Available online at www.sciencedirect.com
SCIENCE DIRECT®
Talanta 60 (2003) 617–621
Talanta
www.elsevier.com/locate/talanta

Intraoperative chemiluminometric assay for simplified localization of parathyroid adenomas during surgery for primary hyperparathyroidism

H. Bergert *, T. Zimmermann, D. Ockert, I. Alldinger, S. Kersting, H.D. Saeger

Department for Visceral-, Thoracic- and Vascular Surgery, University Hospital of the Technical University of Dresden, Fetscherstraße 74, 01307 Dresden, Germany

Received 19 July 2002; received in revised form 14 March 2003; accepted 17 March 2003

Abstract

Hyperparathyroidism is no longer considered an uncommon endocrinopathy. Between 1994 and 2002, 138 patients underwent neck exploration for primary hyperparathyroidism at the Department for Surgery in the University Hospital of Dresden. Common reasons for operation failure are usually hyperplasia or remnant parathyroid tissue, inadequate parathyroidectomy at initial operation or the occurrence of abnormal hyperfunctioning supernumerary glands. To reduce the operation time, as well as the failure rate that accompanies the incomplete excision of hypersecreting parathyroid tissue, we perform an intraoperative monitoring of parathyroid hormone with an immunochemiluminometric assay (Nichols Advantage™ Intact PTH). With modification of this assay, it was possible to perform the assay in the operating theatre and to attain the hormone values within 10 min. In all cases of successful operation with excision of the adenomatous parathyroid gland the PTH values declined to under 20 percent of the initial intraoperative PTH values. Our results declare the intraoperative parathyroid hormone monitoring as a cost-effective method to control the effect of surgical treatment.

Keywords: Primary hyperparathyroidism; Intraoperative parathyroid hormone monitoring; Immunochemiluminometric assay

1. Introduction

Hyperparathyroidism is no longer considered an uncommon endocrinopathy. With widespread use of multi-channel analyzers for serum calcium determination as a part of general medical examination, about one case of primary hyperparathyroidism per 1000 hospital patients per year may be detected. That is why fewer patients with advanced skeletal, urologic and hypercalcemic syndromes are referred to the hospital for surgical treatment compared with the literature more than 20 years ago. About 60% of our treated patients were in an

* Corresponding author. Tel.: +49-351-4582-863; fax: +49-351-4584-317.
E-mail address: hendrik.bergert@mailbox.tu-dresden.de (H. Bergert).

0039-9140/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0039-9140(03)00190-5

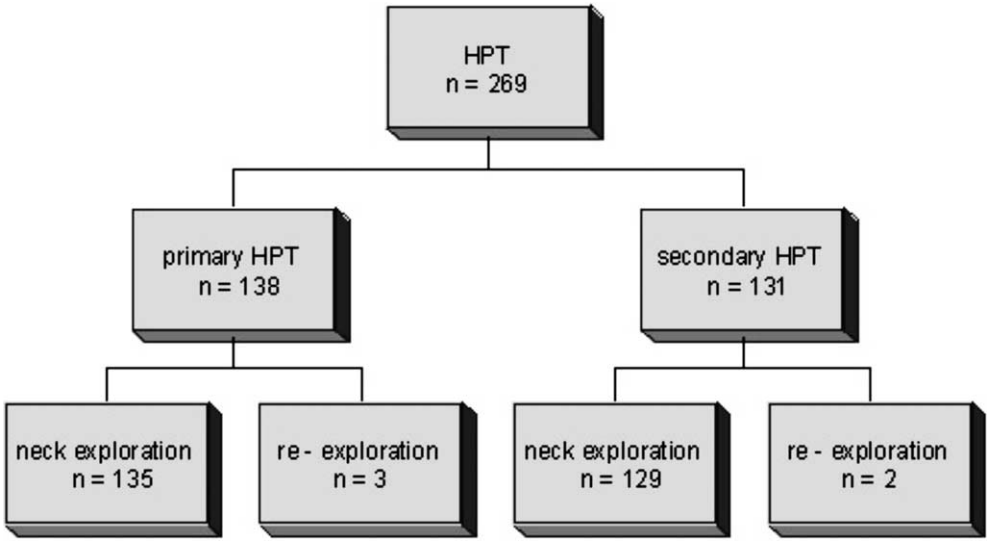

Fig. 1. Operations in hyperparathyroidism (HPT), Department of Surgery, University Hospital Dresden, Germany.

asymptomatic state and the operation is becoming more and more a prophylactic procedure.

