A quick intraoperative parathyroid hormone assay in the surgical management of patients with primary hyperparathyroidism: a study of 206 consecutive cases

E Vignali, A Picone, G Materazzi, S Steffe, P Berti, L Cianferotti, F Cetani, E Ambrogini, P Miccoli, A Pinchera and C Marcocci

Dipartimento di Endocrinologia e Metabolismo, Ortopedia e Traumatologia, Medicina del Lavoro, Dipartimento di Chirurgia and Dipartimento di Matematica, Università di Pisa, Via Paradisa 2, 56124 Pisa, Italy

(Correspondence should be addressed to C Marcocci; Email: c.marcocci@endoc.med.unipi.it)

Abstract

Objective: The traditional surgical approach for patients with primary hyperparathyroidism (PHPT) consists of the identification of at least four glands and in the removal of all hyperfunctioning parathyroid tissue.

Design: To evaluate whether intraoperative parathyroid hormone (PTH) monitoring will allow a more limited surgical procedure by confirming complete removal of all hyperfunctioning tissue.

Methods: Plasma samples were obtained from 206 consecutive patients with sporadic PHPT before skin incision, during manipulation of a suspected adenoma, and 5 min (T-5) and 10 min after removal of abnormal parathyroid tissue. PTH was measured by a quick immunochemiluminescent assay (QPTH). The operative success was defined by a decrease of PTH greater than 50% of the highest pre-excision value.

Results: A > 50% decrease of PTH occurred in 203 patients and was evident at T-5 in the majority of cases. All but three had normal serum calcium the day after surgery and afterwards. PTH concentration did not show a > 50% decrease in the remaining three cases after completion of surgery. One patient had negative neck exploration and remained hypercalcemic; the other two had normal serum calcium at follow-up. Thus, the intraoperative QPTH correctly predicted the outcome of surgery in 201 patients (97.5%) (200 true positive and 1 true negative), and provided three false positive and two false negative results.

Conclusions: The intraoperative QPTH measurement represents a useful tool to assist the surgeon during parathyroidectomy. It indicates whether all hyperfunctioning parathyroid tissue has been removed, limiting the procedure to a unilateral neck exploration in most cases.

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Introduction

The primary goal of parathyroidectomy for patients with primary hyperparathyroidism (PHPT) is to achieve normal levels of serum calcium and parathyroid hormone (PTH), with minimal complications and efficient use of operating time and resources (1). The most important variable that influences the success of parathyroidectomy is the experience of the surgeon. In very experienced hands the rate of cure is about 95% of cases (2), but in less experienced hands the rate of persistence of the disease can be as high as 15% of cases (3).

The standard surgical procedure is to explore one or preferably both sides of the neck and to identify at least four parathyroid glands. If an abnormal gland is found and removed, its parathyroid nature should be confirmed by frozen section. To exclude the possibility of a multiglandular disease, a biopsy of an adjacent normal-appearing parathyroid gland should be performed (1, 4).

This strategy will, however, fail in some cases. Persistent postoperative hyperparathyroidism is generally due to the missing of an ectopic tumor or one of multiple abnormal glands. Moreover in some circumstances it is difficult to distinguish normal from abnormal glands either at the operating table or by frozen section. Indeed in most cases the pathologist can only indicate if the biopsied lump is a parathyroid, but not whether it is normal or abnormal (5, 6).

Based on the assumption that the concentration of circulating PTH should fall if all hyperfunctioning parathyroid tissue is removed, intraoperative monitoring of PTH was proposed to assist the surgeon during neck
exploration (7–9). The clinical utility of this approach has been validated by Irvin and Deriso (9) using a modified two-site immunochemiluminometric assay (ILMA) with a turnaround time from blood collection to availability of the assay result of 10 min (quick PTH assay, QPTH).

In the present study we report our experience on the use of the QPTH assay in a large series of patients with PHPT submitted to cervical exploration and we confirm its utility to improve the success rate of parathyroidectomy.

**Subjects and methods**

**Patients**

The study group included 206 consecutive patients with sporadic PHPT (46 males, aged 22–76 years, mean 51 years, and 160 females, aged 21–82 years, mean 58 years). Before operation all patients had hypercalcemia (range 2.6–3.7 mmol/l) and elevated PTH levels (range 57–1200 ng/l).

