Clinical Performance of a Parathyrin Immunoassay with Dynamically Determined Reference Values

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We compared the clinical performance of a carboxyl-terminal radioimmunoassay for human parathyroid hormone (iPTH), using either a dynamic reference interval (95% confidence limits of serum iPTH concentrations observed in 11 normal individuals during intravenous infusions of Na2EDTA and CaCl2) or a gaussian (2 SD) reference interval derived from 233 normocalcemic individuals. The 2 SD ranges were 3.5 to 9.8 pmol/L for serum iPTH and 2.19 to 2.53 mmol/L for total calcium. The iPTH dynamic interval was lower for calcium concentrations >2.50 mmol/L; it was higher, wider, and continued to increase for calcium values <2.25 mmol/L. Use of the dynamic reference interval increased the clinical sensitivity of our assay from 81% and 61% to 100%, respectively, in primary hyperparathyroidism (n = 47) and hypoparathyroidism (n = 18). Test specificity was maintained at 100% in hypocalcemic disorders but fell to 93% (62/67) in hypercalcemic disorders. Overall, use of the dynamic reference interval improved the assay performance.

Additional Keyphrases: parathyroid hormone • hyper-, hypocalcemia

Immunoradiometric and radioimmunoassays with specificity for the intact molecule or for certain regions and cytochemical bioassays are currently used to measure PTH in human serum (1-4). Even if the cytochemical bioassays can have nearly 100% clinical sensitivity in discriminating normal individuals from those with primary hyperparathyroidism (5-7), their use remains limited in view of the technical complexity involved. The clinical sensitivity of intact-molecule or region-specific assays falls between 80% and 95%, given the same circumstances, without any definitive advantage for amino-terminal, carboxy-terminal, mid-molecule, or intact-molecule assays (1), in either their research or commercial versions (8-10). This apparent failure of immunoassays to distinguish normal individuals from those with primary hyperparathyroidism has never been properly explained, although patients with primary hyperparathyroidism and normal iPTH concentrations are generally correctly classified on the basis of an "inappropriately" high iPTH value for the prevailing calcium (11).

We were interested to see if iPTH reference intervals derived from normocalcemic populations contributed at least partly to this lack of sensitivity of human PTH immunoassays. We have thus generated reference intervals for iPTH in the hypercalcemic and hypocalcemic ranges of serum calcium concentration by running functional tests of the parathyroid glands in normal individuals and have compared the clinical sensitivity of our in-house iPTH assay with both sets of reference values. Our results indicate that the clinical sensitivity of a given PTH immunoassay can be increased to nearly 100% by defining appropriate reference ranges for the hypercalcemic and hypocalcemic regions of serum calcium concentration.

Materials and Methods

Experimental Subjects

*Dynamic studies:* Five normal men and six normal women, 30 to 45 years old, participated in this study. They were all volunteers, and they all signed informed-consent forms. Serum calcium was increased in all by means of a 2-h intravenous infusion of CaCl2, the rate of infusion being 125 μmol per kilogram body weight per hour. A week later, serum calcium was decreased in all by means of a 2-h intravenous infusion of Na2EDTA in isotonic saline, 51 μmol kg−1 h−1. Procaine HCl (12.5 μmol kg−1 h−1) was added to this infusion to prevent arm pain. Blood was sampled for total serum calcium and iPTH measurements before each infusion and every 15 min thereafter throughout the 2-h period.

*Normal individuals:* Ninety healthy hospital employees, 100 voluntary individuals recruited for osteodensitometry studies, and 43 patients who were consulting for minor disorders unrelated to P/Ca metabolism were used to define our normocalcemic iPTH 2 SD reference range. Overall, there were 133 women and 100 men, ages 18-79 y.

*Patients:* Serum was collected from hospitalized subjects or outpatients under evaluation for suspected parathyroid dysfunction. They were classified retrospectively according to their history and medical follow-up. Hyperparathyroid patients suffered from either surgically proven primary hyperparathyroidism (n = 47), malignancies (n = 13), vitamin D intoxication (n = 4), hyperthyroidism (n = 2), or acute pancreatitis (n = 1); furthermore, 11 had had a recent renal transplant. Hypocalcemic patients suffered from surgical or autoimmune hypoparathyroidism (n = 18), pseudohyperparathyroidism (n = 4) or secondary hyperparathyroidism without renal failure (n = 19). Eight patients in this last group had malabsorption syndromes (Crohn disease, gluten enteropathy), two had advanced liver diseases, six suffered from chronic malnutrition, two were hypomagnesemic, and one was in the recovery phase of acute renal failure.

