Biochemical markers of bone turnover and their utility in osteoporosis

Michael P. Caulfield, PhD, and Richard E. Reitz, MD

Osteoporosis, an age-related, degenerative disease of bone is an asymptomatic disease that is diagnosed after an already unacceptable loss of bone has occurred. Alternatively, a compression fracture may lead to the initial diagnosis. Diagnosis is established by bone mineral density (BMD) at the hip and/or spine. BMD is a static measure of bone composition, reflecting its “history,” and detectable changes take an extended period of time (years). With the increasing number of drugs now available for the treatment of osteoporosis (particularly antiresorptive agents), a more rapid method to assess efficacy of therapy is required (months vs. years for BMD). This is driven by the increasing health costs associated with osteoporosis — greater than US$10 billion annually. In the past 10 to 15 years, several assays, which measure biochemical markers of bone turnover, have been developed. While these markers are of limited use for diagnosis, they offer the advantage of a short time frame (months) for assessment of changes in the rates of bone turnover following therapeutic intervention, changes in bone markers predict improvement in bone density and strength. This article reviews the current biochemical assays available for assessment of bone turnover and their potential clinical use.

The skeleton is composed of two types of bone; cortical and trabecular or cancellous bone. Cortical bone is the dense bone present in the shafts of the long bones and the vertebral end plates. Cortical bone accounts for about 80% of the bone present in the body and gives strength to the skeleton. Trabecular bone accounts for about 20% of the total bone and is the “honeycomb network” of bone present at the ends of the long bones and the core of the vertebrae. The honeycomb nature and connectivity of the trabecular bone allows it to adapt to the stresses that are applied to these regions of bone. Due to its honeycomb nature, however, the surface area of the trabecular bone is roughly equivalent to that of cortical bone and accounts for 50% of the bone being actively turned over at any given time.

Bone turnover involves two distinct phases: bone resorption and bone formation. Bone resorption is initiated by an activation signal, such as parathyroid hormone (Figure 1). Upon activation, mononuclear osteoclast precursor cells fuse to form large multinucleated cells called osteoclasts. Osteoclasts resorb bone by forming a sealed extracellular compartment (lacunae) between the cell and bone into which the osteoclast secretes acid and proteases. The acid and enzymes within the lacunae are responsible for the solubilization of the mineral matrix and degradation of the protein matrix. The total resorption phase proceeds for a period of about 50 days. After resorption is complete, osteoblasts move into the cavity and commence laying down osteoid (protein matrix). The osteoid is composed primarily of type I collagen, but it also contains other noncollagenous proteins, such as osteocalcin. After the protein matrix has been deposited, formation continues by calcification of the osteoid. Formation continues until the resorption cavity has been filled with new bone. The complete turnover cycle takes about 200 days. Bone density increases during the first three decades of life, reaching a peak bone mass in the 30- to 35-year age group. (The higher an individual’s peak bone mass, the slower their approach to osteoporosis; i.e. they have more bone to lose.) Following the attainment of peak bone mass, bone is lost at a slow rate. The rate of bone loss increases in women following the menopause, when bone resorption exceeds formation, resulting in an overall net loss of bone.

With trabecular bone’s increased surface area and increased resorption, it occurs after menopause that relatively more bone is lost from these areas, resulting in weakened trabeculae. In severe osteoporosis, the trabeculae may actually be completely lost. The thinning or loss of trabeculae is the ultimate cause of decreased bone strength (as well as decreased density) and increased risk of spine and hip fracture.

For a bone marker to be useful in assessing the rate of bone turnover and monitoring therapy, the following attributes are required:

- demonstrate a difference in the rate of bone turnover pre- and post-menopause;
- demonstrate minimal analytical variation;
- demonstrate a significant change in response to treatment, and
- measure change in a short time frame (months vs. years).

Biochemical markers of bone turnover are generally divided into two subclasses: bone formation and bone resorption markers. Table 1 outlines the different biochemical markers available for routine testing of bone turnover and indicates how each of the different assays meets the criteria above.

