Potential Clinical Utility of a New IRMA for Parathyroid Hormone in Postmenopausal Patients with Primary Hyperparathyroidism

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Background: A new commercially available (so-called second-generation) IRMA for parathyroid hormone (PTH) separately detects intact PTH and its N-truncated fragments; however, no studies have compared the first- and second-generation IRMAs for PTH in patients with primary hyperparathyroidism (PHPT) to assess their respective diagnostic accuracies.

Methods: We concomitantly investigated 39 postmenopausal patients with PHPT and a control group of 70 healthy postmenopausal women matched for age, renal function, and vitamin D status. In all individuals, PTH was measured with a classic IRMA (PTH-S; DiaSorin Inc.), which uses antibodies directed against epitopes 1–34 and 39–84, and a new method (Scantibodies Laboratory Inc.), which uses antibodies against epitopes 1–4 and 39–84 (PTH-W) and epitopes 7–34 and 39–84 (PTH-T). We also assayed serum PTH in 10 PHPT patients every 24 h for 5 days after successful surgery.

Results: The different assays gave serum PTH values that were >2 SD higher than values for the control population in 59% (PTH-S), 77% (PTH-W), and 82% (PTH-T) of patients with PHPT. However, ROC curve analysis showed no significant differences among the three PTH assays, demonstrating overlapping diagnostic sensitivities. In PHPT patients, the correlation among the assays was highly significant ($r = 0.91–0.92; P < 0.001$). The ratio PTH-W:PTH-T × 100 showed a gaussian distribution in both PHPT patients and controls, whose mean (SD) values [63.4 (13.3)% vs 64.5 (9.5)%, respectively] did not differ significantly. After parathyroidectomy, the mean percentages of variation in PTH detected with all of the assays were quite similar.

Conclusions: The distribution of the PTH-W:PTH-T ratio in patients and controls suggests that PHPT does not markedly influence the rate at which biologically inactive fragments are generated by central or peripheral cleavage of PTH. The similar postoperative curves seem to contradict the hypothesized effect of acute hypocalcemia in modulating the central secretion of hormonal fragments. Our results indicate that the three investigated assays have similar diagnostic sensitivities in PHPT.

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Biologically active parathyroid hormone (PTH)4 includes the 84-amino acid peptide produced by the parathyroid glands as well as fragments of various lengths generated by cleavage of the intact molecule within the glands. Different fragments also originate from peripheral metabolism of the intact PTH molecule, i.e., in the liver. Native PTH and its N- and C-terminal fragments are then eliminated by the kidneys (1–3). Because the half-life of the intact molecule is a few minutes, whereas the half-lives of the C-terminal fragments are hours, considerable amounts of C-terminal and mid-molecular fragments accumulate in the blood of patients with renal failure (4, 5).

The classic concept that the biological activity of PTH is exerted exclusively by its N-terminal peptide through interaction with the PTH/PTHrP receptor has been chal-
lenged by recent findings in patients with renal failure. In fact, the PTH C-terminal fragment containing amino acids 7–8, which has been hitherto considered biologically inactive, may actually contribute to the apparent PTH resistance in renal failure because the effects of the intact molecule on the PTH/PTHrP receptor appear to be dose-dependently antagonized by this fragment (2, 6–8).

Several available IRMAs for PTH cross-react with the above-mentioned large C-terminal fragments (9). It has been demonstrated that when serum from both healthy and uremic individuals is fractionated by HPLC, two distinct immunoreactive peaks are obtained that can be detected equally by many commercially available IRMAs for PTH (5, 8, 10).

In an attempt to overcome these shortcomings, Gao et al. (9) introduced a new IRMA in which the labeled antibody recognizes only the first four amino acids of the PTH molecule. This enables separate detection of intact PTH and its N-truncated fragments. Thus, in light of these new discoveries about possible independent activities of C-terminal PTH fragments, a new scenario has been revealed for studies on the pathophysiology of PTH secretion and action, especially in patients with renal failure (11, 12).

