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Primary Hyperparathyroidism

Challenges and pitfalls in management

Janneke E. Witteveen

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Primary Hyperparathyroidism

Challenges and pitfalls in management

PROEFSCHRIFT

ter verkrijging van de graad van Doctor aan de Universiteit van Leiden,
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Life is short
Break the rules
Forgive quickly
Kiss slowly
Love truly
Laugh uncontrollably
and never regret anything
that made you smile

Mark Twaine, 1835-1910
ter nagedachtenis aan Kim Megens

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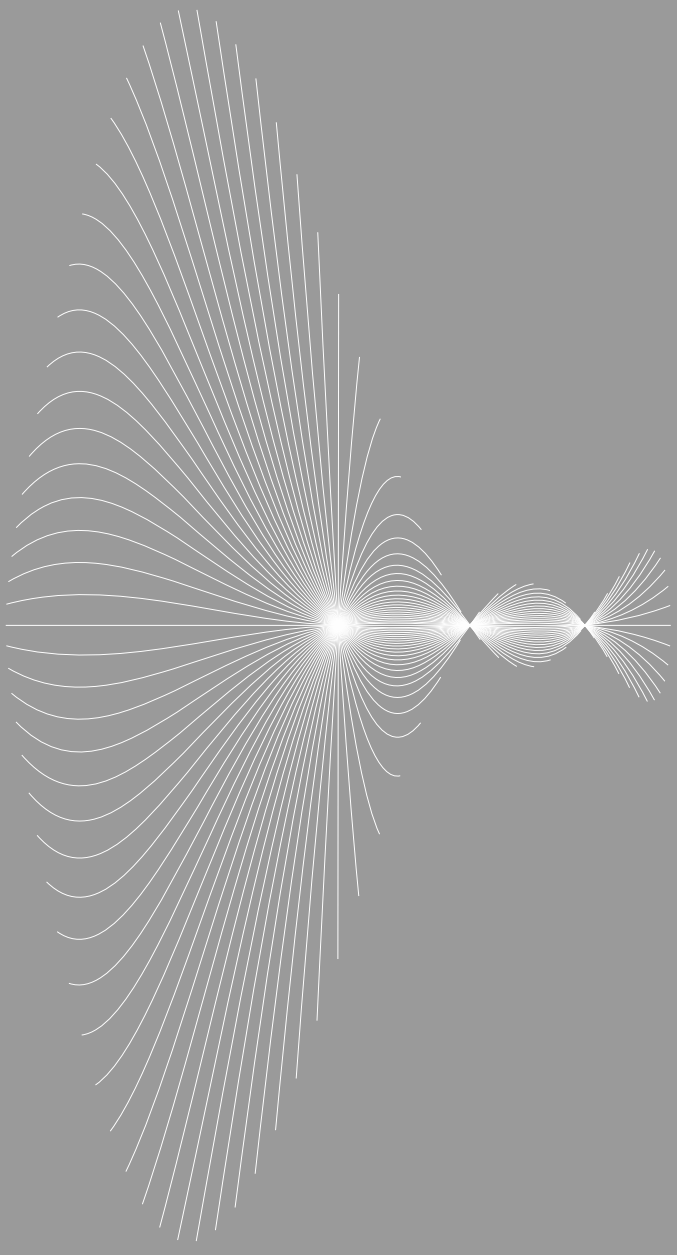

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1. PARATHYROID GLANDS

Anatomy

The parathyroid glands were the last classical endocrine glands to be identified, with the first description made in a rhinoceros by Richard Owen in 1850 (1). The large size of this species probably enabled to macroscopically distinguish the parathyroids from the adjacent thyroid gland (1). Three decades later the parathyroid glands were finally described in humans by Sandström (Figure 1) (1,2). Parathyroid glands vary from 2-7 mm in length, 2-4 mm in width and 0.5-2 mm in thickness (3). Although the majority of normal adults (84%) have 4 glands, 1-7% have 3 glands and 3-13% have 5 glands (4-7). The superior glands arise from the fourth branchial (pharyngeal) pouch and descend into the neck together with the thyroid gland. The superior glands are thus most commonly found near or within the thyroid capsule. The inferior glands, together with the thymus, arise from the third branchial pouch and can be found anywhere from the lower thyroid pole to deep in the mediastinum.

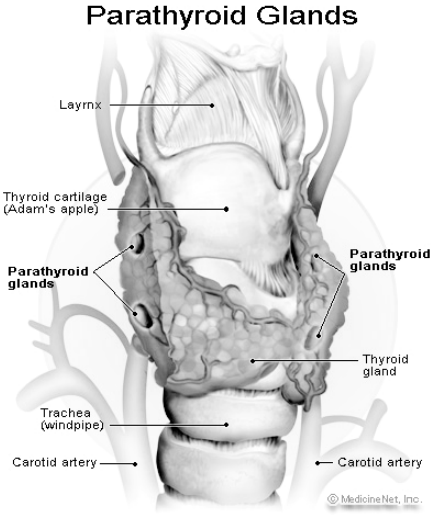

Figure 1: Anatomy of the parathyroid glands

Physiology

The parathyroid glands are essential for normal calcium homeostasis via the production of parathyroid hormone (PTH), which is exclusively produced by the chief cells of the parathyroid glands. PTH production depends on the number of parathyroid chief cells and the secretion of PTH per cell. PTH secretion depends on the setpoint of calcium-dependent secretion, on the secretory capacity of the chief cells and on the number of chief cells that are in the secreting phase. PTH secretion is episodic and rhythmic and once in the circulation, PTH has a very short half life of only a few minutes, enabling very flexible and rapid changes in circulating serum calcium levels. PTH is broken down by Kupfer cells in the liver and PTH fragments are secreted via the kidney together with still intact PTH.

PTH secretion is regulated by extracellular calcium ions, which bind to the calcium sensing receptors (CASR), which are located on the chief cells of the parathyroid gland. Binding of calcium to the CASR activates the receptor, which leads to inhibition of PTH secretion, of *PTH* gene expression and thus PTH synthesis, and of parathyroid cell proliferation (8). PTH secretion is also inhibited by binding of active vitamin D ($1,25(OH)_2D$) to the vitamin D receptor, which is also located in the chief cells of the parathyroids.

In contrast, hypocalcaemia results in a release of available PTH from its intracellular reservoir, in a decrease in intracellular break down of PTH, in a increase of PTH biosynthesis and in a decrease of the secretory rest phase of the parathyroid cell (9). Long-term hypocalcaemia eventually results in four-gland parathyroid hyperplasia.

Pathophysiology of hyperparathyroidism

Hyperparathyroidism is the disease state resulting from one or more parathyroid glands producing too much PTH. This disease state is associated with an increase in the calcium-dependent setpoint of PTH secretion, an increase in the number of PTH secreting cells and an increase in parathyroid parenchymal cells.

Hyperparathyroidism can be secondary to a decrease in serum calcium concentration caused by a negative calcium balance, due to vitamin D deficiency,

renal insufficiency, gastro-intestinal disorders, or hypercalciuria of renal origin. Long-term stimulation of the parathyroids by persistent hypocalcaemia is eventually associated with monoclonal changes, resulting in autonomous "tertiary" hyperparathyroidism. In this case the production of PTH is no longer regulated by circulating serum calcium concentrations, which is analogous to the case in primary hyperparathyroidism.

In primary hyperparathyroidism (PHPT) one or more parathyroid glands are by definition autonomous. The increase in the calcium-dependent setpoint of the chief cells, leads to increased PTH secretion despite increased levels of serum calcium (Figure 2). The most important determinant of the calcium-dependent setpoint for PTH secretion is the number and function of CASR. Although, mutations in the *CASR* gene and downregulation of CASR expression, have been described in 31% of parathyroid carcinomas, the majority of patients with PHPT have normal expression and function of the CASR (10,11).

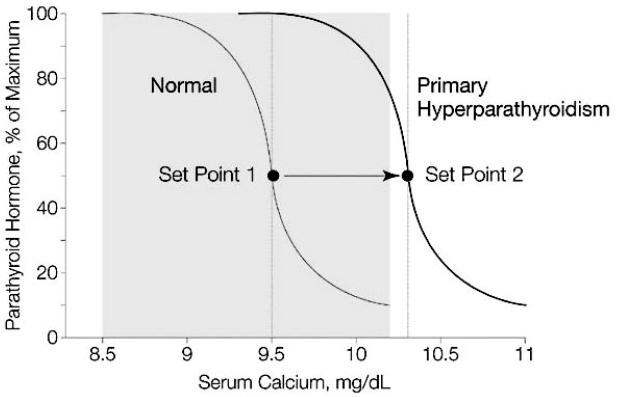

Figure 2: Changes in the calcium-dependent setpoint leading to PHPT

II. PRIMARY HYPERPARATHYROIDISM

Incidence

The estimated incidence of PHPT is 0.4% in adult men, 1.7% in adult women and 2-3% in women above the age of 60 years (12-14).

Clinical features

Over the past few decades, the clinical profile of PHPT, originally described as "bones, stones, groans and psychic moans", has drastically changed to an almost asymptomatic disease, due to the introduction of automated calcium screening. Chronic exposure of the skeleton to high circulating PTH concentrations increases bone turnover in favour of bone resorption, ultimately leading to decreased bone mineral density, predominantly at cortical sites, and increased risk of fractures. PTH has been shown to influence bone metabolism by stimulating RANKL secretion, an osteoclast activator, and by suppressing OPG secretion, the natural decoy receptor for RANKL (15-25).

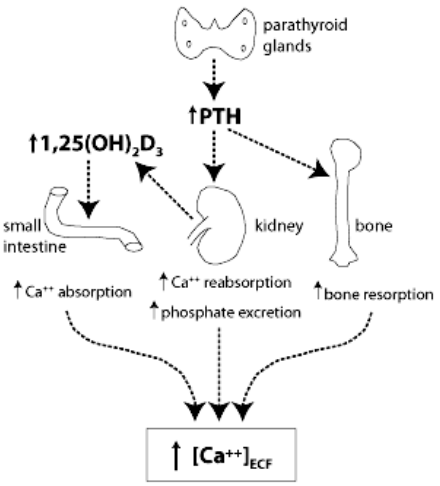

Figure 3: The effect of increased PTH concentrations on bone, kidney and intestine

In the kidney, high circulating PTH levels increase tubular reabsorption of calcium and decrease tubular reabsorption of phosphate. However, approximately 30% of patients with PHPT demonstrate hypercalciuria, with increased risk for nephrocalcinosis and nephrolithiasis. Renal function may deteriorate due to hypercalcaemia and associated dehydration and to recurrent renal stones. PTH also stimulates 1α-hydroxylase enzyme synthesis in the kidney, which is essential for the

conversion of 25-hydroxyvitamin D, into its active metabolite, 1,25-dihydroxyvitamin D. Active vitamin D stimulates the intestinal absorption of calcium.

The net result of increased circulating PTH concentrations on bone, kidney and intestine is an increase in extracellular serum calcium concentration. Clinical manifestations of hypercalcaemia include polyuria, polydipsia and constipation. Hyperparathyroidism may also be associated with non specific symptoms, such as tiredness, mood swings, depression, irritability, forgetfulness, bony pain and muscle weakness.

Pathological changes

Primary hyperparathyroidism can most commonly occur sporadically or more rarely in the context of a genetic syndrome, such as MEN-I (OMIM 131100), MEN-IIa (OMIM 171400) or hyperparathyroidism-jaw tumor (HPT-JT; OMIM 607393) (26). The disease is caused by a solitary parathyroid adenoma in more than 80% of cases, hyperplasia in 15-20% of cases and parathyroid carcinoma in 0.1 to 5% of cases (27-31).

A parathyroid adenoma is characterized by the presence of an encapsulated benign neoplasm usually involving a single gland with an adjacent rim of normal glandular tissue (32).

Parathyroid hyperplasia is characterized by an absolute increase in parathyroid parenchymal cell mass resulting from proliferation of chief cells, oncocytic cells and transitional oncocytic cells in multiple parathyroid glands in the absence of a known stimulus for PTH hypersecretion such as renal failure or vitamin D deficiency (32). A diagnosis of hyperplasia is also favoured when synchronous multiglandular disease is found at surgery.

Parathyroid carcinoma is a malignant neoplasm derived from parathyroid parenchymal cells, which, according to the criteria of the World Health Organization, is characterized by vascular invasion, perineural space invasion, capsular penetration with growth into adjacent tissues and/or metastases (32). The

presence of a trabecular pattern, fibrous bands and/or multiple mitoses are considered to be only minor diagnostic criteria.

Localization studies

Localization studies are not required in case of traditional bilateral neck exploration, because experienced surgeons have an 86–100% chance of excision of all pathological tissue as the procedure entails visualization of all four parathyroid glands (33,34). However, localization studies become mandatory when a more focused unilateral or minimally invasive approach is opted for.

Technetium 99m sestamibi (Tc99m-MIBI), particularly when complemented with single emission computed tomography (Tc99m-MIBI-SPECT), is currently the imaging technique of choice for the preoperative localisation of parathyroid adenomas (33,35). Tc99m-MIBI is a monovalent lipophilic cation that diffuses passively through cell membranes and accumulates almost exclusively in mitochondria following negative membrane potentials (36). In primary hyperparathyroidism, the increased uptake of Tc99m-MIBI is based on an abundance of mitochondria in parathyroid oxyphil cells, which sequester sestamibi intracellularly, and on increased perfusion of hyperactive parathyroid glands. The sensitivity of Tc99m-MIBI-SPECT for localising hyperactive parathyroid glands ranges from 66% to 90% and the positive predictive value ranges from 83 to 100% (33,37-44).

In experienced hands, an ultrasound (US) of the neck is a highly sensitive technique, which is more convenient and less expensive than Tc99m-MIBI-SPECT and particularly more sensitive for intrathyroidally located parathyroids, which is a difficult location for other non-invasive localization techniques. US, however, is of limited value in the case of retrosternal or posteriorly located parathyroid glands. The reported sensitivity is highly variable, ranging from 47% to 89%, depending on parathyroid pathology and the presence of thyroid nodules (33,45-48).

The use of Computed Tomography (CT)-scans and of Magnetic Resonance Imaging (MRI)-scans is recommended in the case of a suspected ectopic mediastinal parathyroid gland, to better define size and location of the lesion

relative to adjacent structures (49). The sensitivity of CT-scans for localising hyperactive parathyroid glands remains, however, lower than that of Tc99m-MIBI-SPECT and US, despite recent developments in equipment and in diagnostic protocols and algorithms (45). A very promising localization method is the novel four-dimensional CT scan (4D-CT), which provides both functional and highly detailed anatomic information about parathyroid tumors. This method is reported to have a sensitivity of 70% for localising hyperactive parathyroid glands (50). MRI is time-consuming, expensive, difficult to interpret and a number of patients cannot undertake the procedure because of indwelling pacemakers and other metallic implants (51).

Selective venous sampling (SVS) of parathyroid hormone (PTH) is an invasive technique that can be used when the above mentioned noninvasive localization studies are negative or disconcordant. During SVS a catheter is introduced under local anaesthesia via a sheath in the right femoral vein by an intervention radiologist and is guided by fluoroscopy to each of the jugular, subclavian and brachiocephalic veins, the azygos vein and to the vena cava superior and inferior, from which serial blood samples are obtained for PTH measurement. SVS is based on the anatomical assumption that regional drainage of each parathyroid gland occurs via the veins of the thyroid plexus into the adjacent superior, middle and inferior thyroid veins (52). However, there are many variations in the venous anatomy of the parathyroid glands due to embryological differences, but most often also due to previous surgical interventions disturbing local anatomy and venous drainage. Despite these anatomical variations, SVS has been reported to be successful in predicting the side of a pathological parathyroid gland in 39-93% of patients with primary hyperparathyroidism (49,52-60) and in 66-75% of patients with negative noninvasive studies (57,58,61). SVS is also successful in identifying the anatomical location of an ectopic parathyroid gland, with a sensitivity 66-100% (62,63).

Management of primary hyperparathyroidism

In primary hyperparathyroidism (PHPT), surgical removal of all pathological parathyroid glands is the only approach that provides definitive and durable cure.

Cure rate is 94-100% for bilateral neck as well as less invasive surgery, more than 6 months after parathyroidectomy (PTx) (33,38,40-44,64-66) with a complication rate of 0.4%-7.7% in the hands of experienced surgeons (41,43,44,65,66). The risks of surgery for PHPT consist in 13-20% of patients in hypoparathyroidism, in 1-6% of patients in transient or permanent vocal cord damage due to unilateral or bilateral damage to the nervus laryngeus recurrens and in <1% to mortality.

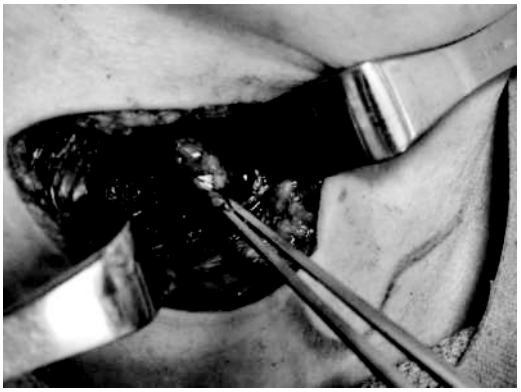

Figure 4: Resection of an enlarged parathyroid gland during minimally invasive neck surgery

Surgery can be performed by standard bilateral neck exploration, which consists of visualization of all four parathyroid glands followed by either excision of a single enlarged parathyroid gland, or subtotal or total parathyroidectomy with auto transplantation if more than one parathyroid gland is found to be enlarged.

Unilateral neck exploration and minimally invasive neck exploration both require pre-operative localization of the enlarged parathyroid gland and the use of intra-operative PTH (IOPTH) measurement to predict that the pathological parathyroid tissue has been fully excised. The "Miami criteria" are used to interpret IOPTH measurement: a PTH decrease of more than 50% after excision of all pathological parathyroid tissue (67,68). If the IOPTH does not drop below 50% or if a pathological gland is not found during minimally invasive surgery, the procedure is conversed to bilateral neck exploration. IOPTH monitoring has a sensitivity of up

to 99% (69,70) and has been shown to significantly increase cure rate by about 10% (69,71).

Cinacalcet is the only registered medical treatment for PHPT and is available for clinical use in the Netherlands since the summer of 2009. Cinacalcet is a calcimimetic approved by the European Medicines Agency Committee for Medicinal Products for Human Use for "the reduction of hypercalcaemia in patients with primary hyperparathyroidism for whom parathyroidectomy would be indicated on the basis of serum calcium levels (as defined by the relevant treatment guidelines), but in whom parathyroidectomy is not clinically appropriate or is contraindicated" (72). Calcimimetics increase the sensitivity of the calcium sensing receptor (CASR) on parathyroid cells to extracellular calcium concentration (73-79), thereby significantly decreasing serum PTH and calcium concentration. This agent has been reported to normalize serum calcium concentration in ~88% of patients with PHPT (76-79).

