Using biochemical markers of bone turnover in clinical practice

ABSTRACT

Biochemical markers of bone turnover provide clinically useful evidence of the normal and pathologic processes that reflect bone cell activity in the skeleton. Understanding the behavior of markers of bone formation and bone resorption should aid in managing patients with a variety of skeletal disorders.

KEY POINTS

Biomarkers of bone formation and resorption reflect the overall osteoblastic and osteoclastic activity in the skeleton and in some situations may serve as surrogates for histologic examination of bone.

Biomarkers of bone turnover can be used to document the effects of therapeutic agents in some patients with osteoporosis and possibly reduce the need for frequent bone density testing.

In cancer patients with bone metastases, biomarkers of bone resorption provide evidence of the efficacy of antiresorptive therapy. The baseline levels also have prognostic value: patients with the highest levels have the worst prognosis.

A variety of biochemical assays that reflect the activity of osteoblasts (the bone-forming cells) and osteoclasts (the bone-resorbing cells) have been developed for clinical use (FIGURE 1). They have helped increase our understanding of the bone remodeling cycle, the pathogenesis of skeletal disorders, and the response of these disorders to therapy.1-4

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Although no guidelines to date recommend their widespread use in clinical practice, we believe they will eventually be accepted. For example, markers of bone resorption are excellent indices of disease activity in patients with osteoporosis due to menopause, immobilization, or autoimmune processes, as well as Paget disease of bone or bone metastases. Normalization of the test results can be used to help establish the efficacy of treatment.

Similarly, markers of bone formation are excellent indices of disease activity in Paget disease, osteomalacia and rickets, osteoblastic bone metastases, and to a lesser extent in renal osteodystrophy. Again, successful treatment is associated with normalization of the tests.

This review summarizes some aspects of bone physiology and the pathogenesis of various metabolic bone disorders as a guide for clinicians considering using biochemical markers of osteoblast and osteoclast activity in patient management.

MARKERS OF BONE FORMATION

Osteoblasts are mononuclear cells that attach to bone surfaces and form new bone, most commonly at sites that recently underwent resorp-
BONE TURNOVER MARKERS

Markers of bone formation are measured in serum; markers of resorption are measured in urine or serum.

Procollagen type I propeptides
Procollagen type I propeptides are cleaved from the ends of the procollagen molecule and can be detected in the circulation. Those from the amino-terminal end are called PINPs; those from the carboxy-terminal end are called PICPs. Although these propeptides are also synthesized in the skin, tendons, ligaments, cornea, blood vessels, fibrocartilage, and many other tissues, their main source is bone. The level of each of the propeptides in blood is thought to reflect the amount of newly synthesized collagen.

MARKERS OF BONE RESORPTION

Osteoclasts are multinucleated cells that resorb bone. They initiate bone remodeling and help shape growing bone and so are more numerous in children. They also liberate skeletal calcium to maintain a normal serum calcium concentration. Postmenopausal women who are estrogen-deficient tend to produce more osteoclasts, which accounts for the bone loss that can occur after menopause.

Markers of bone resorption are measured in serum or urine. The most direct indicators are fragments of bone collagen produced by osteoclast activity.

Hydroxyproline
Hydroxyproline is an amino acid common to and characteristic of all forms of collagen, and urinary hydroxyproline excretion is the oldest test of bone resorption. However, this test lacks specificity for bone resorption because excreted hydroxyproline also comes from other tissues, particularly from skin collagen (which can turn over rapidly in certain disorders), from newly synthesized collagen that is not incorporated into tissue, and from dietary collagen and gelatin. Because it is less specific than newer tests, it is no longer widely used.

Collagen cross-links
Urinary pyridinoline and deoxypyridinoline are more specific markers of bone resorption.

Pyridinolines are cross-linking amino acids that strengthen collagen fibrils in the extracellular matrix. They are found in the main fibril-forming collagens (types I, II, and III) of many tissues. Pyridinoline is the major chemi-
The bone remodeling cycle and markers of bone turnover

The bone remodeling cycle begins with osteoclastic bone resorption, which occurs over about 10 days, followed by osteoblastic bone formation, which evolves over 3 months. The biochemical markers of bone turnover reflect the activity of osteoclasts and osteoblasts.

