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Bone turnover markers

This article forms part of our 'Tests and results' series for 2013, which aims to provide information about common tests that general practitioners order regularly. It considers areas such as indications, what to tell the patient, what the test can and cannot tell you, and interpretation of the results.

Keywords

bone remodelling; biological markers; osteoporosis

Bones are constantly remodelled to cope with the body's calcium requirements and to repair microscopic damage. The entire skeleton is replaced every 10 years in adults, and around 10% of the skeleton is involved in bone remodelling at any one time.

Bone turnover markers are a non-invasive way of assessing this process. A useful marker for routine use should change in parallel with gold standard measures of bone turnover, such as bone biopsy and calcium balance studies, be readily available in automated form, differentiate between disease states with high rates of turnover and healthy people, and change in response to therapy. These tests are requested increasingly often as part of routine clinical care, especially for osteoporosis. It is important for all clinicians to be able to interpret the results of these tests and understand their role in patient management.

What is the test?

Several tests can act as bone turnover markers. They are classified as markers of bone resorption or bone formation (*Table 1*). Because clinical information for the impact of these markers on hard endpoints, such as fracture, has been limited by variations in markers and conditions between clinical studies, the International Osteoporosis Foundation recently recommended particular reference markers to be reported in all clinical trials. These are C-terminal telopeptide

of type 1 collagen (CTX) for bone resorption, and procollagen type 1 N propeptide (P1NP) for bone formation.¹

How does the test work?

Bone is constantly being remodelled. The process begins when an area of bone is resorbed by osteoclasts, forming a discrete pit. Osteoblasts then deposit organic matrix, or osteoid, into the pit, and the osteoid then calcifies. An area of bone is completely replaced after 3 months. Because bone resorption and bone formation are tightly coupled, a marker from either group usually reflects bone turnover rate.

Bone comprises about two-thirds mineral and one-third osteoid, most of which is type 1 collagen. Pyridinium cross-linking molecules stabilise the collagen triple helix in mature bone. During resorption, fragments of type 1 collagen enter the circulation, and are then cleared in the urine. The collagen derived bone resorption markers in clinical use do not reflect dietary intake and are relatively specific for bone.

The NTX test is based on an antibody against the N-terminal of collagen, including the cross-linking region; CTX is based on an antibody to an octapeptide at the C-terminal end. The urine crosslinks test is most accurately done by high performance liquid chromatography (HPLC) as the automated tests detect only free deoxypyridinoline (DPD) and are less responsive to treatment.

Bone formation markers may be enzymes or other proteins associated with osteoblast function, or may reflect the formation of type 1 collagen. During bone formation, procollagen is cleaved at the N- and C-terminal ends. Thus, P1NP reflects the rate of new bone formation.

Alkaline phosphatase (ALP) was the earliest marker of bone turnover and is still useful in detecting conditions with gross elevations in bone turnover such as Paget disease. If sequential results are followed carefully, total

Table 1. Bone turnover markers commonly available in Australia

Bone resorption	Also known as:	Specimen	Within person variation (CV)
C-terminal telopeptide of type 1 collagen	CTX, CrossLaps	Serum/plasma	10%
N-terminal telopeptide of type 1 collagen	NTX	Urine, serum	25% (urine) 12% (serum)
Pyridinium crosslinks: deoxypyridinoline, pyridinoline	DPD, PYD	Urine	12%
Bone formation			
Alkaline phosphatase (total)	ALP	Serum/plasma	8%
Alkaline phosphatase (bone specific)	B-ALP	Serum/plasma	10%
Procollagen type 1 N propeptide	P1NP	Serum/plasma	8%
Osteocalcin		Serum	8%

ALP reflects treatment changes in individuals. However, the absolute change is smaller than for the other markers, and the wide population range means results may be mistakenly interpreted as normal in many postmenopausal women. Although the bone ALP test is more specific, there is up to 20% cross reactivity with the liver isoform in some disease states.² Osteocalcin is a vitamin-K dependent protein attached to hydroxyapatite in calcified bone, and is therefore a late marker of bone formation. The usefulness of osteocalcin is limited by a lack of standardisation and the need for prompt and special handling of the specimen due to its instability.

When should the test be requested? What do the results mean?

Assessment of fracture risk

Bone turnover markers increase in proportion to fracture risk, independent of bone mineral density (BMD).^{3,4} In general, turnover markers also tend to be higher in patients with low bone density. However, this correlation is not absolute in individuals and this application of the test is most useful in population studies.

Very high marker levels (more than 1.5 times the upper reference limit) are not typical of postmenopausal osteoporosis and should prompt a search for another cause. For example, after a

fracture, markers may remain increased for up to 6 months. Other causes could include high turnover states such as hyperparathyroidism or hyperthyroidism, Paget disease, malignancy including myeloma, or advanced renal failure.

