group of collagen decomposition products has gained
attention over the last years: fragments with telopeptides including
specific epitopes, such as: beta-collagen C-terminal cross-linked
telopeptides (beta-CTx) and beta-Crosslaps.
Beta-CTx and beta-Crosslaps assays recognize fragments of col-
lagen 1 that have the beta isomerized 8AA-octapeptide (EKHDG
-beta-GGR) which builds an epitope located on C-terminal telo-
peptides. Often the terms beta-CTx and beta-Crosslaps are used
dsynchronously. However, there is a small, test dependent, diffe-
rence: Crosslaps includes fragments that containing at least one
8AA peptide 6,7; beta-CTx includes fragments containing at leas-
two 8AA-peptides (18).
Assays directed towards the 8AA peptide are known to be more
bone specific; serum assays, in particular, detects two 8AA resulting
more specific compared to urine assay that recognises only one
8AA (19).
Automated CTX serum assay has become increasingly available
and it’s replacing urinary NTX because of its simplicity and ro-
bustness. Reference intervals should be age and sex-specific.

Other markers of bone resorption
Two enzymes found in osteoclasts have received attention as mark-
ers of osteoclast activity.
Osteoclasts produce an acid phosphatase isoenzyme which is not
inhibited by tartrate, called type 5 Tartrate Resistant Acid Phos-
phatase band 5b (TRAP 5b). This enzyme is present in the osteo-
clast’s ruffled border membrane and in the resorptive space. In-
creased TRAP 5b levels have been described in high bone tur-
nover states, like Paget’s disease and bone metastasis. Recen-
tly, due to assay evolution, TRAP 5b is becoming one of the BTM
used for prediction of high bone turnover significantly related to
BMD loss (20). Few studies on type 5b TRAP in osteoporosis af-
ected patients have been reported.
Serum cathepsin K is of interest because it is the primary proteolytic
enzyme used by osteoclasts to degrade bone type I collagen du-
ring resorption. Although several studies suggest that it may be
a valuable marker of bone resorption (21), more trials are requi-
red to evaluate its performance.

Recent advances in research
Due to the paramount interest in this field, researches have been
encouraged to identify new potential BTMs. Among these, the fol-
lowing must be mentioned: receptor activator of nuclear-factor kappa-
B ligand (RANKL) and osteoprotegerin (OPG), two cytokine of
the tumour necrosis factor (TNF) family, are osteoclasts products.
Receptor activator of nuclear-factor kappa-B (RANK) is localized
on the surface of osteoclasts and pre-osteoclasts.
Bone resorption is influenced by osteoclasts through the interaction
between RANK, RANKL and by the OPG that inhibit RANK–RANKL
interaction (22-25). OPG and RANKL play a critical role in the reg-
ulation of bone turnover acting on osteoclast activity. The cir-
culating levels of OPG and RANKL are inversely related to BMD
and contribute to the development of osteoporosis in postmeno-
pausal women (26). They may possibly be used as markers of bone
metabolism, although the broad role of RANK ligand signaling in
the immune system may limit its specificity.

Sources of variability
To avoid being mislead, clinicians who use biochemical markers
of bone turnover should be familiar with factors that influence BTMs and, in turn, assay results (27).

The most important biologic factors are diurnal and day-to-day variability in bone forming and bone-resorbing activities. Levels of bone turnover markers are highest in the early morning and lowest in the afternoon and evening (28). Levels of urinary markers can, accordingly, vary up to 30% during the day. It is worth to mention that the expression as a ratio to creatinine, intended to limit such variability, may introduce, in turn, another bias (10).

Fasting blood samples should be obtained in early morning. An increase in dietary calcium intake can lower the levels of bone resorption markers, particularly in people whose calcium intake was previously low (29). Presumably, this effect is mediated by inhibition of parathyroid hormone secretion. In addition to all the mentioned issues, preanalytical conditions are known to be critical: collection, transport, centrifugation and storage should be performed within 4 hours, in refrigerated conditions for most of them.

Several factors such as age and sex (children and post-menopausal women), but also physical activity, can increase bone turnover (30); reference ranges, adjusted for age and sex, are recommended but unfortunately, this information is not always available (31, 32).

The lack of assay standardisation is still a matter of concern, making difficult the comparison of results obtained by different methods and/or in different laboratories. This is the reason why the Consensus of the Belgian Bone Club suggests that patient’s monitoring should be always done in the same laboratory (10).

The use of BTM has further limitations: long-term corticotherapy, limited mobility, bone metastases, acromegaly and thyrotoxicosis may change bone turnover (33). In particular, the use of corticosteroids exceeding 3 months, inhibit bone formation with a fall in osteocalcin, PINP and ALP and increases bone resorption (34, 35). Time plays an important role, when bone metabolism is concerned: bone formation and resorption markers increase as early as a minimum of 4 months after the fracture, reflecting bone healing (36).

The potential use of BTM as a tool to assess fracture risk and to monitor treatment

Fractures risk prediction and BTMs: the currently available evidences

Several prospective cohort studies showed that markers of bone formation or resorption are significantly associated with fracture risk (37-39). Moreover, when women with a low BMD have increased levels of BTMs, fracture risk is further increased. Although men are less extensively investigated, several studies suggest that BTM plays a role in fracture risk prediction. A key point is the definition of the best biomarkers to be used in clinical practice; in this view, a systematic review of all the available data would be of great help. Unfortunately, given the heterogeneity of published data, due to different population, type and number of markers used for monitoring, length of follow up, choice of treatment, just to mention only few of the methodological issues, such filtered information is not yet available and clinicians need to rely on indications derived from primary studies.