Markers of bone resorption
- Hydroxyproline
- Collagen cross-links
  - Pyridinolines (pyridinoline, deoxypyridinoline)
  - Cross-linked telopeptides (NTx, CTx)

Markers of bone formation
- Total alkaline phosphatase
- Bone alkaline phosphatase
- Osteocalcin
- Procollagen type I propeptides (PINP, PICP)

Other markers of bone resorption
Two enzymes found in osteoclasts have received attention as markers of osteoclast activity.

Serum tartrate-resistant acid phosphatase (TRAP) 5b has not been studied extensively in patients but appears to correlate with other markers of bone resorption.

Serum cathepsin K is of interest because it is the primary proteolytic enzyme used by osteoclasts to degrade bone type I collagen during resorption. Several studies suggest it may be valuable as a marker of bone resorption, but more studies are required to evaluate its performance relative to established bone resorption markers.

Receptor activator of nuclear factor kappa (RANK), RANK ligand, and its decoy receptor osteoprotegerin are the pivotal regu-
Bone turnover markers

In children, bone turnover can be more than 10 times greater than in adults because of three physiologic processes interacting in the skeleton: bone modeling, remodeling, and growth. Levels of bone formation and resorption markers therefore are much higher in children than in adults. Unfortunately, no studies have compared all the available markers in the same pediatric reference population.

In puberty, bone growth accelerates, with an increase in bone turnover markers that reflects the effect of hormones that induce the growth spurt.

Postmenopausal women who do not use hormone replacement therapy have higher levels of bone resorption and formation markers than premenopausal women. Levels in postmenopausal women on hormone replacement are no different than in premenopausal women. In postmenopausal women not on estrogen, urinary levels of NTx have been reported to discriminate between normal bone mineral density (lowest NTx levels), osteopenia, and osteoporosis (highest levels). Normal levels of NTx are found in a small percentage of women. This may be explained by the variable levels of serum estradiol in postmenopausal women.

Elderly men, in contrast, have variable findings. However, accelerated bone turnover has been noted in men with full-blown hypogonadism caused by androgen suppression therapy.

Clinical applications of bone turnover markers

In postmenopausal osteoporosis

To assess fracture risk. Osteoporosis is diagnosed on the basis of bone mineral density. Although the lower the bone density the greater the risk of fractures, markers of bone resorption can independently predict hip fractures, and a better marker of risk is the combination of low density in the hip and high levels of markers of bone resorption (CTX and deoxypyridinoline).

Markers of bone formation are somewhat less likely to be elevated than markers of bone resorption, and if they are elevated, they decrease...
as expected in response to therapy that inhibits bone resorption, though more gradually and to a lesser extent than the resorption markers.31–35

To monitor bisphosphonate therapy. Antiresorptive drugs such as bisphosphonates reduce the risk of fracture, as they increase bone density and decrease the rate of bone resorption, as shown in many clinical trials.31–35 Because the rate of bone resorption reaches a nadir within 3 to 6 months of starting bisphosphonate therapy and because the increase in bone density after 1 year is quite modest (about 3%–4%), most of the decrease in vertebral fracture incidence, which becomes apparent during the first year of treatment, probably can be attributed more to normalization of bone resorption (and a less perforated structure) than to the increase in bone density.36 This would suggest that it is more appropriate to document that bone resorption has been inhibited than to measure bone density every year when following patients taking antiresorptive agents.

Furthermore, effective antiresorptive therapy reduces the levels of resorption markers by 50% to 70%,32–35 whereas after 1 year bone density has generally not increased more than the error of the bone density measurement. This observation has led to the suggestion that bone density measurements generally should not be done more often than every 2 years when following the effects of antiresorptive therapy. Even with a 20% to 30% day-to-day variation in levels of bone resorption markers, it is easier to document the efficacy of therapy with resorption biomarkers than with bone density.

To document compliance. Another reason to consider measuring a resorption marker (after 3 months of therapy) is to document compliance, a considerable problem in the treatment of an asymptomatic disorder.