The sensitivity of the preoperative localization diagnostic methods (ultrasound, CT-scan, MRI, Thallium-Technetium-scan, ^{99m}Tc-Sestamibi parathyroid imaging) ranges between 65 and 85% in our own experience (data not shown) and in the literature [1–4], but the sensitivity of the surgical neck exploration itself is between 90 and 97% [5–7].

The surgical management of primary hyperparathyroidism by experienced surgeons is associated with excellent results [5,8]. But several studies have documented recurrence of hyperparathyroidism after adenomatous gland resection [5,7]. The operative strategy for hyperparathyroidism is well known and widely accepted. It is:

- to identify all four parathyroid glands
- if there is only one gland enlarged, then this parathyroid gland should be removed
- an obvious macroscopically normal gland should be taken and sent for perioperative frozen section as well.

Most reoperations for primary hyperparathyroidism have to be performed because of hyperfunction of residual tissue in the neck or in the mediastinum [7].

Although the success rate of parathyroid surgery is between 90 and 97%, there are some typical intraoperative problems [9,10] such as: the small size of adenomatous or hyperplastic glands, the atypically located glands in the mediastinum, thymus or inside of thyroid glands and the occurrence of abnormal hyperfunctioning supernumerary glands (that may be expected in $\approx 6\%$ of the cases).

In order to reduce the surgical operation time as well as the failure rate that accompanies the incomplete excision of hypersecreting parathyroid tissue, we started to perform an intraoperative monitoring of parathyroid hormone.

2. Experimental

2.1. Patients and methods

Between January 1994 and April 2001, 269 patients underwent neck exploration for hyperparathyroidism at our Department for Visceral-, Thoracic- and Vascular Surgery in the University Hospital of Dresden. Some 138 operative interventions were carried out for primary hyperparathyroidism and 131 for secondary hyperparathyroidism. Five operations were per-

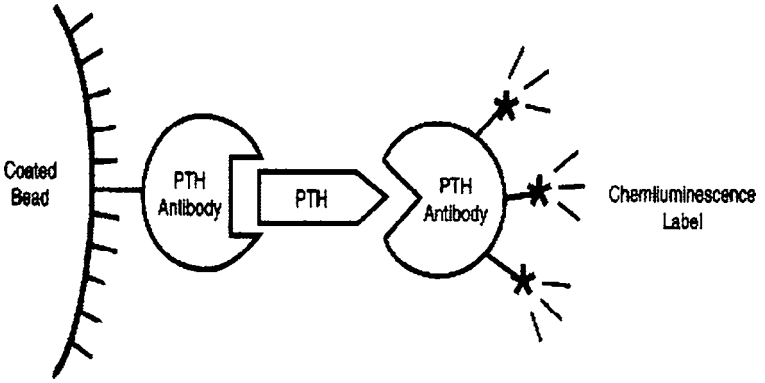

Fig. 2. Method: Chemiluminometric assay for PTH—monitoring.

formed on patients who had undergone previous neck exploration, three in primary and two in secondary hyperparathyroidism (Fig. 1).

At the beginning of the 1990s an immunochemiluminometric assay with an incubation time of about 2 h was developed. We have modified this assay only by reducing the incubation time in a first step from 1 h to 20 min without any changes of agents and a performed analytical sensitivity of 5 pg ml^{-1} (AutoLumat Plus LB 953, Berthold Technologies, Bad Wildbad, Germany). In the last 3 years, PTH was measured using the Quick-IntraOperative™ Intact PTH assay (Nichols Institute Diagnostics) with an incubation time of 7 min and an analytical sensitivity of 1 pg ml^{-1}. With this modification it was possible to perform the assay in the operating theatre and to attain the hormone values within 10–12 min.

Intact parathyroid hormone is a single chain polypeptide of 84 amino-acids and has a short half-life of ≈ 4 min, which confirms a rapid rate of disappearance of the molecule from the peripheral circulation. This method utilizes two antibodies that bind to separate portions of the intact parathyroid molecule and a chemiluminescent label as detection system.

For intraoperative use, 1 ml of blood is drawn from a peripheral vein to an EDTA-containing Vacutainer tube and then pipetted into a microcentrifuge tube and centrifuged for 30 s at 6000 rpm. Aliquots of 300 μl serum are pipetted into glass tubes and 100 μl chemiluminescent acridinium ester labelled polyclonal PTH (1–34) antibody (Quest Diagnostics Laboratory Network, San Juan Capistrano, CA) are then added. In the same step a polyclonal (44–68) PTH antibody (Quest Diagnostics Laboratory Network), which is covalently bonded on a polysterene bead, is pipetted into the sample. In this way the parathyroid hormone is sampled in form of a sandwich-complex (Fig. 2). Tubes are then incubated at 37 °C for 7 min while shaking at 500 rpm in a heater-shaker (QuiCk-Pak™ Kinetic Enhancer). After incubation, the tubes are decanted and after a repeated wash with 2 ml of saline, the bound luminescence in the assay tubes after injection of a trigger solution is measured by the luminometer (Nichols Advantage™, Nichols Institute Diagnostics, CA). The concentration of intact parathyroid hormone is directly proportional to the amount of chemiluminescence.

