New assays for parathyroid hormone (PTH) and the relevance of PTH fragments in renal failure

WILLIAM G. GOODMAN

Department of Medicine, Division of Nephrology, UCLA School of Medicine, Los Angeles, California

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Background. Immunometric assays for parathyroid hormone (PTH) are used extensively to assess bone and mineral metabolism in patients with end-stage renal disease (ESRD) who are treated with dialysis. Results generally correspond to bone histology as documented by bone biopsy, and they are useful in monitoring disease progression. Recent work has shown, however, that older, first-generation immunometric PTH assays detect not only full-length PTH(1-84), but also other amino-terminally-truncated PTH fragments (ntPTH) that may have inhibitory effects on bone cell metabolism and/or contribute to the development of adynamic renal osteodystrophy. New second-generation immunometric PTH assays, by contrast, detect PTH(1-84) exclusively. The diagnostic value of plasma PTH determinations using second-generation immunometric PTH assays and the utility of estimates of the concentration of ntPTH in plasma in patients with ESRD has been assessed only recently.

Methods. Results were reviewed from three published studies that examined the relationship between bone histology and plasma PTH levels as measured both by first- and by second-generation immunometric PTH assays in patients with ESRD. In all three studies, the concentration of ntPTH was estimated from the numerical difference between the results obtained with each assay and a ratio of PTH(1-84)/ntPTH was calculated.

Results. In one report, all patients with adynamic renal osteodystrophy had PTH(1-84)/ntPTH ratio values <1.0, although some patients with high-turnover skeletal lesions also had values <1.0. Estimates of the ratio of PTH(1-84)/ntPTH were found to be a better predictor of adynamic bone than PTH values measured by either assay. By contrast, two other studies failed to confirm these observations. One made use of the same second-generation immunometric PTH assay employed in the original report, whereas the other used a different assay with similar specificity for PTH(1-84). Plasma PTH levels obtained by first- and second-generation assays were highly correlated in these two independent reports.

Conclusion. Plasma PTH levels, as determined by first-generation and second-generation immunometric assays, are highly correlated and have similar diagnostic value for the non-invasive assessment of renal osteodystrophy. The contention that ntPTH estimates and values for the PTH(1-84)/ntPTH ratio are useful in the diagnostic assessment of renal osteodystrophy has yet to be confirmed.

Immunometric assays for parathyroid hormone (PTH) have been used extensively for the past 15 to 20 years to assess bone and mineral metabolism in patients with chronic kidney disease [1]. These double antibody sandwich-type assays circumvent many of the shortcomings of single-antibody radioimmunoassays (RIAs) used previously, thus making it possible to obtain reliable and reproducible measurements of the concentration of PTH in serum or plasma in those with end-stage renal disease. Several studies of large numbers of patients using quantitative bone histomorphometry and tetracycline-based measurements of bone formation have demonstrated that plasma PTH levels, as determined by immunometric assays, are generally reliable predictors of bone histology and the underlying type of renal osteodystrophy in patients undergoing either hemodialysis or peritoneal dialysis [2–7]. Accordingly, plasma PTH measurements are employed extensively not only for the initial diagnosis of renal bone disease but also to monitor its evolution because bone biopsy is not used commonly in routine clinical practice.

Despite these findings, certain therapeutic interventions such as the use of large intermittent doses of calcitriol or other active vitamin D sterols to treat secondary hyperparathyroidism and the use of large oral doses of calcium as a phosphate-binding agent can disrupt the relationship between plasma PTH levels and the rates of bone formation and turnover in patients with end-stage renal disease [8, 9]. Moreover, recent reports have led to considerable uncertainty among clinicians about the overall validity of plasma PTH measurements for the diagnosis and management of renal osteodystrophy [10, 11]. In part, some of these difficulties are related to the introduction of new immunometric PTH assays, which differ fundamentally from those utilized previously, providing substantially different numerical results [12]. Controversy persists, however, about the bio-
logical significance of several large peptide fragments of PTH that are present in the circulation both in normal subjects and in those with renal failure. Some of these peptides have been shown to influence bone cell function and may thus affect skeletal metabolism, but their importance as modifiers of bone formation and turnover in patients with renal bone disease remains uncertain [11].