Each of the formation and resorption markers shown in Table 1 is a product of the action of the osteoclast or osteoblast. The possible exceptions to this are osteoprotegerin (OPG) and receptor activator of nuclear factor-κB ligand (RANKL). Both proteins are synthesized by the osteoblast and are involved in the communication between osteoblasts.
and osteoclasts. RANKL is the ligand for the RANK receptor (present on osteoclasts and osteoclast progenitor cells), and is responsible for stimulating resorption through formation and activation of osteoclasts. OPG is a decoy receptor for the RANKL preventing it from binding to its receptor RANK. The net effect of OPG and RANKL interaction is to regulate osteoclast activation and, hence, bone resorption. Their usefulness in the diagnosis, assessment of bone turnover, and monitoring of therapy has not yet been extensively evaluated.

**Bone formation markers**

Bone formation markers are related to:
- deposition of the protein matrix, osteocalcin (OC), and propeptides of type I collagen (PICP and PINP), and
- calcification of the matrix (bone-specific alkaline phosphatase, BAP).

**Osteocalcin (bone Gla protein, OC).** Osteocalcin, synthesized by the osteoblast, is the major noncollagenous protein in the matrix. It contains γ-carboxylated glutamic acid residues that are known to bind calcium and may be involved in the calcification of the matrix (the exact physiological role of OC is unknown). Intact osteocalcin is labile and undergoes proteolytic cleavage in serum that results in N-terminal, mid-molecule, and N-terminal mid-molecule fragments. Serum values of intact OC decrease with repeated freeze/thaw cycles or upon prolonged storage. The large internal fragment generated by the proteolytic cleavage is more stable than intact osteocalcin. The heterogeneity of osteocalcin, the specificity of the antibody, and the standard material used in different assays for OC make it impossible for different assays to be interchanged.

**Procollagen type I N-terminal peptide (PINP).** Type I collagen is the major protein present in the matrix. It is composed of three chains that are aligned during synthesis to produce a linear molecule continuing a long triple helix. During synthesis, the collagen is produced as a precursor molecule with extensions at both ends of the molecule. Prior to deposition in the matrix, the precursor peptides (PINP and procollagen type I C-terminal peptide, PICP) are removed and released into the circulation. The precursor peptides, PINP and PICP, are surrogate markers for measurement of type I collagen synthesis and deposition. PINP circulates as the intact molecule or its fragments.

**Bone-specific alkaline phosphatase (BAP).** BAP is synthesized by the osteoblast and is presumed to be involved in the calcification of the matrix. BAP is one of a number of different isoenzymes of alkaline phosphatase: bone, liver, kidney, intestine, and placenta. The difference in glycosylation of the bone and liver isoenzymes (products of the same gene) has been exploited to generate specific antibodies against BAP. BAP is a more stable analyte than intact osteocalcin.

**Bone-resorption markers**

Bone-resorption markers are all related to osteoclast resorption of the matrix:
- dissolution of the mineralized matrix (tartrate-resistant acid phosphatase)

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**Table 1. Biochemical markers of bone turnover***

<table>
<thead>
<tr>
<th>Type</th>
<th>Method</th>
<th>Sample Type</th>
<th>Differentiate Pre vs Post Menopause</th>
<th>Analytical Variance</th>
<th>Biologic Variance Within Person</th>
<th>Demonstrate Change with Therapy</th>
<th>Detect Change in Short Time</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Formation markers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>BAP</td>
<td>ELISA, manual</td>
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<td>Good</td>
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<tr>
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<td>+/-</td>
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<td>Good</td>
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<td>Yes</td>
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<td>Fair</td>
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<td>Good</td>
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<td>Yes</td>
</tr>
<tr>
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<td>Good</td>
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<td><strong>Alternate markers</strong></td>
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<td>RANKL</td>
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<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Osteoprotegrin</td>
<td>ELISA</td>
<td>Serum</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

*Adapted from reference 1.