The total time to perform this assay is between 10 and 12 min. About 1 h before starting the operation, the technician has to enter the operating room to perform instrument checks and to

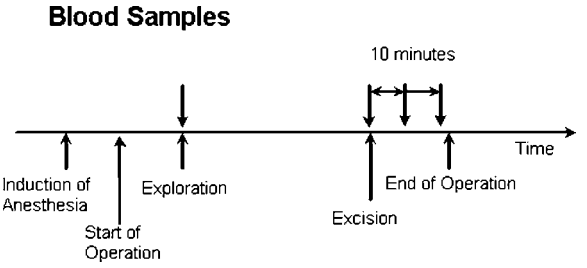

Fig. 3. Intervals of taking blood samples during parathyroid surgery.

Table 1
Patient characteristics and preoperative laboratory values (mean values)

Average age: 43.4 years	Calcium: 3.12 mmol l^{-1} (range: 2.61–5.02)
Female/male ratio: 3:1	Phosphorus: 0.86 mmol l^{-1} (range: 0.25–1.82)
Primary hyperparathyroidism: $n = 138$	Intact PTH–values: 358 pg ml^{-1} (range: 58–1732)
Intraoperative PTH–monitoring: $n = 126$	

generate the calibration curve. With a transportable cart containing all instrumentations required for the assay performance, the technician is able to move between different operating rooms where parathyroidectomies can take place at the same time.

3. Results and discussion

Table 1 demonstrates the patient characteristics and preoperative laboratory parameters in all 138 patients who underwent neck exploration for primary hyperparathyroidism. Bone pain (23%) and nephrolithiasis (11%) were the most common symptoms. About 60% of our patients were in an asymptomatic state of the disease.

For monitoring purposes, blood samples were taken from a peripheral vein at the following times: preoperatively just before induction of anesthesia, after identifying the suspicious parathyroid gland during the neck-exploration, in the moment of the excision and then in two 10-min intervals after excision (Fig. 3).

Initial intraoperative concentrations of PTH ranged from 79 to 1380 pg ml^{-1}. The maximum values are attributed to manipulation of the adenomatous glands during exploration.

It was observed that 10 min after excision, PTH serum values declined to an average value of 89 pg ml^{-1}. In all cases of successful parathyroidectomy (adenoma excision), the PTH values declined to under 20% of the initial intraoperative PTH values 20 min after resection (Fig. 4). A typical change of total hormone values in a successful adenoma resection is shown in Fig. 5. In one case of

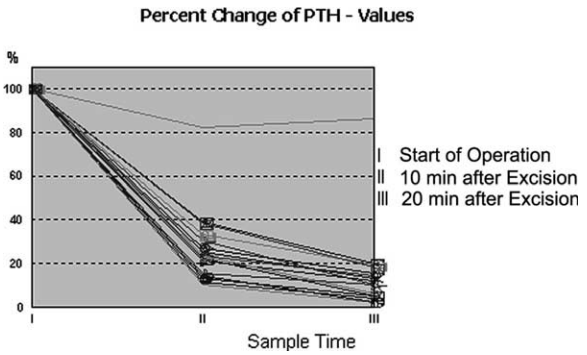

Fig. 4. Percentage decrease of PTH values during operation.

operation failure, the PTH value did not decrease to the baseline value of 20% (Fig. 4) and therefore means that we have not found the hypersecreting gland although we already removed four glands. This female patient had a fifth supernumerary gland in the mediastinum (visible in a ^{99m}Tc-Sestamibi parathyroid imaging performed postoperatively), but she rejected an extended operation with sternotomy. The two other cases of reoperation because of persistent primary hyperparathyroidism were referred to us from other hospitals. In both cases, atypically located glands were found in the thymus and in the thyroid gland.

