

Intra-operative parathyroid hormone measurements – experience of a non-academic hospital

A. N. MORKS, M.D.

Department of Surgery, Reinier de Graaf Hospital, Delft, The Netherlands

T. M. VAN GINHOVEN, M.D., PH.D.

Department of Surgery, Erasmus MC, Rotterdam, The Netherlands

J. M. PEKELHARING, M.D., PH.D.

Department of Clinical Chemistry, Reinier de Graaf Hospital

E. J. J. DUSCHEK, M.D., PH.D.

Department of Endocrinology, Reinier de Graaf Hospital

P. C. SMIT, M.D., PH.D.

P. W. DE GRAAF, M.D., PH.D.

Department of Surgery, Reinier de Graaf Hospital

Summary

Background. Surgery is the treatment of choice for symptomatic primary hyperparathyroidism. The majority of research concerning intra-operative parathyroid hormone (ioPTH) measurements is conducted in university hospitals. Whether ioPTH measurements are feasible and useful in predicting the presence of remaining hyperfunctioning parathyroid tissue in a non-academic hospital remains uncertain.

Methods. Data were collected on all patients with biochemically proven and surgically treated primary hyperparathyroidism treated at the Reinier de Graaf Hospital from August 2002 to December 2007.

Results. Sixty-five patients were included. The mean pre-operative serum calcium level was 2.78 mmol/l (range 2.28 - 3.80 mmol/l, normal range 2.20 - 2.65 mmol/l) and the mean serum parathyroid hormone level 17.0 pmol/l (range 4.0 - 90.3 pmol/l, normal range 1.0 - 5.5 pmol/l). All patients were operated on for primary hyperparathyroidism, using ioPTH measurements during their first operation. Sensitivity and specificity rates of ioPTH measurements were 98% and 89%, respectively. The ioPTH test accurately indicated incomplete removal of all hyperfunctioning parathyroid tissue in 8 patients (12%). Five patients (8%) were re-explored immediately, of whom 4 were successfully treated in this single operative session. One patient was operated on successfully the next day. Two patients were operated on with a successful result during a second admission. In all the ioPTH measurements there was 1 false-positive result (1.5%) and 1 false-negative result (1.5%).

The mean postoperative calcium value for the successfully treated patients was 2.34 mmol/l (range 2.14 - 2.71 mmol/l, normal range 2.20 - 2.65 mmol/l). The mean postoperative PTH level for the successfully treated patients was 3.76 pmol/l (range 0.40 - 7.1 pmol/l).

Conclusion. Our data suggest that ioPTH measurements are feasible and useful in a non-academic hospital.

Primary hyperparathyroidism is usually caused by a single parathyroid adenoma secreting excessive parathyroid hormone (PTH).¹ The standard of care for symptomatic primary hyperparathyroidism is surgical removal of all hyperfunctioning parathyroid tissue. In experienced hands, parathyroidectomy by conventional neck exploration is successful in 95 - 99% of cases, with a very low complication rate.^{2,3} The introduction of radionuclide parathyroid localisation studies and, more recently, intra-operative parathyroid hormone (ioPTH) monitoring has fuelled a trend towards minimally invasive parathyroidectomy (MIP).¹ The fact that more than 90% of patients have only one abnormal parathyroid gland adds to the suitability of MIP as a procedure. Large studies have compared the traditional neck exploration with the minimally invasive approach, showing equal cure and complication rates.⁴⁻⁷ MIP has the potential to be more cost-effective than the traditional method, with shorter operation times and fewer days in hospital.⁸ Minimally invasive surgery is currently the standard of care in patients with conclusive localisation studies (all indicating only one suspect gland at the same location) and no suspicion of multi-glandular disease. It is recommended that ioPTH assays be used during this targeted approach.¹