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**Figure 1. Schematic representation of the bone turnover cycle.**

Quiescence: Bone at rest, lined with a layer of mononuclear cells. Resorption phase: Following activation by hormones (parathyroid hormone, 1,25 dihydroxy vitamin D) or cytokines osteoclast progenitor cells fuse to form osteoclasts and initiate resorption. Reversal phase: Osteoclastic resorption terminates, osteoblasts enter resorption cavity and commence laying down the osteoid (protein matrix). Formation phase: Newly incorporated osteoid is mineralized until resorption cavity is filled.

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degradation of the protein matrix, specifically type I collagen (hydroxyproline, pyridinium crosslinks, telopeptides).

For the bone-resorption markers, in particular, the timing of sample collection is important as resorption markers have a diurnal rhythm, with the marker level being highest in the morning. Because of this, a morning collection is usually recommended. For monitoring therapy, it is important that samples are collected at the same time of day, and that the same marker(s) is used for subsequent samples to help minimize the diurnal and assay variations.

**Tartrate-resistant acid phosphatase (TRAP).** TRAP is an isoenzyme of acid phosphatase. The other major isoenzyme of acid phosphatase is the prostate form, which is tartrate sensitive. TRAP is synthesized by the osteoclast, released into the resorption lacunae, and presumed to help in the dissolution of the mineral matrix. TRAP activity is labile, and the resorption lacunae, and presumed to help in the dissolution of the mineral matrix. TRAP activity is labile, and the resorption lacunae, and presumed to help in the dissolution of the mineral matrix. TRAP has traditionally been determined enzymatically, although an immunoassay has recently been developed (TRAP-5b).

All the other resorption markers are related to degradation products of the mature, modified type I collagen. These modifications occur during bone formation as type I collagen is deposited within the bone matrix. Crosslinks are formed enzymatically among three specific amino acids (hydroxylysine or lysine residues) present within two adjacent but staggered collagen molecules. One amino acid is within the helical region; the other two are within the telopeptide region of the two collagen molecules. Crosslinks occur at both the amino and carboxyl terminal ends of the nonhelical regions (N- or C-telopeptides, respectively). The pyridinium crosslinks formed (deoxyribonucleotidase or Dpd, and pyridinoline or Pyd) act to strengthen the matrix, and are only found in mature collagen. The relative amount of Dpd to Pyd is increased in bone, compared to crosslinks present in other tissues (aorta or cartilage). Importantly, pyridinium crosslinks are not found in type I collagen present in skin.

**Hydroxyproline.** Hydroxyproline is a modified amino acid, primarily present in collagen molecules, and is less specific than the pyridinium crosslinks because it is present in both new and mature collagen in bone and other tissues, including skin. In addition, collagen in the diet can contribute to the urinary concentrations of hydroxyproline. For accurate determination of urinary concentrations of hydroxyproline, it is necessary to carefully control the patient’s diet to reduce the consumption of collagen-containing material. Because of these limitations, it is not as reliable as the newer, more specific markers.

**Free deoxypyridinoline (Dpd).** Urinary-free Dpd is measured using specific antibodies in an ELISA format. The assay is also available on an automated platform. Free Dpd values are corrected for creatinine concentration.

**N-telopeptide (NTx).** The monoclonal antibody for the NTx recognizes both the Pyd and Dpd form of the crosslink when attached to the N-telopeptides. Urine and serum NTx assays are available in an ELISA format. The urine assay is available on an automated platform. Urine results are corrected for creatinine and expressed as nmol bone collagen equivalents (BCE)/mmol creatinine.

**C-telopeptide (CTx).** Serum and urine CTx are measured in independent ELISA assays using a monoclonal antibody raised against an eight amino-acid peptide (Glu.Lys.Ala.His.Asp.Gly.Gly.Arg) involved in the crosslinking of the C-telopeptide region. Antibody recognition is independent of the crosslink structure. The serum assay is available on an automated platform. Urine CTx values are corrected for creatinine concentration.

