Diagnostic Utility of C-Terminal Parathyrin Measurement as Compared with Measurements of N-Terminal Parathyrin and Calcium in Serum

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We compared results obtained from two parathyrin (parathyroid hormone) assays with differing specificities, using sera from 172 normal donors and from 98 patients with disorders of calcium regulation. Intact parathyrin was measured in both assays; the C-parathyrin assay also measured the 53-84 amino acid C-terminal hormone fragment; the N-parathyrin assay also measured the 1-34 N-terminal fragment. The reference interval for the C-parathyrin assay (860-3720 int. units/L; 430-1860 ng/L) was markedly higher than for the N-parathyrin assay (460-1260 int. units/L; 230-630 ng/L), a finding consistent with the longer half-life of C-parathyrin fragments in human circulation. Mean C-parathyrin in primary hyperparathyroid sera—5720 (SD 2760) int. units/L or 2860 (SD 1380) ng/L—clearly exceeded the reference interval and values for sera from patients with non-parathyroid malignancy [1740 (SD 760) int. units/L; 870 (SD 380) ng/L]. Secondary hyperparathyroid patients also had supranormal C-parathyrin values: 6100 (SD 2720) int. units/L; 3050 (SD 1360) ng/L. We found no consistent correlation between parathyrin and serum calcium in any clinical group. The two parathyrin assays showed about equal diagnostic power, but their results could not be used interchangeably in sequential monitoring of patients.

Additional Keyphrases: reference intervals • hypo- and hyperparathyroidism • cancer • hormones • disorders of calcium regulation • hypercalcemia

Measurement of parathyrin (parathyroid hormone, PTH) is clinically useful in the differential diagnosis of several disorders of calcium regulation (1). This hormone is present in serum in at least three forms (2): (a) intact hormone (84 amino acids), (b) hormone fragments that include the carboxy-terminal amino acid sequence (C-PTH), and (c) a fragment including the first 34 amino acids from the amino terminus (N-PTH). Antibodies to PTH differ in their relative reactivity toward these several species. Some investigators have proposed that the diagnostic utility of PTH assays can be specifically related to cross-reactivity studies with the active and inactive fragments (3).

The present study was undertaken to determine the relationship between assay results obtained with an antibody reacting primarily with N-PTH (and intact hormone) and another antibody reacting primarily with C-PTH (and intact hormone). These assays are more completely described in an accompanying paper (4) and in a previous report (5).

Sera obtained from 171 normal adult donors and from 98 patients with disorders of calcium regulation were assayed for PTH with each antibody and for calcium by atomic absorption spectroscopy.

Methods and Materials

Procedures

C-terminal parathyrin assay: The C-PTH assay we used is described in the accompanying paper (4) and is a sequential double-antibody radioimmunoassay that cross-reacts with both C-fragments and intact hormone. We used the same assay buffer (pH 7.4, containing 100 mL of human serum per liter), intact bovine parathyrin standards, and iodinated PTH (4), for both PTH assays.

N-terminal parathyrin assay: The N-PTH assay used in the present study cross-reacts with both the N-fragment (1-34) and intact hormone (5). We have modified it from the originally reported charcoal-adsorption separation to a double-antibody precipitation. Briefly, the N-PTH assay mixture consists of 0.5 mL of sample or standard, 0.3 mL of 125I-labeled bovine PTH (30 pg), and 0.1 mL of guinea pig antiPTH antibody (no. 211/32, 1/840 000 final dilution; Burroughs-Wellcome), which is incubated for 24 to 26 h at 25 °C. Rabbit anti-guinea pig serum is added and incubation continued for an additional 2.5 to 3 h, then 1.0 mL of assay buffer is added and the mixture is centrifuged. The radioactivity of the resulting pellets is measured with a gamma counter. Each standard curve point is assayed in triplicate and each sample or pool in duplicate plus individual determinations of non-specific binding (4). Results are calculated as picogram equivalents of bovine intact hormone per milliliter of serum, and standards are calibrated to the World Health Organization's reference preparation of bovine PTH as international bioassay units (int. units/L).

Serum calcium was assayed by atomic absorption spectroscopy (6).

Selection of Patients and Donors

We assayed sera from 111 well adults to establish a reference interval in the C-PTH assay and sera from 61 well adults for the N-PTH reference interval. Serum samples from patients with surgically documented primary hyperparathyroidism, secondary hyperparathyroidism due to chronic renal failure, renal transplants, malignancy (non-parathyroid) with hypercalcemia, hypoparathyroidism following parathyroidectomy, pseudohypoparathyroidism, osteomalacia, or thyrotoxicosis were obtained from hospitals in the Los Angeles area.