Technical details about new immunometric assays for PTH and their use in the clinical management of patients with chronic renal failure are the primary focus of the current discussion. The role of PTH fragments as potential modifiers of bone metabolism in chronic renal failure is also addressed.

**EVOLUTION OF PTH ASSAYS**

RIA methods were used almost exclusively in early PTH assays [13, 14]. These systems employed single antibodies directed at epitopes located in the mid- or carboxy-terminal regions of the hormone in competitive displacement assays with isotopically labeled peptides, usually highly purified full-length PTH or synthetic PTH fragments. Because the portions of the molecule targeted by these antibodies were some distance from the amino-terminal end, most RIAs for PTH cross-reacted with, and thus detected a variety of amino-terminally-truncated PTH-derived peptides, or carboxy-terminal fragments, as well as the full-length hormone comprised of 84 amino acids, or PTH(1-84).

Most, if not all, amino-terminally-truncated PTH fragments have generally been thought to lack biological activity because they do not contain the amino-terminal portion of the hormone responsible for binding to and activating the type 1 PTH receptor, or PTH1R. Some of these peptide fragments arise from the degradation of PTH(1-84) within parathyroid cells, whereas others are generated by the metabolism of PTH(1-84) in peripheral tissues, most notably liver [15, 16]. They are removed from the circulation predominantly by glomerular filtration and thus accumulate in the plasma of patients with renal failure [16–18]. Because these peptide fragments were detected by conventional RIAs for PTH, plasma PTH values were commonly elevated, frequently markedly so, in patients with end-stage renal disease. Measurements often were not reproducible, and values failed to consistently predict the underlying type of renal bone disease [19, 20].

The subsequent development of immunometric PTH assays made it possible to reliably and reproducibly measure the concentration of PTH in serum or plasma in patients with end-stage renal disease [1, 21]. These assays utilize two antibodies directed toward distinct epitopes in different portions of the PTH molecule. One antibody, which is tagged with $^{125}$I or a chemiluminescent agent, serves to label peptides that are immobilized for quantification by a second capture antibody attached to a solid phase such as a plastic bead. Only large peptides capable of interacting with both antibodies, presumably PTH(1-84), are measured in immunometric PTH assays. Small peptides that lack one or both targeted epitopes are not detected.

Until recently, immunometric PTH assays were thought to detect predominantly full-length, biologically active PTH(1-84). It is now apparent, however, that several widely utilized immunometric PTH assays detect one or more peptides distinct from PTH(1-84) [12, 22, 23]. Some of these run on high-performance liquid chromatography at the same position as synthetic PTH(7-84) [22, 24, 25]. Thus, first-generation immunometric PTH assays overestimate the concentration of PTH(1-84) in serum or plasma. In contrast, recently developed second-generation immunometric PTH assays measure PTH(1-84) exclusively and do not detect large amino terminally truncated peptides such as synthetic PTH(7-84). The specificity of second-generation immunometric PTH assays for PTH(1-84) is determined by labeling antibodies that are directed toward epitopes located in the most amino-terminal portion of the molecule [12]. Indeed, the capacity for detecting synthetic human PTH(1-34) by the labeling antibody used in one second-generation immunometric PTH assay is eliminated almost completely by deleting the first amino acid residue.

The ability to measure PTH(1-84) specifically and exclusively represents an important technical advance. Second-generation immunometric PTH assays should thus prove to be useful in studies designed to better characterize the physiology of PTH secretion and hormone metabolism. Ultimately, they may also provide better diagnostic discrimination in certain clinical disorders of bone and mineral metabolism, but sufficient data to address these issues are not yet available.

The impact of recent advances in PTH assay methods on the diagnosis and management of patients with renal osteodystrophy remains to be determined. It is not yet known whether second-generation immunometric PTH assays provide additional diagnostic information compared to first-generation assays in the biochemical assessment of patients with renal osteodystrophy. Considerable additional work will be required to address this issue.