To help decide when to restart bisphosphonate therapy. After long-term treatment with a bisphosphonate, the drug may be retained in the skeleton for years. This seems particularly true of alendronate (Fosamax).37 After 5 years of continuous alendronate treatment, bone resorption continues to be suppressed near the maximal level, in some patients for years after they stop taking the drug.38

Once the bone resorption marker begins to approach the pretreatment level, it would signal a possible need to restart the therapy. If a pretreatment level was not measured, an estimate of significant bone resorption would be signaled when the resorption marker is more than 20% above the mean premenopausal level. For urinary NTx this would be more than 42 nmol bone collagen equivalents/mmol creatinine.

![FIGURE 2. The combination of the assessment of bone mineral density (BMD) and the bone resorption rate to predict hip fracture risk in the elderly. Low BMD was defined according to the World Health Organization guidelines, ie, by a value lower than 2.5 standard deviations (SD) below the young adult mean (T score less than −2.5). High bone resorption was defined by carboxy-terminal cross-linking fragments of collagen type I (CTx) or free deoxypyridinoline (D-Pyr) values higher than the upper limit (mean + 2 SD) of the premenopausal range. Women with both low hip BMD and high bone resorption were at a higher risk of hip fracture than women with either low hip BMD or high bone resorption.](image-url)
To monitor teriparatide therapy. It has been reported that the long-term efficacy of the anabolic drug teriparatide (Forteo) is predicted by measuring bone markers 1 to 3 months after the start of therapy. The rise of serum procollagen propeptides (both types) correlated well with an increase in lumbar bone density at 18 months. A rise in serum PICP of at least 46 ng/mL at 1 month and a rise in serum PINP of at least 17.2 ng/mL at 3 months almost always predicted a significant increase in bone mineral density at 18 months.

In glucocorticoid-induced osteoporosis

Glucocorticoid therapy causes bone loss and an increased incidence of fractures when given in high doses or for prolonged periods by the oral, parenteral, or inhaled routes. The pathogenesis of the bone loss has been explored by measurements of bone turnover markers. During glucocorticoid therapy, levels of bone formation markers are generally low and those of bone resorption markers are either normal or low. Presumably, the reduction in bone resorption is not enough to overcome the reduction in bone formation, and bone loss ensues. In children, the effects on bone formation are particularly profound, as linear growth may be retarded.

Giving a bisphosphonate during glucocorticoid therapy is quite effective in increasing bone density and preventing fractures. Patients who receive alendronate have lower levels of bone formation and resorption markers than do untreated subjects. Presumably, bone resorption is inhibited more than bone formation, accounting for the skeletal benefits.

In immobilization-induced osteoporosis

Studies of normal volunteers placed on bed rest indicate that urinary CTx and NTx excretion increase significantly after 24 hours, no match the results of teriparatide therapy in postmenopausal women with osteoporosis treated with teriparatide (Forteo). The relationships between changes in biochemical markers and changes in lumbar spine BMD were evaluated by Spearman rank correlation analysis.

CHEN P, SATTERWHITE JH, LICATA AA, ET AL. EARLY CHANGES IN BIOCHEMICAL MARKERS OF BONE FORMATION PREDICT BMD RESPONSE TO TERIPARATIDE IN POSTMENOPAUSAL WOMEN WITH OSTEOPOROSIS. J BONE MINER RES 2005; 20:962-970. WITH PERMISSION FROM THE AMERICAN SOCIETY FOR BONE AND MINERAL RESEARCH.
doubt reflecting a rapid increase in osteoclast activity.49 In a 16-week study of bed rest in volunteers, markers of bone formation were reduced and markers of bone resorption increased, demonstrating the mechanisms for the profound and rapid loss of bone in immobilized patients.50

In a long-term cross-sectional study of paraplegic men with spinal cord injuries, bone turnover patterns changed over time.51 During the first year after injury, urinary deoxypyridinoline excretion was markedly elevated, whereas blood total alkaline phosphatase and osteocalcin levels were normal to slightly elevated. Over a 30-year period after injury, the bone resorption marker returned to normal levels in most patients and the bone formation markers were normal. Fracture incidence rose but leveled off after 20 years.