Fate of complications after successful surgery

In PHPT, the importance of achieving cure has been highlighted in a number of studies focusing on the effect of parathyroidectomy on bone mineral density, nephrolithiasis and neurocognitive function (80-82). Patients who are cured after PTx demonstrate a significant postoperative increase in bone mass, with BMD remaining significantly higher than baseline values for up to 15 years after parathyroidectomy (81-83). This is in contrast to patients treated conservatively, in whom significant cortical bone loss and increased fracture risk is observed in the long-term. As reported in a number of published studies, patients with nephrolithiasis at presentation demonstrate no recurrence of renal stones after successful PTx, in contrast to a 75-100% chance of recurrent nephrolithiasis in conservatively treated patients (80,83). Symptomatic and also "asymptomatic" patients undergoing parathyroidectomy have also been reported to experience a significant improvement in various parameters of quality of life compared to patients who did not have surgery (84-88).

III. PERSISTENCE OR RECURRENCE OF PHPT

Definitions

Persistence of hyperparathyroidism is defined as high serum calcium and PTH concentrations or inappropriate concentrations of one parameter with respect to the other, documented directly post-operatively or within the first 6 months after parathyroidectomy, and persisting thereafter. Recurrence of hyperparathyroidism is defined as the recurrence of elevated serum calcium and PTH concentrations after successful PTx and a period of normalization of both parameters of at least 6 months after surgery.

Etiology

Persistent hyperparathyroidism is reported to occur in 2-7% of patients after parathyroidectomy (33,89,90). Excluding insufficient experience of the operating surgeon, persistent PHPT may be caused by a pathological gland which is missed during initial surgery (6.3-69%), a second adenoma (6.3%), non-recognition or incomplete excision of multiple gland hyperplasia (9-29%), an ectopically located hyperactive gland undetected at initial surgery (11-77%), supernumerary glands (4.2-8%) or rarely parathyromatosis from parathyroid cell spillage during initial surgery (0.7-6.3%) (90-98). The diagnosis of persistent hyperparathyroidism may be delayed because of initial suppression of multiple pathological glands by a dominant enlarged gland, falsely suggesting cure immediately post-operatively. These pathological glands eventually overcome their suppressed state leading to overt hyperparathyroidism.

In a 5 year follow-up of 91 patients successfully operated for PHPT, Westerdahl *et al* reported a recurrence rate of hyperparathyroidism of 4%, largely due, however, to previously undiagnosed germline mutations in the *MEN-1* gene and, consequently, multiple gland pathology (99). Other causes of recurrence of PHPT are metastases from a parathyroid carcinoma or newly developed adenomas, most of the times arising in hyperplastic parathyroid glands (91-93).

Location of ectopic parathyroid glands

In 24-77% of patients who undergo revision surgery for PHPT missed glands are found in ectopic locations, such as the tracheoesophageal groove (2.8-33%), thymus (8.3-27%), retroesophageally (1.8-10.4%), intrathyroidally (4-10%), the carotid bifurcation (8.3%), the mediastinum (2.6-7.3%), the aortic-pulmonary window (1.3-6.3%), the carotid sheath (2.1-5.5%) and parapharyngeally (2.1%) (56,93,94,100,101).

Most ectopias of the superior gland are acquired ectopias due to the effect of gravity on an enlarged gland, causing the gland to travel down the prevertebral plane towards the posterosuperior mediastinum (102). Congenital ectopias of the superior glands, are due to undescended glands, found behind the pharynx, above the superior thyroid pole or at the posteromedial aspect of the thyroid lobe, while an excessively migrated gland can be found intrathyroidal (102). Most inferior gland ectopias are congenital ectopias caused by abnormal embryological migration of the parathymic complex, extending from the angle of the mandible to the pericardium (102). An undescended inferior parathyroid gland can be found along the carotid sheath, from the angle of the mandible to the lower thyroid pole (103), while an excessively migrated gland can be found in the anterior mediastinum, within the thymus or at the posterior aspect of the thymic capsule.

Supernumerary glands develop from accessory parathyroid fragments caused by the rapid caudal and medial migration of the thymus, which may drag a fragment of the third branchial pouch, the origin of the inferior parathyroid glands (102). Supernumerary glands may also be found in close contact with the vagus nerve or sometimes within it (104). There is a close spatial relationship between the vagus nerve and the third and fourth pharyngeal pouches, the origin of the inferior and superior parathyroid glands, as a portion of this nerve is derived from the fourth branchial arch.

Localization studies

A second (or subsequent) neck exploration is technically more challenging than initial surgery and may be associated with an up to 3-fold increase in morbidity

(33,93,105,106). Localization studies are, therefore, highly recommended prior to revision surgery in order to decrease operation time, improve surgical outcome and reduce the risk of complications due to lengthy surgical explorations (33,91,93,105,106).

The sensitivity of Tc99m-MIBI-SPECT prior to revision surgery varies from as low as 27% to as high as 94%, depending on the selection of patients, the pathology of the parathyroid gland(s) and the methods used to determine the accuracy of the technique (49,94,96,97,100,101,107-111). Tc99m-MIBI-SPECT has a lower sensitivity in patients with multiple hyperplastic parathyroid glands compared to patients in whom a single adenoma is found at surgery (94% vs. 43%) (112). The sensitivity of Tc99m-MIBI-SPECT is also decreased in patients who have had previous resection of pathological parathyroid gland(s) compared to patients who have had previous negative neck exploration (89% vs. 27%) (108).

Ultrasound of the neck (US) is reported to have a sensitivity of 17%-90% prior to revision surgery (49,94,96,97,101,107,108,110-113). In contrast to Tc99m-MIBI-SPECT, US is not influenced by the pathology of the parathyroid gland (112).

When both Tc99m-MIBI-SPECT and US are negative or when results of these localisation techniques are disconcordant, an MRI-scan, CT-scan or selective venous sampling for PTH (SVS) can be helpful to identify residual pathological parathyroid gland(s). The sensitivity of MRI-scan prior to revision surgery is 33-89% (49,97,110-112), that of CT-scan is 33-50% (49,97,110) and that of SVS is 50-91% (49,94,97,111,114). 4D-CT has shown a promising sensitivity of 80% prior to revision surgery in patients with PHPT who have had previous unsuccessful parathyroidectomy (108).

Outcome of revision surgery

Despite the challenges of revision surgery, the reported cure rate remains high after re-exploration ranging from 82% to 100% (49,56,94,96,100,101,107-109,111,115,116). However, the highest cure rates (98-100%) are found in patients who had previous thyroid surgery or negative neck explorations and in patients who have solitary adenomas (101,109,115) and the lowest cure rates (82-83%) are found

in patients with persistent hyperparathyroidism after removal of one or more pathological parathyroid glands (94,108). Patients with multiple gland hyperplasia and patients who have had 3 previous neck explorations are less likely to achieve cure after further surgery (96,107).

Complications of revision surgery

Re-operations are associated with an increased risk of complications due to distortion and scarring of surgical planes caused by previous interventions. The overall complication rate after revision surgery is reported to be none to 29% (49,56,94,96,101,107,108,117,118). The most common complications are hypoparathyroidism (2.6-12.9%) and permanent or transient recurrent laryngeal nerve injury (0.08-25% and 2.3-13.5%, respectively) (49,56,94,97,100,101,107,111,117-119). Other possible complications are post-operative bleeding requiring blood transfusion (1.1-2.1%), mandibular nerve injury (0.4-1.6%), wound hematoma (0.08-2.2%), pneumothorax (1.6-4.4%), pneumonia (0.4%-2.2%) and deep venous thrombosis or pulmonary embolism (1-2.2%) (56,94,100,101,108,117). Rare complications are Horner syndrome after sternotomy, chylous fistulas, arrhythmia, myocardial infarction, wound erythema and renal failure (56,108,117).

IV. OUTLINE OF THIS THESIS

Part I. Cure after parathyroidectomy for primary hyperparathyroidism

Primary hyperparathyroidism is the most common cause of hypercalcaemia in the outpatient setting. Surgical removal of all pathological parathyroid glands is the only approach that provides definitive and durable cure in these patients. Patients who are cured after surgery show a significant postoperative increase in bone mass (81-83), no recurrence of renal stones (80,83) and improvement in various parameters of quality of life compared to patients who had no or unsuccessful surgery (84-88). In ***Chapter 2*** we assessed the prevalence of persistent and recurrent hyperparathyroidism after surgery for primary hyperparathyroidism in our hospital

over a 24 year period. We also evaluated the fate of clinical symptoms and complications of PHPT after successful surgery.

Part II. Localization studies in persistent primary hyperparathyroidism

The need for preoperative localization of pathological parathyroid gland(s) before initial surgery depends on the chosen surgical approach. In case of traditional bilateral neck exploration localization studies are not deemed necessary, but they become mandatory prior to more focused unilateral or minimally invasive surgery (33,34). Accurate localization studies are even more important before revision surgery for persistent hyperparathyroidism, as a second (or subsequent) neck exploration is technically more challenging than initial surgery (33,93,105,106). In ***Chapter 3*** we evaluated the predictive value of Tc99m-MIBI-SPECT in patients with persistent PHPT prior to revision surgery compared to the predictive value of this technique in patients with sporadic PHPT prior to initial surgery.

We also evaluated the predictive value of selective venous sampling for PTH in patients with persistent PHPT prior to revision surgery compared to the predictive value of Tc99m-MIBI-SPECT in the same patient group. Results of this study are described in ***Chapter 4***.

Part III. Complications of the surgical management of primary hyperparathyroidism

PHPT is associated with decreased bone mineral density, due to the stimulating effects of increased circulating levels of PTH on osteoclastogenesis by the stimulation of RANKL secretion, an osteoclast activator, and the suppression of OPG secretion, the natural decoy receptor for RANKL (15-25). The increased bone turnover in favour of bone resorption induced by the pre-operative hyperparathyroid state, leads to demineralization of bone particularly at cortical sites. Following successful surgery, the rapid decrease in serum PTH levels and the pre-operative high bone turnover state, lead to a transient hypocalcaemia, due to increased influx of calcium, phosphate and magnesium into bone. Restoration of mineralization of

bone and eventual decrease in bone turnover lead to a significant increase in bone mass (81-83).

Post-operative severe persistent hypocalcaemia is usually due to a hungry bone syndrome, a syndrome seldom seen in patients with sporadic PHPT. The syndrome is predominantly observed in patients with severe hyperparathyroidism-associated increased bone turnover, specially those with parathyroid carcinoma. Data on this rare complication of the treatment of primary hyperparathyroidism are scarce and its management remains a challenge. In ***Chapter 5*** we performed a systemic review of the literature on hungry bone syndrome, summarizing its main features and suggestions for its prevention and management.

Part IV. Aspects of osteocyte function in primary hyperparathyroidism

Some of the effects of PTH on bone are exerted by the specific binding of PTH to its receptor PTHR1 in osteocytes, resulting in inhibition of the expression of the *Sost* gene (120-123). This gene encodes for the protein sclerostin, which decreases bone formation by inhibition of the Wnt signaling pathway in osteoblasts (124,125). Intermittent PTH administered to post-menopausal women has been shown to decrease circulating sclerostin levels (126). To assess whether chronic hypersecretion of PTH also inhibits sclerostin secretion, we measured serum sclerostin concentrations in patients with untreated PHPT and in patients cured after successful parathyroidectomy for PHPT. Results are reported in ***Chapter 6.***

Binding of PTH to the PTHR1 on osteocytes has been reported to upregulate Fibroblast Growth Factor 23 (FGF23) mRNA production (127,128), a stimulator of tubular phosphate transport and a suppressor of 1α-hydroxylase production by the kidney (129,130). Reports on the effect of chronic high circulating levels of PTH, as characteristic of PHPT, on FGF-23 levels are scarce and not always concordant (130-132). In ***Chapter 7*** we evaluated the effect of chronic excess of PTH on FGF23 concentrations by measuring FGF23 concentrations in patients with PHPT before surgery and in those with euparathyroidism cured after successful parathyroidectomy for PHPT.

Part V. Clinical and molecular aspects of parathyroid carcinoma

In ***Chapter 8*** we report the many challenges and pitfalls encountered in the management of a patient with multiple metastases from parathyroid carcinoma over a 17 year period.

Several genes have been discovered to play a role in the etiology of PHPT, among which the *MEN-1*, *HRPT2* and *CASR* genes. Mutations in the *HRPT2/CDC73* gene, which encodes for the protein parafibromin (133), are associated with the hyperparathyroidism-jaw tumor syndrome (HPT-JT; OMIM 607393). Patients with a HPT-JT syndrome have a 15-24% chance of developing parathyroid carcinoma (29,134-136) and loss of heterozygosity of chromosome 1q, the location of the *HRPT2/CDC73* gene, is reported to be found in 55% of parathyroid carcinomas (1,17,19-21). Downregulation of the CASR has been reported in patients with the HPT-JT syndrome, as well as in patients with sporadic carcinoma (137).

The aim of the study described in ***Chapter 9*** was to evaluate the role of mutations in the *HRPT2/CDC73* gene, loss of parafibromin staining or downregulation of CASR expression as determinants of prognosis in patients with an established diagnosis of parathyroid carcinoma.

Part VI. Management of patients with persistent primary hyperparathyroidism

In patients with persistent primary hyperparathyroidism who had undergone multiple surgeries, re-explorations are associated with a more than 3-fold increased risk of morbidity (33,93,105,106), due to scarring of the operation field and disruption of the normal patterns of drainage by previous surgeries. In ***Chapter 10*** we assessed the demographic, clinical, biochemical, surgical and pathological characteristics of patients with persistent PHPT to identify predictive factors for cure after revision surgery.

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No recurrence of sporadic primary hyperparathyroidism when cure is established 6 months after parathyroidectomy

Janneke E. Witteveen, Job Kievit, Hans Morreau, Johannes A. Romijn, Neveen A.T. Hamdy

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ABSTRACT

Objective: Cure rate for primary hyperparathyroidism (PHPT) is reported to be 94-100% 1 year after surgery, but recent data suggest recurrence in 4% of patients 1-5 years postoperatively. The aim of our study was to establish the cure rate and its maintenance in the long-term after parathyroidectomy (PTx) in patients with sporadic primary hyperparathyroidism.

Design: Evaluation of recurrence in patients with sporadic hyperparathyroidism who underwent PTx 1-24 years prior to the study.

Patients & Methods: We identified 111 patients who underwent initial PTx between 1984 and 2008 and had no *MEN-1*, *MEN-2* or *CASR* mutation; parathyroid carcinoma; a history of lithium use; or renal failure. Thirty-eight patients were lost to follow-up or were unwilling or unable to participate in the study. Cure was defined as maintenance of normal serum calcium and parathyroid hormone concentrations 6 months after PTx.

Results: Cure was achieved in 68 of 73 patients studied (93%) and was sustained in all for 6±5 years.

Conclusion: The cure rate of sporadic PHPT after initial surgery is 93%. When cure is achieved, this is sustained in 100% of the patients for up to 24 years post-operatively. Our data suggest that closer early follow-up is advocated in all patients undergoing PTx to definitively establish cure and to provide a safety net for those with residual gland pathology. The data do not support the need for long term follow-up when cure is established 6 months after PTx.

INTRODUCTION

In primary hyperparathyroidism (PHPT), surgical removal of all pathological parathyroid glands is the only approach that provides a definitive and durable cure. Cure rate has been reported to be in the range of 94-100% for bilateral neck, as well as less invasive surgery, more than 6 months after parathyroidectomy (PTx) (1-10). Residual postoperative biochemical features of PHPT may be due to either recurrent or persistent PHPT. In a 5 year follow-up of 91 patients successfully operated for PHPT, Westerdahl *et al.* reported a recurrence rate of 4%, largely due to previously undiagnosed germline mutations in the *MEN-1* gene and consequent multiple gland pathology (11). Persistent hyperparathyroidism is usually due to a pathological parathyroid gland missed at initial surgery. Such a gland is often small, usually forming a part of unrecognized multiple gland hyperplasia (12,13). The diagnosis of persistent hyperparathyroidism may be delayed because of the initial suppression of multiple pathological glands by a dominant enlarged gland, falsely suggesting cure immediately post-operatively. These pathological glands eventually overcome their suppressed state leading to overt hyperparathyroidism.

In PHPT, the importance of achieving cure has been highlighted in a number of studies focusing on the effect of PTx on bone mineral density (BMD), nephrolithiasis and neurocognitive function (14-16). Patients who are cured show a significant post-operative increase in bone mass, with BMD remaining significantly above baseline for up to 15 years of follow-up (17). This is in contrast to BMD changes in patients treated conservatively, in whom significant cortical bone loss and increased fracture risk are observed. As reported in a number of studies, patients with nephrolithiasis at presentation demonstrate no recurrence of renal stones after successful PTx, in contrast to a 75-100% chance of recurrent nephrolithiasis if not operated (14,17). Symptomatic and also 'asymptomatic' patients undergoing PTx have been reported to experience a significant improvement in various parameters of quality of life compared to patients who had no surgery (18-22). The aim of our study was to establish the cure rate and its

maintenance in the long-term after initial PTx in patients with sporadic PHPT who underwent surgery in our hospital between 1984 and 2008.

PATIENTS AND METHODS

Study population

All patients with a diagnosis of PHPT who had a PTx in the Leiden University Medical Center (LUMC) from March 1984 to May 2008 were identified from our hospital records and were considered for study. Patients who were not initially operated in our institution and patients with parathyroid carcinoma, renal failure, or a history of lithium use were excluded from the study. Patients with a *MEN-1, MEN-2* or *CASR* mutation were also excluded from the final analysis, although genetic testing may have been undertaken some years after initial PTx. Details on the outcome of these 32 patients with a documented mutation are shown in Figure 1. One hundred and eleven patients with sporadic PHPT were thus eligible for the study and were recalled for a follow-up visit at the outpatient clinic of the Department of Endocrinology and Metabolic Diseases.

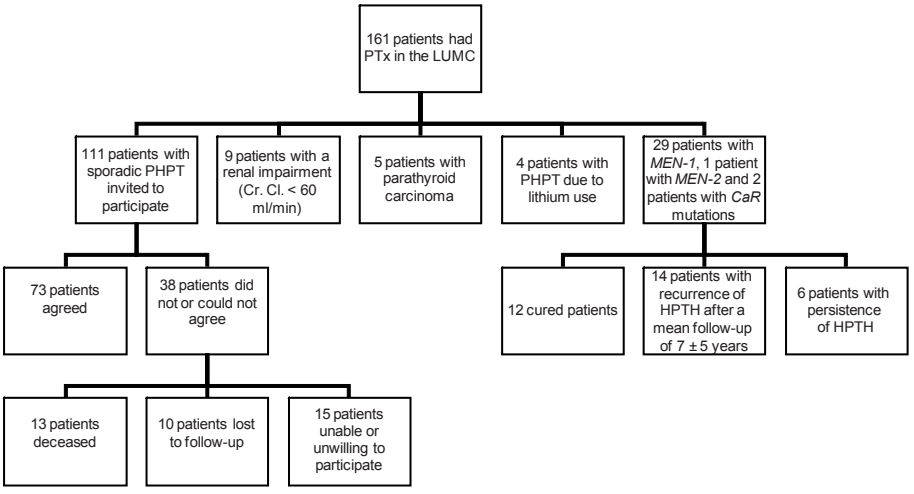

Figure 1. Study flow diagram

Methods

A detailed medical history was obtained from all patients with special emphasis on symptoms related to hypercalcaemia and non specific symptoms of hyperparathyroidism, such as tiredness, mood swings, depression, feeling irritable, being forgetful, feeling weak, bone pain, muscle weakness, constipation and polyuria/polydipsia. These symptoms represent 10 of the 13 items of the validated Pasieka's 'Parathyroid Assessment of Symptoms Score (PAS)' (21,23). Blood was collected from all patients. Operative and pathology data were obtained from patients' hospital records. Fracture history was obtained by direct questioning and was confirmed by reviewing available radiological data spanning the period before and after PTx.