Monitoring the effects of treatment

Bone mineral density is a common surrogate marker of osteoporosis treatment efficacy. However, due to the relatively small effect of treatment relative to the precision of the test, it is not practical to repeat BMD at intervals shorter than 2 years. Also, fracture risk reduction on treatment is far greater than would be predicted by the BMD increase achieved.⁵ Fewer than half of patients prescribed a bisphosphonate are taking the medication after 1 year.⁶ For these reasons, it is helpful to assess the effects of, and compliance with, treatment within a few months. Some studies show improved adherence to treatment when turnover marker results were provided to patients, although this finding is not universal.

Bone resorption markers typically fall by over 40% within 3 months of starting bisphosphonate therapy. This is followed by a reduction in bone formation markers over the next 6–12 months.⁷ Denosumab produces a very rapid fall in resorption markers, which remain suppressed, with a slower fall in formation markers.⁸ A similar

though smaller effect can be seen with oestrogen and other agents acting on the oestrogen receptor.⁷ In addition, the magnitude of the change in markers on antiresorptive therapy is related to the reduction in fracture risk.⁹ If bone turnover markers do not fall on antiresorptive therapy, it could be due to non-compliance with therapy, malabsorption, or poor absorption, for example by eating too soon after taking oral bisphosphonates. Alternatively, there may be an undetected cause of secondary osteoporosis needing investigation.

Teriparatide, in contrast, stimulates new bone formation, and P1NP should double within the first month of treatment and continue to increase over the first 6 months.

There is a minor (18%) fall in P1NP on strontium ranelate.¹⁰ Because this change is small relative to within individual variation, the signal-to-noise ratio is so high that markers are not useful for monitoring individuals on this drug.

Monitoring after cessation of bisphosphonates (drug 'holidays')

There is no consensus on how long bisphosphonate therapy should continue. Several groups have proposed a 'drug holiday' may be appropriate in lower risk patients after 5 years of continuous treatment, based on evidence of continued fracture prevention and reduced risk of rare complications including atypical fracture.¹¹ It is attractive to use markers to guide when to recommence therapy, for example when they increase to the upper half of the premenopausal range, however there is no definite evidence to support this.

Some oral surgeons propose using CTX to assess the risk of osteonecrosis of the jaw (ONJ) after invasive dental procedures in bisphosphonate treated patients. In a small study of 200 extractions, the risk of ONJ was higher if CTX was less than 200 pg/mL.¹² Although this evidence is limited by small numbers, larger studies are in progress.

Precautions

Bone turnover markers vary from day-to-day in individuals (*Table 1*). Although this variability is less with serum markers compared to urine, and is reduced further using fasting early morning

specimens, this variability should be taken into account when assessing serial results. A significant change in results is generally defined as 2.8 times the biological variation. Thus, significant changes in CTX and P1NP are 30% and 21% respectively.

Most bone turnover markers are renally excreted, therefore increase in renal failure. The main exception is ALP.

There are two automated P1NP assays: 'total P1NP' which detects both the trimeric and monomeric forms of the peptide, and 'intact P1NP' which detects only the trimeric form. Because trimeric P1NP is cleared by the liver and monomeric P1NP is cleared by the kidney, total P1NP increases in kidney failure but intact P1NP does not.¹³ It is therefore important to know which assay your laboratory uses.

As for any test where serial results are being used to guide treatment, the patient should be tested at the same laboratory to minimise variation. Not only may the result vary between laboratories, but there is no general agreement on whether age and gender specific reference ranges or target ranges should be provided. At the Institute of Medical and Veterinary Science in Adelaide, we suggest a target for CTX based on BMD data in clinic patients, which plausibly is also the upper reference limit for stable premenopausal women.¹⁴ However, there is no fracture data from any source.

What should I tell my patient?

Bone turnover is highest in the early morning, decreasing significantly by lunchtime. In addition, meals suppress markers, particularly of bone resorption. Blood should be collected fasting in the early morning, and repeat specimens should be collected at the same time of day.

If urine is required, collecting the second urine specimen of the morning, while remaining fasting, reduces variation. Results are expressed as a ratio to creatinine, to correct for urine concentration differences.

What won't the test tell you?

The goal of osteoporosis treatment is to reduce the risk of fracture. Although turnover markers can contribute to the assessment of fracture risk, clinical assessment is vital to assess the risk

of falls, and results that do not fit the clinical picture may be due to secondary causes of bone loss or recent fracture.

Guidelines

The Royal Australian College of General Practitioners guideline for osteoporosis management recognises the response of bone turnover markers to treatment, particularly in the first few months after initiating bisphosphonates or teriparatide, but does not yet recommend their routine use.¹⁵

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