Bone Protein and Peptide Assays in the Diagnosis and Management of Skeletal Disease

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The measurement in blood of bone proteins is an important adjunct to imaging procedures for clinical assessment of the skeleton. The discovery of new bone proteins and their structural characterization has led to immunochemical procedures of improved sensitivity and specificity for their quantification. An appreciation of the advantages and disadvantages of these evolving methodologies will assist in their clinical application.

Although the skeleton is generally regarded as a relatively inert organ system (1), it is, in fact, actively metabolizing, albeit at a much slower rate than other organs. Quantitatively, it has been estimated that 10% of the skeleton turns over annually, a proportion that some organ systems turn over each day. Nevertheless, like other organs, the metabolism of the skeleton is signaled by the products of its cellular components. Thus, just as the metabolic activity of the cells of the liver is signaled by the serum concentration of aspartate aminotransferase, so is the metabolic activity of the skeleton signaled by the serum concentration of the products of its metabolically active cells, the osteoblast and the osteoclast. Consequently, the products of bone cells can be measured in serum and used in the clinical assessment of the skeleton (1, 2).

In this paper I review and update the application of serum measurements of bone-specific proteins to clinical assessment of skeletal status in patients with metabolic bone diseases. I will focus on those bone proteins (and peptides) that are produced exclusively by skeletal cells: bone Gla (γ-carboxyglutamic acid) protein (BGP), bone-specific alkaline phosphatase (BAP), bone-specific acid phosphatase, and collagen-related proteins and peptides, including amino acid catabolites and procollagen peptides.1 Not included in this review are proteins that are produced by bone cells but also by other cells as well. In addition to matrix Gla protein, this latter group includes proteins whose names unfortunately imply exclusive or major bone origin, e.g., osteopontin (bone sialoprotein) and osteonectin (2). Although also produced by osteoblasts, these proteins have a significant if not primary contribution to their pool by nonskeletal tissues; this limits their application in skeletal assessment (1, 3).

In clinical practice, serum measurements of bone proteins are complemented by a wide variety of additional studies, including biochemical measurements of calcium, phosphorus, and such hormones as parathyрин, calcitonin, and vitamin D metabolites; tests of renal function; and skeletal procedures such as radiology, bone scans, and computer-assisted imaging procedures (1). These clinical tools will be only tangentially discussed here and the reader can consult recent reviews of these topics. However, their importance in a comprehensive evaluation of the patient with bone disease cannot be overemphasized.

The clinical goal of assay procedures related to the skeleton should be to assess separately the processes of bone formation and bone resorption. Bone formation and bone resorption underlie both normal and abnormal skeletal metabolism (1). These two processes are closely coupled in health and remain coupled in many skeletal diseases. Understanding the separate roles of bone formation and bone resorption will help to answer questions about metabolic abnormalities of the skeleton that intervene in disease states. For example, in patients with osteoporosis, is the primary disorder that of increased bone resorption or decreased bone formation? At least as important if not more so will be the elucidation of the effect of therapeutic regimens on bone formation and bone resorption. For example, again in osteoporosis, can sequential therapeutic regimens be devised that stimulate bone formation without stimulating bone resorption or inhibit bone resorption without inhibiting bone formation? Only serum markers seem applicable to the frequent and quantitative measurements that will be necessary to answer these questions.

The general discussion will focus on the major skeletal diseases, Paget's disease, osteoporosis, osteomalacia, and osteitis fibrosa cystica, and the bone disease of hyperparathyroidism. Less-common diseases will be considered for specific issues. Less-common urinary measurements of the above-mentioned analytes will not be detailed, although they too can have specific applications (1–3).

Alkaline Phosphatase

Background

The most commonly used serum marker to assess osteoblast function is the measurement of alkaline phos-
phatase (EC 3.1.3.1) enzymatic activity (1, 4, 5). Although this procedure can be valuable in some circumstances, its lack of specificity is a serious drawback. Serum alkaline phosphatase activity can come from tissues other than bone, and its concentration in blood may include contributions from the liver, the gastrointestinal tract, the kidney, the placenta, certain tumors, and other sources. Furthermore, it has not been possible to quantify the relative contribution of these various tissue sources to the serum alkaline phosphatase activity. Because the protein structure of many of these forms of alkaline phosphatase is similar if not identical, biochemical procedures designed to differentiate the bone form from other forms of alkaline phosphatase have not been technically convenient or clinically reliable (1). This is not surprising because the distinguishing characteristics of BAP are due primarily to its post-translational modification by bone cells (5).