Laboratory Methods

Serum calcium was measured by automated colorimetry (12). Total proteins were also measured in each case and total serum calcium was corrected for total protein concentration (13) in those patients whose protein values were above or below the reference interval (60-78 g/L). Serum iPTH was measured by a carboxyl-terminal radioimmunoassay. Antiserum C-52, presaturated with a molar excess of hPTH(44-68) (Bachem, Torrance, CA) to eliminate its midmolecule reactivity, was used at 1/50 000 dilution with [125I](Tyrβ2)hPTH(52-84) as tracer (14). Standard
hPTH(39-84) (Peninsula Laboratories, Belmont, CA) was used after verification of content by amino acid analysis. This standard was used to measure the iPTH content of all sera. Standard hPTH(1-84) 79/500 (National Institute for Biological Standards and Control, Holly Hill, Hampstead, London, England) was also used as standard to illustrate the behavior of intact hormone in the assay. The interassay and intra-assay coefficients of variation, calculated from a sample run in triplicate at 50% binding, were 8.9% and 3.3%, respectively. All other aspects of the assay have been previously published (15).

Statistical Analysis

The distribution of serum iPTH and calcium values among normal individuals, analyzed by the method of Lilliefors (16), was not significantly different from gaussian. Thus the reference intervals for iPTH and calcium were defined as two standard deviations above and below the mean. To define the dynamic reference interval of iPTH during functional tests of the parathyroid glands, all the iPTH and calcium results of the 11 normal individuals studied were simultaneously analyzed by use of a logistic model corresponding to a four-parameter sigmoidal equation (17). To compensate for heteroscedasticity of the sigmoid curve, a weighting factor of 1/\[\text{iPTH}\] was applied to all points. The 95% confidence limits of this curve were calculated according to Rodbard et al. (18, 19).

Results

Figure 1 depicts the characteristics of the iPTH radioimmunoassay used for these studies. Standard hPTH(39-84) was five to six times more potent on a molar basis in displacing [125I][Tyr52]hPTH(52-84) tracer than was hPTH(1-84). The lowest concentration significantly different from zero was 0.2 fmol of hPTH(39-84) per tube, equivalent to 1.3 pmol per liter of serum.

Table 1 summarizes mean serum iPTH and calcium values for normal men and women and the 2 SD reference ranges of both analytes for the overall population.

Figure 2 (left) depicts the dynamic interval of serum iPTH values observed in 11 normal individuals during induced hypercalcemia or hypocalcemia. In Figure 2 (right), these results are compared with the 2 SD reference ranges derived from our 233 normal individuals. The 95% confidence limits of the maximal response to hypocalcemic stimulation (total calcium, \(C_{\text{a}} = 1.75 \text{ mmol/L}\)) were 11.4 to 42.3 pmol/L. Those of the non-suppressible fraction of circulating iPTH (\(C_{\text{a}} = 3.50 \text{ mmol/L}\)) were 1.7 and 6 pmol/L. At the extremities of the 2 SD reference box the limits were 5.7 to 20 pmol/L (\(C_{\text{a}} = 2.19 \text{ mmol/L}\)) and 2.4 to 8.5 pmol/L (\(C_{\text{a}} = 2.56 \text{ mmol/L}\)).

Application of the 2 SD or dynamic reference intervals of iPTH values to the study of patients with hypercalcemic disorders is illustrated in Figure 3 and Table 2. When hypercalcemic iPTH values were assessed with use of the 2 SD reference range, nine of the 47 patients with surgically proven primary hyperparathyroidism had iPTH values within this range. When the same iPTH values were assessed by using the dynamic interval, all patients with primary hyperparathyroidism had above-normal values for iPTH. All patients with cancer or hypercalcemia from other causes had values for iPTH below the upper limit of the 2 SD reference range; however, four of the former and one of the latter had values slightly above the dynamic interval. Two patients with vitamin D intoxication had results below either reference interval. The 11 patients with hypercalcemia after recent renal grafts had values well above either range.

The same approach was also used to study patients with hypocalcemia (Figure 4 and Table 2). Seven of the 18 patients with hypocalcemic hypoparathyroidism had iPTH concentrations within the 2 SD reference range, but all were below the dynamic range. All patients with pseudohypoparathyroidism and 17 of 19 patients with secondary hyperparathyroidism without renal failure had values for iPTH above the 2 SD reference range but within the dynamic interval, whereas two had values only slightly above.

