Novel Parathyroid Hormone (1-84) Assay as Basis for Parathyroid Hormone Monitoring in Renal Hyperparathyroidism

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Hypothesis: Cross-reactivity of parathyroid hormone (PTH) fragments with immunometric “intact” PTH assays limited the use of intraoperative PTH monitoring in renal hyperparathyroidism. A new assay generation measuring whole PTH (1-84) should be able to predict complete or incomplete resection of hyperfunctioning parathyroid tissue.

Design: Consecutive series for evaluation of intraoperative PTH monitoring using a second-generation assay.

Setting: University hospital section of endocrine surgery.

Patients: Twenty-two patients received hemodialysis; 9 patients showed good and 4 patients reduced graft function after kidney transplantation.

Interventions: Total parathyroidectomy, central neck dissection, bilateral thymectomy, and immediate autotransplantation was the standardized approach in 35 consecutive patients. Blood samples were drawn before incision and at 5-minute intervals after excision of the last gland. Stored samples were analyzed using a “second-generation” assay (Bio-Intact PTH [1-84]; Nichols Institute Diagnostics, San Clemente, Calif). Parathyroidectomy was classified as total, subtotal, or insufficient according to first-generation intact PTH values in the first postoperative week.

Main Outcome Measures: Intraoperative ability to predict total, subtotal, or incomplete parathyroidectomy.

Results: Independent of renal function, Bio-Intact PTH dropped into the normal range in all patients with total and subtotal resections after a maximum of 20 minutes. It indicated insufficient parathyroidectomy in 4 (80%) of 5 patients. One failure was caused by devascularization of remaining parathyroid tissue. An intraoperative differentiation between total and subtotal resection was not possible.

Conclusions: Intraoperative monitoring with quick, second-generation assays for PTH (1-84) seems to be a valuable new tool in surgery for renal hyperparathyroidism because a more accurate differentiation between sufficient and insufficient parathyroidectomy may be achieved. An intraoperative decision about the need for immediate or delayed autotransplantation seems impossible because a differentiation between total or subtotal parathyroidectomy cannot be made. Because of possible devascularization of parathyroid tissue, Bio-Intact PTH monitoring can only be interpreted in the context of the operative findings.

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Surgery for renal hyperparathyroidism (RHPT) aims at sufficient reduction of hyperfunctioning parathyroid tissue. However, a total loss of parathyroid hormone (PTH) secretion should be avoided to restore normal bone metabolism. To define the extent of surgery necessary to minimize the risk of persistent or recurrent RHPT caused by supernumerary glands left in situ, and to determine the need for immediate or delayed autotransplantation of parathyroid tissue, it would be desirable to monitor the decrease of the exaggerated PTH secretion intraoperatively.

See Invited Critique at end of article

It has been shown recently that intraoperative PTH monitoring is of limited value during surgery for RHPT using “first-generation,” quick intact PTH (iPTH) immunometric assays because complete or incomplete resection of hyperfunctioning parathyroid tissue cannot be predicted correctly. The potential reason for
this is an overestimation of whole PTH (1-84) values due to cross-reactivity of currently available first-generation PTH assays to non–PTH (1-84) fragments that accumulate in renal failure. Therefore, second-generation PTH immunometric assays claiming to detect the biologically active PTH (1-84) molecule without cross-reactivity to PTH fragments have been developed.

The aim of this study was to evaluate a second-generation PTH assay concerning its potential ability to predict incomplete resection. The influences of hemodialysis or renal function after kidney transplantation were considered, thus outlining standards for the use of second-generation quick PTH assays for intraoperative monitoring of parathyroid function in RHPT.

**METHODS**

**PATIENTS**

This study included 35 consecutive patients undergoing primary surgery for RHPT. The median ±SD age at surgery was 48.4 ± 12.7 years. Twenty-two patients (63%) with RHPT were receiving continuous hemodialysis (secondary HPT). Thirteen patients (37%) had persistent RHPT after kidney transplantation, the 9 in group B showing good graft function (serum creatinine levels, <2 mg/dL) and 4 in group C showing reduced graft function (serum creatinine levels, >2 mg/dL). All patients were followed up postoperatively at regular intervals for at least 6 months for up to 4 years (mean ±SD follow-up, 34 ± 13 months).