Clinical Application

The measurement of serum alkaline phosphatase enzymatic activity remains one of the most common of all clinical tests and continues to be used for skeletal assessment (6, 7). In diseases where there is significant skeletal involvement, alkaline phosphatase remains a useful clinical test. Thus, this measurement is commonly used in the patient with Paget's disease, where high concentrations reflect the increased bone formation that is commonly present. Serum alkaline phosphatase activity can also be above normal in other diseases where there is a dramatic and significant increase in osteoblastic activity, including osteitis fibrosa (the bone disease of hyperparathyroidism) and osteomalacia (6–8). However, because serum alkaline phosphatase activity originates from a variety of organ sources, its measurement lacks specificity. The clinical impact of this lack of specificity is evident in the patient with skeletal disease and liver disease, where routine testing cannot reveal the source of the serum alkaline phosphatase. More subtle but equally important limitations are present in patients with less dramatic biochemical skeletal changes such as osteoporosis; in these patients, any changes in BAP are obscured by the small contribution that they make to the circulation pool of the enzyme (1).

Many assay procedures have been developed in attempts to specifically identify by biochemical means BAP in serum. These procedures have generally relied on the electrophoretic characteristics conferred on BAP by its post-translational modifications by the osteoblast (1, 5). Although these procedures have improved the bone-specificity of the alkaline phosphatase measurements, they remain technically difficult and have not received wide acceptance (1, 6, 7). Recent advances in the molecular biology and biochemistry of alkaline phosphatase have led to the development of immunochemical methods for the measurement of BAP (5, 9). These approaches have used the unique specificity for defined epitopes of monoclonal antibodies raised against crude extracts of BAP. In one procedure, two separate RIAs were used simultaneously for assay of BAP in serum (4, 6): one of the assays made use of an antibody that recognized BAP and liver alkaline phosphatase equally; the antibody in the other assay recognized BAP with a fivefold greater affinity than the liver isoenzyme. The serum concentration of BAP was calculated from the two assay results. This method appeared to give better clinical correlations between skeletal disease and alkaline phosphatase measurement than did enzymatic measurements of alkaline phosphatase (4, 6).

A more direct approach for the measurement of BAP was recently developed by using two antibodies for the bone isoenzyme in a two-site assay format (9). These two antibodies had distinct recognition sites for BAP and showed minimal cross-reactivity for alkaline phosphatase activity. Application of this assay to patients with Paget's disease demonstrated increased serum concentrations of BAP (10, 11). The correlation between BAP and total serum alkaline phosphatase activity was highly significant. By contrast, this BAP assay did not react with the increased liver isoenzyme seen in patients with liver disease. The relative novelty of BAP assays makes it difficult at this time to compare their clinical applications with those of BGP. It is clear that the two measurements do not always correlate and may even become dissociated, especially in Paget's disease (11). However, the differences between the two measurements, when fully understood, may ultimately lead to their more sophisticated application to clinical studies. In any case, although these new immunochemical assay procedures will need extensive clinical validation, they hold considerable promise for skeletal assessment in patients with bone disease (Figure 1).

Bone Gla Protein

Background

BGP is the major noncollagen protein of bone (1). Also named osteocalcin, BGP has 49 amino acids; it is pro-

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Fig. 1. Comparison of serum total alkaline phosphatase activity (TAP) with immunoassay measurements of bone Gla protein (BGP, ◦) and bone-specific alkaline phosphatase (BAP, ○) in Paget's disease.

TAP was measured with p-nitrophenyl phosphate, the upper limit of normal in this assay being 100 U/L (1, 9). BGP was measured by a radioimmunoassay, upper limit of normal, 5 nmo/L (12, 13). BAP was measured by a two-site immunoradiometric assay, upper limit of normal, 50 U/L (9, 10). The correlation (r) between BAP and TAP was 0.92 (P < 0.001); between BGP and TAP, 0.26 (P < 0.10); and between BGP and BAP, 0.34 (P < 0.05) (adapted from ref. 11).
duced exclusively by the osteoblast and its dental counterpart, the odontoblast (2). The function of BGP has not been discovered, although considerable experimentation has been directed toward investigating a role for BGP in bone formation or resorption. Nevertheless, the presence of BGP in serum and its essentially exclusive production by the osteoblast (the odontoblast contribution to serum concentrations being negligible) make the measurement of serum BGP uniquely valuable for assessing skeletal metabolism (1–3).

The direct rationale for the assay of serum BGP is as a measurement of bone anabolism (1, 12). As an osteoblast product, BGP represents the activity of the cell responsible for bone formation. Thus, it is reasonable to assume that when there is increased bone formation, the serum BGP concentrations will be increased (13). This hypothesis is generally supported by clinical studies of BGP, as detailed subsequently. However, it is also possible that certain forms of BGP are measurements of bone resorption. This possibility is based on the incorporation of BGP into bone matrix upon synthesis by the osteoblast; when the matrix is resorbed by the osteoclast, BGP may be released into serum. These forms of BGP are likely to be fragments of the molecule. Some data, discussed subsequently, support this hypothesis (14, 15).