In MIP only the gland suspected to be abnormal is explored, leaving the others unexamined. There is therefore the possibility of missing hyperfunctioning tissue that may cause persistent disease and require re-operation. To reduce this uncertainty, ioPTH measurements can be used. The half-life of this hormone is approximately 5 minutes,⁹ so once all the hyperfunctioning tissue is removed serum PTH levels drop quickly, providing instantaneous feedback to the surgeon. Failure of PTH to decline indicates the presence of remaining hyperfunctioning parathyroid tissue and warrants further exploration. This poses the question of which cut-off point should be used to define an adequate decrease.¹⁰ The Miami criterion is commonly used, i.e. a 50% decrease between the pre-operative sample and the sample drawn 10 minutes after removal of the abnormal gland.¹¹

The majority of research concerning ioPTH measurements published in recent years has been conducted in university hospitals and referral centres,^{4,5,11-13} results from non-academic hospitals remaining scarce.¹⁴ This gives the impression that ioPTH measurements are mainly, or only, performed in an academic setting, and whether ioPTH measurements are logistically feasible and useful in a non-academic hospital setting remains uncertain.¹⁵ We therefore analysed our results from a non-university hospital operating on approximately 15 patients per year.

Methods

Data were collected at the Reinier de Graaf Hospital in Delft from August 2002 to December 2007. All patients with a biochemically proven diagnosis of PHPT (hypercalcaemia with a concomitant increase or inappropriately high level of serum PTH) who underwent surgery were included. Patient demographics and clinical values were recorded pro- and retrospectively. Before 2005, medical records of the participating patients were retrieved using operation-specific codes and relevant hospital coding systems. Thereafter, results were collected prospectively. Only patients with primary hyperparathyroidism who had ioPTH measurements during their first surgical treatment were included. Patients on lithium therapy, those who did not have ioPTH measurements performed, and those who had previously undergone parathyroid gland surgery were excluded from further analysis.

Before surgery

Baseline patient characteristics (gender, age, medical history) and pre-operative laboratory values (serum calcium and PTH) were collected. All patients received pre-operative localisation studies consisting of nuclear scintigraphy (^{99m}Tc-sestamibi scan) and/or ultrasound investigation and/or spiral computed tomography (CT). All procedures, whether via conventional neck exploration or the minimally invasive approach, were performed under general anaesthesia by one of two dedicated endocrine surgeons, P.W.d.G. and P.C.S. The minimally invasive approach, involving a small incision, was the operation of choice if a single adenoma was suspected.

During surgery: ioPTH assessment

Measurements of ioPTH were available from the beginning of 2002 and were fully operational during the study period. Levels were measured before incision after induction of anaesthesia, directly before extirpation of the targeted gland, and 3, 6, 9 and 12 minutes after gland removal. Blood samples drawn via a peripheral venous catheter were sent by pneumatic post to the laboratory for analysis. Results were available in the operating theatre 40 minutes later. The ioPTH assessment was carried out using the Siemens Immulite 2500 analyser. Ethylenediaminetetra-acetic acid (EDTA) plasma was added to beads coated with affinity-purified polyclonal goat antibodies directed against PTH 44-84. After washing, affinity-purified polyclonal goat antibodies directed against PTH 1-34 conjugated to a marker enzyme were added and the amount of bound enzyme was measured. The reference values were 1.6 - 6.8 pmol/l. Depending on the surgeon's decision, the patient was brought to the recovery room after being extubated or remained in the operating theatre until the test results were available. The majority of the patients were kept intubated in theatre while the ioPTH results were awaited. Patients who were brought to the recovery room were extubated. If no

satisfactory drop in ioPTH was evident, the patient was re-intubated straight away and a further exploration was performed. An adequate decline in PTH levels was defined as a decrease in the PTH level 12 minutes after removal of the targeted gland of 50% or more compared with the pre-incision level (although the half-life of PTH is approximately 5 minutes, we use 12 minutes as the cut-off point). If the decrease in PTH was less than 50%, further exploration was done immediately. A false-positive result was defined as an adequate decrease of the ioPTH level with postoperative hypercalcaemia (persistent disease). A false-negative result was defined as an inadequate decrease of the ioPTH level despite the fact that the patient was normo- or hypocalcaemic postoperatively.

