New Biochemical Marker for Bone Disease: Is It a Breakthrough?

Direct biochemical markers of bone metabolism historically include serum alkaline phosphatase (EC 3.1.3.1) activity to indicate new bone formation as well as urinary hydroxyproline to assess changes associated with increased bone resorption or bone degradation as a consequence of bone diseases such as Paget disease or osteoporosis (1). Unfortunately, however, both of these markers have inadequate sensitivity and specificity for bone disease, so that clinicians have largely ignored their routine use for clinical care. Nonetheless, research has continued in an attempt to identify biochemical markers having good specificity for bone disease and capable of reflecting the accelerated breakdown of bone with disease—as distinct from normal bone turnover, which includes a certain amount of bone remodeling as well as bone breakdown as part of normal bone metabolism throughout life. In the past 5 years, osteocalcin (2) and procollagen peptide (3) have emerged as bloodborne markers for assessing bone metabolism in disease. Although lending improved specificity to the picture of bone turnover, these serum markers do not appear to be the answer to the issue of an analyte that can specifically identify accelerated bone loss in (e.g.) osteoporosis and Paget disease and also signal the presence of bone metastases with malignancy, in which the metastatic tumor causes a rapid loss of bone matrix.

In the past few years, researchers on markers of bone loss have refocused their efforts in the direction of bone collagen as a potential source of biochemical markers released into the circulation when the matrix of bone begins to deteriorate, i.e., when the bone density breakdown exceeds the normal remodeling process and negative bone loss begins. It was recognized over a decade ago (4, 5) that the collagen cross-link pyridinoline (PYD), a 3-hydroxypyridinium compound, was present only in extracellular collagen fibrils; although this product of cross-link maturation was present primarily in cartilage, it was also present in bone. More recently (6), a relatively specific bone collagen cross-link has been identified, deoxypyridinoline (DPD), which shows promise in specifically reflecting bone collagen breakdown; this may thus be the bone-specific marker that has so far eluded bone investigators. The concentration of these cross-links in adult bone type I collagen favors PYD to DPD in a molar ratio of ~3.5 to 1. Neither PYD nor DPD is present in the collagen of skin, the most abundant contributor to collagen-breakdown products found in the circulation; thus, these two compounds provide a clear improvement in specificity over that of hydroxyproline, which is abundant in both cartilage and skin collagen. As to nomenclature, these two cross-links are collectively referred to in the literature as the pyridinium cross-links.

The clinical evaluation of these two cross-links as markers of metabolic bone disease has been championed since the mid-1980s by Simon Robins from Aberdeen, Scotland, a co-author of one of the articles in this issue of Clinical Chemistry (7). Robins’ laboratory was one of the first to develop an HPLC method (8) to measure these cross-links in the urine of both healthy subjects and patients with metabolic bone disease (9), where breakdown of bone is expected to be accelerated. His studies have since catalyzed intense activity in this area, in that several laboratories, including our own, have also established HPLC assays for the two pyridinium cross-links to diagnose abnormal human bone resorption with disease and malignancy as well as to monitor specific treatments of patients with bone disease.

The advantages of the urinary cross-link assays over the measurement of urine hydroxyproline for assessing accelerated loss of bone matrix are significant. Most importantly, because these cross-links are made only in extracellular collagen fibrils, their release comes only from the breakdown of a mature matrix and not from newly formed collagen, as occurs with new bone formation. Second, DPD is found almost exclusively in type I bone collagen, lending markedly improved specificity over that of hydroxyproline, which is released from virtually every source of connective tissue in the body, including skin. Third, the cross-links released from bone matrix are not metabolized further to other products; hence, they should directly reflect the extent of resorbing bone. Finally, unlike hydroxyproline, pyridinium cross-links are not influenced by dietary intake and foods containing collagen. The lack of dietary influence lends further support to the improved specificity seen with the cross-link measurements. These and other technical advantages have propelled the measurement of urinary pyridinium cross-links to the forefront of specific methods for assessing the presence of an accelerated rate of bone breakdown in various forms of bone disease.