The new method has also been used to investigate PTH concentrations in elderly individuals (13). However, data on patients with primary hyperparathyroidism (PHPT) are lacking; to date, preliminary results have been reported only in abstract form (14).

We investigated the diagnostic accuracy of the new method described by Gao et al. (9) in a discrete number of patients with PHPT and in age-matched healthy postmenopausal women and compared it with that of the standard IRMA used in our laboratory for years.

Patients and Methods

Patients

A total of 39 consecutive postmenopausal women with primary hyperparathyroidism [mean (SD) age, 63.9 (8.2) years; mean menopausal duration 16.9 (10.0) years] were prospectively enrolled and all included in the study.

In 24 asymptomatic patients, hypercalcemia was occasionally detected in the course of the standard biochemical evaluation performed in our center before the screening measurement of bone mineral density. Ten patients presented with bone pain, 3 were referred to our center by their general practitioners for metabolic evaluation of nephrolithiasis, and 2 were referred for etiologic definition of hypercalcemia.

Diagnosis of PHPT was made according to conventional clinical and laboratory criteria. All patients without indication for surgery or who refused parathyroidectomy had a history of at least 1 year of prolonged hypercalcemia without evidence of nonparathyroid etiology and had unsuppressed serum concentrations of intact PTH. Familial hypocalciuric hypercalcemia was excluded because in all cases the ratio of calcium clearance to creatinine clearance was >0.01 (15). No patients took medications (including estrogen) known to influence skeletal or mineral metabolism. In 11 patients nephrolithiasis was shown by ultrasonography. Osteoporosis was diagnosed according to densitometric criteria in 18 patients, 10 of whom presented with previous vertebral, hip, or Colles fractures.

In 10 patients the diagnosis of PHPT was confirmed by surgical excision of a parathyroid adenoma. In these latter cases, blood samples were collected preoperatively, 2 h after surgery, and then daily for 6 days; total and ionized calcium, phosphorus, creatinine, total alkaline phosphatase activity, and PTH concentrations were assessed in all blood samples to evaluate the timing of functional recovery of the residual glands.

Because the incidence of PHPT peaks by the time of menopause (16), we concomitantly studied 70 healthy postmenopausal women [mean (SD) age, 65.6 (10.9) years; mean menopausal duration, 17.1 (11.2) years] as a control group. In each woman, history, physical examination, and laboratory tests excluded disorders of bone or mineral metabolism, none of the women in this group took drugs affecting mineral metabolism or bone turnover.

All patients were studied in our referral center between March and May 2002, after giving informed consent. The study was approved by our local Ethics Committee.

Methods

In both patients and controls, fasting blood samples were drawn between 0800 and 0900 in the morning into red and purple-top Vacutainer Tubes for the determination of total calcium (tCa), ionized calcium (iCa; only in PHPT patients), phosphorus, magnesium, creatinine, total alkaline phosphatase activity (AP), 25-hydroxyvitamin D (25OHD), and PTH (by the three methods under study). We also collected 24-h and short fasting urine for measurement of 24 h urinary calcium, phosphorus, and creatinine, or fasting urinary calcium:creatinine ratio and tubular maximum reabsorptive rate for phosphate/gluomeral filtration rate determinations.

tCa, phosphorus, magnesium, creatinine, and AP were measured within 4 h on a multichannel automated analyzer (AutoAnalyzer RA 500; Technicon). In PHPT patients, iCa was measured after sampling by use of an ion-specific electrode (Nova 8; Nova Biochemical). Samples for all radioimmunologic assays were immediately centrifuged, aliquotted, and stored at −20 °C, and assayed within 3 months by one specifically trained research associate who was blinded of the patients’ history and the results of other laboratory tests.

25OHD was measured by RIA (DiaSorin Inc.), as reported previously (17). Intra- and interassay CV were 8.1% and 10%, respectively.