After surgery

All normo- and hypocalcaemic patients were considered cured. Persistent disease was defined as hypercalcaemia in the first 3 months after surgery. Recurrent disease was defined as a return of hypercalcaemia more than 3 months postoperatively. Serum calcium levels were obtained the evening after surgery, the next day, and during outpatient visits planned approximately 2 weeks postoperatively at the department of surgery, and 3 and 12 months postoperatively at the department of endocrinology. In the case of temporary hypocalcaemia, calcium supplementation was prescribed. Persistent hypercalcaemia was an indication for a second operation or referral to an endocrinologist for further analysis.

Pathological evaluation

Pathology reports were analysed. Pathological distinction between adenoma and hyperplasia is difficult. Adenomas are characterised by an encapsulated hypercellular lesion, a predominantly single-cell population and absence of stromal adipocytes. Outside the capsule there may be a thin rim of residual normal or atrophic parathyroid tissue. Hyperplasia is characterised by a hypercellular lesion, a heterogeneous population of proliferating cells and a reduction of stromal adipocytes.^{16,17}

Results

During the period August 2002 - December 2007, 79 patients with hyperparathyroidism were operated on and included in this study. After exclusion criteria were applied, a total of 65 patients with primary hyperparathyroidism (50 women and 15 men, mean age 63 years, range 29 - 84 years) were included in the analysis. The mean serum calcium level was 2.78 mmol/l (range 2.28 - 3.80 mmol/l, normal range 2.20 - 2.65 mmol/l) and the mean serum PTH level 17.0 pmol/l (range 4.0 - 90.3 pmol/l, normal range 1.0 - 5.5 pmol/l). Six patients were medically treated for their hyperparathyroidism and were therefore normocalcaemic. All 65 patients were operated on for the first time with the diagnosis of primary hyperparathyroidism. In total 65 primary operations were performed, leading to 5 further explorations in the same operative session after an inadequate decrease in the ioPTH value.

Pre-operative imaging studies

Before surgery, 63 of the 65 patients (97%) underwent ^{99m}Tc-sestamibi scintigraphy (Fig. 1). Ultrasonography was performed in 62 of the 65 patients (95%) and CT in 30 (45%). A combination of all three imaging modalities was used before surgery in 28 cases (43%).

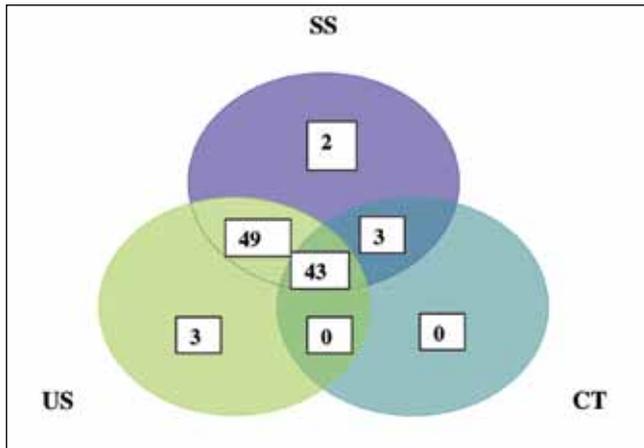


Fig. 1. Pre-operative localisation studies performed (numbers represent percentages) (SS = sestamibi scanning, CT = computed tomography, US = ultrasonography).

All primary operative procedures (Fig. 2)

A total of 65 primary operations were performed. The minimally invasive procedure (MIP) was chosen in 56 patients suspected of having uniglandular disease (86%). Eleven MIPs (17%) were converted to a unilateral or bilateral approach. Reasons for conversion were an inadequate decrease in ioPTH and anatomical difficulties encountered during MIPs (large thyroid lobes and inability to find the adenoma at the expected location). Nine primary conventional neck explorations (14%) were performed for various reasons including previous neck surgery, multinodular struma and suspected multiglandular disease.

Adequate decrease of ioPTH measurements

In 56 of the 65 first operations for PHPT (86%) there was an adequate decrease in the PTH level. In this group, 1 patient had persistent hypercalcaemia after surgery despite an adequate decrease of ioPTH levels (false-positive result). During the MIP, which was directed towards the right lower parathyroid gland as indicated by sestamibi scanning, an abnormal parathyroid gland was found and removed. Pathological analysis of the extirpated gland showed a parathyroid adenoma, and unfortunately this patient was diagnosed with a metastasised malignant tumour (non-parathyroid) and was lost to follow-up. When last seen, the patient had a normal calcium level with a high level of PTH.