Serum Biochemistry

Serum concentrations of calcium (reference range 2.15-2.55 mmol/l), albumin (reference range 34-48 g/l), phosphate (reference range 0.90-1.50 mmol/l), and creatinine (reference range 44-80 μmol/l) were measured using semi-automated techniques. Creatinine clearance was calculated using the Cockcroft formula. Serum concentrations of intact parathyroid hormone (PTH: reference range 1.5-8 pmol/l) and 25-hydroxycholecalciferol (25 (OH) D3) (reference range 30-120 nmol/l) were measured using standard RIAs. Vitamin D status was judged to be inadequate when 25(OH)D3 concentrations were <50 nmol/l (24,25).

Radiological investigations

BMD was measured at the lumbar spine (L1-L4) and femoral neck, using dual energy X-ray absorptiometry (DXA, Hologic QDR 4500; Waltham, MA, USA), in 46 patients who had a baseline BMD measurement before PTx. World Health Organization criteria were used to define osteopenia (*T*-score between -1 and -2.5) and osteoporosis (*T*-score <-2.5) (26). Lateral X-rays of the thoracic and lumbar spine were performed in 35 patients, when medically indicated, in case of height loss or back pain, and in those who had baseline X-rays before PTx.

Fifty one patients, including those with persistent PHPT and those with a history of nephrolithiasis or nephrocalcinosis, and representing 70% of the study population, had an ultrasound of the kidneys to document the presence or absence of nephrocalcinosis and/or nephrolithiasis.

Surgical procedure

The indication criteria for surgery were based on the 1990 (27) and subsequent 2002 (28) NIH Consensus guidelines. The 1990 guidelines were broadly followed in our institution in the period preceding their publication. Before the introduction of intra-operative PTH (IOPTH) measurement in our hospital in 1997, almost all the patients underwent bilateral neck exploration with visualization of all the four parathyroid glands. This led either to the excision of a single enlarged parathyroid gland, or to subtotal or total PTx with auto transplantation if more than one parathyroid gland was found to be enlarged. If all parathyroids could not be visualized and no enlarged parathyroid was found, dissection of the ipsilateral anterior compartment extending from the level of the hyoid bone superiorly to the suprasternal notch inferiorly and hemithyroidectomy were undertaken on the side of the missing parathyroid. After standardizing the use of IOPTH, a more selective surgical approach was opted for, and less invasive neck exploration was undertaken in patients with positive preoperative localization studies. IOPTH monitoring consisted of two initial baseline measurements with an interval of 15-20 minutes, followed by five measurements at 3-minute intervals after excision of the pathological parathyroid(s). Surgery was considered successful if IOPTH decreased by more than 50% within 7 minutes of excision of a pathological parathyroid gland(s).

Definition of cure, persistence and recurrence of hyperparathyroidism

Successful surgery or cure was defined as normal serum calcium and PTH concentrations as measured more than 6 months after PTx. Persistence of hyperparathyroidism was defined as high serum calcium and PTH concentrations or inappropriate concentrations of one parameter with respect to the other, documented

directly post-operatively or within the first 6 months after PTx, and persisting thereafter. Recurrence of hyperparathyroidism was defined as the recurrence of elevated serum calcium and PTH concentrations after successful PTx and a period of normalization of both parameters of at least 6 months after surgery.

Statistical analysis

Statistical analysis was performed using the SPSS 16.0 for Windows (Chicago, IL, USA). Results are expressed as mean ± S.D. unless otherwise stated. χ^2 test and Student's *t*-test were used as appropriate for categorical variables and continuous variables. A probability level of random difference of $P<0.05$ was considered significant.

The study was approved by the local ethics committee, and informed consent was obtained from all patients prior to inclusion in the study.

RESULTS

Demographic data

Seventy-three of the 111 patients who were invited to participate in the study agreed to take part. Of the remaining 38 patients, 13 had died, 10 were lost to follow-up and 15 were physically unable or unwilling to take part in the study (Figure 1).

Fifty-six women and 17 men, with a mean age of 56 ± 10 years at the time of the diagnosis were included in the study *(*Table 1). At the time of the first presentation, only 18 of the 73 patients (25%) were asymptomatic. Although only 7 patients had symptoms related to hypercalcemia such as polyuria, polydipsia, and constipation, nonspecific symptoms were common. Tiredness was reported in 44%, muscle or bone pain in 22%, and depressive symptoms in 16% of the patients. Twenty- seven patients (37%) had symptoms related to renal stones and six patients (8%) had sustained a documented fracture, two vertebral and four non-vertebral after some degree of trauma. Mean pre-operative serum calcium concentration was 2.76 ± 0.20 mmol/l and mean PTH level was 19.5 ± 15.7 pmol/l. Renal impairment,

as defined by a creatinine clearance of <60 ml/min, was documented in 21% of patients. Renal stones were documented on ultrasound of the kidneys in 41% of patients and 38% had BMD evidence for osteoporosis on DXA.

Sixty-three of the 73 patients (86%) had undergone parathyroid localization studies before PTx, using Tc99m-MIBI-SPECT scan and/or ultrasound scan of the neck. Forty-seven of the 73 patients (64%) had undergone bilateral neck exploration, 12% had unilateral neck exploration and 23% had a minimally invasive procedure. Surgery was guided by IOPTH monitoring in 53 patients (73%). In 14 of the 73 patients (19%), PTx was combined with a hemithyroidectomy. The decision for this procedure was based on pre-operatively identified thyroid pathology in seven patients and negative bilateral neck exploration in the other seven patients. However, an intra-thyroidal parathyroid gland was found in only 3 of the 14 patients.

Since 2001, due to the availability of IOPTH monitoring and the increased popularity of less invasive surgical procedures, the percent of bilateral neck explorations decreased from 76 to 56% and that of the more focused surgical approaches increased from 24 to 44%. Parallel to the use of less invasive surgical approaches, the use of pre-operative localization studies increased from 77 to 95% and the use of IOPTH monitoring increased from 43 to 93% (Figure 2). A single adenoma was removed at surgery in 56 of the 73 patients (77%), and one or more hyperplastic glands were removed in 16% of the cases.

Short-term follow-up, within one year of parathyroidectomy

At the time of first follow-up, within 3 months of surgery, 70 of the 73 patients (96%) had evidence for biochemical cure of hyperparathyroidism. At the time of the second post-operative follow-up, 6 months after PTx, 68 of the 73 patients (93%) had evidence for cure. Five patients (7%) demonstrated persistence of hyperparathyroidism; in two of whom this was originally overlooked because of a transient normalization of serum calcium and PTH concentrations post-operatively. In these two patients, a second laboratory measurement demonstrated elevated

Table 1. Demographic, laboratory, clinical and surgical data of patients who underwent initial surgery for sporadic primary hyperparathyroidism

	Total patients N=73	Achieved cure after initial PTx N=68	Persistence after initial PTx N=5	*P* value
Gender (men:women)	17:56	16:52	1:4	0.857
Age at diagnosis (years)	56 ± 10	56 ± 11	56 ± 6	0.910
Age at follow-up (years)	63 ± 11	63 ± 11	69 ± 5	0.199
Years after PTx	6 ± 5	6 ± 5	12 ± 5	0.013
Follow-up time after PTx (months)	39 ± 56	33 ± 50	129 ± 66	0.000
Biochemistry prior to intial surgery				
s-calcium (mmol/l)	2.76 ± 0.20	2.77 ± 0.20	2.68 ± 0.08	0.313
PTH (pmol/l)	19.5 ± 15.7	20.2 ± 16.0	9.9 ± 2.0	0.161
phosphate (mmol/l)	0.88 ± 0.18	0.89 ± 0.19	0.85 ± 0.15	0.676
Creatinine clearance (ml/min)	72 ± 14	72 ± 14	66 ± 5	0.068
u-calcium (mmol/24 h)	11.05 ± 4.80	10.96 ± 4.89	12.13 ± 3.67	0.604
Clinical presentation				
Polyuria & polydipsia	6/73 (8%)	6/68 (9%)	0/5	0.488
Constipation	1/73 (1%)	1/68 (1,5%)	0/5	0.785
Tiredness	32/73 (44%)	31/68 (46%)	1/5 (20%)	0.266
Muscle or bone pain	16/73 (22%)	15/68 (22%)	1/5 (20%)	0.914
Depressive symptoms	12/73 (16%)	12/68 (18%)	0/5	0.304
Complications				
Renal impairment	15/73 (21%)	14/68 (21%)	1/5 (20%)	0.925
Nephrolithiasis	30/73 (41%)	28/68 (41%)	2/5 (40%)	0.959
Osteoporosis	28/73 (38%)	27/68 (40%)	1/5 (20%)	0.382
Fractures	7/73 (10%)	6/68 (9%)	1/5 (20%)	0.413
Pre-operative localization studies				
US and/or MiBi-SPECT	62/73 (86%)	58/68 (85%)	4/5 (80%)	0.671
Type of surgery				
Bilateral neck exploration	47/73 (64%)	44/68 (65%)	3/5 (60%)	0.832
Unilateral neck exploration	9/73 (12%)	8/68 (12%)	1/5 (20%)	0.589
Minimally invasive	17/73 (23%)	16/68 (24%)	1/5 (20%)	0.857
Combined with thyroidectomy	14/73 (19%)	13/68 (19%)	1/5 (20%)	0.961
Combined with thymectomy	5/73 (7%)	4/68 (6%)	1/5 (20%)	0.228
Use of IOPTH monitoring	53/73 (73%)	50/68 (74%)	3/5 (60%)	0.513
Pathology				
Adenoma	56/73 (77%)	53/68 (78%)	3/5 (60%)	0.360
Hyperplasia	12/73 (16%)	11/68 (16%)	1/5 (20%)	0.824
No pathological glands found	5/73 (7%)	4/68 (6%)	1/5 (20%)	0.228

s: serum, u: urinary, renal impairment=creatinine clearance <60 ml/min

serum calcium and PTH concentrations at respectively 3 and 5 months post-operatively. There were no significant differences in demographic or biochemical data before or after PTx between patients who were cured after initial surgery and those (n=5) who demonstrated persistence of PHPT (Table 1).

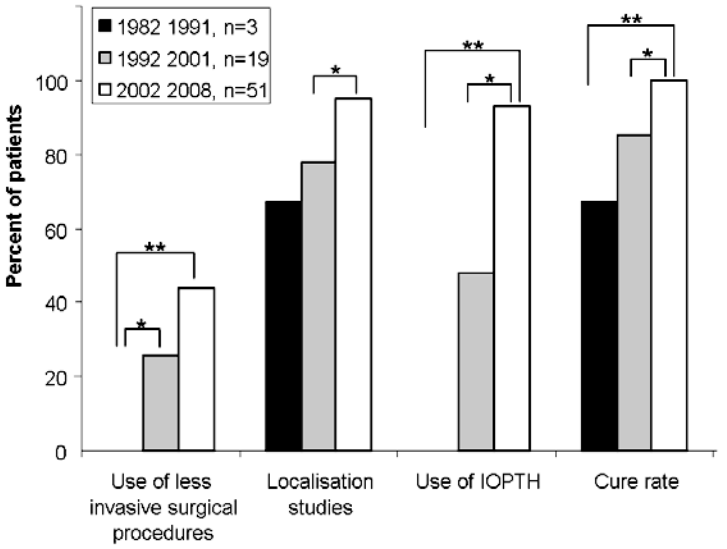

Figure 2. Pattern of change in surgical procedures, use of pre-operative localization studies and IOPTH monitoring, and cure rate over 24 years of follow-up. In the first decade of the study (1982-1991) all patients underwent bilateral neck exploration and IOPTH monitoring was not yet available. * $P<0.05$, ** $P<0.001$.

Long-term follow-up

Patients were evaluated after a mean of 6 ± 5 years and up to 24 years after PTx, with the majority of the patients (68%) being assessed more than 5 years after PTx. Median age at follow-up was 63 years compared to 55 years before PTx. Cure was sustained for the length of follow-up in all 68 patients in whom cure was established at 6 months after initial surgery. Regardless of age, 29% of the patients complained of tiredness, only two of whom were older than 70 years, 22% of muscle or bone pain, and 15% of depressive symptoms, which persisted despite complete cure. The mean corrected serum calcium concentration was 2.27 ± 0.13

mmol/l, with a mean serum PTH concentration of 4.4 ± 2.4 pmol/l. Two of the five patients with persistent HPTH had undergone successful revision surgery, resulting in cure after excision of one or more additional hyperactive parathyroid glands, one of which was situated retrosternally. The third patient is being treated conservatively because of the mild asymptomatic nature of her PHPT and the failure of localization studies to identify the site of the hyperactive gland(s). A conservative approach was also originally opted for in the fourth patient because of the mild asymptomatic nature of his persistent PHPT, but he is now scheduled for revision surgery as he demonstrated significant symptomatic and biochemical progression of his HPTH 9 years after his initial surgery (Figure 3). The fifth patient still has persistent PHPT after unsuccessful full neck and mediastinal explorations, and is being treated successfully with the calcimimetic cinacalcet.

Predictive factors for cure after parathyroidectomy

There were no significant differences in gender and age at presentation between patients who achieved and maintained cure and patients with persistence of HPTH after initial PTx (Table 1). Clinical presentation (symptomatology and skeletal and/or renal complications) was also not significantly different in patients in whom HPTH persisted post-operatively. None of the laboratory investigations, including serum calcium, PTH, phosphate, urinary calcium, and creatinine clearance, showed a significant difference between cured and noncured patients. There was also no significant difference in the availability of pre-operative localization studies, type of surgical approach, or use of IOPTH monitoring between groups. No pathological tissue was found at initial surgery in one of the five patients with persistent HPTH, but this was also the case in 4 patients who were subsequently cured. It is of note that all five patients with persistent HPTH had undergone surgery before 2001 and all had undergone localization studies in the form of ultrasound of the neck and/or Tc99m-MIBI-SPECT scan. Three of the five patients also had IOPTH monitoring (Table 2), in 2 of whom IOPTH monitoring indicated cure, with a decrease in PTH levels of 96 and 71,6% respectively after excision of a pathological parathyroid gland. In both patients, this was associated with a transient normalization of serum

calcium and PTH concentrations lasting 3 and 5 months respectively after PTx. In the third patient with persistent HPTH, only one hyperplastic gland could be identified and removed during surgery, but there was no significant decrease in IOPTH levels and hyperparathyroidism persisted post-operatively.

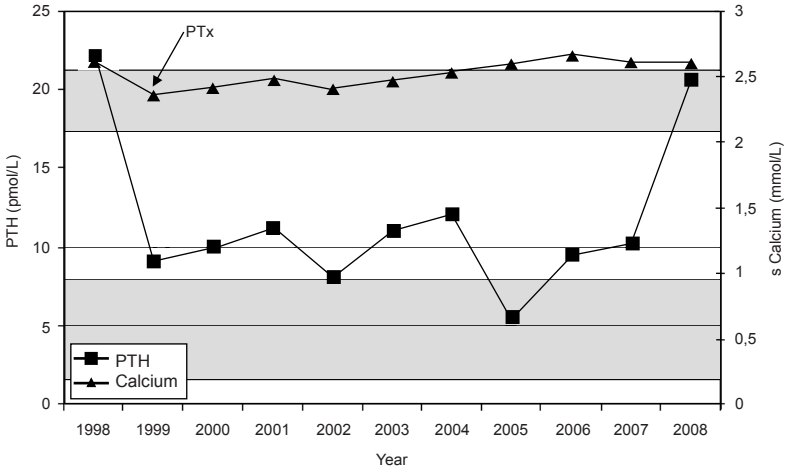

Figure 3. Serum calcium and PTH concentrations in a patient conservatively treated for mild asymptomatic persistent hyperparathyroidism after incomplete parathyroidectomy (PTx). Clear demonstration of progression of biochemical features associated with clinical manifestations of overt hyperparathyroidism 9 years after surgery, outlining the importance of long-term follow-up in these patients.

Fate of symptoms/complications of primary hyperparathyroidism after cure

After successful parathyroidectomy, none of the 68 cured patients had specific symptoms related to hypercalcaemia, particularly no polyuria and polydipsia (Figure 4). Although there was a trend towards a decrease in reported tiredness (*P*=0.05), there was no significant change in a number of other non-specific symptoms, such as depressive symptoms, muscle pain or muscle weakness after cure. The frequency of nephrolithiasis decreased significantly from 41% to 10%, *(P*=0.000). There was also a significant improvement in BMD, with only 16% of patients having evidence for osteoporosis a mean of 6 years after PTx compared to

Table 2. Surgical and pathological data of patients with sporadic primary hyperparathyroidism with persistent HPTH after initial surgery

		Localisation studies						
Age at presentation (years)	Gender	US scan	MiBi-SPECT scan	Surgical procedure	IOPTH monitoring	Pathology excised parathyroid	Transient cure	Outcome of revision PTx
49	F	No	Yes, Positive	Bilateral	No	Adenoma	None	Persistence
57	F	No	No	Bilateral	No	No pathology	None	Cured
66	F	Yes, Positive	No	Unilateral	Yes	Adenoma	5 months	Cured
56	M	Yes, Positive	No	Minimally invasive	Yes	Adenoma	3 months	Awaits revision PTx
53	F	Yes, Negative	Yes, Negative	Bilateral	Yes	Hyperplasia	None	Persistence

40% before surgery P=0.002). After parathyroidectomy, 4 patients, all of whom were post-menopausal women aged 59 to 78 years, sustained 4 documented fractures, one vertebral and 3 non-vertebral (2 hip fractures and 1 Colle's fracture). 64% of patients who had renal impairment prior to PTx demonstrated a 35% improvement in their renal function after successful surgery. In the remaining patients with renal impairment at initial surgery, this remained stable in 21% and further decreased by 22% in 14% of patients who achieved and maintained cure. Renal function decreased over time of follow-up in 4 patients who had normal renal function pre-operatively.

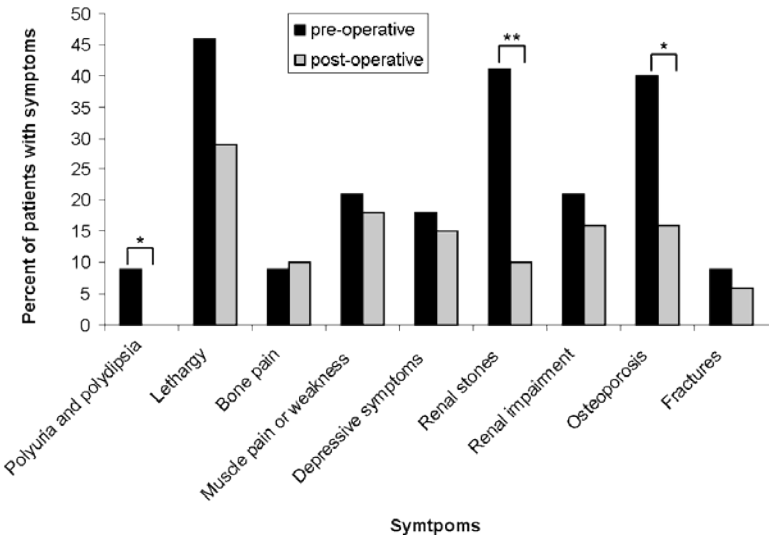

Figure 4. Change in symptomatology and in renal and skeletal complications after successful surgery for sporadic primary hyperparathyroidism, after a mean 6 years follow-up. * P<0.05, ** P <0.001.