Serum PTH was measured by three immunoradiometric assays. The first one (PTH-S; N-tact PTH SP; DiaSorin Inc.) uses two affinity-purified polyclonal antibodies, one specific for the N-terminal 1–34 portion of the PTH
molecule and the second specific for the 39–84 sequence of the hormone. The lower detection limit of the assay is 1.2 ng/L. The intra- and interassay CV were 3.0% and 5.5% at 30 ng/L and 2.8% and 4.7% at 180 ng/L, respectively. The other two IRMAs for PTH (Duo PTH; Scantibodies Laboratory, Inc.) both use the same polyclonal-antibody-coated solid-phase for the 39–84 region of the PTH molecule. The specificities of these two assays are attributable to the specificities of the detection antibodies. The first assay, PTH Whole (PTH-W), uses a second, 125I-labeled polyclonal antibody directed to the 1–4 portion of PTH, i.e., specific for the N-terminal region capable of binding to the PTH/PTHrP receptor. The second IRMA in the Duo Kit, PTH Total (PTH-T), uses a 125I-labeled antibody specific for the 7–34 region of the hormone, and thus is capable of binding to both the intact molecule and its 7–84 fragment. The N-terminally (1–6) truncated PTH fragment (PTH N-tr) concentrations are obtained by subtracting the PTH-W value from the PTH-T value. The lower detection limits are 1.1 ng/L for PTH-W and 1.3 for PTH-T. The intra- and inter assay CV for PTH-W were 3.5% and 5.8% at 30 ng/L and 2.8% and 4.2% at 180 ng/L, respectively, and the intra- and inter assay CV for PTH-T were 4.1% and 4.9% at 30 ng/L and 3.1% and 3.4% at 180 ng/L, respectively. The results are continuous variables, with the upper limits of the reference interval defined as the mean + 2 SD.

**Statistical Analysis**

Statistical analysis was performed by use of SPSS, Ver. 10 (SPSS Inc.). Descriptive statistics are expressed as the mean (SD). After a test for normality, mean age, menopausal duration, and 25OHD concentrations of patients and controls were compared by an unpaired t-test or Mann–Whitney U-test, as appropriate. Statistical significance was set as P < 0.05. Finally, ROC curves (with 95% confidence intervals) were designed to identify the PTH assay that provided the best differentiation between healthy individuals and PHPT patients. The comparison among ROC curves was performed by the Wilcoxon signed-rank test.

**Results**

The main biochemical indices of calcium homeostasis (Table 1) showed that PHPT patients and matched healthy women did not differ for age, renal function, and vitamin D status. The mean (SD) tCa concentration in PHPT patients was 1.49 (0.14) mmol/L.

In our evaluation of the overall clinical diagnostic sensitivities of the assays, PTH values were more than 2 SD above the distribution for healthy individuals in 59% of patients by the PTH-S assay, in 77% of patients by the PTH-W assay, in 82% of patients by the PTH-T assay, and in 67% of patients by the PTH N-tr assay (Fig. 1). On the other hand, as illustrated in Fig. 2, the ROC curves of the four assays did not significantly differ in terms of areas under the curves, indicating that the four methods showed overlapping diagnostic sensitivities. The areas under the curves (with 95% confidence intervals) were 0.916 (0.859–0.972) for the PTH-S assay, 0.960 (0.917–1.003) for the PTH-W assay, 0.965 (0.921–1.008) for the PTH-T assay, and 0.898 (0.824–0.973) for the PTH N-tr assay.

The correlation matrix of the various PTH assays tested in patients with PHPT is shown in Table 2. The four assays showed very similar correlations with the main indices of mineral metabolism (data not shown).