All patients underwent parathyroidectomy in the period from March 1997 to May 2001 at the Department of Surgery of the University of Pisa. One hundred and thirty patients, selected on the basis of preoperative imaging (neck ultrasound and/or ⁹⁹mTc-sestamibi) indicating the presence of a single adenoma, absence of goiter, and no previous neck surgery, underwent minimally invasive video-assisted parathyroidectomy (10), and 76 underwent a standard cervical approach. A suspected enlarged parathyroid gland was removed and submitted to frozen section only if the QPTH intraoperative measurement did not show a significant decrease (see below); normal-looking glands were not routinely biopsied. Serum calcium was checked the day after surgery and weekly afterwards for 3–4 weeks in all patients. The follow-up ranged from 1 month to 3 years (mean 10 months).

All patients gave their informed consent and our internal Review Board approved the study.

**Blood sample collection**

Blood samples, drawn from a peripheral vein or occasionally from the internal jugular vein, were obtained at the following times: (i) T-beginning (T-b), after induction of anesthesia and before skin incision; (ii) T-manipulation (T-m), during manipulation of the suspected parathyroid adenoma; and (iii) 5 min (T-5) and 10 min (T-10) (±0.5 min), after removal of each suspected parathyroid gland.

Blood samples were collected in tubes containing EDTA and immediately centrifuged, for 30 s at 3000 rpm, in a Costar Microcentrifuge (Costar Corporation, Cambridge, MA, USA) and assayed. Paired plasma samples were stored at −20°C for PTH measurement using the standard 2 h ILMA (see below). On some occasions we were unable to draw blood samples at all scheduled time points, but in all cases the T-b and at least either the T-5 or the T-10 samples were available.

**QPTH assay**

PTH was measured using a two-site ILMA (Nichols Institute Diagnostic, San Juan Capistrano, CA, USA), as modified by Irvin & Deriso (9). The incubation time was decreased from 2 h to 7 min, by increasing the incubation temperature from 20°C to 45°C and the shaking speed from 180 to 400 r.p.m., using a Lab-Line Environ Blok Shaker (Lab-Line Instruments, Melrose Park, IL, USA). A portable luminometer (Luminometer (Stratec Electronic GMBH, Birkenfeld, Germany) and, more recently, Lumat LB-9507 (Berthold Technologies GMBH, Bad Wildbad, Germany)) was used to assess relative light units (RLU). Since April 2000 a specific device provided by Nichols (Quick Pack System) was used for tube incubation and RLU reading. The assay was performed in or close to the operating room. Each sample was run in duplicate. The turnaround time from blood sampling to report of results to the surgeon was 10 min. The lower and upper limits of detection were 10 ng/l and 2200 ng/l respectively. The intra-assay coefficient of variation was 8.5%. The QPTH assay was validated by measurement of PTH in duplicate samples (n = 43), using the standard PTH assay.

To define the operative success we used the same criterion proposed by Irvin et al. (8), i.e. a decrease of PTH following removal of the enlarged parathyroid gland greater than 50% of the highest pre-excision value. We suspected a surgical failure or incomplete removal of all hyperfunctioning parathyroid tissue if both measurements at T-5 and T-10 did not show a >50% decrease compared to the highest pre-excision value. In these cases the neck exploration was continued and additional samples were collected for the QPTH assay 5 and 10 min after the excision of any other abnormal parathyroid gland.

**Results**

To validate the QPTH assay, 43 duplicate samples were assayed in the same day by the QPTH assay and by the standard 2 h ILMA procedure. A good correlation was found between the results obtained by the two methods ($r^2 = 0.98$, $P = 0.0001$).

A >50% decrease of PTH compared to the highest pre-excision value occurred in 203 patients during the surgical procedure. In 195 of them the fall was observed after the excision of the suspected parathyroid adenoma; in the remaining eight patients PTH values remained substantially unchanged or decreased less than 40%. The frozen section of the removed tissue...
showed fat tissue in three cases and an enlarged parathyroid gland in five. In all of these patients the surgical procedure was continued. An additional suspected lump was identified and its removal was followed in all cases by a >50% fall in PTH concentration (Table 1). Pathological examination confirmed the removal of abnormal parathyroid tissue in all patients; five had a double parathyroid adenoma.

A >50% decrease of PTH (72.8 ± 12.3%, mean ± S.D., range 51–98%) was evident at T-5 in 173 of the 195 patients (88.7%) in whom a blood sample at this time point was available. The measurement at T-10 in these patients showed a further decrease or unchanged PTH values (80.4 ± 10.4%, range 55–98%). In the remaining 22 patients the PTH at T-5 remained unchanged or decreased less than 20% in two, between 20 and 40% in four and between 40 and 50% in 16. All these 22 patients (Fig. 1), as well as the eight patients lacking the T-5 sample, showed a >50% decrease of PTH at T-10 (71.1 ± 10.7%, range 52–81%).