The sera had been stored for one month to two years at −70 °C before analysis. Aliquots of each serum were thawed and analyzed by both PTH methods on the same day.

Results

We separated sera from 21 healthy adults into two aliquots and analyzed them, using each PTH antibody. As shown in Figure 1, results from the C-PTH assay were up to fivefold higher than N-PTH results. However, there was no consistent relation between C-PTH and N-PTH results (both assays also measure the intact hormone), so a factor could not be used to convert results from one assay to the other. The results shown in Figure 1 are consistent with the normal ranges found for the
C-PTH (860–3720 int. units/L; 430–1860 ng/L, n = 111) and N-PTH (460–1260 int. units/L; 230–630 ng/L, n = 61) antibodies used. During the C-PTH normal range study, blood from 17 of the donors was sampled more than once during two years, and all such C-PTH values (Figure 2) fell within the established normal range. In most cases, the range of values found for any individual was within the ±2 CV (between-run) limits of assay precision. Blood from three normal donors was sampled for C-PTH assay every hour from 0900 to 1200 hours via indwelling catheters. These results showed no time-de-}

Fig. 1. Comparison of results (int. units/L; ng/L) for serum C-PTH and N-PTH for normal healthy adults

dependent trends and all values were within the assay precision limits.

Figure 3 shows results of serum calcium determinations from normal donors, together with calcium and C-PTH values for 29 patients later confirmed at surgery to have primary hyperparathyroidism. These two groups of results are well separated. Results for serum calcium and N-PTH in normal persons and primary hyperparathyroid patients showed a distribution similar to that described in previous reports (5). We saw no correlation between results for serum calcium and C-PTH in the normal or primary hyperparathyroid groups.

Serum from 17 patients with chronic renal failure was an-
alyzed for N-PTH and C-PTH. Most of the results (Figure 4) are above the reference intervals for both PTH assays. As with other samples analyzed, PTH results were generally higher in the C-assay than the N-assay, but not by a constant factor. Two patients with results that were normal by the N-PTH assay had above-normal C-PTH results. C-PTH results from the same patients are plotted vs serum calcium in Figure 5, which also shows the lower PTH values for patients after successful renal transplantation.

Figure 6 shows results for N-PTH and C-PTH in patients with hypercalcemia due to primary hyperparathyroidism or secondary to non-parathyroid malignancy. Mean PTH values by either assay were higher for patients with documented primary hyperparathyroidism than for those with malignancy. Three of the 17 primary hyperparathyroid patients had N-PTH and C-PTH concentrations overlapping the non-parathyroid malignancy range, while four more had N-PTH overlapping the non-parathyroid malignancy range. All PTH values from patients with non-parathyroid malignancy were within or below the respective normal ranges for the two assays. As shown in Figure 7, calcium values tended to be similar for the two groups, ranging from 2.5 to 4.9 mmol/L (102–196 mg/L) in non-parathyroid disease and from 2.6 to 4.1 mmol/L (104–164 mg/L) in primary hyperparathyroidism. Sixteen of the 18 non-parathyroid malignancy patients shown would be correctly classified if the decision line shown in Figure 7 were used.

Table 1 shows PTH results in patients who were surgically hypoparathyroid. Calcium concentrations, where measured, in these patients are below the reference interval (which is 2.2–2.6 mmol/L, or 88–104 mg/L). One-half of the C-PTH results were below the reference interval (880–3720 int. units/L; 430–1860 ng/L) and the others were in the lower quarter of the range. Two of the N-PTH results were below the reference interval (460–1260 int. units/L; 230–630 ng/L) and two were within the range.

Sera from patients with other disorders of calcium regula-

Table 1. Serum PTH and Calcium in Six Hypoparathyroid Patients

<table>
<thead>
<tr>
<th></th>
<th>C-PTH</th>
<th>N-PTH</th>
<th>Calcium, mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>ng/L</td>
<td>int. units/L</td>
<td>ng/L</td>
<td>int. units/L</td>
</tr>
<tr>
<td>494</td>
<td>988</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>326</td>
<td>652</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>315*</td>
<td>630</td>
<td>128</td>
<td>256</td>
</tr>
<tr>
<td>549*</td>
<td>1096</td>
<td>162</td>
<td>324</td>
</tr>
<tr>
<td>719*</td>
<td>1438</td>
<td>488</td>
<td>976</td>
</tr>
<tr>
<td>386*</td>
<td>772</td>
<td>581</td>
<td>1162</td>
</tr>
</tbody>
</table>