The frequency distributions of the PTH-W:PTH-T ratio, which represents the percentage of biologically active PTH with respect to the total immunoreactive intact PTH, followed an almost gaussian distribution in both patients

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<thead>
<tr>
<th>Table 1. Main biochemical indices for patients with PHPT and age-matched healthy postmenopausal women.</th>
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<td>Mean (SD)</td>
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<td>tCa, mmol/L</td>
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<tr>
<td>Phosphorus, mmol/L</td>
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<tr>
<td>Magnesium, mmol/L</td>
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<tr>
<td>Creatinine, µmol/L</td>
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<td>CIcr, mL/s</td>
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<td>AP, U/L</td>
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<tr>
<td>PTH-S, ng/L</td>
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<tr>
<td>PTH-W, ng/L</td>
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<td>PTH-T, ng/L</td>
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<td>PTH-Ntr, ng/L</td>
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*NS, not significant; CIcr, creatinine clearance.

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**Fig. 1.** Serum PTH concentrations in patients with primary hyperparathyroidism, as measured by the different assays. Boxes show mean values (± 2 SD) of healthy age-matched postmenopausal women. PTH S, DiaSorin PTH assay; PTH W, PTH Whole assay (Scantibodies); PTH T, PTH Total assay (Scantibodies); PTH N-tr, N-truncated fragments of PTH (Scantibodies).
and controls (Fig. 3). The mean values [63.4 (13.3)% vs 64.5 (9.5)% for patients and controls, respectively] did not differ significantly.

In PHPT patients, we found no significant correlation between PTH-W:PTH-T ratio and iCa, AP, or 25OHD.

The mean postsurgical percentage variations in PTH, as detected by the four assays under study, are shown in Fig. 4. The patterns of the two patients with creatinine clearance <40 mL/min are illustrated separately in the top panel of Fig. 4; patients with normal renal function were grouped according to whether they needed (Fig. 4, middle panel) or not (Fig. 4, bottom panel) therapy with active vitamin D metabolites after surgery. As illustrated, the pattern and timing of recovery of PTH secretion were quite similar as measured by the four investigated assays except for PTH N-tr in patients with renal failure.

**Discussion**

In recent years the introduction of assays using two different antibodies with affinities for distinct epitopes within the PTH molecule have allowed accurate measurement of intact PTH, improving diagnostic and clinical performance in parathyroid and skeletal disorders. Even more recently, Gao et al. (9) developed a new method encompassing two assays capable of separately identifying both the intact biologically active molecule (PTH-W) and the 7-34 portion of the PTH molecule (PTH-T). The latter fragment is not able to bind to PTH receptor, having lost the first 6 amino acids (1). Moreover, it is possible to estimate the concentrations of PTH fragments truncated at the N-terminal region (PTH N-tr), which accumulate in substantial amounts in the blood of patients with renal failure, where they account for up to 50% of immunoreactivity. In the past, the latter fragments were considered to be biologically inactive; however, a possible physiologic role for these fragments has recently been hypothesized after a receptor specific for the C-terminal region of PTH was discovered in cells of an osteoblastic/osteocyte lineage (6). Furthermore, in animal models, the administration of human PTH(1-84) and human PTH(1-34) did not give quite overlapping responses. These findings

### Table 2. Linear correlation among the three assays for PTH in patients with PHPT (n = 39).

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<tr>
<th></th>
<th>PTH-S</th>
<th>PTH-W</th>
<th>PTH-T</th>
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<tr>
<td>PTH-W</td>
<td></td>
<td></td>
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<tr>
<td>r</td>
<td>0.908</td>
<td></td>
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<tr>
<td>P</td>
<td>&lt;0.001</td>
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<td>PTH-T</td>
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<tr>
<td>r</td>
<td>0.918</td>
<td>0.921</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<tr>
<td>PTH-N-tr</td>
<td></td>
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<tr>
<td>r</td>
<td>0.705</td>
<td>0.589</td>
<td>0.857</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
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suggest that the intact molecule may also interact with the receptors for the C-terminal region of PTH, which in turn exert antagonistic effect on the biological actions of 1–84 PTH (2, 6). Such results further promoted interest for new assays capable of distinguishing the intact molecule from its large C-terminal fragments.

