Biochemical Markers of Bone Turnover: Why Theory, Research, and Clinical Practice Are Still in Conflict

In metabolic bone diseases other than osteoporosis (Paget disease, rickets and osteomalacia, primary and secondary hyperparathyroidism), biochemical markers of bone turnover have been important clinical tools in patient management for more than 3 decades. In these diseases, bone metabolism is often so abnormal that even crude, nonspecific markers such as serum alkaline phosphatase and urine hydroxyproline have been sufficiently reliable to allow clinicians to make appropriate management decisions without difficulty or error. When noninvasive measurements of bone mineral density (BMD) became available, the most common metabolic bone disease, osteoporosis, began to receive attention from researchers, pharmaceutical companies, and clinical practitioners. BMD technology from the beginning had very acceptable accuracy and precision errors such that diagnosis and monitoring of disease progression or regression were straightforward (1, 2). However, there was and remains one major drawback to BMD alone in the management of osteoporosis: bone turnover is generally so slow (particularly in relation to the other metabolic bone diseases listed above) that it may take up to 2 years in most circumstances to be certain with 95% confidence that any change in BMD is more than can be accounted for by method imprecision (3–5). When studying large groups of patients in a clinical trial, this is not a practical problem because significant group changes can be detected as early as 6 months after an intervention has started (6–9). This is of limited benefit to individual patient care.

To overcome this, researchers turned to biochemical markers of bone turnover as adjunctive tools in patient management. It became clear very early in this endeavor that total alkaline phosphatase and urine hydroxyproline lacked both the sensitivity and specificity to be of much value. Newer markers had to be developed, which required a more in-depth understanding of basic bone biochemistry and physiology. Over the past 15 years, this challenge was met with rapid development of markers of bone resorption and bone formation that were more specific to bone and bone collagen. We now have a broad array of resorption markers (e.g., pyridinium cross-links of collagen and the amino- and carboxy-terminal telopeptides of these cross-links) and formation markers [e.g., bone-specific alkaline phosphatase (BAP), procollagen extension peptides, osteocalcin]. Much effort has gone into assay development and refinement such that several bone turnover markers are now available on fully automated platforms (10, 11).

Clinical trials quickly confirmed the validity of these assays. Treatment with a drug designed to inhibit bone resorption clearly segregated those on active treatment from those on placebo. This change could be detected as early as a few weeks in tightly controlled small studies (12, 13) and very clearly by 3–6 months in large controlled clinical trials (6–9). Moreover, the change in markers was substantially greater than the change in BMD. However, when clinicians cautiously introduced these markers into practice, the results were disappointing. Further research highlighted the marked diurnal variation in several markers and the very broad biologic variability of most of the markers (14), particularly the urine-based markers of bone resorption (15–17). Further analytic refinements, the development of serum assays for resorption markers, and close attention to preanalytic issues (18) could only partially overcome these obstacles to more routine clinical use. Clinicians continued to complain that they could not reproduce the results reported from carefully conducted research studies. In particular, they remained skeptical that a baseline measurement of bone turnover can provide legitimate information about prospectively measured rates of bone loss as has been demonstrated repeatedly in well conducted studies (19–24). Nor were clinicians convinced that a reduction in a marker of bone resorption in a patient treated with an antiresorptive drug provided any meaningful information about whether the patient was or was not responding appropriately to therapy. They were certainly reluctant to make any changes in therapy solely on the basis of response or lack of response to markers. In great part, this problem stemmed from a limited understanding of the role of markers as adjunctive tools in osteoporosis.

There was and is continued clamoring for a demonstration that there is a quantitative association between a change in bone turnover markers and a change in BMD (25, 26). Many reports fueled this thinking by pointing out that such an association could not be found. What was not recognized is that turnover markers reflect global skeletal activity, whereas BMD measurements assess only a very small portion of the skeleton. In healthy subjects, total bone mineral content in the lumbar spine is only ~50–100 g and only 25–50 g in the proximal femur. Bone turnover markers assess the activity of the total skeletal bone mineral content of 1500 g.

This conflict between the outstanding research on biochemical markers of clinical turnover and the unacceptable clinical application of these markers is brought into sharp focus in the article by Seibel et al. (27) in this issue of Clinical Chemistry. These investigators distributed pooled serum and urine samples to 79 laboratories in five European countries, selecting only laboratories with good, ongoing experience with bone turnover marker assays. Each laboratory had to routinely perform 20 or more tests per week on 2 or more marker assays. Seventy-three laboratories completed the trial, and the results were surprising: disappointing but very illuminating.