Despite the limitations of currently available data, several reports indicate that plasma PTH values obtained using second-generation immunometric assays are highly correlated with values determined by first-generation assays [12, 23, 26]. Although there is some variation among assays from different commercial sources, available results indicate that plasma PTH levels measured by second-generation immunometric PTH assays are approximately 40% to 50% lower than those determined using first-generation assays across a wide range of
plasma PTH concentrations [11, 12, 23, 26]. Such findings suggest that PTH(1-84) represents slightly more than half of the immunoreactivity detected by first-generation immunometric PTH assays. The remainder of the immunoreactivity detected in first-generation PTH assays reflects the presence of a variety of amino terminally truncated peptide fragments.

**PTH MEASUREMENTS AND BONE METABOLISM IN RENAL FAILURE**

The utility of PTH measurements for the diagnosis and management of bone disease in patients with end-stage renal disease depends largely on the extent to which results have been validated by bone histomorphometry. Abundant quantitative histologic data are available from clinical studies of renal osteodystrophy using first-generation immunometric PTH assays, but relatively little information is available using second-generation immunometric assays that measure PTH(1-84) exclusively. As such, the diagnostic value of recently introduced immunometric PTH assays has yet to be determined.

When measured by first generation immunometric assays, plasma PTH levels generally predict the underlying type of renal bone disease in patients with end-stage renal disease who have not been treated with vitamin D sterols or who are receiving small daily oral doses of vitamin D, specifically calcitriol [2–7]. Values two- to four-fold above the upper limit of normal correspond to relatively normal rates of bone formation as documented by the technique of double tetracycline labeling in bone biopsy specimens. For the most widely utilized first-generation immunometric assays, plasma PTH levels in patients with normal rates of bone formation usually range from 150 to 250 pg/mL, or 15 to 25 pmol/L. Higher levels are seen in those with bone biopsy evidence of secondary hyperparathyroidism, whereas plasma PTH concentrations below 150 pg/mL, or 15 pmol/L, suggest the presence of adynamic renal osteodystrophy. Plasma PTH levels less than 100 pg/mL, or 10 pmol/L, provide even stronger biochemical evidence of adynamic bone, particularly if serum calcium concentrations are also elevated [3].

It should be recognized, however, that most reports describing the relationship between plasma PTH levels and bone histology in patients with end-stage renal disease antedate the use of large, intermittent intravenous doses of vitamin D to treat secondary hyperparathyroidism [2–7]. As such, these somewhat dated results may not accurately reflect the consequences of current therapeutic strategies on bone formation and turnover in patients with secondary hyperparathyroidism. Large, intermittent doses of calcitriol and the use of large oral doses of calcium to manage phosphorus retention have each been implicated as causes of adynamic renal osteodystrophy. Both therapeutic interventions reduce osteoblastic activity and diminish bone formation in patients undergoing long-term dialysis [8, 9, 27, 28]. Indeed, bone formation may decrease substantially in some patients with secondary hyperparathyroidism who are treated with large intermittent doses of calcitriol even though plasma PTH levels remain as high as 400 or 500 pg/mL, or 40 or 50 pmol/L [8].

The mechanisms responsible for the disparity between plasma PTH levels and bone formation during intermittent calcitriol therapy remain uncertain. It may, however, reflect direct inhibitory actions of vitamin D, either alone or together with vitamin D–associated changes in calcium metabolism, on osteoblastic function [8, 9, 27, 28]. Such observations underscore the importance of more precisely defining the relationship between plasma PTH levels and bone formation within specific therapeutic contexts. Although PTH is the predominant regulator of bone formation and turnover in patients with end-stage renal disease, other factors also affect osteoblastic activity through PTH-independent pathways [8, 9, 27–29]. Thus, diabetes mellitus, corticosteroid therapy, osteoporosis, bone aluminum toxicity, and growth hormone therapy each directly affect osteoblastic activity and bone formation. Failure to adequately consider these issues may partially account for results from studies that challenge the diagnostic value of first-generation immunometric PTH assays as predictors of bone histology in patients with end-stage renal disease [10].

Three reports have examined the relationship between bone histology and plasma PTH levels as determined by first-generation and second-generation PTH assays in patients with renal osteodystrophy [11, 30, 31]. Although neither PTH assay was shown to be superior as a predictor of bone histology and bone turnover, technical differences between first- and second-generation PTH assays were used in all three studies to estimate the concentration of amino terminally truncated PTH peptides in plasma. Several reports indicate that amino terminally truncated PTH peptides exert inhibitory effects on osteoblasts and other bone cells [32, 33]. Moreover, the accumulation of excess amounts of these peptides has been suggested to contribute to the skeletal resistance to PTH that characterizes end-stage renal disease, possibly by interacting with a putative C-PTH receptor, distinct from PTH1R, which is capable of high-affinity binding to mid- and/or carboxyterminal regions of the PTH molecule [11, 26].