Our study compares a new assay with the conventional IRMA used in our laboratory, which uses two different polyclonal antibodies for the 1–34 and the 39–84 portions of PTH. It should be noted that in the study by Gao et al. (9) the new method was matched with another assay that detected predominantly, but not exclusively, the intact molecule. Furthermore, to the best of our knowledge, our study is the first investigation of a discrete sample of PHPT patients published in detail; previously available data were published only as an abstract (14).

Our results confirm that even with the newest assays simultaneous measurement of PTH and calcium is mandatory for the diagnosis of PHPT and other parathyroid disorders (1, 18) because increased concentrations of PTH are not detected in 100% of patients, regardless of the assay used. Moreover, analysis of the ROC curves showed that the investigated assays did not significantly differ in terms of diagnostic sensitivity, although a slightly higher percentage of patients had values in the PTH-W and PTH-T assays that were above the reference intervals. Furthermore, the very good correlations among the PTH-S, -W, and -T assays (r = 0.91–0.92; P < 0.001) indirectly confirm their almost equal potential diagnostic utility, whereas the correlations with PTH N-tr were somewhat weaker. The diagnostic sensitivity was lower than in a previous report (14), probably because we included in our sample many asymptomatic patients or patients with mild PHPT. In this currently most common presentation of the disease, PTH concentrations are usually only slightly increased. On the other hand, the validity of our results is corroborated by the concomitant investigation of the control group of healthy women. We therefore avoided several pitfalls potentially generated by the use of the ‘historical’ reference interval of a center, attributable to methodologic, technical, or seasonal discrepancies.

Overall, the correlations between PTH N-tr values and the biochemical indices of PTH activity were lower than those for the PTH-S, -W, and -T assays (data not shown). The correlation between PTH N-tr values and AP (r = 0.318; P < 0.05) was lower than those observed for the PTH-S, -W, and -T assays. This seems to indicate that even in PHPT patients the accumulation of C-terminal fragments of PTH could decrease bone turnover rate (2, 6, 7); likewise it has been speculated in patients with chronic renal failure (2).

Because of the short half-life of the hormone, several previous reports dealt with the decrease in immunoreactive PTH after adenoma excision (19, 20). The current study investigated the relatively long-term recovery of residual parathyroid glands made atrophic by excess PTH secretion from the adenoma. Our results showed that in patients with normal or slightly reduced renal function, provided that the surgery had not damaged glandular tissue or its vascular supply, functional recovery of parathyroid glands started from the second and lasted up to the sixth day after surgery. In patients with persistent postsurgical hypocalcemia, however, PTH values measured by the four methods remained near the lower limit of the reference interval, which implies that residual glands were not able to react to the hypocalcemic challenge. The similar postoperative behavior of PTH measured by the different assays seems to contradict the hypothesis that serum calcium can modulate the secretion both of the intact hormone and its fragments at the glandular level. This also appears to be confirmed by the
lack of correlation between the PTH-W:PTH-T ratio and iCa values (data not shown).

On the other hand, as shown in Fig. 3, we found a wide and very similar distribution of the PTH-W:PTH-T ratio in both healthy women and PHPT patients. This reflects the remarkable individual variations in the rate at which both glandular secretion and peripheral clearance, which seem not modified by the disease.

We have identified the following limitations of our study: (a) we exclusively included postmenopausal women; therefore, our results could not be generalized; (b) the patients sample was not homogeneous in term of clinical presentation and biological effects on target organs; therefore, our population might differ from those investigated in other centers or in other countries; and (c) we can not exclude that a statistical difference may be demonstrable in a larger sample of patients.

In conclusion, our study showed that PTH-W and PTH-T measurements did not significantly improve diagnostic sensitivity in patients with PHPT. The assessment of N-truncated fragments did not provide clinically meaningful information in PHPT patients with normal or slightly altered renal function compared with those obtained by assessing the intact molecule. At present, this should be considered in conjunction with the increased cost of repeated measurements. However, the relevance of this new method could be reconsidered in light of our growing knowledge about the interaction of the C-terminal fragments of PTH with both the classic and the recently discovered specific PTH receptors (6, 7, 11).

References