Parathyroidectomy was judged within the first postoperative week using the commercially available first-generation iPTH assays defined earlier. It was considered total in patients who had iPTH levels less than 10 pg/mL (subgroup 1), considered subtotal for iPTH levels between 10 and 65 pg/mL (subgroup 2), and considered insufficient for iPTH levels greater than 65 pg/mL (subgroup 3).

Persistence was defined as the presence of the typical biochemical findings, symptoms, and signs of RHPT immediately postoperatively or within a period of up to 6 months. An occurrence of elevated iPTH levels and clinical symptoms later than 6 months postoperatively was defined as recurrence.

Groups A, B, and C were analyzed separately regarding differences between subgroups 1, 2, and 3 concerning absolute Bio-iPTH (1-84) and relative Bio-iPTH (1-84) decay after removal of the last parathyroid gland. In Figures 1, 2, and 3, the percentages of patients of each group and subgroup reaching the normal range of Bio-iPTH (1-84) levels are presented in 5-minute steps up to 30 minutes after excision of the last gland.

**BLOOD SAMPLING**

Blood samples were drawn from a peripheral artery. The first sample was taken right after induction of anesthesia but before any kind of neck manipulation. Further samples were collected every 5 minutes over a maximal period of 50 minutes after the removal of the last gland.

Sample aliquots were stored at −80°C. Because of the lack of a quick whole PTH (1-84) assay, the hormone levels were analyzed after surgery by a Nichols Advantage Chemiluminescent System (Nichols Institute Diagnostics, San Clemente, Calif). The Bio-Intact PTH (1-84) assay (Bio-iPTH; Nichols Institute Diagnostics) was used to detect the biologically active PTH (1-84) molecule without cross-reactivity to non–PTH (1-84) fragments.

This assay uses a polyclonal antibody toward the first 6 amino acids of the N-terminal part of the PTH molecule. The second antibody is directed toward the mid- and C-terminal part of the molecule (amino acid sequence 39-84). The test shows an in assay variation of less than 4% and an interassay variation of less than 10%. The normal range is 8 to 50 pg/mL, the analytical sensitivity is 1.5 pg/mL, and the functional sensitivity is 4 pg/mL.

For intraoperative monitoring and during postoperative follow-up, PTH levels were determined using commercially available first-generation iPTH assays running on Elecsys 1010, Elecsys 2010, and Modular autoanalyzers (Roche Diagnostics, Mannheim, Germany). These systems are routinely used in our institution. Normal iPTH values range from 15 to 65 pg/mL.

**DEFINITION OF PATIENTS**

According to their kidney function, patients were divided into 3 groups. Group A consisted of 22 patients (63%) receiving hemodialysis. Thirteen patients (37%) had persistent RHPT after kidney transplantation, the 9 in group B showing good graft function (serum creatinine levels, <2 mg/dL) and 4 in group C showing reduced graft function (serum creatinine levels, >2 mg/dL). All patients were followed up postoperatively at regular intervals for at least 6 months for up to 4 years (mean ±SD follow-up, 34 ± 13 months).

Parathyroidectomy was judged within the first postoperative week using the commercially available first-generation iPTH assays defined earlier. It was considered total in patients who had iPTH levels less than 10 pg/mL (subgroup 1), considered subtotal for iPTH levels between 10 and 65 pg/mL (subgroup 2), and considered insufficient for iPTH levels greater than 65 pg/mL (subgroup 3).

Persistence was defined as the presence of the typical biochemical findings, symptoms, and signs of RHPT immediately postoperatively or within a period of up to 6 months. An occurrence of elevated iPTH levels and clinical symptoms later than 6 months postoperatively was defined as recurrence.