**Applications of bone markers**

Osteoporosis is diagnosed using BMD (Table 2). Biochemical markers have not been approved for this application. The markers can be used, however, in conjunction with BMD to help assess the state of bone turnover in an individual. Once a BMD score has been obtained, the level of a turnover marker can help in deciding if an individual is losing bone slowly (low level of marker), ideally toward the middle to low-end of the pre-menopausal reference range. Alternatively, she may have a faster rate of bone turnover with a marker above or in the upper end of the pre-menopausal reference range. Post-menopausal reference ranges for the markers are not generally used in assessing bone turnover, as it is known that the rate of bone turnover and, hence, the level of markers increases after the menopause. Therefore, a result within a post-menopausal reference range might be misleading in helping assess the rate of

### Table 2. Use of bone mineral density for osteoporosis diagnosis

<table>
<thead>
<tr>
<th>BMD Score*</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;2.5†</td>
<td>Severe osteoporosis</td>
</tr>
<tr>
<td>1.0 to 2.5</td>
<td>Osteopenic (low bone mass)</td>
</tr>
<tr>
<td>&gt;1</td>
<td>Normal</td>
</tr>
</tbody>
</table>

*SD below the young adult mean.† With one or more fractures.
turnover. For this reason, the pre-menopausal bone marker ranges are used and ideally should include women over the age of 30 where turnover has reached a steady state.7

Once an individual has been identified as being osteoporotic or having low bone density with rapid loss of bone,8 then therapeutic intervention can be used to help prevent further bone loss and improve BMD. Therapeutic approaches to the treatment of osteoporosis can be classified as either antiresorptive (estrogens, selective estrogen receptor modulators or SERMs, bisphosphonates, calcitonin, and vitamin D) or anabolic — parathyroid hormone. Neither therapeutic approach cures osteoporosis, but both help prevent bone loss (antiresorptive) or stimulate new bone synthesis (anabolic). The scope of this article does not permit a review of the merits of each of these different therapies and their mechanisms of action, nor does it give examples of how individual markers respond to the individual agents. Therefore, we will just consider therapies as a class (antiresorptive or anabolic) and the response of the different markers as a class (formation or resorption).

Figure 2A shows a representative response of formation and resorption markers to an antiresorptive therapy. Antiresorptive agents inhibit the resorption process directly and, therefore, have a rapid effect on the bone resorption marker. Effects of therapy can be detected within one month and reach a new steady state by three months. Formation markers respond more slowly, as these are affected secondarily and have to complete the turnover cycle, replacing the bone already resorbed before initiation of therapy. Formation markers typically reach a new steady state by six months. There is now evidence that this new steady state is maintained for prolonged periods (up to seven years) if therapy is continued.9

Figure 2B shows a representative response of turnover markers with anabolic therapy. As there is no direct intervention in the process of bone turnover with this type of therapy, the detection of an effect takes slightly longer. As the anabolic agent stimulates bone formation, however, these markers increase prior to the concomitant increase in the resorption markers.10

It is important to note that these representative curves are the average response of a population being studied. The success of response to therapy can be assessed by a rapid change (three to six months) in marker level following initiation of treatment (Figure 2). For a successful response to therapy, the decrease (antiresorptive) or increase (anabolic) shown by the marker needs to translate into an increase in bone BMD in one to two years and a reduction in fracture risk. Various studies have been performed to assess bone markers’ ability to indicate the positive effect on BMD. In one study, a successful response to therapy (antiresorptive) was reported as a decrease (baseline vs. six months of therapy) of approximately 15% for serum OC and 30% to 45% for urine CTx and NTx, respectively.11 Other studies evaluating changes in marker levels in response to therapy with concordant increase in BMD have been reported.12-15 An alternative way to assess successful therapeutic intervention is by a reduction in fracture risk. Similar reductions in bone markers, as those mentioned above, were also found to be associated with a decrease in future risk of fracture.7,16-18

In summary, BMD is the recommended method for diagnosis of osteoporosis. Biochemical markers of bone turnover have demonstrated their utility in assessing the rate of bone turnover in individuals with low bone mass, and in helping identify individuals suitable for therapeutic intervention. The most useful attribute of bone markers, however, is after the initiation of therapy where the bone markers respond rapidly to the intervention permitting an early evaluation of the efficacy of therapy and compliance. Studies have shown that an early response to therapy by the bone markers predicts a positive increase in bone mineral density, an increase in bone strength, and a decreased risk of osteoporotic related fractures.16-18

References