The first problem encountered was the great diversity in available assays for the same analytes. Among the resorption markers, 29 laboratories reported results of simultaneous determination of total urinary pyridinoline and urinary deoxypyridinoline using HPLC methods.
Two laboratories used a commercially available HPLC assay, whereas the remainder used in-house methods. The interlaboratory CV was almost 30% and was not changed when results were normalized for creatinine excretion. This is disappointing, but not overly surprising given that most laboratories used in-house assay methods.

The situation with osteocalcin assays was even worse. Eighteen laboratories used an RIA method from one of three different commercial sources. Among these, 18 laboratories used five different RIAs. Can that alone explain 68% and 71% interlaboratory CV for the low and high serum pools respectively? Probably yes, but that does not negate these unacceptable data.

Far more troubling are the results for serum BAP. Of the 42 laboratories reporting results, 22 used an enzyme immunoassay method from one manufacturer. For the serum pool with a low value for BAP, the interlaboratory CV was a staggering 25% with the IRMA assay and 17% with the enzyme immunoassay. For the high BAP serum pool, the corresponding values were an equally unacceptable 16% and 20%, respectively.

Putting all of this together makes it easier to understand how both researchers and clinicians can be both correct and in genuine conflict. Any investigator paying appropriate attention to detail will insist that all reagents for a single experiment come from the same batch. This should provide as tight an interassay CV as possible, as has routinely been reported from these studies. In retrospect, it is unfortunate that Seibel et al. (27) did not collect batch information as part of their data set. It would be almost inconceivable that the disappointing results for BAP could have been obtained with the same IRMA or enzyme immunoassay batches. Insistence on single-batch reagent sets works in the research laboratory, but this cannot help the clinical chemist offering a service to nonbelieving clinicians. The clinicians have clearly been right all along (just as clearly as I have been wrong all along): what seems to work so well in the research laboratory just does not work in clinical practice.

This situation is of course not unique to biochemical markers of bone turnover. Carefully conducted clinical research has demonstrated the vital importance of glycohemoglobin (HbA1C) determinations in the intermediate and long-term management of patients with diabetes mellitus. Yet considerable debate and controversy still surround the optimal methods and limitations in standardization of HbA1C (28–31). This causes confusion for the clinician who cannot always have the measurement done in the same laboratory for an individual patient, and several assays might be used for different patients of the practice. This does not negate the value of the measurement, but causes the clinician to take extra precaution when interpreting the data in individual patients. Of course, lack of standardization of important analytes can be found in other examples of establishing diagnoses and monitoring disease progression or regression. It is to be somewhat expected that research reports with new analytes precede the quality-control aspects necessary for widespread use.

Is there a solution in sight for the markers of bone turnover? Probably not without much agitation on the part of the investigators who have put so much effort into bone-turnover marker research. The clinical chemist is not going to put the effort needed for standardization unless there is a clamoring from clinicians, and that is not likely to happen. The clinicians will read this important report and simply say, “I told you so!” The manufacturers may be sufficiently embarrassed (and ultimately financially disturbed) by these findings to make the necessary adjustments to both optimize their assay conditions and dramatically reduce batch-to-batch variation and convince the clinical chemist that they have succeeded: a long, expensive road to hoe.

Could this important report by Seibel et al. (27) sound a death knell for biochemical markers of bone turnover? Au contraire! The response should be quite the opposite! The science to date is impeccable. I believe the markers are an essential adjunct to the management of patients with metabolic bone diseases including osteoporosis. For osteoporosis specifically this will be underscored by the recent exciting information about the effectiveness of intermittent recombinant human parathyroid hormone as the first available true bone formation stimulation agent (32). All currently FDA-approved osteoporosis drugs are antiresorptive agents. The clinician will need a set of tools beyond BMD to help decide which class of drug (formation-stimulation, resorption-inhibition) and which combination of drugs from different classes are best suited to the needs of the individual patient. The clinician will need a set of tools to monitor and ensure that the selected drug regimen is optimized for the individual patient. Those tools will be biochemical markers of bone turnover. Clearly the onus is now on the manufacturers of assays to optimize their in-house quality control and demonstrate to the research and, ultimately, the clinical community that they have reached acceptable standards with reagents and reagent sets in which the assay methods have been optimized as already noted. Clinical chemists will have to demonstrate that their application of these methods meets acceptable proficiency standards. While this is going on, the previously skeptical clinician will need to critically review the published research concerning what biochemical markers of bone turnover can and cannot do when measured with proper standardization, accuracy, and precision. We will all need to keep open minds as this quality improvement is progressing and be in a position to put bone turnover markers into practice just as soon as this final crucial piece of the puzzle has been solved to everyone’s satisfaction!

References


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