Skeletal Alkaline Phosphatase Activity in Serum

When alkaline phosphatase (ALP) activity was first identified in bone (1), the initial descriptions hypothesized that the enzyme was involved in bone formation, hydrolyzing phosphate-esters and thereby providing supplemental phosphate for deposition in hydroxyapatite. Seven decades later, we are still proposing functions for this enzyme. Despite compelling evidence that skeletal ALP is somehow essential to the bone formation process (2), the precise biochemical function of the enzyme is unknown. We do know, however, that skeletal ALP is an ectoenzyme of osteoblasts (3–6). Anchored to the outer plasma membrane by a glycan linkage to phosphatidyl-inositol, skeletal ALP can be released to circulation by the action of a glycan-inositol phosphate-specific hydrolase and (or) by matrix vesicle formation (3–8).

Previous clinical studies have shown that quantitative measurement of skeletal ALP activity in serum can provide a useful index of the rate of bone formation (9). The application of such assays has been limited, however, because of a long-standing problem—how to distinguish skeletal ALP activity in the circulating mixture of ALP isoenzymes. The report by Gomez et al. in the current issue of this journal (10) directly addresses this problem with the first description of a new immunoassay that allows the measurement of skeletal ALP activity in human serum with a remarkable combination of sensitivity and specificity.

The issue of specificity is central to the measurement of skeletal ALP activity in serum because human serum normally contains ALP isoenzyme activities derived from skeletal, hepatic, intestinal, and (during pregnancy) placental tissues (3–6). Although the placental and intestinal ALP isoenzyme activities can be distinguished with relative ease (2, 4), it is much more difficult to distinguish the skeletal and hepatic ALP isoenzyme activities because these isoenzymes are the products of a single gene and differ only with respect to posttranslational modifications (4–6). A variety of methods have been developed to distinguish skeletal from hepatic ALP activity—methods based on differential sensitivity to inactivation by heat (11), differential binding to wheat germ agglutinin (12), and physical differences in size and charge that allow for partial separation by electrophoresis on polyacrylamide gels (13). Unfortunately, none of these methods affords the combination of sensitivity, specificity, ease of application, and reliability that is required for routine application as a bone formation index. This frustrating situation has been improved by the recent development of an immunoradiometric assay that can measure skeletal ALP immunoreactive protein (but not activity) with acceptable specificity, i.e., with <10–14% cross-reactivity with hepatic ALP (14).

The current paper by Gomez et al. (10) describes an isoenzyme-specific immunocapture assay that does not require the use of radioactivity and allows measurement of skeletal ALP activity. The assay uses an isoenzyme-specific monoclonal antibody to coat the plastic surface of a 96-well microtiter plate. Skeletal ALP is bound to the antibody in a manner that does not interfere with its enzymatic activity and, as the assay is described, skeletal ALP activity is measured colorimetrically during a 5-min incubation at 25 °C. Longer incubation times might allow detection of even lower concentrations of skeletal ALP activity. This assay appears to be unusually specific for skeletal ALP isoenzyme activity, showing only 3–8% cross-reactivity toward hepatic ALP, as estimated in studies with heat-inactivated sera from patients with cirrhosis. The method is also characterized by relatively low intra- and interassay variations (4–6% and 4.5–7%, respectively). If further studies confirm the current report of these characteristics, particularly the isoenzyme specificity, this assay will be applicable for the assessment and management of osteoporosis, as well as other bone diseases.

Although this new immunoassay for skeletal ALP has many positive attributes, we have one concern regarding the reported specificity. It seems clear that the skeletal ALP preparations (i.e., Pagetic sera) that were used to evaluate specificity were authentic. In contrast, the hepatic ALP materials, prepared from cirrhotic sera (which had been heated to inactivate contaminating skeletal ALP), have not been established as a source of specific hepatic ALP. Thus, in future work, it will be necessary to further define the ALP moieties found in cirrhotic sera. In the meantime, it would be of interest to use bile as an alternative source of hepatic ALP (15).