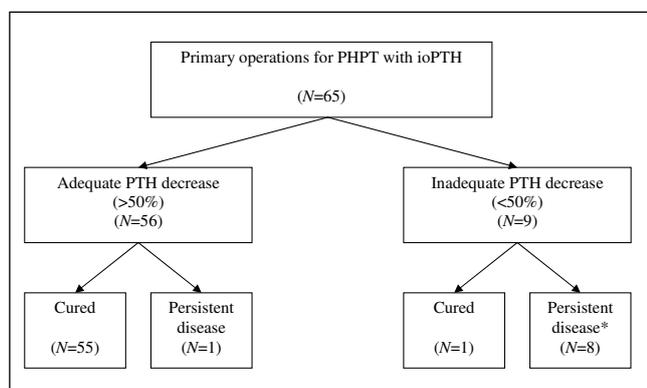


Fig. 2. Flow-chart indicating the results of all primary operations performed using ioPTH measurements (* indicates the presence of remaining hyperfunctioning parathyroid tissue). For outcomes after re-exploration and/or re-operation, see Fig. 3.

Inadequate decrease of ioPTH measurements (Fig. 3)

In 9 cases (14%) there was a decrease of less than 50% in ioPTH levels. Five patients were re-explored in the same operative session and in 4 cases remaining hyperfunctioning tissue was identified and removed. This avoided a second hospital admission and general anaesthetic. These patients remained normocalcaemic during follow-up. The 'uncured' patient was operated on by means of a targeted approach to the left lower parathyroid, based on the result of the sestamibi scan. His medical history comprised previous thyroid gland surgery. After removal of what appeared to be thyroid tissue, the ioPTH levels failed to decrease and the surgical procedure was converted to a conventional neck operation. The procedure was difficult because of the previous neck surgery, and no parathyroid glands could be identified. This patient was referred to a tertiary centre. Because of his low urinary calcium levels, the diagnosis of hypocalcaemic hypercalcaemia is under consideration.

Four patients were not re-explored in the same operative session. One patient was operated on the next day for logistical reasons. This patient was the last on the operating schedule, and due to surgical emergencies the operation had to be re-scheduled to the following morning. Parathyroid tissue was found and the patient remained normocalcaemic during follow-up. Two patients were operated on later. In one of these cases the surgeon had removed an adenoma located at the site indicated by the localisation studies (ultrasound and CT scanning; the sestamibi scan did not show a hot spot). Despite an inadequate ioPTH decrease, he decided against further surgery. However, the patient appeared to have persistent disease and was operated on again during a second admission. The other patient underwent bilateral neck exploration at the initial surgery. The surgeon had removed a lymph node and was unable to identify suspicious parathyroid glands. He could not perform a more extensive surgical search for remaining parathyroid tissue after the ioPTH levels were known (inadequate decrease). The surgical procedure was therefore ended without performing a direct re-exploration. New localisation studies were needed, and the patient was cured after removal of an adenoma during a second MIP. Treatment was successful in both these patients, who were found to be normocalcaemic at follow-up. In the 4th patient

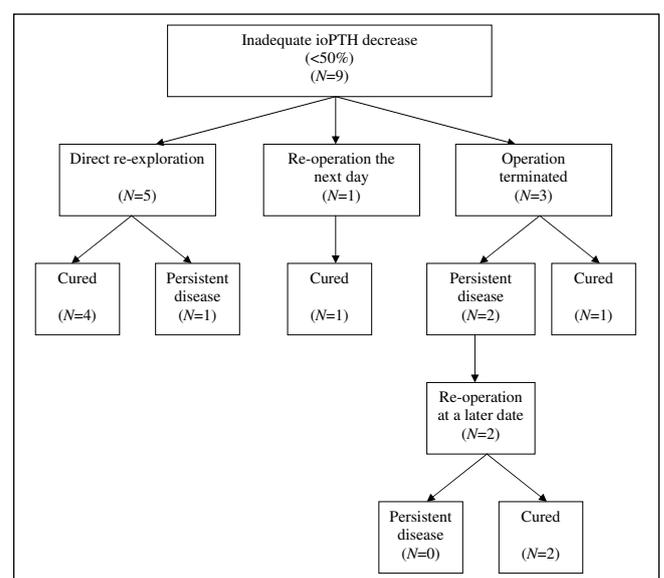


Fig. 3. Flow-chart indicating the results of all operative procedures with an inadequate ioPTH decrease.