As with any new method, however, many technical issues need to be addressed and resolved before this method becomes available for routine use in clinical laboratories. For example, no reference standards are available; every laboratory now performing cross-link measurements is using different standards, none of which has been compared or exchanged among the various research groups. The source of the standards is another problem: standards are being isolated and pre-
pared individually and differently by each laboratory that performs cross-links assays and are being prepared from different sources, including animal (sheep) and human (femoral, cortical) bone. There is no indication whether these different sources or isolation techniques affect the characteristics or stability of the standards for either PYD or DPD. What is clear is that the values being reported by different laboratories differ widely for samples from the "same" type of patient, whether normal or diseased, regardless of whether the results are normalized to urine creatinine excretion or not. There is also no consensus as to whether a spot urine collection is an adequate sample or 24-h urine collection is necessary. One recent report (10) demonstrated a marked diurnal variation in cross-link excretion throughout a 24-h period. This may relate to renal function or, perhaps more likely, to changes in bone turnover that correspond to the subject's activity, e.g., being recumbent or standing. In our own studies, we have observed that cross-link excretions in non-bone-disease situations are higher in hospitalized, recumbent patients than in age-matched, ambulatory, clinic patients (11). It has been recognized for years that prolonged recumbency in bed-ridden, hospitalized patients elicits an increase in alkaline phosphatase activity because of increased bone turnover. Finally, good normative data are lacking for older populations of healthy subjects. Because the majority of patients with bone disease are elderly, it is important to assess age-matched control subjects in any study that demonstrates abnormal changes in urinary cross-link excretion with disease.

Currently, HPLC is the only valid analytical method that can separate PYD from DPD; it is considered the "standard" method for cross-link determinations. However, several laboratories and commercial diagnostic companies are gearing up to develop immunoassays for possible measurement of cross-links in blood as well as urine. The immunoassay development so far has been in the direction of total pyridinoline concentrations because the polyclonal antibodies produced thus far cannot adequately distinguish PYD from DPD. As with the urine cross-link measurements, however, these assays are still in the formative stages of development and require both technical and clinical evaluation before final acceptance as a specific and sensitive measure of bone metabolism is attained. In other words, much work remains to be done in terms of assay standardization, the availability of international reference preparations, and acceptable control material before the application of this technology can settle into clinical laboratory usage. Pharmaceutical houses that have recently developed a new generation of promising bisphosphonate compounds for treating bone diseases are anxiously awaiting the routine availability of methods for cross-link measurements, whether in blood or urine, to monitor the effectiveness of these newer forms of therapy for bone disease. Statistics indicate that bone diseases, especially osteoporosis, are on the rise, given the improved survival of our elderly population with the availability of better health care; this emphasizes the added importance and need for specific biochemical markers to help with both the early diagnosis of bone disease and monitoring its therapy.

From my perspective, a method capable of identifying patients who have cancer and micrometastatic disease would have an additional substantial impact. Detection of these patients before the clinical symptoms of bone metastases develop would cut into the lead time of the disease and allow for more aggressive therapy. In preliminary studies (11) with the urine cross-link assays in cancer patients, we noted several cancer patients who had a negative bone scan result and yet had modest increases in urinary cross-link excretion. If this biochemical method can identify the subset of patients who are in early metastatic disease, this test will be of enormous use in terms of its impact on survival and therapy of cancer patients. As with all new methods, however, we need to move cautiously through the period of euphoria that a new test engenders. Rigorous clinical trials will put into proper perspective the urine cross-link method, which, from the studies published to date, certainly holds considerable promise. Time will tell whether such methods constitute a breakthrough in the use of biochemical markers for bone disease.

References


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2170 CLINICAL CHEMISTRY, Vol. 38, No. 11, 1992