DISCUSSION

In our population of patients with sporadic PHPT, we observed an overall cure rate of 93%, which has risen to 100% over the last 7 years with the increasing use of

pre-operative localization studies and IOPTH monitoring. We demonstrated that when cure is achieved in these patients, this is sustained in all for up to 24 years of follow-up.

Cases of 'recurrent HPTH' reported by Westerdahl *et al.* (11) and Hedback *et al.* (29) were shown to be due to initially unsuspected *MEN-1* or *MEN-2* mutations or due to secondary or tertiary hyperparathyroidism, all potentially associated with multiple gland disease. Data from our study, in which we excluded patients with known germ cell mutations in the *MEN-1* or *MEN-2* gene or other genes controlling parathyroid growth and PTH secretion, support the notion that 'recurrent' HPTH does not occur in sporadic PHPT when cure is established 6 months after PTx. This is in clear contrast to the outcome in patients with a known germ cell mutation, who were excluded from the study, in whom hyperparathyroidism recurred post-operatively in 44% of the cases (Figure 1). In this context, DNA analysis is strongly advocated in all the cases of 'recurrent' HPTH to assess the possible presence of a specific germ cell mutation as this holds significant clinical implications for the management of these patients.

Following adequate surgery, persistent HPTH is most commonly due to multiple gland disease with additional pathological glands often missed during initial surgery. Transient normalization of serum calcium and PTH concentrations occurs post-operatively in multiple gland disease due to the suppression of the activity of smaller glands by a dominant large gland. Pathological small parathyroid glands may take sometimes up to 6 months to recover and become hyperactive in their own right. Failing to consider this possibility may result in premature discharge of patients from follow-up because of the erroneous impression of permanent cure. This is illustrated in one of our patients with persistent HPTH, who demonstrated progression of HPTH 9 years after initial PTx. Solorzano *et al.* also showed that patients with normal calcium and inappropriately high PTH values after surgery have, in fact, persistence of HPTH which can take up to 2 years to become symptomatic (30). Follow-up is therefore strongly advocated for more than 6 months after PTx to definitively establish cure and provide a safety net for those patients with pseudo-cure, who have residual gland pathology.

In addition to the essential requirement of an experienced surgeon, pre-operative localisation studies and IOPTH monitoring appear to significantly contribute to a successful outcome of surgery. In our hands, implementation of these measures has resulted in a 100% cure rate over the last 7 years.

In contrast to the widely observed shift in clinical presentation of PHPT from a symptomatic to a largely asymptomatic one, more than 70% of our patients were symptomatic and had renal impairment, renal stones or osteoporosis with or without fractures. Our institution is a tertiary referral center treating the more severe forms of PHPT, and patients with more severe forms of the disease are thus overrepresented in our study. However, this bias also represents the strength of our study, as we do show that despite the severity of hyperparathyroidism, cure can be achieved and sustained and is associated with significant beneficial effects on kidneys and bones.

We observed a significant decrease in the frequency of nephrolithiasis, an improvement in renal function in those with pre-operative renal impairment, and a general improvement in BMD after successful surgery. Other studies have also shown this positive effect of PTx on BMD, supporting the notion that osteopenia and osteoporosis are at least partially reversible after PTx (15-17). In our study, the limited number of X-rays performed in the study population combined with the small number of fractures sustained before or after PTx precludes a meaningful analysis of an effect of PTx on fracture risk.

The retrospective nature of the analysis of changes in neurocognitive function represents a limitation of our study, particularly with a time span of up to 24 years from presentation. It was notable, however, that a high proportion of patients, who were permanently cured, still had residual nonspecific symptoms, such as tiredness and depressive symptoms. This suggests that to some extent these symptoms may have been falsely attributed to PHPT, although irreversible changes in neurocognitive function may have also occurred as a result of long-term exposure to high circulating levels of PTH.

Our findings from this study hold significant clinical implications. The absence of recurrence of sporadic PHPT, when cure is established 6 months post-

operatively, strongly suggests that long-term follow-up of these patients is not necessary. In contrast, close follow-up is advocated within the first 6 months after PTx to definitively establish cure and to provide a safety net for those with residual gland pathology.

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3

Limitations of Tc99m-MIBI-SPECT imaging scans in persistent primary hyperparathyroidism

Janneke E. Witteveen, Job Kievit, Marcel P.M. Stokkel, Hans Morreau, Johannes A. Romijn, Neveen A.T. Hamdy

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ABSTRACT

Background:

In primary hyperparathyroidism (PHPT) the predictive value of technetium 99m sestamibi single emission computed tomography (Tc99m-MIBI-SPECT) for localizing pathological parathyroid glands before a first parathyroidectomy (PTx) is 83-100%. Data are scarce in patients undergoing reoperative PTx for persistent hyperparathyroidism. The aim of our study was to determine the value of Tc99m-MIBI-SPECT in localizing residual hyperactive parathyroid tissue in patients with persistent PHPT after initial excision of one or more pathological glands.

Method:

We retrospectively evaluated the localizing accuracy of Tc99m-MIBI-SPECT scans in 19 consecutive patients with persistent PHPT who had a scan before reoperative parathyroidectomy. We used as controls 23 patients with sporadic PHPT who had a scan before initial surgery.

Results:

In patients with persistent PHPT, Tc99m-MIBI-SPECT accurately localized a pathological parathyroid gland in 33% of cases before reoperative parathyroidectomy, compared to 61% before first PTx for sporadic PHPT. Tc99m-MIBI-SPECT accurately localized intra-thyroidal glands in 2 of 7 cases and a mediastinal gland in 1 of 3 cases either before initial or reoperative parathyroidectomy.

Conclusion:

Our data suggest that the accuracy of Tc99m-MIBI-SPECT in localizing residual hyperactive glands is significantly lower before reoperative parathyroidectomy for persistent PHPT than before initial surgery for sporadic PHPT. These findings should be taken in consideration in the preoperative workup of patients with persistent primary hyperparathyroidism.

INTRODUCTION

Primary hyperparathyroidism is one of the most common endocrine disorders. Surgical removal of all pathologic parathyroid tissue is the only therapy that leads to definitive and durable cure. The need for preoperative localization of pathological parathyroid gland(s) before initial surgery depends on the chosen surgical approach. In case of traditional bilateral neck exploration, localization studies are not deemed necessary because experienced surgeons have, on average, a 98% chance of excising all pathological tissue, as the procedure entails visualization of all four parathyroid glands (1,2). However, localization studies become mandatory when the surgeon opts for a more focused unilateral or minimally invasive approach. Accurate localization studies are even more important before reoperative parathyroidectomy for persistent hyperparathyroidism, as a second (or more) neck exploration is technically more challenging than initial surgery and may be associated with as much as a threefold increase in morbidity (1,3-5). Persistent hyperparathyroidism is reported to occur in 2-7% of patients who have had a parathyroidectomy (1,6,7). Excluding insufficient experience of the operating surgeon, persistent PHPT may be caused by a second adenoma, multiple gland hyperplasia, an ectopically located hyperactive gland undetected at initial surgery, or, rarely, parathyromatosis from gland spillage during initial surgery (8).

Technetium 99m sestamibi (Tc99m-MIBI), particularly when complemented by single emission computed tomography (Tc99m-MIBI-SPECT), is a widely used imaging technique for the preoperative localization of parathyroid adenomas (1,9). Tc99m-MIBI-SPECT has a sensitivity ranging from 66 to 90% and a positive predictive value ranging from 83 to 100% for pathological parathyroid glands (1,10-17). However, the test is also known to have lower sensitivity for small adenomas weighing <500 mg (15,18) and in the presence of multiple gland pathology (1,9,15,17-20).

Tc99m-MIBI was originally developed for myocardial perfusion imaging. It is a monovalent lipophilic cation that diffuses passively through cell membranes and accumulates almost exclusively in mitochondria following negative membrane

potentials (21). The imaging technique has been used to detect benign tumours as well as several primary malignancies and metastatic tumours (22). In primary hyperparathyroidism, the mechanism by which Tc99m-MIBI can localize a pathological parathyroid gland is based on the increased uptake of the radiopharmaceutical by functionally hyperactive glands; on the abundance of mitochondria in parathyroid oxyphil cells, which sequester sestamibi intracellularly; and on the increased perfusion of hyperactive parathyroid glands. However, imaging studies are often negative in persistent hyperparathyroidism. Potential contributory factors to false negative scans are a postoperative disturbance in the perfusion of remaining pathological glands and a predominance of parathyroid chief cells, which are poorer in mitochondrial content compared to oxyphil cells (18).

In patients undergoing reoperative parathyroidectomy for persistent hyperparathyroidism, the accuracy of Tc99m-MIBI-SPECT in localizing hyperactive parathyroid tissue has been largely assessed in mixed patient groups, including patients in whom no parathyroid tissue was found at initial surgery (23,24), patients with a known *MEN-1* (multiple endocrine neoplasia type 1) mutation (25), and patients with secondary or tertiary hyperparathyroidism (26,27). Data on the ability of Tc99m-MIBI-SPECT to detect and localize residual hyperactive parathyroid tissue in patients with persistent primary hyperparathyroidism are particularly scarce. The available data do suggest, however, that the predictive value of this technique falls dramatically from 80% or higher before initial surgery for single gland disease (SGD) to as low as 50% before reoperative parathyroidectomy for persistent PHPT (28-30). Because reoperative parathyroidectomy may be associated with increased morbidity, accurate localization of residual hyperactive tissue becomes of paramount importance in patients with persistent PHPT (28-30). Correct preoperative localization of a hyperactive parathyroid gland by Tc99m-MIBI-SPECT would allow for more focused and efficient exploration, thus reducing the risk of damage to the recurrent laryngeal nerve(s) and significantly increasing the likelihood of cure.

The aim of the present study was to determine the ability of Tc99m-MIBI-SPECT to detect and localize residual hyperactive parathyroid glands prior to reoperative parathyroidectomy in patients with persistent PHPT.

PATIENTS AND METHODS

Patients

Using hospital records, we identified 19 consecutive patients who required reoperative parathyroidectomy for persistent hyperparathyroidism and who had parathyroid localization studies with Tc99m-MIBI-SPECT before surgery. These were 14 women and 5 men with a mean age of 55 ± 12 years at diagnosis, who had undergone between one and three unsuccessful earlier neck explorations. Sixteen of the 19 patients had their initial surgery and earlier reoperations, if applicable, at other institutions and were referred to our unit for further workup before reoperative parathyroidectomy. We compared these patients with 23 patients with sporadic primary hyperparathyroidism due to single gland disease who also had a Tc99m-MIBI-SPECT scan prior to initial surgery. These were, respectively, 21 women and 2 men with a mean age of 59 ± 12 years at diagnosis. All surgical procedures were undertaken by two surgeons with considerable experience in endocrine surgery.

Methods

Demographic data, operative data, preoperative and postoperative laboratory data and histological findings were obtained from patients' hospital records. In the present study, cure is defined as sustained normal serum calcium and PTH concentrations more than 6 months after parathyroidectomy (6). Persistent hyperparathyroidism is defined as persistently elevated serum calcium and PTH concentrations in consecutive samples within and beyond 6 months after surgery (6).

Histological preparations were independently reviewed by an experienced pathologist. A diagnosis of parathyroid adenoma was based on the presence of a benign encapsulated neoplasm usually involving a single gland with an adjacent rim of normal glandular tissue (31). Parathyroid hyperplasia was defined as an absolute

increase in parathyroid parenchymal cell mass resulting from proliferation of chief cells, oncocytic cells, and transitional oncocytic cells in multiple parathyroid glands in the absence of a known stimulus for PTH hypersecretion such as renal failure or vitamin D deficiency (31). A diagnosis of hyperplasia was also being favoured when synchronous multiglandular disease was found at surgery.

TC99M-MIBI-SPECT imaging technique

The Tc99m-MIBI-SPECT scan was performed as follows: after intravenous injection of 500 MBq of Tc99m methoxy-isobutyle-isonitrile (MIBI), planar images of the head and neck region and chest were performed at a matrix size of 256 x 256 (10 minutes per frame). Scintigraphy was performed as a dual-phase single tracer examination. Images were acquired with the patient in the supine position, 15 min and 2 h after injection of the radiopharmaceutical. A gamma camera (Toshiba GCA-7200, Tokyo, Japan) equipped with low-energy high-resolution (LEHR) collimators was used for image acquisition. Single photon emission computed tomography (SPECT) was performed in a 128 x 128 matrix size, using a step angle of 4^{o} and a step time of 35 s per step, 90 minutes after the injection. The filtered back projection was used for image reconstruction, using a Butterworth filter (8 order, subset 12). All Tc99m-MIBI-SPECT scans were reviewed by an experienced nuclear medicine physician who was blinded to the outcome of the surgical procedure.

Surgical procedures

Bilateral neck exploration consisted of visualization of all four parathyroid glands, followed by either excision of a single enlarged parathyroid gland or subtotal or total parathyroidectomy with auto transplantation if more than one parathyroid gland was found to be enlarged. If not all four parathyroid glands could be visualized and no enlarged parathyroid gland was found, dissection of the ipsilateral anterior compartment extending from the level of the hyoid bone superiorly to the suprasternal notch inferiorly and hemithyroidectomy were undertaken on the side of the missing parathyroid. Unilateral neck exploration and minimally invasive neck exploration were guided by intraoperative PTH measurement.

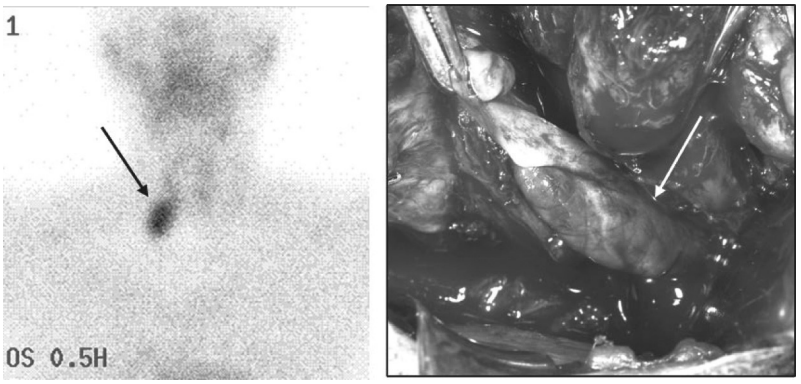

Figure 1. Positive Tc99m-MIBI-SPECT imaging scan demonstrating increased radioactive pharmaceutical uptake in the right lower neck suggesting a pathological parathyroid gland in this location (A), concurring with subsequent operative findings of a 2,5 cm pathological parathyroid adenoma (B).

Analysis of data

The ability of Tc99m-MIBI-SPECT to detect and localize hyperactive parathyroid tissue was evaluated in three different ways, thereby providing the answers to three different questions that are clinically relevant to the operating surgeon.

The first question we addressed is "Is the Tc99m-MIBI-SPECT scan able to detect any parathyroid hyperactivity?" This question relates to the overall sensitivity of the scan for detecting the presence of hyperactive parathyroid tissue in the scanned neck and mediastinal regions (Figure 1). A scan was considered true positive when it correctly identified the presence of an abnormal gland at any site. A scan was considered true negative when no pathological parathyroid gland(s) was found at surgery, and no presence of hyperactive parathyroid tissue was suggested by the scan. A scan was considered false positive when no pathological glands were found at operation, although the scan had suggested the presence of hyperactive parathyroid tissue. A scan was considered false negative when a pathological parathyroid gland was removed at surgery, although the scan had not demonstrated the presence hyperactive parathyroid tissue.

The second question addressed is "Is the scan able to lateralize the area of hyperactive parathyroid tissue?", thus indicating which side of the neck should to be

explored. This question relates to the sensitivity and specificity of the scan in identifying the side of the neck in which hyperactive parathyroid tissue is localized (Figure 2A). A true positive side was defined as a neck side from which a pathological parathyroid gland was removed at surgery, and in which the presence of hyperactive parathyroid tissue was suggested by the preoperative localization test. A true negative side was defined as a neck side in which no pathological parathyroid gland was found at surgery, and in which no presence of hyperactive parathyroid tissue was suggested by the preoperative localization test. A false positive side was defined as a neck side in which no pathological parathyroid gland was found at surgery, although the presence of hyperactive parathyroid tissue was suggested by the preoperative localization test. A false negative side was defined as a neck side from which a pathological parathyroid gland was removed at surgery, although no presence of hyperactive parathyroid tissue was suggested by the preoperative localization test.

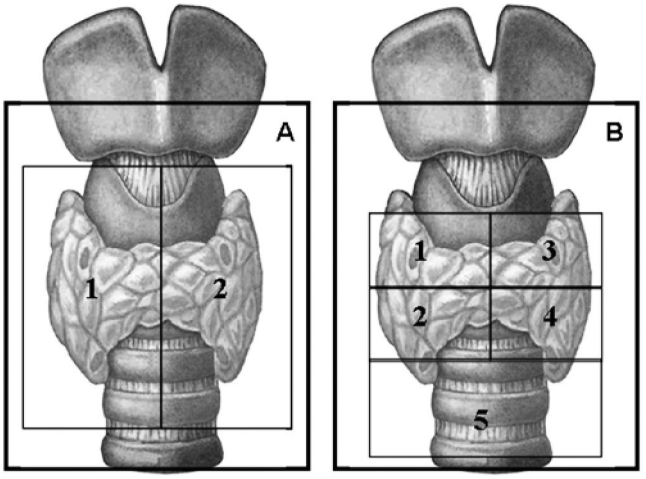

Figure 2. Analysis of the ability of Tc99m-MIBI-SPECT scan to correctly localize a pathological parathyroid gland as confirmed by localization at surgery by dividing the neck into 2 anatomical areas (the right and left side of the neck) (A) or into 5 anatomical areas (the four thyroid quadrants and a 5th area of all possible ectopic localizations in the mediastinum) (B).