Groups A, B, and C were analyzed separately regarding differences between subgroups 1, 2, and 3 concerning absolute Bio-iPTH (1-84) and relative Bio-iPTH (1-84) decay after removal of the last parathyroid gland. In Figures 1, 2, and 3, the percentages of patients of each group and subgroup reaching the normal range of Bio-iPTH (1-84) levels are presented in 5-minute steps up to 30 minutes after excision of the last gland.
RESULTS

GROUP A

Group A consisted of 22 patients receiving hemodialysis. According to the iPTH levels obtained with first-generation iPTH assays during the first postoperative week, parathyroidectomy was considered total in 13 patients (subgroup 1), subtotal in 4 patients (subgroup 2), and insufficient in 5 patients (subgroup 3). Mean ± SD Bio-iPTH levels at baseline were 244 ± 259.9 pg/mL, 208.9 ± 115.2 pg/mL, and 274.5 ± 280.2 pg/mL among subgroups 1, 2, and 3.

As shown in Figure 1, in all patients of subgroups 1 and 2, Bio-iPTH levels dropped into the normal range (<50 pg/mL) within 20 minutes and 10 minutes after excision of the last gland, respectively. Twenty minutes after extirpation of the last gland, mean ± SD Bio-iPTH levels were 20.96 ± 8.22 pg/mL (15.97 ± 8.54% of baseline) among subgroup 1 and 27.23 ± 4.33 pg/mL (21.15 ± 19.31% of baseline) among subgroup 2.

The lowest mean ± SD Bio-iPTH values were 14.1 ± 3.3 pg/mL in subgroup 1 and 15.1 ± 4.5 pg/mL in subgroup 2. Between subgroup 1 and 2, no statistical differences in the absolute or relative Bio-iPTH decay curves were observed at any sampling time.

In subgroup 3, Bio-iPTH levels indicated insufficient parathyroidectomy in 4 (80%) of 5 patients. Mean ± SD Bio-iPTH levels were 68.44 ± 47.54 pg/mL (42.29 ± 36.8% of baseline) 20 minutes after removal of the last gland. In 1 of the 4 patients, Bio-iPTH levels temporarily decreased from 283.7 pg/mL at baseline to 82.7 pg/mL at 10 minutes, 53.6 pg/mL at 20 minutes, and 43.9 pg/mL at 30 minutes and increased again to 60 pg/mL at 35 minutes and 80.4 pg/mL at 40 minutes.

In the fifth patient, only 3 glands could be found, leading to a more extensive cervical exploration to localize the fourth gland. In the course of this exploration, Bio-iPTH steadily dropped from 189.6 pg/mL at baseline to 77.5 pg/mL at 10 minutes, 26.6 pg/mL at 20 minutes, and 19.4 pg/mL at 30 minutes, reaching the lowest level of 13.3 pg/mL at 40 minutes after extirpation of the third gland. Although PTH levels initially stayed in the normal range, biochemistry documented persistence due to the missed fourth gland at the end of the first postoperative week.

Twelve (92%) of 13 patients of subgroup 1 (total parathyroidectomy) had permanent normalization of parathyroid metabolism during follow-up. One patient developed a graft-dependent recurrence 46 months postoperatively.

All 4 patients with subtotal resections (subgroup 2) were cured, whereas all 5 patients classified as insufficient parathyroidectomy (subgroup 3) had persistent hypoparathyroidism by definition. This seemed to be caused by hyperfunctioning parathyroid tissue left in the neck or mediastinum in all patients, although at least 4 glands were removed in 4 patients and 3 glands in 1 patient.

GROUP B

In group B, 8 of 9 patients with kidney grafts and serum creatinine levels less than 2 mg/dL had postoperative iPTH levels indicating total parathyroidectomy (subgroup 1). By definition, 1 patient had subtotal parathyroidectomy (subgroup 2). Insufficient parathyroidectomy (subgroup 3) was not observed.