Follow-up measurement of serum calcium showed decreased levels in all but three patients belonging to the 195 cases who showed a significant fall of PTH after the initial successful excision. The individual pre-operative calcium levels in these three patients were 3.1, 2.9 and 3.7 mmol/l. Two were submitted to video-assisted parathyroidectomy and one to a standard cervical approach. None of these patients showed an increase of PTH at T-m. The PTH concentration at T-5 and T10 was significantly below that of T-0 and the percent decrease was 91 and 94%, 57 and 69%, and 73 and 81% respectively.

In three patients the PTH concentration did not show a >50% decrease compared to the highest pre-excision value at both T-5 and T-10 after removal of a suspected parathyroid lump. In one patient the PTH concentration remained substantially unchanged (±10%); further neck exploration by a standard cervical approach was negative and persistent hypercalcemia was demonstrated. In the other two patients the falls of serum PTH at T-5 and T-10 were 15 and 48%, and 31.5 and 46% respectively. In both cases the surgical procedure was ended, even though it should have been continued on the basis of the QPTH results.

There was no difference in the decreasing rate of calcium between patients treated by video-assisted parathyroidectomy and the standard cervical approach. Transient hypocalcemia (serum levels below 2.0 mmol/l) occurred in 13 of 130 patients (10.0%) of the former and in 10 of 76 patients (13.1%) of the latter subgroup.

In summary, using the criterion of a decrease of PTH concentration >50% compared to the highest pre-excision value, intraoperative QPTH correctly predicted the outcome of surgery in 201 patients (97.5%) (200 true positive and 1 true negative), and provided three false positive and two false negative results. The sensitivity, efficiency and predictive values were 99.0, 97.6 and 98.5% respectively.

The measurement of PTH at T-10 confirmed the persistent fall of PTH in all patients who already had a >50% decrease at T-5.

**Discussion**

The traditional surgical approach for patients with PHPT at first operation usually consists of four-gland explorations with frozen-section biopsy for tissue confirmation (1). However, with the wide use of sensitive preoperative localization studies some surgeons have advocated the use of a unilateral approach.
approach (11–13), or even minimally invasive (14), video-assisted parathyroid surgery (10, 15), the potential benefits of these procedures being the decreased risk of postoperative hypocalcemia, nerve injury and shorter operating time (16–18). The main limitation of these latter procedures is the possibility of a multiglandular disease in up to 15–20% of cases of sporadic PHPT, a condition that cannot often be identified preoperatively (5, 6).

The intraoperative PTH assay as a tool to assist the surgeon during parathyroid surgery was introduced by Nussbaum et al. in 1988 (7), but only recently has this procedure been introduced into clinical practice, following the development of rapid assay methods which used non-radioactive materials (8, 9). Although several reports have confirmed its validity in determining whether all hyperfunctioning parathyroid tissue has been removed, there is not complete agreement on its routine use (19–23).

In the present study we report our experience using a QPTH assay in a large series of patients with sporadic PHPT. Using the criterion proposed by Irvin et al. (8) (i.e. a ≥ 50% decrease compared to highest pre-excision value) the results of the intraoperative QPTH assay correctly predicted the outcome of surgery in 201 out of 206 patients (97.6%). In these patients the demonstration of a significant fall of intraoperative PTH allowed the surgeon to end the operative procedure, thus avoiding the need for a time-consuming and often unnecessary bilateral neck exploration and biopsy of a normal parathyroid gland. The intraoperative monitoring of PTH allowed the easy identification of the five cases of double adenoma in whom the removal of an abnormal gland was not accompanied by a significant fall of PTH. On the other hand, the use of this surgical strategy (to end the operation in the PTH declines >50% of the highest pre-excision value) was not associated with a definitive cure in three patients in whom the PTH concentration significantly declined following the removal of a pathological parathyroid gland, but hypercalcemia recurred soon after operation. These three patients had moderately high preoperative serum calcium levels (mean 3.2 mmol/l vs 2.8 mmol/l of the entire group of patients). The pattern of PTH decrease following surgery was indistinguishable from that of patients successfully operated. We have no reason to believe that in these three cases the QPTH assay gave erroneous PTH values. Indeed, in the follow-up one of these patients was submitted to further investigation because of moderate hypercalcemia (2.9 mmol/l) and severe osteoporosis. A suspected parathyroid adenoma was shown at ultrasound and 99mTc-sestamibi scan. The patient was re-operated and a second parathyroid adenoma was removed. Intraoperative PTH significantly decreased and the postoperative calcium level was normal. We are following the course of the illness in the other two patients; the most probable hypothesis is the presence also in these patients of a second adenoma or a multiglandular disease, whose function was suppressed by the abnormal gland, thus explaining the transient fall of circulating PTH following its removal.