Bisphosphonate therapy in spinal cord injury patients reduces urinary NTx and prevents bone loss.52,53 These agents have also proven effective in reversing hypercalcemia in immobilized patients.54

In inflammatory bowel disease

Patients with inflammatory bowel disease, especially Crohn disease, have low bone mass and are at risk of fractures.55 These complications could be due to glucocorticoid therapy, hypogonadism, vitamin D deficiency, weight loss, and high circulating levels of bone-active cytokines released by inflammatory cells residing in the diseased intestine.

Elevated levels of urinary NTx have been reported to predict bone loss in the lumbar spine after 2 years of follow-up (FIGURE 4).56 The cytokines interleukin 1, interleukin 6, and tumor necrosis factor may be responsible for the increased bone resorption.56

Bone formation markers have not been found to be outside the normal range, although both interleukin 1 and tumor necrosis factor alpha are known to inhibit bone formation.

Bisphosphonate treatment produces an increase in bone density concomitant with decreases in markers of bone resorption and formation.57,58 Of considerable interest is the observation that infliximab (anti-tumor necrosis factor alpha; Remicade) generally produces a rise in bone formation markers, with a smaller and inconsistent effect on bone resorption.59,60

FIGURE 4. The relationship between baseline urinary N-telopeptide cross-linked type 1 collagen (NTx) and the annual percent change of spine bone mineral density in (BMD) patients with inflammatory bowel disease over 2 years.


In rheumatoid arthritis

The incidence of osteoporosis and fractures is also increased in patients with rheumatoid arthritis.61 As patients with inflammatory bowel disease, a variety of factors can contribute to bone loss, including glucocorticoid therapy, hypogonadism, vitamin D deficiency, immobility, and elevated levels of bone-active cytokines.

Generally, studies have reported increased bone resorption based on type 1 collagen markers,62,63 whereas patients with osteoarthritis have levels of these bone resorption markers no different from those of control subjects.62 Although serum total TRAP protein is elevated in rheumatoid arthritis patients, this is probably due to the 5a isoform, the origin of which may be macrophages and dendritic cells.64
Urinary CTx and NTx increase after only 24 hours of bed rest

The influence of abnormalities in bone formation on bone loss is less clear. Levels of bone formation markers have been reported to be normal, elevated, or reduced.

Treatment of rheumatoid arthritis with high-dose glucocorticoid pulse therapy is effective in controlling the symptoms and some manifestations of the immune system in patients with the disorder. The latter effect would be expected to have a beneficial effect on bone metabolism. This appears to be the case, as there are only transient decreases in bone formation markers and no significant reduction in bone density. Similarly, there is only a transient decrease in serum osteocalcin after an intra-articular injection of a glucocorticoid, and no effect on urinary pyridinoline.

As would be expected, bisphosphonate therapy prevents bone loss in rheumatoid arthritis patients treated with glucocorticoids. Both oral and intravenous therapy decrease the levels of bone turnover markers. Infliximab therapy was shown to reduce the levels of bone resorption markers but not of PINP (a bone formation marker).

In primary hyperparathyroidism

Hypersecretion of parathyroid hormone increases osteoclastic activity, with a secondary increase in osteoblastic activity. Bone loss may ensue and an increase in fracture incidence may be a consequence, particularly in postmenopausal women, who have the highest incidence of the disorder.

Before screening chemistry panels became widely used during routine medical evaluations, it was not unusual to find elevated serum total alkaline phosphatase levels in patients discovered to have primary hyperparathyroidism. Today, this finding is not so common, as the disorder is diagnosed at a much earlier stage. Nevertheless, more specific and sensitive markers of bone turnover have made it possible to demonstrate the metabolic abnormalities that reflect the skeletal pathology in patients with primary hyperparathyroidism and its response to various therapies.

On average, patients with untreated primary hyperparathyroidism have high levels of markers of bone resorption and formation, except in the mildest cases. Bone turnover returns to normal within 6 months to a year after successful parathyroidectomy. This response correlates with improvement in bone density, primarily in the lumbar spine.