Group B had mean ± SD Bio-iPTH levels of 70.6 ± 62.8 pg/mL (subgroups 1 and 2) at baseline, which were lower than those in group A. Ten minutes after excision of the last gland, Bio-iPTH levels dropped into the normal range in all patients of subgroup 1. This was the case 5 minutes after the last gland had been removed in the patient of subgroup 2 (Figure 2). Twenty minutes after extirpation of the last gland, mean ± SD Bio-iPTH levels were 13.81 ± 8.79 pg/mL (15.13 ± 6.61% of baseline) among subgroup 1 and 1.8 pg/mL (20% of baseline) in the patient.
with subtotal parathyroidectomy. The lowest mean ± SD Bio-iPTH levels were 12.3 ± 8.4 pg/mL in subgroup 1 and 1.3 pg/mL in subgroup 2. When interpreting the relative or absolute Bio-iPTH decay curves, making a differentiation between subgroups 1 and 2 was not possible. All patients of subgroups 1 and 2 had permanent normalization of calcium and PTH levels.

**GROUP C**

In group C, 2 of 4 patients with reduced renal function after kidney transplantation (serum creatinine levels > 2 mg/dL) belonged to subgroup 1 (total parathyroidectomy) and 2 patients to subgroup 2 (subtotal parathyroidectomy). Mean ± SD Bio-iPTH levels at baseline were 102.5 ± 1.9 pg/mL in subgroup 1 and 127.2 ± 39.8 pg/mL in subgroup 2. The normal range was reached in all patients at 10 and 15 minutes after removal of the last gland in subgroups 1 and 2, respectively (Figure 3). Twenty minutes after excision of the last gland, Bio-iPTH levels were 24.9 ± 1.3 pg/mL (24.3 ± 0.1% of baseline) among subgroup 1 and 33.3 ± 6.65 pg/mL (26.68 ± 0.03% of baseline) in subgroup 2. The lowest Bio-iPTH levels were 15.7 and 17.7 pg/mL in subgroup 1 and 17.7 and 28.8 pg/mL in subgroup 2. The relative and absolute Bio-iPTH decay was similar in both subgroups. A clear difference between subgroups 1 and 2 was not seen at any sampling time.

No differences between both subgroups were encountered during follow-up. Total and subtotal resection provided sufficient reduction of hypersecreting parathyroid tissue.

**COMMENT**

When medical treatment fails, surgery is indicated to normalize PTH hypersecretion in RHT. It is important to achieve sufficient reduction of hyperfunctioning parathyroid tissue while taking into account that in RHPT, the total amount of parathyroid tissue in orthotopic and ectopic locations becomes stimulated. However, to restore normal bone metabolism, a total loss of PTH secretion should be avoided.

Surgical strategies include subtotal (<3/4) parathyroidectomy and total parathyroidectomy, including transcervical thymectomy with immediate or, as a discussed alternative, delayed autotransplantation. All techniques require extensive bilateral neck exploration, which may increase local complications. A limited exploration could minimize these complications. The ability to predict complete or at least sufficient resection could determine the extent of surgical exploration.

After total parathyroidectomy and immediate autotransplantation, recurrence of RHPT may be caused by the parathyroid autograft or by hyperfunctioning ectopic parathyroid tissue left in situ during the primary operation. Thus, a differentiation between total and subtotal parathyroidectomy would be desirable. Only the subgroup with total parathyroidectomy would require immediate autotransplantation, whereas the subgroup with subtotal parathyroidectomy would have enough parathyroid tissue left to avoid a total loss of PTH secretion. If this still happened postoperatively, delayed autotransplantation of cryopreserved parathyroid tissue could be an alternative. In patients with insufficient (incomplete) reduction of parathyroid hyperactivity, immediate parathyroid autotransplantation must be avoided.

In contrast with primary hyperparathyroidism, where adequate resection of the hyperfunctioning parathyroid glands can be documented by intraoperative PTH monitoring using one of the first-generation immunometric quick iPTH assays, this could not be achieved in RHPT and it was impossible to correctly predict insufficient reduction of the hyperactive parathyroid metabolism leading to persistent RHPT. The main reason for this failure of PTH monitoring in RHPT can be attributed to an overestimation of whole PTH (1-84) values due to cross-reactivity to non–PTH (1-84) fragments that accumulate in renal failure.