Discussion

We tried to establish the gaussian (2 SD) reference intervals for serum iPTH and calcium as carefully as possible. The number of patients seemed adequate and their age and sex distribution paralleled the general population seeking medical attention at our hospital (normal workers of the institution, peri-menopausal women (20) and middle-aged men recruited for osteodensitometry studies, and elderly patients consulting for unrelated disorders). The normality of the distribution was checked before the usual ±2 SD ranges were computed. Nevertheless, it was already apparent from Figure 2 (right) that the gaussian reference box did

| Table 1. Values* for Total Calcium and iPTH, and Range, for Normal Individuals |
|---------------------------------|-----------------|-----------------|------------------|
|                                 | Men             | Women           | All              |
| No.                             | 100             | 133             | 233              |
| Age, y                          | 38.4 ± 15.00    | 43.4 ± 14.9     | 41.2 ± 15.1      |
| Ca, mmol/L                      | 2.37 ± 0.08     | 2.35 ± 0.08     | 2.36 ± 0.08      |
| iPTH, pmol/L                    | 6.00 ± 1.42     | 7.13 ± 1.53     | 6.64 ± 1.59      |
| Gaussian ref. interval          |                 |                 | 2.19–2.53        |
|                                 |                 |                 | 3.5–9.8          |

*Mean ± SD is given for values; ranges are ±2 SD.

2440 CLINICAL CHEMISTRY, Vol. 34, No. 12, 1988
not describe adequately the distribution of these data: there were very few points in the upper right and lower left corners.

The dynamic range was established with 158 pairs of data points coming from 11 normal individuals. This number was too low to establish distinct iPTH 2 SD reference intervals for the whole spectrum of individual calcium values encountered in this study. This is why we chose instead to calculate the mean sigmoidal response curve and its 95% confidence limits with weighting in a manner analogous to the calculation of RIA curves (18, 19). With this approach, the actual relationship between iPTH and calcium concentrations was taken into consideration and the influence of outliers was minimized, particularly near the "normal" calcium values where an improvement in clinical discrimination was required. It can be seen from Figure 2 (right) that the dynamic interval more adequately described the distribution of values in healthy individuals, although the upper limit of this interval still seemed relatively too high for normal calcium values. A higher number of data points or the use of values for ionized instead of total calcium might have improved this part of the curve.

Nevertheless, comparison of the two ranges led to interesting observations. The upper limit of the iPTH dynamic interval was always lower than that of the 2 SD range in hypercalcemia, and this difference (inappropriate iPTH values) was already apparent at the upper limit of the normal reference interval for calcium. Similar observations could be made at the lower end of normal calcium values, where the lower limit of the iPTH dynamic range was already 1.5 times higher than that of the 2 SD reference range. Again, the range delimited by the lower limits of both ranges denoted inappropriately low serum iPTH concentrations. It was of interest that, even with major differences between the two ranges, only two of the 233 normal individuals had iPTH values outside both ranges.

The iPTH radioimmunoassay used in these experiments was a carboxyl-terminal assay desensitized for mid-molecule reactivity (14). Even if, under clinical circumstances, our assay acted mainly as an assay for large carboxyl-terminal fragment, it remained very useful in studying variations in CaCl₂ or Na₂EDTA infusions, mainly because the quantity of circulating large carboxyl-terminal fragments seems as well regulated by serum calcium as is intact iPTH(1-84), at least in patients with normal renal function (21).

The use of the dynamic range increased the clinical sensitivity of this assay to 100% in detecting primary hyperparathyroidism and idiopathic or surgically induced hypoparathyroidism. This improvement was obtained partly at the expense of our assay specificity in hypercalcemia, but not in hypocalcemia. Four patients with malignancies and one with vitamin D intoxication had iPTH values above the upper limit of the dynamic interval. The phenomenon whereby iPTH values tend to increase as a function of increasing serum calcium in patients with cancer-related

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Table 2. Clinical Performance of the Gaussian and Dynamic Reference Intervals for iPTH Values in Hypercalcemic and Hypocalcemic Disorders

<table>
<thead>
<tr>
<th>Diagnoses</th>
<th>Gaussian No. outside/total (and % outside)</th>
<th>Dynamic No. outside/total (and % outside)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHP</td>
<td>38/47 (80.9)</td>
<td>47/47 (100)</td>
</tr>
<tr>
<td>Cancer</td>
<td>0/13 (0)</td>
<td>4/13 (30.8)</td>
</tr>
<tr>
<td>Other</td>
<td>0/7 (0)</td>
<td>1/7 (14.3)</td>
</tr>
<tr>
<td>R. Gift</td>
<td>11/11 (100)</td>
<td>11/11 (100)</td>
</tr>
<tr>
<td>HypoP</td>
<td>11/18 (61.1)</td>
<td>18/18 (100)</td>
</tr>
<tr>
<td>Pseudo</td>
<td>4/11 (100)</td>
<td>0/4 (0)</td>
</tr>
<tr>
<td>2ary hyper</td>
<td>1/19 (100)</td>
<td>2/19 (10.5)</td>
</tr>
</tbody>
</table>

PHP: Primary hyperparathyroidism; Other, vitamin D intoxication, hypothyroidism, pancreatitis; R. Gift, renal graft; HypoP, hypoparathyroidism; Pseudo, pseudohypoparathyroidism; 2ary hyper, secondary hyperparathyroidism without renal failure.