Nevertheless, the following data (3) are of some interest: u-CTX appears to be an independent factor (i.e. not related to BMD value) to define hip fracture risk in women; a decrease in carboxylated s-OC/total s-Oc ratio is associated with increased risk of subsequent fracture, in men; the serum increase of s-ICPT is associated with an increased risk of osteoporotic fractures independent of BMD in the male Australian population.

We can affirm that although BTMs and particularly those of bone resorption, may have some utility in predict fracture outcome, a clear conclusion cannot be drawn yet (3).

Monitoring of osteoporosis treatment and BTMs: the currently available evidences

Clinicians are in great need of a tool to monitor osteoporosis treatment in order to choose the best therapy, the best dose and the optimal dose frequency. As previously mentioned, BTMs have been considered the ideal choice, compared to BMD, given the rapid changes following therapy.

Markers of bone resorption decrease within days or weeks of starting treatment with antiresorptive agents. Although there is a general agreement on the rational for BTM use, is not easy to interpret clinical research aimed to define its routine use. Many variables need to be taken into account: mechanism of action of the drug (antiresorptive versus anabolic), drug doses, route of drug administration, specific response of the single marker. As a general rule, a baseline assessment is required, followed by repeated measurements at some times during treatment.

The extent of the observed change, in turn, is influenced by drug efficacy (level of change) and by imprecision of the measurement and, finally, by intra-individual variability. The concept of Least Significant Change (LSC) was introduced (40) in order to be confident that a consistent change in markers value has occurred. The key point is, however, to observe a change in the primary outcome: the number of fractures. Several trials indicate that the larger the decrease of in BTMs, following anti-resorptive treatment, the larger the reduction in fracture risk (Table 1). The FIT trial (37), the HORiZON (38) trial, the MORE (39) trial report the decrease in BTMs following treatment, expressed as Odds ratio or Hazard ratio. Interestingly, although all of these trials focused on different drugs (alendronate, zoledronic acid, raloxifene respectively) the magnitude of the effect for each marker and for each marker type (resorption or formation) was similar (ranging from -59.2% to -40.8% change in BTM).

Interpretation of BTM

The treatment of osteoporosis induces large and rapid changes in BTMs. Several studies have described a significant relationship between the reduction in BTMs following anti-resorptive therapy and the reduction in vertebral and non-vertebral fracture risk (37-39, 41, 42), supporting the use of BTMs for monitoring osteoporosis treatment (43). A baseline assessment with repeated measurements at defined points during therapy is mandatory. In order to effectively use markers, it is important to appreciate the LSC: only a decrease higher than the LSC can be interpreted as a potential biological effect (44).

Recent guidelines have suggested that a decrease of at least 30% for serum markers and 50%-60% of urinary markers need to be documented in order to suggest that a biological event has occurred. The ability to detect changes between the two values with confidence is also related to the imprecision of the measurement, as well as biological (intra-individual) variability, which may be influenced by factors such as time of day, fasting, compliance to instructions.

Many studies have shown that the intra-individual variability is around 10% for serum markers and 30% for urine markers, and the signal-to-noise ratio is better for serum markers (10), so serum samples are preferred to urine samples when measuring BTMs. This indication, as well as the need to define age and sex related reference ranges, has important consequences for follow up. The LSC for each BTM, considering the within-subject and between-subject variation, need to be accurately defined.

Serum CTX and s-PINP show responsiveness to treatment and low within-subject variability. Thus, their measurement usually enables the identification of the majority of responders to treatment using the LSC approach (45, 46).

Laboratories must guarantee that the analytical variability is well documented and under control to minimize the contribution to marker LSC.
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Table 1 - The performance of BTMs in monitoring treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Trial</th>
<th>Author</th>
<th>Sample size (fractures)</th>
<th>Sample size (months)</th>
<th>BTM Measurement</th>
<th>% change in BTM</th>
<th>Follow-up for fracture (years)</th>
<th>Fracture endpoint</th>
<th>Outcome (95%CI)</th>
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<tr>
<td>Alendronate</td>
<td>FIT 2004 (37)</td>
<td>Bauer 6,087</td>
<td>s-PINP 12</td>
<td>−40.8</td>
<td>Vertebral</td>
<td>OR 0.77</td>
<td>0.66-0.90</td>
<td>Vertebral</td>
<td>0.002 (0.43-0.88)</td>
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<td></td>
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<td>s-CTX 12</td>
<td>−59.9</td>
<td>Mean 3.6</td>
<td>Hip</td>
<td>0.78</td>
<td>0.51-1.19</td>
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<td>Hip</td>
<td>0.80</td>
<td>0.73-0.85</td>
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<td>Hip</td>
<td>0.90</td>
<td>0.80-1.00</td>
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<td>−50.9</td>
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<td>Hip</td>
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<td>0.61-1.21</td>
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<td>−53.3</td>
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<td>Hip</td>
<td>0.77</td>
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<td></td>
<td>s-CTXc 12</td>
<td>−53.3</td>
<td>Mean 3.6</td>
<td>Non-vertebral</td>
<td>0.75</td>
<td>0.71-1.37</td>
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<td>Zoledronic</td>
<td>HORIZON 2009 (38)</td>
<td>Delmas 1,270</td>
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<td>Any clinical</td>
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References

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