the operation was terminated despite an inadequate decrease in ioPTH measurements. During this operation a gamma probe indicated removal of all hot (hyperfunctioning) parathyroid tissue, with no activity left in the area operated on. The surgeon decided to end the operation, and calcium levels have remained within the normal range during long-term follow-up.

The ioPTH measurements accurately predicted remaining hyperfunctioning parathyroid tissue in 8 patients. The sensitivity (55/56) and specificity (8/9) of ioPTH measurements in the Reinier de Graaf Hospital are 98% and 89%, respectively.

Pathological examination of tissue from cured patients

Pathological examination of tissue obtained during the operation showed 60 (95%) of the 63 cured patients to have had single-gland parathyroid adenomas and 1 (1.5%) to have had hyperplasia. In 1 patient (1.5%) both ultrasonography and CT showed multiglandular disease. Only one gland was identified on ^{99m}Tc-sestamibi scintigraphy. Pathological examination confirmed the presence of multiglandular disease (adenoma).

In 1 case (1.5%) only, pre-operative ultrasonography showed an abnormal gland; CT and ^{99m}Tc-sestamibi scintigraphy showed no abnormality. At surgery two glands were removed, pathological examination of which showed normal parathyroid tissue without the presence of hyperplasia or adenoma. The ioPTH measurements showed an adequate decrease and the patient was normocalcaemic at follow-up. No cured patient was diagnosed with malignant disease.

Complications

Postoperative complications included 1 permanent recurrent nerve injury (1.5%) after conventional neck exploration, 4 cases of temporary hoarseness (6%), 1 wound infection (1.5%) and 12 cases of transient postoperative hypocalcaemia (18%).

Follow-up

The mean duration of follow-up of the 65 patients was 126 weeks (range 2 - 440 weeks). Sixty-one (94%) patients were followed up for more than 3 months. The mean calcium level of all cured patients was 2.34 mmol/l (range 2.14 - 2.71 mmol/l). The patient with a slightly increased calcium level (2.71 mmol/l) had a low normal PTH value and is therefore considered cured. The 2 patients with persistent disease are being medically treated for their hypercalcaemia. Their calcium levels at follow-up were 2.46 mmol/l and 2.15 mmol/l. The mean postoperative PTH level in cured patients was 3.76 pmol/l (range 0.40 - 7.1 pmol/l). Five of them had a PTH level higher than the upper limit of the normal range. Their calcium levels were 2.47 mmol/l or lower, and they were therefore considered to be cured.

Discussion

Many reported studies investigating the use of ioPTH measurements were conducted in university hospitals. In this study, we evaluated the use of ioPTH measurements in a non-academic hospital with relatively few patients with PHPT. We conclude that ioPTH measurement is feasible and useful in a non-academic setting. The sensitivity and specificity of the ioPTH measurements in our hospital are 98% and 89%, respectively. The ioPTH test

accurately indicated incomplete removal of all hyperfunctioning parathyroid tissue in 8 patients. Four of these 8 patients were successfully treated during the same operative session, and 1 direct re-exploration led to the conclusion that referral to a tertiary centre was necessary. One patient was re-operated on successfully the next day, because it was impossible to do an immediate re-exploration for logistical reasons. This indicates the importance of adequate planning of these operations. We consider the possibility of immediate re-exploration to be the main advantage of using ioPTH.