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Fig. 2. The gaussian (mean ± 2 SD) and dynamic (95% confidence limits) ranges of iPTH values derived from the study of 233 normal individuals (right) and from 11 normal individuals made hypercalcemic and hypocalcemic by means of CaCl₂ or Na₂EDTA intravenous infusions (left). The dynamic range is also compared with the 2 SD range in the right-hand figure.

Fig. 3. iPTH values obtained for hypercalcemic patients, analyzed as a function of the 2 SD range defined in 233 normal individuals (right) or as a function of the dynamic range defined in 11 normals during functional tests of the parathyroid glands (left).

On the right, the upper and lower limits of the 2 SD range are indicated by hatched lines. On both graphs, the limit of detection of the assay is also indicated by the lowest hatched line. PHP, primary hyperparathyroidism; Other, vitamin D intoxication, hyperthyroidism, pancreatitis; R. Gift, renal-graft patients.

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Fig. 4. iPTH values obtained for hypocalcemic patients, analyzed as a function of the 2 SD range defined in 233 normal individuals (right) or as a function of the dynamic range defined in 11 normals during functional tests of the parathyroid glands (left).

On the right, the upper and lower limits of the 2 SD range are indicated by hatched lines. On both graphs, the limit of detection of the assay is also indicated by the lowest hatched line. HypoP, hypoparathyroidism; Pseudo, pseudohypoparathyroidism; 2ary hyper, secondary hyperparathyroidism without renal failure.

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CLINICAL CHEMISTRY, Vol. 34, No. 12, 1988 2441
hypercalcemia has also been observed in some other iPTH radioimmunoassays (22, 23). An impaired renal function with some dehydration could explain in part why the non-suppressible fraction of circulating iPTH is higher in some of these patients as compared with our hydrated volunteers. Co-existing primary hyperparathyroidism, residual secondary hyperparathyroidism accompanying chronic debilitating conditions, or long-term vitamin D deficiency could also explain the results (24). The patient with vitamin D intoxication and the iPTH value above the dynamic interval had primary biliary cirrhosis. We found, finally, that our capacity to distinguish these hypercalcemic patients from those with primary hyperparathyroidism on iPTH values alone was limited to 75% (19/26), a reflection of the limitations of a carboxyl-terminal assay in these situations. Of the two patients who were intoxicated with vitamin D and whose iPTH values were suppressed below the lower limit of the dynamic interval, one had been parathyroidectomized and the other was lost to follow-up before his basal parathyroid status could be assessed.

Our four patients with pseudohypoparathyroidism had iPTH values above the 2 SD reference range but within the dynamic interval. This suggested that none of them had an increased parathyroid function. This may have been related to therapy with 1,25(OH)2D3 in two of the four cases. In the absence of renal insufficiency, only two hypocalcemic patients had an iPTH concentration slightly above the upper limit of the dynamic interval. This may have reflected the fact that some of the other patients were hypomagnesemic, a factor known to impair iPTH secretion (25, 26) or that some of these patients were being chronically treated for their diseases. Studies of more patients are needed before a particular significance can be attached to hypocalcemia or normocalcemia concomitant with iPTH values above the upper limit of the dynamic interval.

The utilization of a different reference range did not change the intrinsic characteristics of our carboxy-terminal assay, as in renal insufficiency for example (results not shown). It merely gave a better appreciation of the existing relationship between serum calcium and iPTH concentrations. This dynamic interval was obtained during acute experiments in well-hydrated normal individuals, so it is possible that it may not perfectly reflect more chronic changes in partly dehydrated patients. With recently introduced intact PTH assays (2, 3) the use of a dynamic reference interval would probably give near-perfect specificity and specificity. Nevertheless, we found that even with a less sophisticated assay, the dynamic interval was far more informative than the 2 SD “box” approach, and we now use it on a regular basis. It can be obtained easily in a limited number of healthy volunteers with minor discomfort. “On site” monitoring of ionized calcium in whole blood during Na2EDTA and CaCl2 infusions is now easily available with reliable instruments, making this approach less hazardous. Aliquots of samples obtained during these experiments could also be kept for eventual evaluation of new assays. This would be more economical and probably more informative than the comparison of a limited number of samples with reference laboratory assays (27–29).

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References