* Patient being treated with vitamin D.
Table 2. Serum PTH and Calcium in Patients with Calcium Regulatory Disorders

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Calcium, mmol/L</th>
<th>N-PTH</th>
<th>C-PTH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Int. units/L</td>
<td>ng/L</td>
<td>Int. units/L</td>
</tr>
<tr>
<td>Pseudohypo-parathyroidism</td>
<td>2.2</td>
<td>6308</td>
<td>3154</td>
</tr>
<tr>
<td>Osteomalacia</td>
<td>2.5</td>
<td>4632</td>
<td>2316</td>
</tr>
<tr>
<td>Osteomalacia with hyperphosphatemia</td>
<td>2.4</td>
<td>1134</td>
<td>567</td>
</tr>
<tr>
<td>Thyrotoxicosis</td>
<td>3.0</td>
<td>478</td>
<td>239</td>
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<tr>
<td>Thyrotoxicosis</td>
<td>3.0</td>
<td>714</td>
<td>357</td>
</tr>
</tbody>
</table>

tion were examined for N-PTH, C-PTH, and calcium; results are shown in Table 2. The pseudohypoparathyroid patient showed serum calcium concentrations at the low limit of the normal range, and increased C-PTH. Two of the three osteomalacia patients had increased PTH values, and the two patients whose serum calcium was measured had results that were within the reference interval. In the two thyrotoxicosis patients, calcium was increased and C-PTH and one N-PTH were below the reference interval; the other N-PTH value was within it.

Discussion

Several investigators have sought to correlate the results from PTH antibody-specificity studies with clinical utility, in an effort to use N-PTH or C-PTH cross-reactivity to predict the ability of an antibody to discriminate among clinically distinct classes of patients (3, 7). More recently, several investigators have suggested that such a view may be an overgeneralization (1, 8). In the present study, we examined sera from 172 normal donors and 92 patients with disorders of calcium regulation, using two PTH antibodies that have been characterized as cross reacting primarily with the C-terminal region (4) or with the N-terminal region (5) of the molecule. The intact hormone contains both regions; thus, each antibody reacts to the intact hormone in addition to selected fragments, according to its specificity. Parallel standard curves were found with intact human PTH in both assays (4). Because both the intact hormone and the N-terminal fragment are thought to be physiologically active (9), the N-PTH assay in this report could perhaps more properly be designated an assay of “active” PTH.

The strategy of using discrimination among clinical groups to characterize an assay deserves some discussion. If only patients who meet very rigorous diagnostic criteria are included in the study, the assay will appear to discriminate better than if patients with early or mild disease (more typical of those seen in routine clinical practice) are included. Thus, the characteristics of the normal populations (i.e., age, health, sex) and hyperparathyroid patients (duration of disease, size and number of diseased glands) may be more influential than is antibody specificity. This point is illustrated by studies involving a particular C-PTH antibody and two identically defined patient populations. One investigator found only 10% overlap with normal values of values for primary hyperparathyroid patients (10); another found 40% overlap (11) and also found some results from hyperparathyroid patients to be as low as from hypoparathyroid patients—results clearly at variance with the earlier (10) study. The most likely explanation for this discrepancy is that the patient groups, although identically defined, differed in the two studies. These limitations in experimental design also influence the clinical data presented in this report.

Our reference intervals for the C-PTH-specific antibody (860-3720 int. units/L; 430-1860 ng/L) and for the N-PTH-specific antibody (460-1260 int. units/L; 230-630 ng/L) are consistent with reported ranges calculated against bovine-derived intact hormone with use of other antibodies relatively specific for N- and C-PTH (1). The range for C-PTH is about double the range for N-PTH and intact hormone, because the biologically inactive C-fragments have a longer half-life than does either active hormone form (intact and N-fragment), and thus tend to accumulate in the serum as metabolic by-products (8, 12). Some of the donors were sampled as many as six times during the two-year study. Resampling patients with suspected primary hyperparathyroidism has been proposed to improve the discrimination among clinical groups based on PTH results alone (13). As shown in Figure 2, the range of values we found from resampling these normal donors is apparently narrower than the range observed by Almquist et al. (13) in resampling 26 patients with suspected hyperparathyroidism. Perhaps there is a tendency toward more erratic PTH production in patients whose calcium regulation is disturbed.