In patients who do not undergo surgery, alternative means of preventing bone loss include estrogen replacement in estrogen-deficient postmenopausal women, bisphosphonates, and cinacalcet (Sensipar). Estrogen, relaxin (Elista), and alendronate all reduce levels of bone resorption and formation markers, and estrogen and alendronate increase bone density. Although cinacalcet usually restores the serum calcium to the normal range and prevents bone loss, it only reduces serum parathyroid hormone levels by about 20%, and both bone resorption and formation markers increase above baseline. This could be related to fluctuations in serum parathyroid hormone that occur during each day of therapy.

In osteomalacia and rickets

Osteomalacia and rickets of any cause are characterized by increased osteoblastic activity. If the underlying cause is vitamin D deficiency, genetic or acquired defects in calcitriol synthesis, or vitamin D resistance, then hyperparathyroidism with increased bone resorption is a secondary feature.

Serum total alkaline phosphatase activity has been a useful marker of disease activity for many years, although the newer markers, except for serum osteocalcin, are potentially more sensitive. The insensitivity of osteocalcin as an index of osteoblastic activity is unexplained but could be related to the state of differentiation of the osteoblasts. Bone resorption markers are elevated in vitamin D deficiency but are not widely used in clinical practice, as serum parathyroid hormone is an excellent indirect means of assessing the presence of increased bone resorption and the response to therapy.

In renal osteodystrophy

Bone disease associated with renal failure is termed renal osteodystrophy and is quite heterogeneous. Microscopic examination of a bone biopsy specimen is still considered the gold standard for diagnosis, and measurement
in serum of intact parathyroid hormone is an important guide to diagnosis and response to therapy.

Nevertheless, recent studies suggest that serum markers of bone formation and resorption may be of additional help in assessing bone turnover. At present it is not certain whether any of the newer markers are superior to serum total alkaline phosphatase activity. Future studies that correlate bone histology with bone turnover markers should clarify the value of the various markers.

In cancer
Bone metastases are a common complication in cancer patients. They are classified as osteolytic, osteoblastic, or mixed on the basis of radiographic features. Biochemical markers of bone turnover have proven useful in assessing the magnitude of the metastases, the response to therapy, and even the prognosis for survival.

Osteolytic metastases, which are common in breast cancer, are associated with increases in bone resorption markers, and after treatment with intravenous bisphosphonates the levels can decrease nearly 70%. Patients with higher levels of urinary NTx had a higher risk of skeletal complications and disease progression than patients with low levels across multiple tumor groups, including multiple myeloma.

In osteoblastic metastases. Prostate cancer patients, who typically have predominantly osteoblastic lesions, have elevations of serum total alkaline phosphatase activity and other markers of bone formation. In addition, they have elevated bone resorption markers. Urinary NTx decreased markedly but serum bone-specific alkaline phosphatase decreased only slightly after treatment with intravenous zoledronic acid (Zometa), whereas androgen ablation therapy has inconsistent effects on bone turnover. High levels of these markers again predict poor prognosis.

In hormone-suppression therapy. Two of the most successful cancer therapies, aromatase inhibitors for breast cancer and androgen ablation for prostate cancer, accelerate bone loss through marked suppression of gonadal steroids. Bone resorption and formation markers increase and bone loss ensues, with resorption exceeding formation. Estrogen suppression appears mainly responsible in both sexes, since raloxifene prevents bone loss in prostate cancer patients.

Bisphosphonates are highly effective in preventing bone loss in either sex. A single infusion of zoledronic acid in androgen-ablated prostate cancer patients can prevent bone loss for at least 1 year.

In Paget disease of bone
Paget disease of bone evolves over many years, from an early osteolytic phase to dominance of secondary osteoblastic activity. In patients with extensive polyostotic disease, bone resorption and formation marker levels may be higher than in almost any other skeletal disorder. An exception is serum osteocalcin, which once again usually does not accurately reflect the rate of bone formation.

Bisphosphonates, given orally or intravenously, produce an early decrease in bone resorption followed by a fall in bone formation. In clinical practice it appears adequate to use the least expensive test, serum total alkaline phosphatase activity, to assess disease activity and the response to therapy.

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