The third question is “Is the scan able to accurately localize the region of the neck where the hyperactive parathyroid tissue is present?”, thus providing an even smaller operative field for the surgeon to focus upon. This third question takes accuracy of localization of the imaging modality a step further by identifying the localization of the hyperactive parathyroid tissue in any of 5 designated anatomical areas of the neck (four thyroid quadrants, and a fifth “elsewhere” area combining all possible ectopic localizations in the mediastinum) (Figure 2B). With respect to the risk of damaging the recurrent laryngeal nerve, a correct answer to question 2 would limit the risk to one nerve only, whereas a correct answer to question 3 would provide additional information on the magnitude of this risk by identifying the specific region to be explored. A true positive region was defined as a region from which a pathological parathyroid gland was removed at surgery, and in which the presence of hyperactive parathyroid tissue was suggested by the preoperative localization test. A true negative region was defined as a region in which no pathological parathyroid gland was found at surgery, and in which no presence of hyperactive parathyroid tissue was suggested by the preoperative localization test. A false positive region was defined as a region in which no pathological parathyroid gland was found at surgery, although the presence of hyperactive parathyroid tissue was suggested in this region by the preoperative localization test. A false negative region was defined as a region from which a pathological parathyroid gland was removed at surgery, although no presence of hyperactive parathyroid tissue was suggested in this region by the preoperative localization test.

True negative and false positive scans, sides or regions could only be determined in patients who were cured after the surgery by the finding and removal of a pathological parathyroid gland elsewhere in the neck or mediastinum (this latter condition precluding comprising the sensitivity and specificity by false negative surgery).

In all three analyses widely accepted definitions for sensitivity and specificity were used to address each of the three different questions posed. Sensitivity was thus judged to be the power of the test to identify the presence of pathological parathyroid glands at any site regardless of accuracy of localization (question 1), on

the correct side of the neck (question 2), or in the exact region of neck (question 3). Specificity was judged to be the power of Tc99m-MIBI-SPECT to recognize the absence of a pathological parathyroid gland in the truly negative side of the neck (question 2) or in the exact region of the neck (question 3).

Statistical analysis

Statistical analysis was performed using the SPSS 16 software (SPSS Inc., Chicago, IL). Results are expressed as mean ± SD unless otherwise stated. Baseline characteristics of the patient groups were compared using the one-way analysis of variance (ANOVA) for continuous variables, and the chi-square statistic was used for comparison of categorical variables. A probability level of random difference of $P<0.05$ was considered significant.

RESULTS

Demographic data

Patients who underwent reoperative parathyroidectomy for persistent PHPT did not differ significantly in age and gender from patients who underwent initial surgery for sporadic PHPT. Laboratory data before operation did not differ significantly between the two patient groups (Table 1).

Operative data and postoperative cure

Bilateral neck exploration was more frequently performed in patients with persistent hyperparathyroidism undergoing reoperative parathyroidectomy (84%) than in patients with a single adenoma undergoing initial surgery (56%) (Table 1).

At least one and up to three hyperactive parathyroid glands had been removed at initial surgery in patients with persistent hyperparathyroidism. The 18 pathological parathyroid glands removed at reoperative parathyroidectomy were removed from ectopic locations (n=8, 44%): intrathyroidal (n=4), thymus (n=2), mediastinum (n=1) and a location ventral to the trachea (n=1). Other glands were removed from expected anatomical locations in the right lower quadrant (n=4), right upper

quadrant (n=3), left lower quadrant (n=2), and left upper quadrant (n=1) of the neck.

Table 1. Demographic, laboratory, operative and pathology data of patients who had a Tc99m-MIBI-SPECT scan prior to initial surgery for sporadic PHPT or prior to reoperative parathyroidectomy for persistent PHPT

	Persistent PHPT (n=19)	Sporadic PHPT (n=23)	*P* value
Age (years ± SD)	55 ± 12	59 ± 12	0.274
Sex (M/F)	5:14	2:21	0.127
Pre-operative laboratory data			
s-Corrected Calcium (mmol/L)	2.88 ± 0.35	2.80 ± 0.23	0.399
s-PTH (pmol/L)	16.2 ± 9.3	24.5 ± 20.2	0.131
Post-operative laboratory data			
s-Corrected Calcium (mmol/L)[a]	2.46 ± 0.28	2.29 ± 0.13	0.027
s-PTH (pmol/L)[b]	10.0 ± 11.7	4.0 ± 2.7	0.043
Operative data			
Bilateral exploration	16 (84%)	13 (56%)	0.053
Unilateral exploration	3 (16%)	5 (22%)	0.625
Minimally invasive	0	5 (22%)	0.030
Pathology data			
Adenoma	8 (42%)	19 (83%)	0.037
Hyperplasia	9 (47%)	4 (17%)	0.152
No pathological glands	2 (11%)	0	0.210
Gland diameter (cm ± SD)	1.21 ± 0.93	2.03 ± 1.40	0.062

s: serum, [a] reference range 2.15-2.55 mmol/L, [b] reference range 1.5-8 pmol/L

Complete cure was eventually achieved in 11 of the 19 patients (58%). Of the remaining 8 patients, 6 had no further surgery, 1 had 1 further operation, and 1 had 3 further operations, which resulted in improvement in the severity of the hyperparathyroidism but not in complete cure as defined in our study. In all 8 patients the decision to withhold further surgery was based on a combination of lack of severe symptoms, mild biochemical features of hyperparathyroidism, and the increased morbidity risk associated with further surgery in the context of prior

extensive neck exploration, which had to be extended to mediastinal exploration in some cases.

The 23 patients with PHPT who had a Tc99m-MIBI-SPECT scan prior to initial surgery for sporadic PHPT were all cured, and cure was sustained for a mean duration of 47 months (range 6-188 months) after PTx. Pathological parathyroid glands removed at surgery were predominantly located in the right (n=10, 43%) or left (n=7, 30%) lower thyroid quadrants, and only 1 pathological gland was located in the right upper quadrant of the neck. Five successfully removed ectopic glands had been in subcapsular thyroidal (n=2), intrathyroidal (n=1), or mediastinal (n=2) locations.

Histological data

On histological examination, 42% of the patients with persistent hyperparathyroidism had a second adenoma and 47% had hyperplastic parathyroid gland(s) (Table 1). A single adenoma was removed in 83% of the patients who underwent initial surgery for sporadic PHPT. The average diameter of a pathological parathyroid gland found at reoperative parathyroidectomy for persistent PHPT was smaller (1,21 cm) compared to the average size of glands found at initial surgery for sporadic PHPT (2,03 cm).

Value of Tc99m-MIBI-SPECT scans prior to reoperative parathyroidectomy for persistent PHPT

In total, 18 hyperactive parathyroid glands were removed at reoperative parathyroidectomy from 19 patients with persistent PHPT. One hyperactive parathyroid gland was removed in 16 of these patients, 2 glands in 1 patient, and no pathological glands could be found in the other 2 patients despite extensive bilateral neck and mediastinal explorations. Tc99m-MIBI-SPECT demonstrated increased radiotracer uptake, suggesting parathyroid hyperactivity in only 9 of the 19 patients (47%) (Figure 3). At reoperative parathyroidectomy 15 patients had a pathological

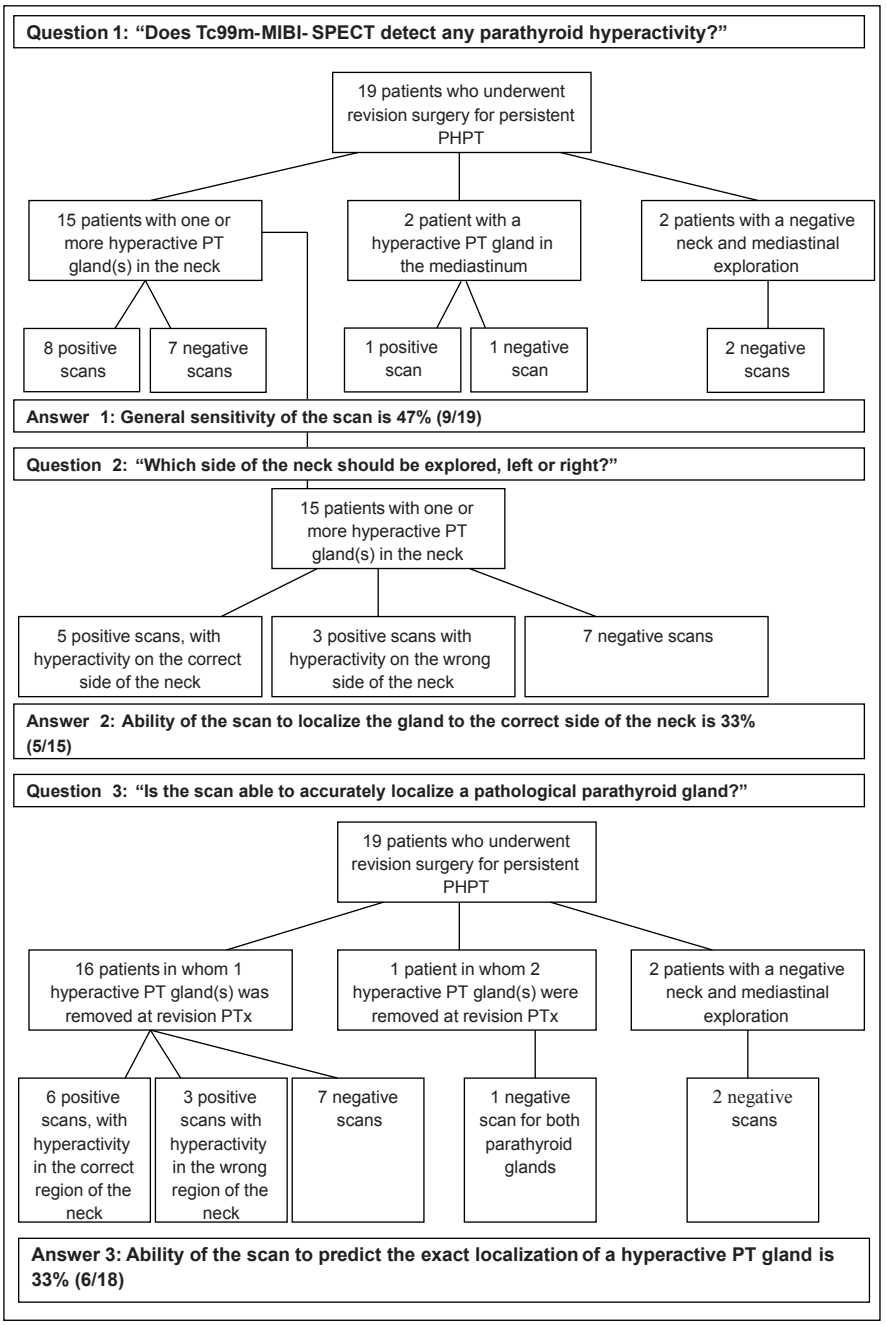

Figure 3. Flowchart of the answers to the three clinical questions addressed in 19 patients who had preoperative parathyroid localization studies with Tc99m-MIBI-SPECT prior to reoperative parathyroidectomy for persistent PHPT.

gland removed from the neck region. Tc99m-MIBI-SPECT was able to identify the correct side of the neck in 5 of these 15 patients (33%) (Table 2; Figure 3).

Tc99m-MIBI-SPECT was able to detect and accurately localize only 6 of the 18 pathological glands removed at reoperative parathyroidectomy either from the neck or from ectopic localizations (33%) (Table 3; Figure 3). Two of these glands, an adenoma and a hyperplastic gland, were found in normal anatomical locations in the left upper and left lower thyroid quadrants, and 4 glands were found in ectopic locations in the neck, one adenoma was in the thymus, one adenoma and one hyperplastic gland were in the thyroid, and one adenoma was located ventral to the trachea, caudally in the neck.

Of the 12 pathological glands not accurately localized by Tc99m-MIBI-SPECT, 67% were hyperplastic glands and 33% were adenomas. Only 4 of the 12 glands missed at Tc99m-MIBI-SPECT were found in an ectopic location at surgery: one hyperplastic gland was in the thymus, one adenoma and one hyperplastic gland were in the thyroid gland, and one hyperplastic gland was in the mediastinum. Pathological glands with negative imaging were smaller, with a mean diameter of 9 mm, ranging from 5-16 mm and had a histological predominance of chief cells.

Value of Tc99m-MIBI-SPECT scans prior to initial surgery for sporadic PHPT

All 23 patients with sporadic PHPT had one hyperactive parathyroid gland removed at initial surgery and achieved cure. However, Tc99m-MIBI-SPECT scan demonstrated increased radiotracer uptake, suggesting parathyroid hyperactivity in only 15 of the 23 patients (65%) (Figure 4). In 21 of the 23 patients the hyperactive parathyroid gland was removed from the neck. Tc99m-MIBI-SPECT was able to identify the correct side of the neck in 14 of these patients (67%) (Table 2; Figure 4). In 2 of the 23 patients a hyperactive gland was removed from the mediastinum, but Tc99m-MIBI-SPECT was able to detect only one of these glands (Figure 4).

Tc99m-MIBI-SPECT was able to accurately localize 14 of the 23 pathological parathyroid gland removed at surgery (61%) (Table 3; Figure 4). Of the 14 glands accurately localized glands by Tc99m-MIBI-SPECT, 12 (86%) were adenomas located in the right lower thyroid quadrant in 9 cases (76%), in the left lower thyroid

quadrant in 2 cases (17%), and in the mediastinum in 1 case (7%). The remaining 2 accurately localized glands were hyperplastic parathyroid glands located in the left lower thyroid quadrant. Of the 9 pathological glands that were not localized or that were inaccurately localized by Tc99m-MIBI-SPECT, 5 were found in the neck in expected anatomical locations at surgery and 4 were ectopically located: 3 intrathyroidally and 1 in the mediastinum. Intrathyroidal parathyroid glands were found in 2 cases by visualization during extensive bilateral neck exploration and in 1 case during unilateral neck exploration combined with intraoperative PTH (IOPTH) measurement. The mediastinal gland was found during thoracic extension of a full bilateral neck exploration during which no pathological parathyroid glands were found in the neck.

Table 2. Predictive value of Tc99m-MIBI-SPECT for the localization of pathological parathyroid glands in the correct side of the neck

	Sensitivity	Specificity	PPV	NPV
Before initial surgery for sporadic PHPT (*n*=23)	67%	100%	100%	75%
Before reoperative parathyroidectomy for persistent PHPT (*n*=19)	33%	80%	63%	53%

PPV: positive predictive value, NPV: negative predictive value

Table 3. Predictive value of Tc99m-MIBI-SPECT for the localization of pathological parathyroid glands in the exact region of the neck

	Sensitivity	Specificity	PPV	NPV
Before initial surgery for sporadic PHPT (*n*=23)	61%	99%	93%	91%
Before reoperative parathyroidectomy for persistent PHPT (*n*=19)	33%	95%	67%	84%

PPV: positive predictive value, NPV: negative predictive value

Limitations of Tc99m-MIBI-SPECT imaging studies

Localization ability for pathological parathyroid glands in anatomically expected versus ectopic locations

Combining all patients included in this study, pathological parathyroid glands were most frequently found in the right (37%) and left (32%) lower thyroid quadrants (Figure 5). Thirteen of the 41 pathological glands removed at surgery (32%) were found in ectopic locations: intrathyroidally (n=7), in the thymus (n=2), in the mediastinum (n=3), and ventral to the trachea in the neck (n=1*)*. An intrathyroidal parathyroid gland was accurately localized preoperatively in 2 of 7 cases (29%), a mediastinal gland in 1 of 3 cases (33%), a gland in the thymus in 1 of 2 cases (50%), and a gland ventral to the trachea in one case (100%).

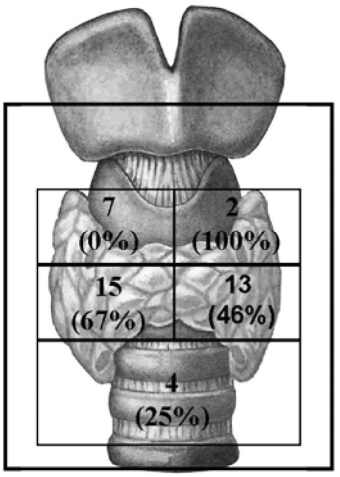

Figure 5. Combined number of pathological parathyroid glands identified at initial (n=23) and at revision surgery (n=18) in each of the four quadrants of the neck and the mediastinum. The sensitivity of Tc99m-MIBI-SPECT for localization in each of these four quadrants of the neck and the mediastinum is shown in parentheses.

Tc99m-MIBI-SPECT scan had a lower sensitivity for pathological parathyroid glands identified at surgery in the upper quadrants of the neck (2/9=22%), than for glands found in the lower quadrants of the neck (16/28=57%) (Figure 5). None of the 7 pathological parathyroid glands in the right upper thyroid quadrant could be

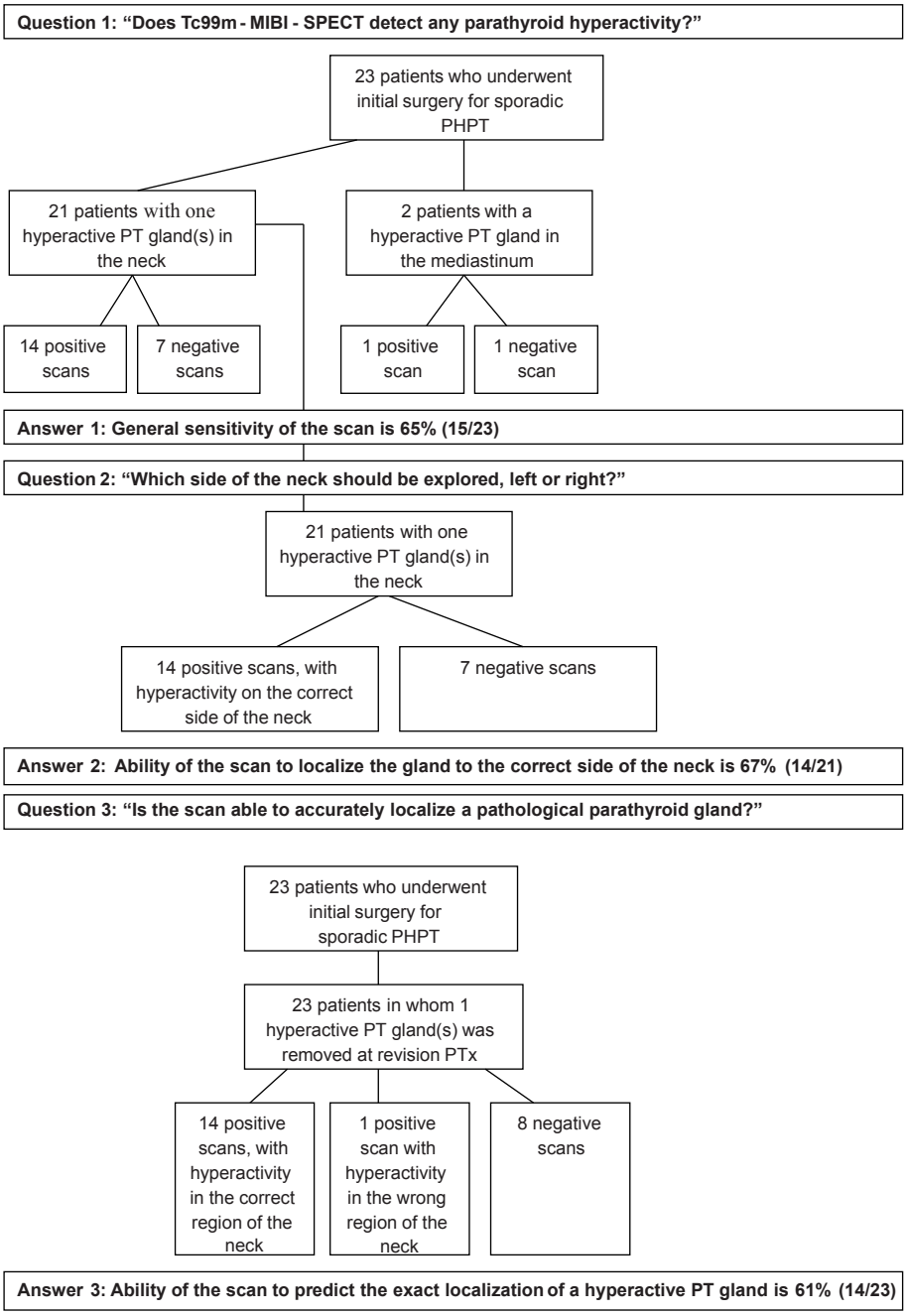

Figure 4. Flowchart of the answers to the 3 clinical questions addressed in 23 patients who had preoperative parathyroid localization studies using Tc99m-MIBI-SPECT prior to initial surgery for sporadic PHPT.

accurately localized pre-operatively. One of the 7 pathological glands in the right upper thyroid quadrant showed uptake in the right lower quadrant, and the other 6 glands showed no radiotracer uptake on Tc99m-MIBI-SPECT.