Like most metabolic bone diseases, osteoporosis is a disease of insufficient bone volume. A bone volume deficit develops only when the rate of bone formation is exceeded by the rate of bone resorption, and effective treatment requires that this imbalance be corrected or reversed. The diagnosis of osteoporosis depends on measurements of bone density, but bone density changes too slowly (in most situations, a year or more must elapse before a change can be detected) to afford the clinician with an acceptable way to monitor the early response to treatment. Furthermore, density measurements do not provide a physiological picture of
the skeletal imbalance between the rates of bone formation and resorption. Histomorphometric measurements of bone biopsies can provide a local assessment of the rates of bone formation and resorption (i.e., at the sampling site), but the procedure is limited by its invasiveness and the imprecision of the methodology.

Biochemical assessments of serum and urine markers of the bone formation and resorption processes hold great promise as a means to the required information, but relatively few such assays have been developed thus far (16). Ideally, such assays should be noninvasive, accurate, sensitive, specific, convenient, and inexpensive. Although no such ideal assays exist, several methods are available that can be used to estimate the rates of bone formation and resorption. Bone resorption assays consist of methods that measure byproducts of the bone resorption process, e.g., hydroxyproline (17, 18), galactosyl hydroxylysine (18), pyridinoline or deoxypyridinoline (18, 19), telopeptides of collagen (20), and an enzyme believed to be unique to the osteoclast, an isoenzyme form of tartrate-resistant acid phosphatase activity (21). Bone formation assays include methods to measure byproducts of the bone formation process, e.g., procollagen peptides (22); proteins believed to be unique to the osteoblast, e.g., osteocalcin (17); and, the object of our attention, skeletal ALP (11-14).

Among these various assays of bone formation, skeletal ALP has several advantages. Skeletal ALP activity in serum has a relatively long half-life and is not greatly affected by diurnal variation (2, 23), in contrast to serum osteocalcin, which shows diurnal variation as great as 30% (24). Furthermore, skeletal ALP activity in serum has been validated as a bone formation index (9, 15, 25), except in conditions of calcium deficiency and osteomalacia (26). And, finally, skeletal ALP activity is not particularly sensitive to inactivation by freezing and thawing or long-term frozen storage. The only real impediment to the clinical utility of measuring skeletal ALP activity in serum as an index of the rate of bone formation has been the lack of a method that combines specificity with sensitivity. Electrophoretic methods (13) do not always completely separate skeletal from hepatic ALP activity and are subject to interference from intestinal ALP, although the inclusion of wheat germ agglutinin in the (agarose) electrophoresis procedure may minimize these problems (27, 28). Heat inactivation methods can be designed to maximize specificity (11, 29), but only at the cost of sensitivity. Precipitation with wheat germ agglutinin has been reported to separate skeletal from hepatic ALP activity (12, 29), albeit perhaps not completely (15, 30). It is therefore, not surprising that the recent development of a sensitive radioimmunoassay for skeletal ALP was greeted with such interest, despite a cross-reactivity of 10–14% toward hepatic ALP activity (14, 15, 31–33).

Almost all biochemical assays of bone formation and resorption markers are applicable to studies of group differences, and this includes assays for skeletal ALP. In contrast, bone density assays are applicable not only to groups of patients, but also to individual patients because the within-subject biological variability and the analytical imprecision of the bone density assays are small. Because biochemical assays typically have within-subject variabilities and analytical imprecisions (CVs) of at least 20–30%, they have not yet been successfully applied to individual patients in clinical practice. It will be interesting to learn whether the immunoassay of Gomez et al. (10) exhibits a sufficiently low biological-plus-analytical variation to be applicable to individual patients.

Although the immunoassay of Gomez et al. appears to be more specific than the current immunoradiometric assay (with comparable sensitivity), we should reserve judgment of such relative advantages until the assay is applied and tested in clinical settings. Will the interassay variations be as low in other laboratories? Will the cross-reactivity toward hepatic ALP exceed the reported low values of 3–8% when other preparations of hepatic ALP are tested in place of the cirrhotic sera that were used for this initial study? Will the biological-plus-analytical variation be sufficiently low to make this test applicable to individual patients? The answers to these questions will determine whether the assay reported by Gomez et al. becomes an alternative method or becomes the method of choice for the quantitative measurement of skeletal ALP activity in serum.

References
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John Farley
David J. Baylink

Departments of Medicine and Biochemistry
Loma Linda University, and
J.L. Pettis Memorial Veterans Medical Center
Loma Linda, CA

1 Address for correspondence: 151 Research Service, J.L. Pettis Memorial Veterans Medical Center, 11201 Benton St., Loma Linda, CA 92357. Fax 909-796-1680.