4. Conclusions

Our results declare the intraoperative parathyroid hormone monitoring as a cost-effective

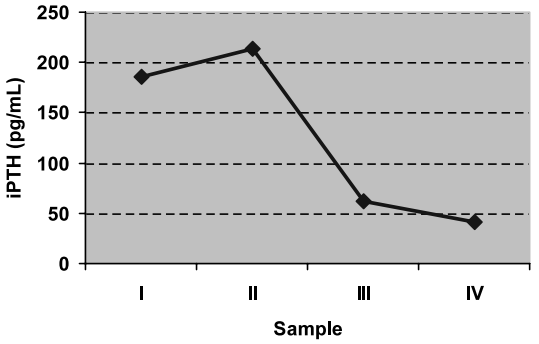

Fig. 5. Intraoperative change of total iPTH values in a typical case of primary hyperparathyroidism at start of operation (I), during cervical exploration (II), 10 min (III) and 20 min (IV) after resection.

method to control the effect of surgical treatment. The costs for an intraoperative PTH monitoring amount to between 70 and 100 Euro and can be reduced if more than one patient undergoes the operation on the same day, as resources can be shared. We use the monitoring as our 'biochemical frozen section'. A decrease of the PTH concentration to $<40\%$ of the baseline values 10 min after excision and to $<20\%$ 20 min after adenoma resection predicts the success of parathyroid surgery. Other authors propose for a decrease of $>50\%$ in PTH values 10 min after resection of the diseased gland as a strong predictor of successful surgery [11–17], which is quite comparable with our results. For us, it is important to measure an additional PTH value (20 min) after resection to ensure the decreasing trend.

Our results in the intraoperative parathyroid hormone monitoring in surgery of primary hyperparathyroidism are well established now in our operating theatre and may:

- decrease of operative time
- help to localize inaccessible glands
- allow unilateral neck exploration in primary hyperparathyroidism with significant reduction of average operative time (we changed our operation performance in that way and we gave up to send an obvious macroscopically normal gland as reference to the pathologists for frozen section)
- identify multiple gland involvement
- improve the success rate of parathyroid surgery.

The role of intraoperative PTH monitoring for secondary hyperparathyroidism is not as well defined as it is for primary hyperparathyroidism [18]. The ability to detect inadequate degrees of parathyroid resection during secondary parathyroidism remains to be proven. In these cases long-term follow-up is necessary to assess if an absolute PTH percentage decrease exists which is predictive for successful operation or operation failure.

References

[1] J. Giron, E. Ouhayoun, M. Dahan, J. Berjaud, J.P. Esquerre, J.P. Senac, J.J. Railhac, Eur. J. Radiol. 21 (1996) 167.
[2] (a) A. Malhotra, C.E. Silver, V. Deshpande, L.M. Freeman, Am J. Surg. 124 (1996) 331;
(b) J.A. Bergmann, R. Pallant, Ear Nose Throat J. 172 (1998) 404.
[3] G.L. Irvin, D.M. Carneiro, J. Am. Med. Assoc. 284 (2000) 934.
[4] M.J. O'Doherty, A.G. Kettle, C.P. Wells, R.E.C. Collins, A.J. Coakley, J. Nucl. Med. 33 (1992) 313.
[5] O.H. Clark, Adv. Endocrinol. Metab. 6 (1995) 1.
[6] G.I. Irvin, Am. J. Surg. 162 (1991) 299.
[7] M. Rothmund, P. Wagner, Ann. Surg. 207 (1988) 310.
[8] G.W. Summers, Ear Nose Throat J. 75 (1996) 434.
[9] J.A. Edis, L.A. Ayala, R.H. Egdahl, Manual of Endocrine Surgery, Springer, New York, 1975, p. 3.
[10] C. Wang, Ann. Surg. 183 (1976) 271.
[11] L.R. Johnson, G. Doherty, T. Lairmore, J.F. Moley, L.M. Brunt, J. Koenig, M.G. Scott, Clin. Chem. 47 (2001) 919.
[12] L.J. Sokoll, H. Drew, R. Udelsman, Clin. Chem. 46 (2000) 1662.
[13] L.J. Sokoll, R. Udelsman, D.W. Chan, Diagn. Endocrinol. Metab. 17 (1999) 295.
[14] H. Chen, L. Sokoll, R. Udelsman, Surgery 126 (1999) 1016.
[15] G.L. Irvin, G. Sfakianakis, L. Yeung, G.T. Deriso, L.M. Fishman, A.S. Mollinari, J.N. Foss, Arch. Surg. 131 (1996) 1074.
[16] G.L. Irvin, A.S. Molinari, C. Figueroa, D.M. Carneiro, Ann. Surg. 229 (1999) 874.
[17] M. Tommasi, A. Brocchi, A. Benucca, G. Manca, D. Borelli, P. Cicchi, G. Capelli, Int. J. Biol. Mark. 10 (1995) 206.
[18] B.M. Clary, S.C. Garner, G.S. Leight, Surgery 124 (1998) 559.