A decrease in the PTH level of 50% or more 12 minutes after removal of the abnormal gland(s) compared with the pre-incision level was considered an adequate response. Since the half-life of PTH is less than 5 minutes, theoretically we could have used 6 minutes as the cut-off point. However, because it is known that some people have a slow decline in ioPTH values we therefore prefer to use the 12-minute cut-off. Many centres use the Miami criterion,¹¹ which is almost the same as our definition, the only difference being that it uses the PTH level 10 minutes after removal of the targeted gland. With 12 minutes we are on the safe side of the Miami criterion. Other studies report decisions based on a 30-minute end-point.¹⁸ Nevertheless, our sensitivity and specificity rates are comparable to those found in larger series in tertiary referral centres.^{11,19,20}

The percentage of false-positive results (1.5%, *N*=1) is comparable to or lower than results presented in the literature. There was 1 false-negative result (1.5%), in which ioPTH measurements suggested incomplete removal of hyperfunctioning parathyroid tissue. Although there was an inadequate ioPTH decline, that fact that a gamma probe indicated removal of all hyperfunctioning tissue led the surgeon to decide to end the operation. The patient appeared to be cured, with normal calcium and PTH levels during follow-up 2 years later. However, this patient was potentially exposed to an unnecessarily extended surgical dissection.

Use of ioPTH measurements requires a well-structured protocol involving many people. Our protocol includes sending an e-mail to the laboratory stating the name of the patient, and the operation date and time. A laboratory technician is on standby at the time of surgery to receive and analyse the samples immediately.

Besides the convenience for the patient of avoiding re-admission, ioPTH measurements also save the costs of a second hospital admission, additional outpatient clinic visits, a second general anaesthetic and operative fees. Unfortunately our database does not enable us to conclude whether ioPTH measurements are cost-effective. To analyse the cost-effectiveness properly, a prospective cost-benefit analysis should be done.

In conclusion, the use of ioPTH measurements remains a point of discussion and several centres report good results without the use of ioPTH measurements.²¹⁻²⁴ However, our data suggest that ioPTH measurements are feasible and useful in a medium-sized non-academic hospital, using a well-structured protocol.