Clinical Application

Radioimmunoassays of BGP have proven clinically useful in the assessment and management of patients with metabolic bone diseases (1–3, 12, 13). Serum BGP concentrations are increased in patients with various bone diseases characterized by increased osteoblastic activity. These include Paget’s disease, osteomalacia, osteitis fibrosa, renal osteodystrophy, and occasionally skeletal metastases (12, 13). In most of these disorders, serum BGP measurements correlate with other indices of bone formation, e.g., serum alkaline phosphatase, bone histomorphometry, bone scans (where applicable), calcium kinetic studies, and roentgenographic, densitometric, and imaging procedures (6, 7, 12, 13). There are, however, exceptions to these generalizations. In patients with renal osteodystrophy, renal failure complicates the measurement of BGP in serum (16). BGP, BGP metabolites, or related species are retained in serum and have disproportionately high concentrations (14–16). In patients with skeletal metastases, concentrations of serum BGP are not consistently increased and may not identify the affected patient (17). In patients with Paget’s disease, dissociation between serum BGP and serum alkaline phosphatase may limit the diagnostic applications of the former (13, 18). Nevertheless, with these reservations, BGP can be a useful clinical tool in managing patients with various skeletal diseases. Early clinical studies of BGP as measured by RIA (12, 13, 19) established the key aspects of this analyte that make it useful in skeletal assessment: it is increased in diseases characterized by increased bone formation; it is specific for bone, unlike serum alkaline phosphatase activity; and it responds rapidly, within hours, to perturbations in skeletal homeostasis (Table 1). Subsequent studies continue to confirm these initial observations.

In conditions where the increase of bone formation is more subtle, the application of BGP measurement is more limited. Increased concentrations of BGP have been observed in patients with excess thyroid hormone, growth hormone, vitamin D, and parathyrin; disease states characterized by decreased amounts of these hormones have decreased concentrations of BGP (20–24). Furthermore, the administration of calcitonin, glucocorticoids, and estrogens reportedly decreases serum BGP (13, 25–28). Inconsistent results have been reported for the effects of alcohol and other drugs on BGP (29–31). Variable BGP results have been reported in patients with osteoporosis (both high and low turnover), and reports are contradictory about the effects of age on serum BGP measurements, with normal, increased, and decreased concentrations having been reported in relation to these variables, sometimes even from the same laboratory (2, 6, 12, 13, 32–35). Most laboratories report higher BGP concentrations in males than in females (2, 12, 13). Some laboratories have reported a circadian pattern for serum BGP; others have not (22, 36, 37). Equally inconsistent results have been reported of BGP measurements across the menopause, across the menstrual cycle, and during pregnancy (29, 38–40). Whereas in adults gonadal steroids seem to have only subtle effects on BGP, significant increases of BGP have been reported during the gonadal changes of puberty (41, 42).

Nevertheless, even subtle changes can still be clinically valuable for the given patient, and serial BGP measurements can be used in concert with other clinical tools to evaluate treatment regimens in osteoporosis (6, 43–46). Furthermore, BGP measurements can be used to document the subtle skeletal involvement that can occur in rheumatoid arthritis and to monitor the further inhibition of bone formation that steroid treatment can produce (26–28). Although some of the published discrepancies regarding BGP may represent clinical differences, others may represent technical differences in the radioimmunoassays (47, 48). Newer assay formats may help to resolve some of these problems (49–51).

| Table 1. Diseases Characterized by Abnormal Concentrations of BGP in Serum |
|----------------------------------|-----------------|
| Increased serum BGP             | Paget’s disease |
|                                  | Hyperparathyroidism |
|                                  | Hyperthyroidism |
|                                  | Osteomalacia |
|                                  | Renal osteodystrophy |
|                                  | Acromegaly |
| Decreased serum BGP             | Hypothyroidism |
|                                  | Hypoparathyroidism |
|                                  | Growth hormone deficiency |

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Collagen Products

Type I collagen is a major product of the osteoblast (1). The measurements of urinary and, in some cases, serum hydroxyproline have long been used to assess bone resorption. Fragments of BGP may also serve this function (14, 15). However, despite improved methodology, the measurements of collagen-related substances such as hydroxyproline (and hydroxylsine) remain technically difficult procedures that are not suitable for routine use (52, 53). In addition, they are not specific indices for bone function and can be influenced by a variety of nonnonskeletal diseases and factors, including diet (52–54).