Localization ability for adenomas versus hyperplastic pathological glands

In the present study, Tc99m-MIBI-SPECT scan was able to accurately localize 16 of 26 adenomas (64%) and 4 of 16 hyperplastic glands (25%) found at surgery.

Localization ability in relation to pathological parathyroid gland size

Pathological parathyroid glands with a diameter greater than 1.5 cm were more frequently accurately localized by Tc99m-MIBI-SPECT than glands with a diameter smaller than 1.5 cm. In patients undergoing initial surgery for sporadic PHPT, Tc99m-MIBI-SPECT had a sensitivity of 71% for glands with a diameter >1.5 cm compared to a sensitivity of 44% for glands with a diameter <1.5 cm. In patients who underwent reoperative parathyroidectomy for persistent PHPT, Tc99m-MIBI-SPECT had a sensitivity of 50% for glands with a diameter >1.5 cm and a sensitivity of 14% for glands with a diameter <1.5 cm.

Localization ability in relation to parathyroid pathology

In patients undergoing initial surgery, 81% of pathological glands, whether adenomatous or hyperplastic, had a predominance of chief cells and only 19% had a predominance of oxyphil cells. In patients undergoing reoperative parathyroidectomy for persistent PHPT, 59% of pathological glands had a predominance of chief cells, 12% had a predominance of oxyphil cells and the remaining glands had a combination of cell types. In patients who underwent initial surgery for sporadic PHPT, Tc99m-MIBI-SPECT was more sensitive in localizing pathological parathyroid glands with a predominance of oxyphil cells compared to those with a predominance of chief cells (100% vs 47% respectively). This higher sensitivity for pathological parathyroid glands with a predominance of oxyphil cells could not be replicated in patients who underwent reoperative parathyroidectomy for persistent PHPT.

DISCUSSION

Our findings from this study underline the limitations of the Tc99m-MIBI-SPECT imaging technique in the accurate preoperative localization of residual pathological parathyroid glands before reoperative parathyroidectomy in patients with persistent hyperparathyroidism. Advances in imaging technology have promoted the development of more focused surgical approaches in the surgical management of primary hyperparathyroidism. The preoperative localization of a pathological parathyroid gland is, however, a prerequisite for a unilateral or minimally invasive surgical approach. Although more than 80% of patients with sporadic primary hyperparathyroidism will have a single adenoma, it has been estimated that 15-20% will have a double adenoma or four-gland hyperplasia (32,33). Ten percent of individuals may also have supernumerary glands (5-7 glands) (34-36), so that it is not surprising that some of these pathological glands may be missed at initial surgery. It has indeed been reported that 1-7% of patients with primary hyperparathyroidism will have persistent hyperparathyroidism after initial surgery (6,37,38). When this is the case, it becomes essential to accurately localize the residual hyperactive glands if morbidity from reoperative parathyroidectomy is to be significantly decreased (39).

Limited data suggest that the localizing value of Tc99m-MIBI-SPECT is much lower for the preoperative localization of residual active parathyroid glands before reoperative parathyroidectomy (28-30). Findings from the present study confirm this notion and emphasize that the ability of Tc99m-MIBI-SPECT to detect and to accurately localize residual hyperactive glands is significantly jeopardized in patients with persistent hyperparathyroidism before reoperative parathyroidectomy compared to its detection and localization ability for pathological parathyroid glands before initial surgery for sporadic PHPT. Several factors may play a role in this decrease in the scan's ability to detect and accurately localize hyperactive parathyroid glands in persistent PHPT. Compared to patients with sporadic PHPT cured after initial surgery, patients with persistent hyperparathyroidism had a lower proportion of adenomas (44% vs. 74%), a higher proportion of hyperplastic glands

(55% vs. 26%), more ectopically located glands (44% vs. 22%), and smaller pathological gland size (1.21 cm vs. 2.03 cm). Published data do indeed suggest a lower predictive value for Tc99m-MIBI-SPECT in cases of parathyroid hyperplasia, ectopic glands, and glands of small size (10,15,18,19,40,41). Patients with persistent HPTH are also at a higher risk from parathyromatosis due to potential spillage during sometimes multiple earlier surgical procedures.

Using Tc99m-MIBI imaging, the radiopharmaceutical uptake of pathological parathyroid glands depends not only on perfusion of the hyperactive gland but also on mitochondrial activity of the hyperactive cells. In the parathyroid gland, oxyphil cells have an abundance of mitochondria compared to that of chief cells. Studies focusing on the causes for negative Tc99m-MIBI-SPECT imaging suggest an influence of the presence of mitochondria-rich cells on outcome. Mihai *et al.* (18) and Erbil *et al*. (42) found that Tc99m-MIBI-SPECT scans were more frequently positive than negative in the case of adenomas rich in mitochondria-abundant oxyphil cells. In contrast, Westerdahl and Bergenfelz (19) found no difference in the positive or negative imaging of predominantly oxyphil-rich adenomas.

Parathyroid adenomas are mainly composed of chief cells, and it could be argued that sestamibi may not be taken up by these pathological glands. This is not the case, however, as demonstrated by the frequent finding of both chief cells and oxyphil cells, although in different percentages, in parathyroid adenomas. When both types of cells are found, the net number of mitochondria-rich cells would depend in large part on the size of the pathological gland. The same applies for hyperplastic glands. In keeping with published data, we also observe a clear relationship between gland size and localizing accuracy of Tc99m-MIBI-SPECT. The accuracy of a Tc99m-MIBI-SPECT scan is thus determined mainly by size and amount of oxyphil cells rather than by the predominance of a cell type or the adenomatous or hyperplastic nature of the gland.

Our findings from this study demonstrate that the ability of the widely used Tc99m-MIBI-SPECT to detect and accurately localize pathological parathyroid glands is significantly limited in patients with persistent hyperparathyroidism before reoperative parathyroidectomy. Although the precise cause for this remains unclear,

it is likely to be multifactorial, and probably includes disturbance in the local vascular supply by previous surgery, as well as differences in gland pathology and size ultimately affecting radiopharmaceutical uptake. It is also conceivable that a previous scan may also have been unable to localize hyperactive parathyroid gland(s) in a specific individual prior to initial surgery. Because most patients in this series did not have preoperative scans before their initial surgery, however, addressing the question of the general ability of Tc99m-MIBI-SPECT to localize a hyperactive parathyroid gland in a specific individual is impossible and represents a limitation of the study.

Our data hold significant implications in the management of patients with persistent hyperparathyroidism. From the practical point of view, and in keeping with recent guidelines of the European Association of Nuclear Medicine (43), our data suggest that it is always worth performing a Tc99m-MIBI-SPECT scan as a first preoperative localization study in patients with either sporadic or persistent hyperparathyroidism. Should the scan fail to demonstrate increased uptake in neck or mediastinum, no effort should be spared to attempt further localization of residual hyperactive glands prior to surgery to minimize morbidity. For instance, it is possible to use the invasive but more reliable PTH venous sampling technique (our own unpublished observations), followed by high resolution 4 dimensional CT-scan of the area of interest. Should the scan be able to lateralize the lesion to the right or left side of the neck, it would be reasonable to undertake more limited unilateral neck exploration under the umbrella of intraoperative PTH measurements. Whether the recently advocated use of double-tracer subtraction parathyroid scintigraphy (43) is able to provide better detection and localization accuracy for residual hyperactive parathyroid glands than Tc99m-MIBI-SPECT imaging in the difficult group of patients with persistent hyperparathyroidism remains to be established.

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4

The role of selective venous sampling in the management of persistent hyperparathyroidism revisited

Janneke E. Witteveen, Job Kievit, Arian R. van Erkel, Hans Morreau, Johannes A. Romijn, Neveen A.T. Hamdy

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ABSTRACT

Introduction:

Localization studies are mandatory prior to revision surgery in patients with persistent hyperparathyroidism in order to improve surgical outcome and reduce the risk of lengthy explorations. However, in this case noninvasive localization studies are reported to have a poor sensitivity. The aim of our study was to determine the accuracy of selective PTH venous sampling (SVS) in localizing residual hyperactive parathyroid glands in patients with persistent or recurrent hyperparathyroidism.

Patients & Methods:

We retrospectively evaluated the localizing accuracy of 20 PTH SVS performed prior to revision surgery in 18 patients with persistent or recurrent primary hyperparathyroidism (n=11) or autonomous (tertiary) hyperparathyroidism (n=7). Tc99m-MIBI-SPECT was also performed in all patients prior to revision surgery. Operative and pathology data were obtained from hospital records.

Results:

SVS was able to accurately localize 15 of the 20 pathological glands removed at revision surgery, representing a sensitivity of 75%. This sensitivity is significantly higher than that of Tc99m-MIBI-SPECT, which was only 30% (P=0.012).

Conclusion:

Our findings demonstrate that SVS is a valuable localization study in patients with persistent or recurrent hyperparathyroidism, with a sensitivity significantly higher than that of Tc99m-MIBI-SPECT. Our data suggest that SVS represents a useful addition to the pre-operative work-up of these patients prior to revision surgery.

INTRODUCTION

In primary hyperparathyroidism (PHPT) parathyroidectomy is reported to have a cure rate of 94%-100% (1-10) with a complication rate of 0-2.7% in the hands of experienced surgeons (3,5,7-9). In contrast, revision surgery for persistent hyperparathyroidism poses a far greater challenge, due to distortion and scarring of surgical planes caused by previous interventions. In addition, the likelihood of supernumerary parathyroid glands, ectopic localizations and parathyromatosis (inadvertent seeding of parathyroid cells during previous surgeries) is also increased. For these reasons, preoperative localization studies are highly recommended prior to revision surgery for persistent hyperparathyroidism in order to decrease operating time, improve surgical outcome and reduce the risk of complications due to lengthy explorations (10-14). However, localizing residual hyperactive parathyroid tissue often represents an elusive task, particularly following previous surgery for PHPT. The most widely used localization study, Tc99m-MIBI-SPECT, has indeed been shown to hold significant limitations prior to revision surgery for persistent PHPT. Explanations for the poor localizing ability of Tc99m-MIBI-SPECT in persistent PHPT, as low as 50%, include the small size of residual pathological glands, higher probability of hyperplasia and potential distortion of the vascular supply to the residual hyperactive glands due to previous surgery (15-19).

Selective venous sampling (SVS) of parathyroid hormone (PTH) has been shown to be valuable in localizing hyperactive parathyroid glands (20-22), but this invasive technique has generally fallen out of favor since the introduction of non-invasive radionucleotide tests, initially thallium-technetium subtraction scanning (23), and subsequently the more sensitive Tc99m-MIBI-SPECT (10). The diagnostic value of SVS is based on the assumption that regional drainage of each one of the 4 parathyroid glands is into the adjacent superior, middle and inferior thyroid veins, respectively (20). Despite potential anatomical variations, SVS is successful in predicting the side of a pathological parathyroid gland in 39-93% of patients with PHPT (20-22,24-31) and, more importantly, in 66-75% of patients

with negative noninvasive studies (21,27,32). The major limitation of SVS, however, is that it pinpoints the area of venous drainage of a hyperactive gland rather than its exact anatomical location. There may indeed be many variations in regional venous anatomy of the parathyroids due to embryological differences. While each parathyroid gland thus tends to drain ipsilaterally and inferiorly, contralateral drainage has also been described (20). Variations in regional venous drainage may also occur as a result of previous surgical interventions, usually due to ligation of draining veins. In previously published studies, the predictive value of SVS was judged to be positive when a positive gradient was documented on the side of the neck where a pathological gland was found at subsequent surgery (15,27,29). The aim of the present study was to evaluate whether SVS for PTH could contribute to a more accurate preoperative localization of residual hyperactive parathyroid tissue in patients with persistent hyperparathyroidism.

PATIENTS AND METHODS

Patients

Using our hospital records, we selected all patients who had undergone Tc99m-MIBI-SPECT and selective venous sampling for PTH prior to revision surgery for persistent or recurrent PHPT or autonomous tertiary hyperparathyroidism (THPT) due to end-stage-renal failure between February 1994 and January 2009. Eighteen patients who had undergone a total of 20 revision surgeries were considered eligible and were included in the study. Fifteen of the 18 patients (83%) had their initial parathyroidectomy at another hospital and were referred to our hospital for revision surgery. All revision surgeries were undertaken by two surgeons with considerable experience in endocrine surgery.

Methods

Demographic data, operative data and pre- and post-operative laboratory data were obtained from patients' hospital records. All Tc99m-MIBI-SPECT scans were

reviewed by an experienced nuclear medicine physician, blinded to the outcome of the subsequent revision surgery.

In our study, cure was defined as sustained normal serum calcium and PTH concentrations more than 6 months after parathyroidectomy (33). Persistent hyperparathyroidism was defined as persistently elevated serum calcium and PTH concentrations in consecutive samples within and beyond 6 months after surgery (33).

Parathyroid selective venous sampling technique

Parathyroid venous sampling was performed by an experienced intervention radiologist as follows: a 5 Fr MP catheter, with a selective end hole, was introduced via a sheath in the right femoral vein under local anaesthesia and guided by fluoroscopy to each of the jugular, subclavian and brachiocephalic veins, the azygos vein and the vena cava superior and inferior. Blood samples were obtained from several levels along these veins at close distances (1-2 cm), covering the venous drainage of normal anatomical locations as well as potential ectopic locations of parathyroid glands. A total of 30-40 blood samples were collected in whole blood tubes with spray-coated potassium EDTA and immediately put on ice before transportation to the laboratory for PTH measurement. The various sampling sites and any anatomical variation in venous drainage system found at the time of sampling by injecting contrast (Iomeron 300, Bracco Imaging, Konstanz, Germany) were accurately recorded during the procedure.

At the end of the procedure, the catheter was withdrawn and pressure was applied at the point of entry in the femoral vein for approximately 10 minutes until haemostasis was achieved. Regular checks were undertaken for the following 1-2 hours after which the patient was discharged home.

Blood samples were centrifuged and the separated serum was assayed in one batch using an immunochemiluminescent assay (Immulite 2500, Siemens, Deerfield, Il., USA)**.** Using this assay, the normal range of serum iPTH concentration is 1.5-8 pmol/L. The measured PTH concentrations were plotted at the corresponding anatomical sites on the sampling map (Figure 1).

Laboratory screening before the procedure included a coagulation screen and evaluation of renal and thyroid function to establish the safety of contrast administration. If anticoagulation was used, this was temporarily reversed and wherever possible NSAIDs and acetylsalicyl acid were discontinued for a few days before the procedure

Similar to other venous catheterisation procedures, potential complications of SVS include hematoma formation, venous thrombosis, perforation of blood vessels, pseudo-aneurysm, wound infection and side-effects of use of contrast material including anaphylactic reactions and deterioration of renal function (20,34). Patients with a glomerular filtration rate of less than 45 ml/min who underwent SVS were prehydrated using normal 0.9% saline solution.

A selective venous sampling for PTH was deemed to be positive when a gradient of >50% was found between PTH concentrations at a specific anatomical site of sample collection compared to peripheral blood samples obtained at the time of procedure (17,21).

For the purpose of the study, data of each selective venous sampling were reanalyzed by an experienced endocrinologist, blinded to the previously predicted localization and to the outcome of the surgical procedure.

Non-invasive pre-operative localization studies

The Tc99m-MIBI-SPECT scan was performed as follows: following intravenous injection of 500 MBq of Tc99m methoxy-isobutyle-isonitrile (MIBI), planar images of the head and neck region and chest were performed at a matrix size of 256 x 256 (10 minutes per frame). Scintigraphy was performed as a dual-phase single tracer examination before each of the 20 revision surgeries. Images were acquired in the supine position, 15 minutes and 2 hours after injection of the radiopharmaceutical. A gamma camera (Toshiba GCA-7200, Tokyo, Japan) equipped with low-energy high resolution (LEHR) collimators was used for image acquisition. Single photon emission computed tomography (SPECT) was performed in a 128 x 128 matrix size, using a step angle of 4^{o} and a step time of 35 seconds per step, 90 minutes after the

injection. The filtered back projection was used for image reconstruction, using a Butterworth filter (8 order, subset 12).

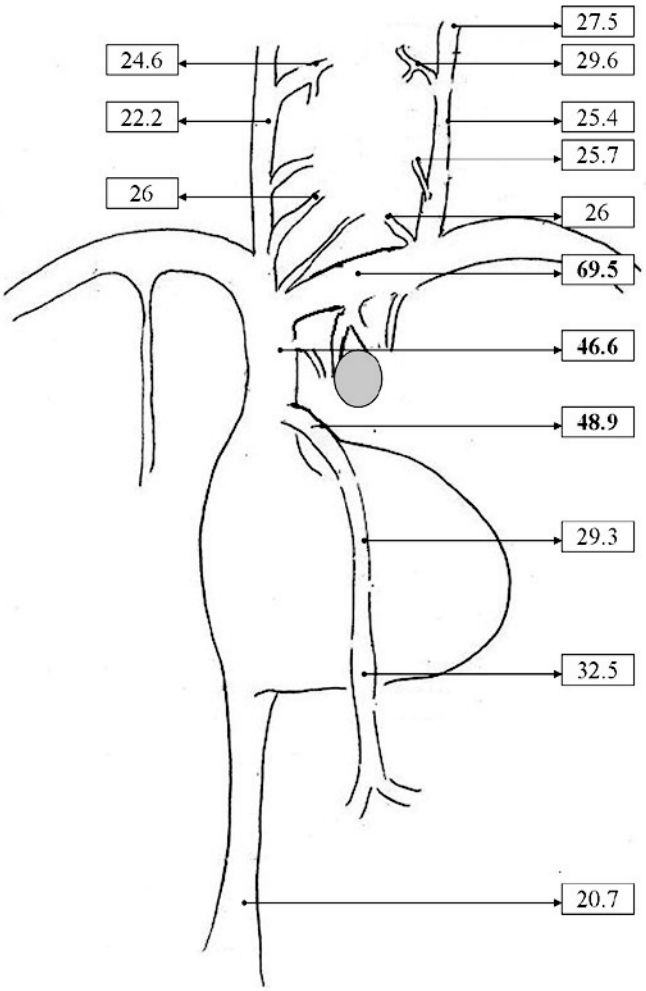

Figure 1: PTH concentrations (pmol/L) plotted on the sampling map demonstrating a gradient in the brachiocephalic vein, the superior vena cava and the azygos vein, suggesting a pathological parathyroid gland in the mediastinum, which corresponded with the subsequent operative finding of a pathological parathyroid gland in aorto pulmonary window (shaded area).