A. N. Morks and T. M. van Ginhoven contributed equally to this manuscript.

REFERENCES

1. Sitges-Serra A, Bergenfelz A. Clinical update: sporadic primary hyperparathyroidism. *Lancet* 2007;370:468-470.

2. Lee JA, Inabnet WB 3rd. The surgeon's armamentarium to the surgical treatment of primary hyperparathyroidism. *J Surg Oncol* 2005;89(3):130-135.
3. Ruda JM, Hollenbeak CS, Stack BC Jr. A systematic review of the diagnosis and treatment of primary hyperparathyroidism from 1995 to 2003. *Otolaryngol Head Neck Surg* 2005;132(3):359-372.
4. Bergenfelz A, Kanngiesser V, Zielke A, Nies C, Rothmund M. Conventional bilateral cervical exploration versus open minimally invasive parathyroidectomy under local anaesthesia for primary hyperparathyroidism. *Br J Surg* 2005;92(2):190-197.
5. Bergenfelz A, Lindblom P, Tibblin S, Westerdaal J. Unilateral versus bilateral neck exploration for primary hyperparathyroidism: a prospective randomized controlled trial. *Ann Surg* 2002;236(5):543-551.
6. Udelsman R. Six hundred fifty-six consecutive explorations for primary hyperparathyroidism. *Ann Surg* 2002;235(5):665-670; discussion 670-672.
7. Smit PC, Borel Rinkes IH, van Dalen A, van Vroonhoven TJ. Direct, minimally invasive adenectomy for primary hyperparathyroidism: An alternative to conventional neck exploration? *Ann Surg* 2000;231(4):559-565.
8. Russell CF, Dolan SJ, Laird JD. Randomized clinical trial comparing scan-directed unilateral versus bilateral cervical exploration for primary hyperparathyroidism due to solitary adenoma. *Br J Surg* 2006;93(4):418-421.
9. Davies C, Demeure MJ, St John A, Edis AJ. Study of intact (1-84) parathyroid hormone secretion in patients undergoing parathyroidectomy. *World J Surg* 1990;14(3):355-359; discussion 360.
10. de Vos tot Nederveen Cappel R, Bouvy N, de Herder W, de Rijke Y, van Toor H, Bonjer J. Novel criteria for parathyroid hormone levels in parathyroid hormone-guided parathyroid surgery. *Arch Pathol Lab Med* 2007;131(12):1800-1804.
11. Carneiro DM, Solorzano CC, Nader MC, Ramirez M, Irvin GL 3rd. Comparison of intraoperative iPTH assay (QPTH) criteria in guiding parathyroidectomy: which criterion is the most accurate? *Surgery* 2003;134(6):973-979; discussion 979-981.
12. Weber KJ, Misra S, Lee JK, Wilhelm SW, DeCresce R, Prinz RA. Intraoperative PTH monitoring in parathyroid hyperplasia requires stricter criteria for success. *Surgery* 2004;136(6):1154-1159.
13. Smit PC, Thijssen JH, Borel Rinkes IH, van Vroonhoven TJ. [Peroperative parathyroid hormone assay: assurance of successful surgical treatment of primary hyperparathyroidism] Peroperatieve parathormoonbepaling: zekerstelling van geslaagde chirurgische behandeling van primaire hyperparathyreoïdie. *Ned Tijdschr Geneesk* 1999;143(14):742-746.
14. Clerici T, Brandle M, Lange J, Doherty GM, Gauger PG. Impact of intraoperative parathyroid hormone monitoring on the prediction of multiglandular parathyroid disease. *World J Surg* 2004;28(2):187-192.
15. Fuchs SP, Smits AB, de Hooge P, Muller AF, Gelissen JP, van Dalen T. [Minimally-invasive parathyroidectomy: a good operative procedure for primary hyperparathyroidism even without the use of intraoperative parathyroid-hormone assessment or a gamma probe] Minimaal invasieve parathyreoïdectomie: ook zonder het gebruik van intraoperatieve parathormoonmeting of gammaprobe een goede behandeling voor primaire hyperparathyreoïdie. *Ned Tijdschr Geneesk* 2005;149(26):1463-1467.
16. Sathe PA, Madiwale CV, Kandalkar BM, Bandgar TR, Shah NS, Menon PS. Primary hyperparathyroidism: a clinicopathological experience. *Indian J Pathol Microbiol* 2009;52(3):313-120.
17. Johnson SJ, Sheffield EA, McNicol AM. Best practice no 183. Examination of parathyroid gland specimens. *J Clin Pathol* 2005;58(4):338-342.
18. Halevy A, Stepansky A, Halpern Z, et al. Quick parathormone assay in the surgical management of hyperparathyroidism. *Isr Med Assoc J* 2003;5(11):775-777.
19. Mozzon M, Mortier PE, Jacob PM, Soudan B, Boersma AA, Proye CA. Surgical management of primary hyperparathyroidism: the case for giving up quick intraoperative PTH assay in favor of routine PTH measurement the morning after. *Ann Surg* 2004;240(6):949-953; discussion 953-954.
20. Carneiro-Pla DM, Solorzano CC, Irvin GL 3rd. Consequences of targeted parathyroidectomy guided by localization studies without intraoperative parathyroid hormone monitoring. *J Am Coll Surg* 2006;202(5):715-722.
21. Gil-Cardenas A, Gamino R, Reza A, Pantoja JP, Herrera MF. Is intraoperative parathyroid hormone assay mandatory for the success of targeted parathyroidectomy? *J Am Coll Surg* 2007;204(2):286-290.
22. Pang T, Stalberg P, Sidhu S, et al. Minimally invasive parathyroidectomy using the lateral focused mini-incision technique without intraoperative parathyroid hormone monitoring. *Br J Surg* 2007;94(3):315-319.
23. Jacobson SR, van Heerden JA, Farley DR, et al. Focused cervical exploration for primary hyperparathyroidism without intraoperative parathyroid hormone monitoring or use of the gamma probe. *World J Surg* 2004;28(11):1127-1131.
24. Mihai R, Palazzo FF, Gleeson FV, Sadler GP. Minimally invasive parathyroidectomy without intraoperative parathyroid hormone monitoring in patients with primary hyperparathyroidism. *Br J Surg* 2007;94(1):42-47.