In our hands the rate of success of an unilateral approach combined with intraoperative PTH monitoring was 98.1%, which is similar or even greater than that obtained by an experienced surgeon performing bilateral neck exploration (3). The four patients with unsuccessful parathyroid surgery had positive preoperative parathyroid imaging (data not shown).

Our series consisted of consecutive patients with sporadic PHPT. The high cure rates associated with the removal of a single abnormal parathyroid gland (documented by the fall of intraoperative PTH levels and normal postoperative serum calcium) raises the question of the true incidence of multiglandular disease, and particularly diffuse hyperplasia, in sporadic PHPT. Indeed, in the present series a multiglandular involvement occurred in 3.9% of cases, a figure that appears rather low compared to that established by morphology (24), even if familial cases were excluded, but comparable to that reported by Bogs et al. (23) and Molinari et al. (5) using the same biochemical approach as we used (QPTH).

The performance of the QPTH assay in the present series was similar to that reported by other authors, even though the procedure and methodology may differ (8, 9, 19–23).

There is no complete agreement on which PTH level should be considered as the reference value to evaluate the fall of PTH after removal of an abnormal gland. Moreover, it is debated whether it is useful to obtain a blood sample during the visualization/manipulation procedure, which may increase the serum PTH concentration because of the squeezing of the parathyroid tissue. In our study the percent fall of PTH was calculated using, as suggested by Irvin et al. (8), the highest pre-excision value, which in 88 patients (43%) corresponded to the PTH level measured during the visualization/manipulation procedure. In these patients the mean percent increase at T-m was 61.6%. If we considered as the reference the PTH concentration at T-b, instead of the highest pre-excision value, a significant fall occurred only in 171 patients and the number of false negative results would increase from 2 to 34. Therefore, our data further support the use of the highest pre-excision value, rather than the basal value, as a reference for evaluating the intraoperative changes of PTH. It is worth noting that when there is an increase at T-m the decrease of PTH at T-5 could simply reflect the fall due to the short half-life of the hormone. In these cases, as well as in cases of borderline decrease at T-5, measurement at T-10 should be performed.

In few instances the PTH concentration started to decrease at the T-m, possibly because of vascular
damage during manipulation. In all these cases the PTH concentration further decreased after removal of the adenoma.

The choice of a 50% fall as criterion for successful parathyroidectomy is based on the experimental data provided by Brasier et al. (25), who monitored serum PTH in eight PHPT patients cured by parathyroid adenectomy. On the basis of our data we suggest a change in the interpretation of the intraoperative QPTH results. In fact, by assuming as significant a >40%, rather than a >50%, decrease of PTH after the removal of the suspected parathyroid adenoma, the number of false negatives will decrease from two to zero, without changes in the false positive results. The sensitivity and efficiency of the QPTH measurement will increase to 100 and 99.5% respectively, whereas the predictive value will remain unchanged. Moreover, the number of patients cured by surgery, in which it would have been sufficient to limit the measurement at T-5, will increase from 88.7 to 96.9% of cases.

The measurement of intraoperative PTH is a necessary requisite to perform minimally invasive, video-assisted parathyroid surgery, a procedure in which the operative strategy cannot rely on standard neck exploration. Patients with PHPT ask for such a procedure with increased frequency and we have recently shown that it is associated with a shorter operative time, a better cosmetic result, a less painful postoperative course, and a cost (including the intraoperative PTH assay) comparable to that of the standard cervical approach (16, 17).

In conclusion, our study confirms that the measurement of intraoperative PTH represents a useful tool to assist the surgeon during parathyroid surgery. The main advantages are: (i) the demonstration at the operating table that all hyperfunctioning parathyroid tissue has been removed; (ii) the possibility of performing a unilateral neck exploration in the majority of cases, without a reduction in the rate of cure; (iii) the avoidance of frozen sections; and (iv) the safe use of minimally invasive, parathyroid surgery.

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