We saw no negative correlation between serum calcium and C-PTH in the normal donors, in contrast to reports with other C-PTH antibodies (10). If, as has been suggested (14), one of the mechanisms for feedback control of PTH activity by increasing serum calcium is increased cleavage of active intact hormone to inactive C-fragments and small inactive N-fragments within the gland, a negative correlation would not be expected. Also in contrast to other reports (10), we did not see a positive correlation between serum calcium and C-PTH in our patients with primary hyperparathyroidism. Although some of the C-PTH results in the hyperparathyroidism patients overlapped the normal range, these patients could be distinguished from normal donors on the basis of their high results for serum calcium.

In patients with chronic renal failure, serum C-PTH as well as N-PTH usually exceeded the reference interval. Many of these patients have a low serum calcium concentration as a result of their primary renal disease (15). PTH secretion is stimulated by chronic hypocalcemia and hyperphosphatemia; the high resulting parathyroid activity may contribute to the toxic symptoms of uremia (16) and may interfere with the success of subsequent renal transplantation (15). Another complication of long-standing renal failure is that chronically stimulated hyperplastic parathyroid glands may become autonomous and continue to secrete large amounts of PTH after the hypocalcemia has been corrected. Monitoring the serum PTH is therefore recommended in the management of chronic renal failure (15).

Results from C-PTH specific assays generally are much higher in these patients than N-PTH results because the normal clearance of C-PTH by the kidney is severely compromised by disease (17). Therefore, patients with increased C-PTH but normal N-PTH values may not be subject to the deleterious effects of high hormone concentrations, because the C-fragments are inactive by-products of secretion. Criteria for the use of one assay or the other remain to be established in clinical studies. From the results in Figure 4 it would seem that the two assays may be used concurrently but not interchangeably during the course of treatment.

PTH assay is frequently used in the differential diagnosis of the etiology of hypercalcemia (18). As a result of the increasing use of multi-test panels in routine health screening, the incidence of hypercalcemia detected has risen to 1% in the general population (19). Of these, 25% are found to have primary hyperparathyroidism and about 50% have malignancies (20). In the present study, all patients with hypercalcemia malignancy tested showed N-PTH and C-PTH results within
or below the reference interval. It has been proposed that the term "ectopic hyperparathyroidism" be used to describe a syndrome in malignancy patients who have hypercalcemia and above-normal PTH results in the absence of hyperplastic or adenomatous parathyroid glands (3, 7). Such non-parathyroid production of PTH was not found in the present series and may be related to cross-reacting substances detected by some other radioimmunoassays (20). Clearly above-normal PTH concentration in the presence of hypercalcemia is believed to be diagnostic of primary hyperparathyroidism. Some overlap of PTH results in malignancy patients with results in patients with primary hyperparathyroidism is expected, because 5–20% of hypercalcemic cancer patients are estimated also to have primary hyperparathyroidism (20, 21). The degree of such overlap in the present study (Figure 6) was not markedly different in the two PTH assays. The more rapid N-PTH assay may be appropriate in the differential diagnosis of life-threatening hypercalcemic crisis such as may occur with hemorrhaging parathyroid adenomas (22) or in non-parathyroid malignancies (20).

The most common treatment of primary hyperparathyroidism is parathyroidectomy (23). The surgery is difficult to perform and can result in inadvertent removal of too much tissue, causing persistent hyperparathyroidism in approximately 15% of cases (24, 25). Because the three to six parathyroid glands may be dispersed throughout the neck and mediastinum, some residual PTH production may remain even after surgery (23). Some measurable PTH was found in the hypoparathyroid patients listed in Table 1, but because half were below the normal range and all were relatively low, the results found were appropriate for hypoparathyroidism.

Pseudohypoparathyroid patients are characterized by low serum calcium with increased PTH because the PTH–receptor interaction is believed to be deficient (25). The PTH and serum calcium results in the patient we examined are consistent with this view. In patients with osteomalacia, serum calcium may be normal or decreased with some increase in PTH (26); again, our findings are confirmatory. Suppression of PTH secretion by the hypercalcemia caused by the direct bone demineralization in thyrotoxicosis (27) is more dramatically expressed in C-PTH results than in N-PTH, but both results are low.

In summary, results for PTH assays that are relatively specific for the N-terminus or for the C-terminus have been compared with serum calcium values in a normal population and in patients with various disorders of calcium regulation. In general, C-PTH results were higher in all categories than N-PTH results. No consistent relationship was found between the results from two assays within any diagnosed group, and the assays probably should not be used interchangeably in any given patient. The ability of the two assays to distinguish among clinical groups was found to be fairly similar in spite of markedly different antibody specificities.

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References