To examine the potential clinical relevance of these observations, the concentration of amino terminally truncated PTH fragments (ntPTH) in plasma was estimated by subtracting PTH values obtained using a second-generation immunometric PTH assay, which detects PTH(1-84) exclusively, from those determined by a first-generation immunometric PTH assay that detects both...
had PTH(1-84)/ntPTH ratio values that were skeletal lesions of secondary hyperparathyroidism also significantly greater than plasma PTH values measured by either first-generation or second-generation PTH assays. All patients with adynamic bone had PTH(1-84)/ntPTH values <1, although some individuals with high-turnover skeletal lesions of secondary hyperparathyroidism also had PTH(1-84)/ntPTH ratio values that were <1. In contrast, Coen et al [30] found no relationship between values for the PTH(1-84)/ntPTH ratio and bone histology among 35 patients undergoing regular hemodialysis. Values for the PTH(1-84)/ntPTH ratio did not differ among patients with various histologic subtypes of renal osteodystrophy, and the results failed to selectively identify patients with adynamic renal osteodystrophy [30]. Similar results were reported by Salusky et al [31] using a different second-generation immunometric PTH assay. As such, the contention that values for the PTH(1-84)/ntPTH ratio and/or estimates of the abundance of ntPTH in plasma affect bone metabolism in patients with renal osteodystrophy remain unsubstantiated.

Overall, currently available data indicate that second-generation immunometric PTH are similar to first-generation PTH assays in their capacity to discriminate between patients with high-turnover or low-turnover skeletal lesions of renal osteodystrophy. The diagnostic precision of new PTH assays has not yet been shown to be greater than previously employed immunometric assays in patients with renal bone disease. Results obtained using first- and second-generation immunometric PTH assays are highly correlated, and the slope of this relationship ranges from approximately 0.5 to 0.6. Thus, values determined by second-generation immunometric PTH assays are usually 50% to 60% of those measured by first-generation assays. It is possible, therefore, to estimate plasma PTH values that would be expected using second-generation immunometric assays in patients with various subtypes of renal osteodystrophy based upon available data using first-generation immunometric PTH assays.

The recommended therapeutic target range of 150 to 250 pg/mL, or 15 to 25 pmol/L, in patients with end-stage renal disease and relatively normal rates of bone formation as determined by first-generation immunometric PTH assays should correspond to values of approximately 75 to 125 pg/mL as measured by second-generation assays. Slightly higher values would be expected if the slope of the linear correlation between first- and second-generation PTH assays were somewhat greater [30]. Values above 125 to 150 pg/mL using second-generation PTH assays are likely to be associated with skeletal changes of secondary hyperparathyroidism, whereas levels below 50 to 75 pg/mL would be expected in patients with adynamic renal osteodystrophy. More definitive statements about the relationship between plasma PTH levels as determined by second-generation immunometric PTH assays and bone histology in patients with end-stage renal disease must await the results of additional studies.

CONCLUSION

New second-generation immunometric PTH assays have been developed. The assays measure full-length biologically active PTH(1-84) exclusively and do not detect amino terminally truncated PTH fragments, a feature that distinguishes them from first-generation immunometric PTH assays. Although only a few studies have been done, second-generation immunometric PTH assays have not been shown to be superior to first-generation assays for the diagnostic assessment of patients with renal osteodystrophy. Plasma PTH levels obtained using first-generation immunometric PTH assays thus provide the most definitive guide to the management of renal bone disease because they are supported by abundant bone histology data. Additional work is needed to further characterize the utility of second-generation immunometric PTH assays as predictors of bone histology in patients with end-stage renal disease.

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Reprint requests to William G. Goodman, M.D., Division of Nephrology, 7-155 Factor Bldg., UCLA Medical Center, 10833 Le Conte Ave., Los Angeles, California 90095.
E-mail: wgoodman@ucla.edu

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