Other markers for collagen turnover may be more promising for this application; these include procollagen, collagen, and derived peptides (53–57). Collagen is synthesized as a precursor molecule, procollagen, that contains both amino- and carboxy-terminal extensions. These extensions are cleaved before collagen becomes incorporated as a fibril into bone matrix. Thus, the measurement of these peptides could serve as an index of osteoblast activity and bone formation. In fact, immunoassays based on antibodies for the terminal extension of type I procollagen can serve this purpose (55, 56). Applications of this assay procedure have demonstrated increased serum concentrations of these peptides in patients with Paget's disease (54). An alternative explanation of these observations is that the procollagen peptides represent the resorptive cleavage and release from bone of the intact molecule. However, acute studies of hormone-induced changes in procollagen peptides do not support this latter hypothesis, but rather support the view that bone formation is being reflected (57).

Although the peptidic derivatives of procollagen reflect bone formation, the major amino acid components of collagen—hydroxyproline and hydroxylsine—are derived from the osteoclastic catabolism of collagen and reflect bone resorption (1, 58). However, excreted hydroxyproline represents only a small fraction of collagen catabolism, and it is not specific for bone. Furthermore, the measurement of collagen catabolites remains technically difficult and has not been widely applied (1, 58). Newer methods of measuring bone-derived collagen crosslinks, which do not appear to reflect nonskeletal sources of collagen, may better reflect bone metabolism (58).

Acid Phosphatase

The simplest rationale for the measurement of bone-specific acid phosphatase (EC 3.1.3.2) in serum is as a marker for bone catabolism (1, 59). Bone acid phosphatase represents the activity of the cell responsible for bone resorption, the osteoclast. However, both biochemical and cellular considerations complicate the enzymatic assay of acid phosphatase (60). The enzymatic activity of acid phosphatase associated with osteoclasts must be distinguished from that from other tissue sources, notably prostate, pancreas, and blood cells (59, 60). This is usually accomplished by determining its mobility on acrylamide gel and by noting the resistance of its activity to tartrate. Thus, bone acid phosphatase is usually referred to as tartrate-resistant acid phosphatase (1). In addition to this biochemical complication, there is still some question about the osteoclast specificity of tartrate-resistant acid phosphatase. This isoenzyme may also be present in the mononuclear precursors of osteoclast; a closely related species has been isolated from the uterus and spleen of patients with hairy cell leukemia and appears to be increased in patients with Gaucher's disease (1, 58). There have been only limited studies of serum acid phosphatase activity in patients with skeletal diseases (1, 60–62). Most studies have focused on the use of serum acid phosphatase in patients with prostatic acid phosphatase (62, 63). Increased serum concentrations of the presumed osteoclast-specific form of the enzyme have been demonstrated by enzymatic assay in patients with Paget's disease, skeletal metastases, primary hyperparathyroidism, and, curiously, in osteopetrosis (1, 60, 62). These observations are consistent with the hypothesis that tartrate-resistant acid phosphatase is a serum marker for osteoclastic bone resorption (1). Recent progress in knowledge of the biochemistry and immunochemistry of this enzyme should lead to the development of clinically applicable procedures for assessing bone resorption in patients with skeletal disease (59, 64, 65). At this time, however, immunochemical assays are not available for bone-specific acid phosphatase.

Matrix Gla Protein

Matrix Gla protein (MGP) is another noncollagenous protein originally isolated from bone (66). Like BGP, this 84-amino-acid protein is vitamin K dependent (1, 66). MGP is secreted by the osteoblast and appears before BGP does in developing bone. However, MGP is also found in cartilage (66), in which it may function as a mineralization inhibitor. MGP may thus be useful in assessing the degree of involvement of cartilage in both diseases of bone and diseases of cartilage. Existing radioimmunoassays can be used to measure MGP production by cartilage in vitro (66). Improved assays promise a unique and valuable tool for studying cartilage in vitro in various disease states. However, the presence of MGP in many other nonskeletal tissues may limit its clinical usefulness.

Conclusions

Practicing clinicians now have at their disposal a wide variety of procedures for assessing the skeleton, including improved radiological and densitometric procedures and newer computer-assisted imaging methods for bone. The newer methods have revolutionized the clinical assessment of the patient with bone disease and have broadened our knowledge of skeletal metabolism. However, these methods are expensive and inconvenient and cannot be performed at sufficiently frequent intervals to assess dynamic changes in the metabolism of the skeleton, especially as might occur during treatment. This void may soon be filled with a panel of biochemical and immunochemical methods that can
help to assess skeletal status. The classical measurement of alkaline phosphatase activity can now be supplanted or at least complemented by measurements of BGP and BAP for assessing bone formation and osteoblast activity. The classical measurement of hydroxyproline may soon be supplanted by measurements of bone acid phosphatase for assessing osteoclast function and bone resorption. The application of the new serum measurements in concert with new imaging procedures should greatly help physicians in the diagnosis and management of patients with skeletal diseases. However, extensive studies will be necessary for the clinical validation of these assay procedures.

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References