New, second-generation PTH assays that claim to detect the biologically active PTH (1-84) molecule without cross-reactivity to PTH fragments have been introduced by Scantibodies Laboratories Inc (San Diego, Calif) and Nichols Institute Diagnostics. Recently, a quick assay for intraoperative Bio-iPTH monitoring became available from the latter company. This might help address the aforementioned demands on a patient and situation-adapted surgical strategy.

This study used the regular Nichols Bio-Intact PTH (1-84) assay for analyzing frozen samples taken during surgery. Retrospectively, the decrease in intraoperative PTH was analyzed to study the possible value of this new assay for intraoperative Bio-iPTH monitoring.

To overcome the drawbacks of former studies describing the use of intraoperative PTH monitoring in patients with RHPT, the surgical strategy was standardized for all patients independent of their renal function (total parathyroidectomy, central neck dissection, bilateral thymectomy, and immediate autotransplantation). The serum PTH samples were drawn to get information concerning the optimal time for discrimination between sufficient and insufficient exploration. Additionally, patients were grouped according to their renal function. A clear definition of total (subgroup 1) and subtotal (subgroup 2) parathyroidectomy was based on iPTH measurements within the first postoperative week and biochemical and clinical long-term follow-up examinations.

The most important issue of intraoperative PTH monitoring is to predict whether the resection was incomplete although 4 glands are removed. This would have the consequence of an additional search for supernumerary ectopic glands.

Independent of the renal function, a clear differentiation between sufficient (total/subtotal) and insufficient parathyroidectomy was possible in 34 (97%) of 35 patients. After sufficient parathyroidectomy, normal Bio-iPTH levels were demonstrated in patients receiving hemodialysis, patients with kidney grafts and reduced graft function, and patients with normal kidney graft function within 20, 15, and 10 minutes, respectively. No auxiliary criteria such as 60% PTH decay at 10 minutes, 82% decay at 15 minutes, and greater than 50% decay at 20 minutes after removal of the last gland are necessary if Bio-iPTH monitoring is used intraoperatively.
Sufficient reduction of hyperfunctioning parathyroid tissue was confirmed by a low probability of recurrence as shown by long-term cure of 29 (97%) of 30 patients of both subgroups 1 and 2. One patient receiving hemodialysis developed recurrence after total parathyroidectomy caused by a graft-dependent recurrence 46 months postoperatively.

In our analysis, 5 patients underwent insufficient parathyroidectomy. All belonged to the group of patients receiving hemodialysis. In 4 of these patients (80%), Bio-iPTH monitoring correctly indicated incomplete resection although 4 glands were removed. The possibility to use a quick Bio-iPTH assay intraoperatively would lead to a search for strongly suspected further ectopic supernumerary hyperfunctioning parathyroid glands. In 1 of these patients, Bio-iPTH stayed above the normal range at 10 minutes (82.7 pg/mL) and 20 minutes (53.6 pg/mL) after excision of the last gland, then temporarily dropped into the normal range at 30 minutes (43.9 pg/mL), and increased again at 35 and 40 minutes (60 and 80.4 pg/mL, respectively). This shows that it seems important to analyze the whole curve and not to stop PTH measurements as soon as the normal range is reached once.

In another 1 (20%) of these 5 incompletely resected patients, Bio-iPTH steadily dropped and stayed in the normal range for 40 minutes after exteriorization of the third enlarged parathyroid gland. To look for the missed fourth gland, a more extensive exploration was performed. In such circumstances, the venous drainage of remaining parathyroid tissue may be devascularized and results of Bio-iPTH monitoring should only be interpreted with caution. Therefore, a decrease of Bio-iPTH into the normal range must not be regarded as a guaranteed sign of complete resection because Bio-iPTH monitoring can only be interpreted in the context of the operative findings. Because in all patients who had undergone sufficient (total/subtotal) parathyroidectomy Bio-iPTH levels dropped into the normal range within a maximum of 20 minutes and 4 of 5 patients after insufficient resection were above the normal range 20 minutes after excision of the last gland, this might be a recommendable point to determine sufficient or insufficient resection. This has to be proven in future prospective studies on intraoperative Bio-iPTH monitoring.