Analysis of data

For the purpose of the analysis, the neck was divided in 5 designated anatomical areas: four thyroid quadrants, and a fifth "elsewhere" area combining all possible ectopic localizations in the mediastinum (Figure 2). A true positive region was defined as a region from which a pathological parathyroid gland was removed at surgery, and in which the presence of hyperactive parathyroid tissue was suggested in the same region by the preoperative localization test. A true negative region was defined as a region in which no pathological parathyroid gland was found at surgery, and in which no presence of hyperactive parathyroid tissue was suggested in this region by the preoperative localization test. A false positive region was defined as a region in which no pathological parathyroid gland was found at surgery, although the presence of hyperactive parathyroid tissue was suggested in this region by the preoperative localization test. A false negative region was defined as a region from which a pathological parathyroid gland was removed at surgery, although no presence of hyperactive parathyroid tissue was suggested in this region by the preoperative localization test.

True negative and false positive scans, sides or regions could only be determined in patients who were cured after surgery by the finding and removal of a pathological parathyroid gland elsewhere in the neck or mediastinum (this latter condition precluding compromising the sensitivity and specificity by false-negative surgery).

Widely accepted definitions for sensitivity and specificity were used. Sensitivity was thus judged to be the power of the test to identify the presence of pathological parathyroid glands in an exact region of the neck. Specificity was judged to be the power of the test to recognize the absence of a pathological parathyroid gland in an exact region of the neck.

Statistical analysis

Statistical analysis was performed using the SPSS 16 software (SPSS inc., Chicago, IL., USA). Results are expressed as mean ± SE unless otherwise stated. The McNemar test was used to assess the difference in localization accuracy between

SVS and Tc99m-MIBI-SPECT scan. A probability level of random difference of $P<0.05$ was considered to be significant.

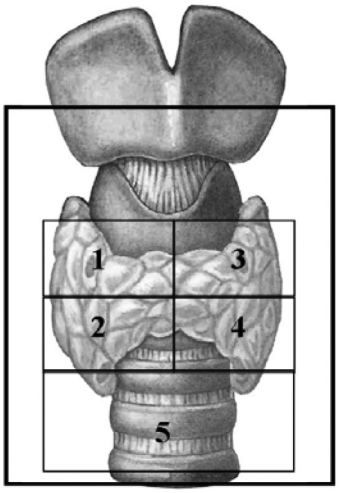

Figure 2: Analysis of the ability of SVS and Tc99m-MIBI-SPECT scan to correctly localize a pathological parathyroid gland as confirmed by localization at surgery by dividing the neck into 5 anatomical areas (the four thyroid quadrants and a 5th area of all possible ectopic localizations in the mediastinum).

Ethical consideration

The methods used in this study were part of the clinical routine work-up of patients undergoing revision surgery in our hospital. The study was approved by the local Ethics Committee and all patients consented to the use of their data.

RESULTS

Pre-operative data

The study population consisted of 18 patients who underwent a total of 20 revision surgeries. Eleven patients had persistent or recurrent PHPT, which was sporadic in 9 patients, due to a *MEN-1* mutation in 1 patient and due to parathyroid carcinoma in 1

patient. Seven patients had persistent or recurrent autonomous tertiary hyperparathyroidism (THPT) due to end-stage renal failure. Patients had an average of 2 previous surgeries, during which an average of 3 pathological parathyroid glands were removed (Table 1).

Operative and pathological data

Surgical approach consisted of bilateral neck exploration in 10 cases, unilateral neck exploration in 8 cases and a sternotomy in 2 cases. Bilateral neck exploration was extended to include mediastinal exploration via sternotomy in 8 cases in which no pathological glands could be found after extensive exploration of the neck.

A single parathyroid adenoma was removed in 5 cases. In 3 of these 5 cases, this was a second adenoma, with an adenoma also removed at initial surgery. All of the 3 second adenomas were found in normal anatomical locations, in the right (n=2) or left (n=1) lower quadrant of the neck. Cure was achieved in 2 of these 3 cases. In the other 2 cases, no pathological glands were removed at initial surgery, and both adenomas were identified in ectopic locations, in the mediastinum (n=1) and high on the left side of the neck on the prevertebral fascia (n=1). Both of these revision surgeries resulted in cure.

One or more hyperplastic gland(s) (n=14) were removed in 12 cases. The majority of hyperplastic glands were identified in normal anatomical locations, in the left upper quadrant (n=4), left lower quadrant (n=3), right upper quadrant (n=1) or right lower quadrant (n=3) of the neck. Three glands were removed from ectopic locations, 2 were in the mediastinum and 1 was intrathyroidal. Only 5 of these 12 revision surgeries (42%) resulted in cure.

A metastasis of a parathyroid carcinoma was removed in 1 case, which resulted in cure lasting for more than 11 months. In the last two cases extensive bilateral neck exploration failed to identify pathological parathyroid glands. Cure was thus achieved in 10 of the 18 patients with persistent or recurrent hyperparathyroidism, 5 after a second surgery, 4 after a third surgery and 1 patient with parathyroid carcinoma was normocalcaemic for almost 1 year after his sixth surgery but has since developed recurrent hyperparathyroidism.

Predictive value of Tc99m-MIBI-SPECT

Tc99m-MIBI-SPECT scans performed before revision surgery were negative in 11 of 20 cases. Scans were only able to detect and accurately localize 6 of 20 surgically removed pathological glands and had thus a sensitivity of 30%.

Selective venous sampling for PTH

An average number of 36 ± 8 samples were collected during each of the 20 selective venous sampling procedures. A positive gradient in PTH concentration of >50% suggesting the presence of a hyperactive parathyroid gland was documented in all 20 cases. The median of the highest PTH concentration found at sampling was 35 pmol/L (range 16-2202 pmol/L) in patients with persistent or recurrent PHPT and 182 pmol/L (range 39-790 pmol/L) in patients with persistent or recurrent THPT (P=0.8). The average gradient was a 4-fold increase in PTH concentration (range 1.5-9), which was not significantly different between patient groups (P=0.7). There were no complications reported for any of the 20 sampling procedures.

Sensitivity of SVS for localizing pathological parathyroid glands

Selective venous sampling (SVS) for PTH was able to accurately localize 15 of 20 pathological parathyroid glands removed at 20 revision surgeries. Ten of these 15 glands: 7 hyperplastic glands and 3 adenomas, were found in normal anatomical locations in the left lower (n=3), right lower (n=4), and left higher (n=3) quadrants of the neck. Five of these 15 pathological parathyroid glands were found in ectopic locations: 4 in the mediastinum and 1 high in the left side of the neck on the prevertebral fascia.

All 5 pathological parathyroid glands that were not accurately localized by SVS were hyperplastic in nature with an average size of 7 mm. Four of these glands were removed from normal anatomical locations and one was intrathyroidal. In only 2 of these 5 glands was a gradient found in the correct side of the neck, but not in the correct quadrant.

Table 1. Demographic, Laboratory, Operative and Pathology Data of patients who underwent Tc99m-MiBi-SPECT and SVS for PTH prior to revision surgery for persistent or recurrent PHPT or autonomous tertiary hyperparathyroidism (THPT)

	11 patients with PHPT (13 operations)	7 patients with THPT (7 operations)	P value
Age at time of SVS (years)	52 ± 3	49 ± 5	0.628
Sex (M/F)	4:7	3:4	0.528
Time after initial operation (months)	121 ± 35	75 ± 26	0.388
Previous operations (No)			
1	5	4	
2	3	2	
3+	5	1	
Glands removed at previous operations (No)			
0	2	0	
1	5	1	
2	1	1	
3+	5	5	
Pathology data at previous operation			
Adenoma	3/13 (23%)	2/7 (28%)	0.787
Hyperplasia	7/13 (54%)	5/7 (72%)	0.444
Carcinoma	1/13 (8%)	0	0.452
No pathological parathyroid glands	2/13 (15%)	0	0.274
Pre-operative laboratory data			
Corrected s-Calcium (mmol/L)	2.72 ± 0.06	2.67 ± 0.08	0.623
s-PTH (pmol/L)	48.9 ± 31.8	50.6 ± 14.7	0.971
Selective venous sampling			
Number of samples	36 ± 3	36 ± 2	0.966
Gradient of increase in PTH	4 ± 0.5	4 ± 1	0.745
Maximum PTH concentration (pmol/L)	214 ± 166	277 ± 106	0.797
Post-SVS operative data			
Bilateral neck exploration	8/13 (62%)	2/7 (29%)	0.160
Unilateral neck exploration	4/13 (31%)	4/7 (57%)	0.251
Sternotomy alone	1/13 (8%)	1/7 (14%)	0.639
Neck exploration combined with sternotomy	4/13 (31%)	2/7 (29%)	0.919
Post-SVS pathology data			
Adenoma	4/13 (31%)	1/7 (14%)	0.417

Hyperplasia	7/13 (54%)	5/7 (72%)	0.444
Carcinoma	1/13 (8%)	0	0.452
No pathological parathyroid glands	1/13 (8%)	1/7 (14%)	0.639

PHPT: primary hyperparathyroidism, THPT: tertiary hyperparathyroidism due to end-stage renal failure, s: serum, PTH: parathyroid hormone, No: number

Sensitivity of SVS in relation to parathyroid gland pathology

SVS was able to accurately localize all 5 adenomas (100%), 9 of 14 hyperplastic glands (64%) and one metastasis from a parathyroid carcinoma (100%) subsequently removed at surgery.

Sensitivity of SVS for pathological parathyroid glands in anatomically expected versus ectopic locations

SVS was able to accurately localize 10 of the 14 pathological glands found in normal anatomical locations (71%) and 5 of the 6 pathological glands found in ectopic locations (83%): mediastinum n=4 and high on the left side of the neck on the prevertebral fascia n=1.

A gradient in both the distal brachiocephalic and the left jugular vein (n=2) accurately corresponded with the finding of a pathological parathyroid gland in the neck at surgery in the 2 cases (100%). A gradient in both the proximal brachiocephalic vein and the vena cava superior (n=3) accurately corresponded with a pathological gland in the right lower quadrant of the neck in 2 of 3 cases (67%) and in 1 case no pathological gland could be found despite extensive neck and mediastinal exploration. A gradient in the vena cava superior and the azygos vein accurately corresponded to the presence of a gland in the mediastinum in 2 of 2 cases (100%).

Sensitivity of SVS in relation to pathological parathyroid gland size

SVS was able to accurately localize 8 of 9 pathological glands (89%) with a diameter greater than 1.5 cm, but only 6 of 11 pathological glands (55%) with a diameter smaller than 1.5 cm, 9 of which were hyperplastic (82%).

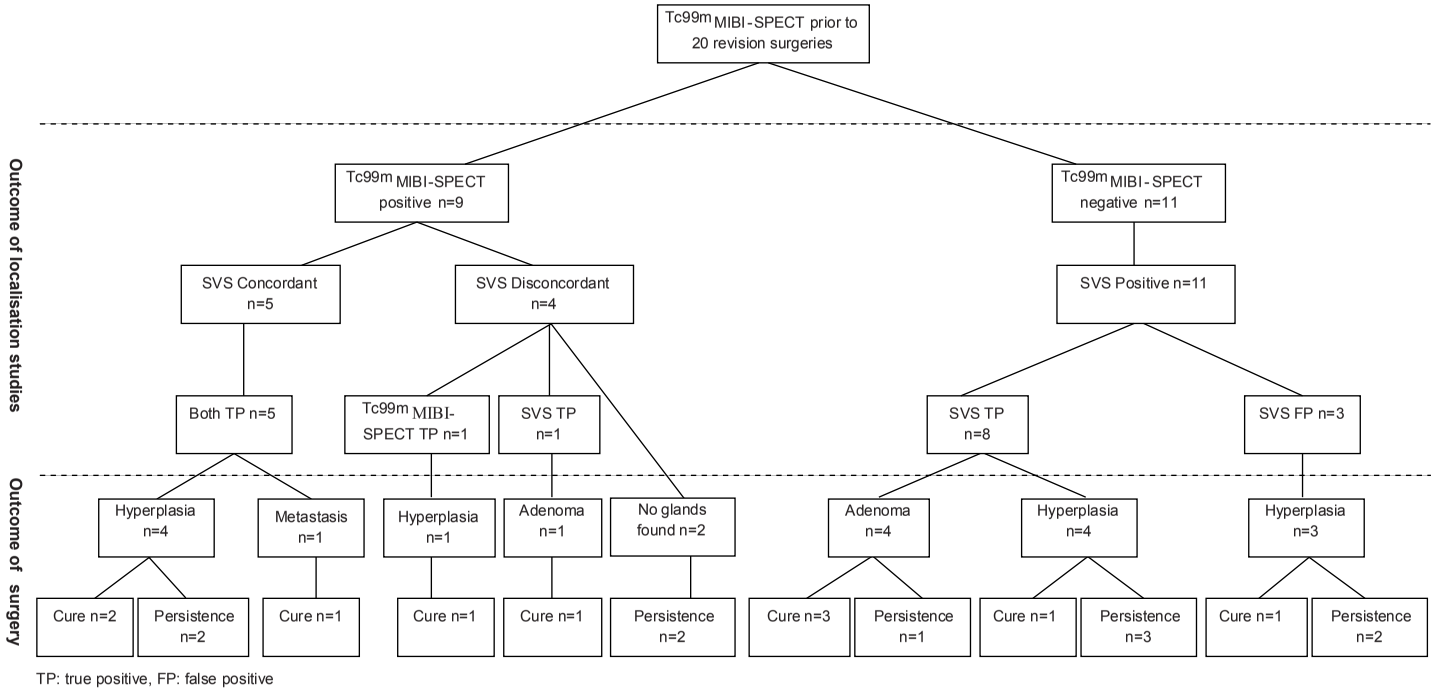

Figure 3: Flowchart of the outcome of Tc99m-MIBI-SPECT compared to SVS in 20 patients who underwent revision surgery.

Sensitivity of SVS for localizing pathological parathyroid glands compared to that of Tc99m-MIBI-SPECT

Tc99m-MIBI-SPECT and SVS were both performed prior to the 20 revision surgeries, during which 1 pathological parathyroid gland was removed in 16 patients, 2 pathological glands in 2 patients and none in 2 patients despite extensive neck and mediastinal exploration. Of these 20 surgically excised glands, 15 (75%) were accurately localized by SVS compared to only 6 (30%) by Tc99m-MIBI-SPECT. SVS was thus able to accurately localize 10 of the 14 pathological glands (71%), which had been inaccurately localized or completely missed by Tc99m-MIBI-SPECT, while Tc99m-MIBI-SPECT was only able to localize 1 of the 6 pathological glands (17%) inaccurately localized by SVS. Localization accuracy was highest when the outcome of Tc99m-MIBI-SPECT and SVS was concordant (n=5) (Figure 3). The overall ability of SVS to accurately localize pathological glands was significantly higher than that of Tc99m-MIBI-SPECT (P=0.012).

DISCUSSION

The present study demonstrates that the ability of selective venous sampling for PTH to accurately localize residual hyperactive parathyroid glands in patients with persistent or recurrent hyperparathyroidism is significantly higher than that of the non-invasive Tc99m-MIBI-SPECT imaging technique. To date, Tc99m-MIBI-SPECT and US of the neck are the most widely used imaging techniques with a sensitivity of up to 90% prior to initial surgery for PHPT (1-5,7,10,35). However, the sensitivity of these techniques has been reported to be as low as 50% before revision surgery for persistent PHPT (15-17).

Because of the invasive nature of SVS and the high costs of the procedure, SVS has been generally used only prior to revision surgery. In keeping with previous observations, the present study shows the better performance of SVS in the accurate detection of residual pathological parathyroid tissue prior to revision surgery compared to that of the widely used Tc99m-MIBI-SPECT (15-17). In our hands, SVS was indeed able to accurately localize 71% of the pathological glands missed

by Tc99m-MIBI-SPECT. Of significant relevance to the operating surgeon is the localizing sensitivity of 100% when concordance is achieved between SVS and Tc99m-MIBI-SPECT, compared to a sensitivity of only 30% when localization is only dependent on Tc99m-MIBI-SPECT.

The disappointing low predictive value of the Tc99m-MIBI-SPECT imaging technique in patients with persistent PHPT is believed to be due to the usually small size of residual parathyroid glands and to their frequent hyperplastic nature (1,6,35-38). Our findings suggest that the sensitivity of SVS is also decreased in the case of pathological parathyroid glands smaller than 1.5 cm compared to glands greater than 1.5 cm (55% vs. 89%), and in the case of hyperplastic compared to adenomatous residual glands (64% vs. 100%), hyperplastic glands being smaller than adenomatous ones (1.45 cm vs 2.3 cm, P=0.21). Although gland size could potentially influence the predictive value of SVS by determining the amount of PTH secreted by the hyperactive parathyroid gland, we were unable to demonstrate a correlation between the gradient in PTH concentration as measured at SVS and the size of the pathological parathyroid gland removed at surgery.

One of the most frequently reported causes of persistent hyperparathyroidism is an ectopic mediastinal location of a pathological parathyroid gland (11,39). Our data and those of others demonstrate a high sensitivity of SVS ranging from 66% to 100% for the localization of these ectopically located glands (17,40). In keeping with previous reports (22), we also observed that SVS was able to accurately localize all ectopically located pathological glands in the mediastinum by the finding of a gradient in the superior vena cava (SVC) alone, or by the finding of a simultaneous gradient in the SVC and in the azygos vein and/or in the brachiocephalic vein. A PTH gradient found only in the brachiocephalic vein remains, however, an interpretational challenge. In contrast to Nilsson *et al.* (22), who suggested that a gradient in the proximal brachiocephalic vein or the SVC corresponded to a mediastinal parathyroid gland in all cases, we observed that a simultaneous gradient in the SVC as well as in the azygos vein was necessary for conclusive evidence for a hyperactive parathyroid gland in the mediastinum. A gradient in the distal brachiocephalic vein was less specific in the study reported by

Nilsson *et al.* (22), corresponding to a mediastinal gland in 86% of cases and to a cervical gland in 14% of cases. Our data suggest, however, that a gradient in the distal brachiocephalic vein corresponded to the localization of a pathological gland in the left side of the neck in 100% of cases.

In persistent or recurrent hyperparathyroidism, previous surgeries may disturb venous anatomy which jeopardizes the localizing ability of radiotracer techniques, such as Tc99m-MIBI-SPECT, and sometimes leads to challenging interpretation of venous sampling data. Notwithstanding, our findings demonstrate that in patients with persistent or recurrent hyperparathyroidism the ability of SVS for PTH to detect and accurately localize pathological parathyroid glands is significantly higher than that of the widely used Tc99m-MIBI-SPECT imaging technique. In experienced hands the SVS procedure is safe and devoid of complications. Concordance of both techniques leads to a reassuring sensitivity of 100%. Our data from this study clearly suggest that SVS for PTH should be reinstated as a valuable tool in the armamentarium of localization studies in the pre-operative work-up of patients with persistent or recurrent hyperparathyroidism.