However, with the new tool of measuring the biologically active PTH (1-84) molecule, it would be impossible to accurately predict whether a patient belongs to subgroup 1 or 2 (total or subtotal parathyroidectomy). Therefore, an intraoperative “titration” of the amount of parathyroid tissue to be resected is not feasible. Based on these preliminary data, it seems impossible to separate patients who need immediate autotransplantation from those who would be candidates for delayed autografting of cryopreserved parathyroid tissue. In all patients, immediate parathyroid autografting was performed, which is theoretically unnecessary in the subtotal group.

This preliminary retrospective study documents that Bio-iPTH monitoring seems to be a valuable new tool in surgery for RHPT. The common use of an intraoperative, quick Bio-iPTH assay seems mandatory because it might facilitate a more accurate differentiation between sufficient and insufficient parathyroidectomy in RHPT, which is not possible with the commonly used first-generation quick PTH assays. Sufficient reduction of hypersecreting parathyroid tissue can be reliably predicted. However, an intraoperative titration of the amount of parathyroid tissue to be resected and a decision about the need for immediate or delayed autotransplantation is impossible because a differentiation between total or subtotal parathyroidectomy cannot be made. Because of the chance of devascularization of remaining parathyroid tissue during extensive neck exploration, Bio-iPTH monitoring can only be interpreted in the context of the operative findings.

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Intraoperative PTH testing has revolutionized the way we treat patients with primary hyperparathyroidism (HPT) by allowing focussed, “minimally invasive” procedures targeting resection of a single parathyroid adenoma. As shown by Irvin et al, Udelsman et al, our group, and others, intraoperative PTH testing (with first-generation assays) allows detection and subsequent resection of additional hyperfunctioning parathyroid glands intraoperatively, thereby improving cure rates following parathyroidectomy. Although the importance of intraoperative PTH testing in patients with primary HPT has been defined, its role in patients with secondary HPT is unclear.

Dr Kaczirek and colleagues have published very important studies addressing this issue. In an article published in the April 2005 issue of Surgery, the authors studied 35 patients with renal HPT undergoing parathyroidectomy and reported that first-generation intraoperative PTH assays were not useful to predict cure following surgery. This is presumably because first-generation intraoperative PTH assays can be falsely elevated due to nonfunctioning PTH fragments often present in patients with renal dysfunction. In this article in the ARCHIVES, the authors retrospectively tested samples from the same 35 patients using a “second-generation” Bio-Intact PTH assay, which has been shown to not cross-react with inactive PTH fragments and which provides a more accurate measure of functional PTH. The authors conclude that the Bio-Intact PTH assay is more accurate for intraoperative PTH monitoring in patients with renal HPT.

In patients with renal HPT, it is essential to distinguish those with compromised renal function (secondary HPT) from those with good renal function after transplantation (tertiary HPT). In patients with tertiary HPT, first-generation intraoperative PTH assays have recently been shown to be reliable and can accurately alter management in 15% of patients undergoing parathyroid surgery. Thus, the remaining question is whether or not second-generation intraoperative PTH assays are beneficial for patients with compromised renal function having parathyroid surgery. Kaczirek and colleagues have clearly shown that the Bio-Intact assay is more accurate in predicting cure, but is it beneficial and practical to use intraoperatively? In the 5 patients who were not cured, if the surgeons had the results of the Bio-Intact PTH assay, could they have altered the operation to achieve surgical cure, despite an extensive bilateral exploration and cervical thymectomy? Second, for patients with primary and tertiary HPT, intraoperative PTH sampling is done 5 or 10 minutes after resection and the analyzer requires 9 minutes to run the first-generation assay: a total of 14 to 19 minutes. With the Bio-Intact assay in patients with secondary HPT, the samples are drawn 20 to 30 minutes after resection and the analyzing time is longer: a total of approximately 40 to 60 minutes. Is it practical to wait an hour with every patient for laboratory results that could potentially alter the management in only a few patients? Third, in this study, each patient required up to a half-dozen arterial blood samples. What is the potential morbidity of this? For these answers, we will have to wait until a prospective study with the Bio-Intact assay is performed. Until then, the role for intraoperative PTH monitoring in patients with secondary HPT still remains unclear.

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