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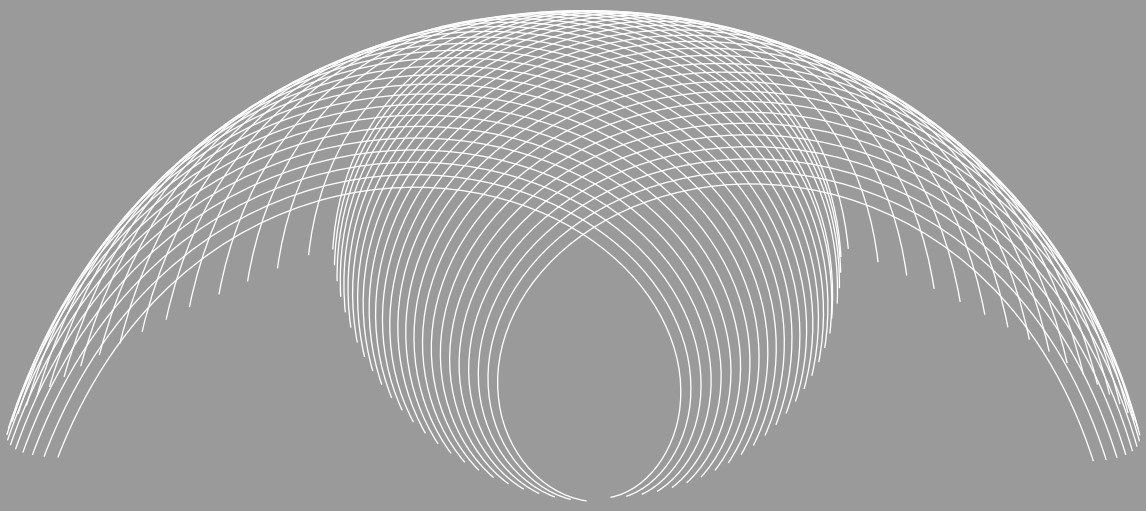
5

Hungry bone syndrome: still a challenge in the post-operative management of primary hyperparathyroidism
A systemic review of the literature

Janneke E. Witteveen, Sjoerd van Thiel, Johannes A. Romijn, Neveen A.T. Hamdy

Submitted



ABSTRACT

The term hungry bone syndrome refers to the rapid, profound and prolonged hypocalcaemia associated with hypophosphataemia, hypomagnesaemia and functional hypoparathyroidism, which follows parathyroidectomy in patients with severe hyperparathyroidism and preoperative high bone turnover. It is a relatively uncommon, but serious adverse effect of parathyroidectomy in patients with severe primary hyperparathyroidism and skeletal manifestations. We conducted a literature search of all available studies reporting a "hungry bone syndrome" in patients who had a parathyroidectomy for primary hyperparathyroidism, to identify patients at risk and address the pitfalls in their management. The severe hypocalcaemia is believed to be due to increased influx of calcium into bone, due to the sudden removal of the effect of high circulating levels of PTH on bone resorption and activation frequency, in the face of continuing bone formation, although there is no good documentation for this. Various risk factors have been suggested for the development of a hungry bone syndrome, including older age, weight/volume of the resected parathyroid glands, radiological evidence of bone disease and vitamin D deficiency. The syndrome is reported in 25-90% of patients with radiological evidence of hyperparathyroid bone disease versus only 0-6% of patients without skeletal involvement. There is insufficient data-based evidence on the best means to treat, minimize or prevent this severe complication of parathyroidectomy. Treatment is aimed at replenishing the severe calcium deficit by using high doses of calcium supplemented by high doses of active metabolites of vitamin D. Adequate correction of magnesium deficiency and normalization of bone turnover are required for resolution of the hypocalcaemia which may last for a number of months after successful surgery. Pre-operative treatment with bisphosphonates has been suggested to reduce postoperative hypocalcaemia, but there are to date no prospective studies addressing this issue.

INTRODUCTION

Patients with primary hyperparathyroidism (PHPT) who undergo parathyroidectomy demonstrate a rapid decrease in serum calcium levels after successful removal of one or more hyperactive parathyroid gland(s). This decrease in serum calcium levels is usually mild and maximal 2 to 4 days post-operatively, independently of the size of hyperactive glands or pathological diagnosis (1-8). Persistence of hypocalcaemia for more than 4 days after parathyroidectomy may be due to intentional or accidental removal of all parathyroid glands, devascularization or trauma to residual parathyroid glands, but is also often due to long-term suppression of residual non-pathological parathyroid glands (1,2,8,9).

The term “hungry bone” syndrome has been coined to the profound (serum calcium <2.1 mmol/l) and prolonged (longer than 4th day post-operatively) hypocalcaemia, which follows parathyroidectomy for severe hyperparathyroidism. This is usually associated with skeletal manifestations, reflected by pre-operative indices of high bone turnover, osteitis fibrosia cystica and/or "brown tumours". The severe hypocalcaemia is believed to be due to the greatly increased skeletal utilisation of calcium, thought to occur as a result of removal of the effect of high circulating PTH levels on bone with immediate arrest of bone resorption in the face of continuing and enhanced bone formation, although there is no good documentation for this.

Literature data on the hungry bone syndrome are scarce despite the still significant prevalence of this clinical problem and despite the challenges associated with its management. This has prompted us to perform a systemic review of the literature on this topic. To this effect, we performed a structured literature search in Medline, Embase and the Cochrane Library for studies reporting a "hungry bone syndrome" in patients who had undergone parathyroidectomy for primary hyperparathyroidism.

METHODS

We searched PubMed, EMBASE, Cochrane Library, Web of Science, CINAHL and Science Direct, using the following search strategy: (("hypocalcaemia"[ti] OR "hypocalcemia"[MeSH Terms] OR "hypocalcemia"[ti] OR Hypocalcemic[ti] OR Hypocalcaemic[ti]) AND (hyperparathyroidism OR parathyroid adenoma OR parathyroid cancer OR "Parathyroid Neoplasms"[Mesh] OR parathyroidectomy OR hyperparathyroid* OR parathyroidectom* OR "Hyperparathyroidism/surgery" [Mesh]) AND (postoperative OR post-operative OR Postoperative Complications OR Postoperative Care OR pretreatment OR pre-treatment OR prevention OR preventive)) OR ("hungry bone" OR "hungry bones")). We restricted our search to publications in the ”English language” and on ”Human subjects”. We also checked the references of relevant articles for additional articles. Abstracts of meetings and unpublished results were not included in the study. The last search was performed on January 17, 2012.

RESULTS

Systematic literature search

The initial search resulted in a total of 364 articles, 144 of which were excluded based on title and abstract, so that a total of 220 potentially relevant papers were retrieved for full assessment (Figure 1). Eligibility criteria included articles reporting a hungry bone syndrome after surgery for primary hyperparathyroidism in adult humans. Exclusion criteria were hypocalcaemia due to any other cause, non-complicated post-operative course, hungry bone syndrome in secondary or tertiary hyperparathyroidism and hungry bone syndrome in children. Comments or Letters to the Editor and articles only displaying a radiological picture were also excluded. One hundred and sixty nine of the 220 publications were excluded based on these exclusion criteria. Consequently, our search strategy ultimately resulted in 51 publications meeting the inclusion criteria of hungry bone syndrome after surgery for primary hyperparathyroidism in adult humans.

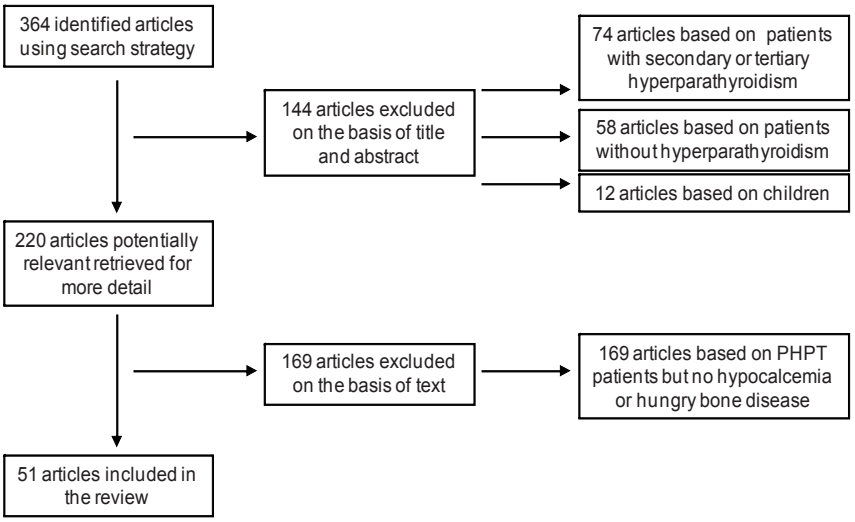


Figure 1. Flowchart of articles included in the systematic review

Pathophysiology of hungry bone syndrome

Bone remodelling consists of a series of cellular events on the bone surface, the function of which is to remove damaged bone through the process of osteoclastic bone resorption, and replacing it with new bone through the process of osteoblastic bone formation. The process of bone resorption which lasts about 2 weeks is followed by a reversal phase of 2-3 weeks, before new bone is formed, which lasts about 3 months. The remodeling space is the total amount of bone that at any time has been resorbed by osteoclasts but not yet reformed by osteoblasts during the coupled remodeling process, because of the delay between resorption and formation. This space depends on the activation frequency of new remodeling sites, which is considerably increased in primary hyperparathyroidism, and on the average difference between the amount of bone resorbed and not yet reformed at each remodeling site, which is largely unchanged in hyperparathyroidism. In severe hyperparathyroidism. The increase in bone resorption leads to mineral depletion of bone and significantly contributes to the hypercalcaemia characteristically observed in patients with hyperparathyroidism (10-14). In patients with hyperparathyroidism and pre-operative high rates of bone turnover, successful parathyroidectomy curbs

osteoclastic resorption, leading to a decrease in the activation frequency of new remodeling sites and to a decrease in remodeling space, leading to a consequent gain in bone mass.

The duration of the hungry bone syndrome is defined as the duration of post-operative hypocalcaemia or time required for normalization of serum calcium following successful parathyroidectomy, which parallels normalisation of bone turnover and may last for up to 9 months, but exceptionally longer in cases of parathyroid carcinoma following radical excision of the tumour. In our experience, the duration of the hypocalcaemia is determined by the height of the increased bone turnover pre-operatively as well as by the time required for recovery of normal function of residual non-pathologic parathyroid tissue (unpublished personal observations).

Clinical manifestations of hungry bone syndrome

Severe hypocalcaemia (serum calcium concentration $\leq$ 2.1 mmol/l) is associated with neuromuscular irritability, clinically manifested by carpopedal spasms, perioral paresthesiae, tingling extremities, Chvostek sign and Trousseau sign (15-26). Patients can also develop generalized convulsions, which can ultimately lead to pathological fractures (27,28), and ultimately if remaining uncorrected to coma and even death. Congestive heart failure, which is reversible after normalization of serum calcium concentration, has also been reported (15,29).

Prevalence of hungry bone syndrome after parathyroidectomy

Data on the prevalence of hungry bone syndrome have been scarce and conflicting after original publications in the eighties from a large case series suggesting that the syndrome develops post-operatively in up to 13% of patients with primary hyperparathyroidism (1,3,30). Recent case series from Asia reported much higher prevalence rates of 24-87% (31-34), whereas a case serie from Saudi Arabia documented a prevalence rate of only 4% (35).

Risk factors for the development of a hungry bone syndrome

Age at time of surgery

Older age at the time of surgery is a risk factor for hungry bone syndrome (1). Brasier *et al.* (1), showed in a group of 198 PHPT patients, that patients who developed hungry bone syndrome were 10 years older than patients with an uncomplicated post-operative course (61 ± 3 vs. 51 ± 1, $P<0.05$).

Table 1. Pre-operative laboratory data

Laboratory investigation	Author	Patients who developed HBS	Patients who did not develop HBS	*P*-value
s-Calcium (mmol/l)	Brasier[1]	3.00 ± 0.05	2.88 ± 0.03	*<0.05*
	Spiegel[36]	3.25 ± 0.05	3.00 ± 0.03	*<0.001*
	Heath[39]	3.94 ± 0.38	2.95 ± 0.15	*<0.01*
	Lee[37]	3.00 ± 0.1	3.00 ± 0.08	*0.7*
s-PTH (pmol/l)	Brasier[1]	10.2 ± 2.00	5.7 ± 0.3	*<0.05*
	Lee[37]	30.7 ± 10	32.9 ± 6	*0.2*
s-alkaline phosphatase (U/l)	Brasier[1]	68 ± 15	38 ± 2	*<0.05*
	Heath[39]	51 ± 37	12 ± 6	*<0.01*
	Lee[37]	248 ± 48	169 ± 31	*0.1*

HBS: hungry bone syndrome, s: serum

Laboratory investigations prior to surgery

Patients who developed hungry bone syndrome had higher pre-operative levels of serum calcium (3.00 ± 0.05 vs. 2.88 ± 0.03 mmol/l, $P<0.05$ and 3.25 ± 0.05 vs. 3.00 ± 0.03 mg/dl, $P<0.001$ and 3.94 ± 0.38 vs. 2.95 ± 0.15 mmol/l, $P<0.01$) and almost 2-fold increased levels of PTH (10.2 ± 2 vs. 5.7 ± 0.3 pmol/l, $P<0.05$) and of alkaline phosphatase (68 ± 15 vs. 38 ± 2 IU/l, $P<0.05$ and 12 ± 6 vs. 51 ± 37 U/dl, $P<0.01$) compared to patients who had an uncomplicated post-operative course (Table 1) (1,4,36). However, Lee *et al.* (37) were not able to demonstrate a significant difference in pre-operative serum calcium levels (3.00 ± 0.1 vs. 3.00 ± 0.08 mg/dl, $P=0.7$), PTH (30.7 ± 10 vs. 32.9 ± 6 pmol/l, $P=0.2$) or of alkaline phosphatase (248 ± 48 vs. 169 ± 31 IU/l, $P=0.1$) levels between 9 patients who developed hungry bone syndrome post-operatively and 14 patients who did not.

Serum magnesium and albumin levels were found to be significantly decreased in patients who subsequently developed a hungry bone syndrome (1.5 ± 0.1 vs. 1.7 ± 0.04 mEq/l, $P<0.001$ and 3.9 ± 0.1 vs. 4.3 ± 0.04 g/dl, $P<0.001$, respectively) (1).

There were no data available on the predictive value of pre-operative bone markers other than alkaline phosphatase, such as procollagen type 1 amino-terminal propeptide (P1NP, a marker of bone formation) and beta-crosslaps (β-CTX, a marker of bone resorption).

Depleted vitamin D status (low levels of 25(OH)D and $1,25(OH)_2D$) has been suggested to be a risk factor for the development of hungry bone syndrome, by some, but not all, authors (1,38,39)

Radiological bone disease prior to surgery

Radiological evidence of PHPT-related bone disease has been reported to be an important risk factor for the development of a hungry bone disease (4,16,17,26,27,31,40), which also reflects prolonged exposure of the skeleton to high circulating PTH levels. Fourteen of 18 available case reports on hungry bone syndrome indeed report skeletal abnormalities, such as subperiostal erosions, lytic lesions, brown tumours, and multiple fractures (15-19,21-27,29,41-45). Osteitis fibrosa cystica was observed in 47-100% of patients who develop hungry bone syndrome (31,36). Hungry bone syndrome was reported in 25-90% of patients with radiological evidence of PHPT-related bone disease versus in 0-6% of patients without skeletal involvement (31,39,40).

Volume and weight of resected pathological parathyroid gland(s)

A large study in 198 patients with PHPT demonstrated that the volume and weight of the removed adenomas were significantly greater in patients who developed hungry bone syndrome compared to patients who had an uncomplicated post-operative course (5 ± 1 vs. 1 ± 0.2 cm^3, $P<0.05$ and 4 ± 1 vs. 2 ± 0.2 gram, $P<0.05$, respectively) (1). Zamboni *et al.* (46) confirmed this finding, by demonstrating that 11 of 16 patients with a single adenoma of > 2 gram developed transient post-

operative hypocalcaemia versus only 3 of 21 patients with a single adenoma of <1 gram (P<0.001).

There are no available data on the relation between histological characterizations of the resected pathological glands (adenoma vs. hyperplasia) and the development of hungry bone syndrome.

Biochemical changes associated with hungry bone syndrome

A rapid decrease in serum PTH levels to a mean of 1.7 ± 0.4 pmol/l follows successful parathyroidectomy in all cases of primary hyperparathyroidism (1). Serum calcium levels drop to levels <2.1 mmol/l within the first 3-4 days, but decrease further after the fourth postoperative day in patients with hungry bone syndrome (1). Serum phosphate levels decrease post-operatively and remain so for the duration of the syndrome (1,17,27,31,37,38,41,47,48). Hypomagnesaemia is frequently encountered (36). Serum alkaline phosphatase levels increase significantly post-operatively and remain elevated for sometimes up to 9 months after surgery (1,17,27,31,38,40,41,43,44,49).

Agarwal *et al.* (31) also reported increased levels of osteocalcin, a marker of bone formation, and decreased urine crosslaps, a marker of bone resorption, in 51 patients one week after surgery, with serum osteocalcin levels normalising only 6 months after successful parathyroidectomy (31). In 3 of 51 patients with extreme osteopenia, bone turnover markers remained elevated for 1 year after successful parathyroidectomy (31).

Radiological changes associated with hungry bone syndrome

Removal of the excessive circulating levels of PTH shuts off bone-resorptive activity and leads to a rapid increase in bone mineral density. Case reports show an increase in bone mineral density of the lumbar spine of 17% at 10 weeks, 10% at 6 months and 27-65% at 1 year after parathyroidectomy (7,44,49,50) and an increase in bone mineral density of the greater trochanter of 33% at 6 months and of 35-131% at 1 year after surgery (7,50).

Table 2. Details of pre-operative treatment with bisphosphonates to prevent post-parathyroidectomy hungry bone syndrome

Author	No. of patients	Radiological evidence of bone involvement	Pre-operative treatment	%HBS
Franca, 2011[50]	6	Osteitis fibrosa cystica	Alendronate 20-30mg/day for 4-6 weeks or pamidronate once once 90 mg iv or ibandronate 150 mg iv	0
Yong, 2010[44]	1	Fracture, extensive osteitis fibrosa cystica, lytic lesions, osteoporosis	Pamidronate twice 90 mg iv	100
Corsello, 2010[45]	1		Zoledronate twice 4 mg	
Gurevich, 2008[52]	1	Not mentioned	Pamidronate 3 times 30 mg and once 90 mg iv	0
Malabu, 2007[35]	46	46% had bone aches	Zoledronate	4
Demirci, 2007[23]	1	Osteoporosis, loss of subperiosteal cortex	Alendronate 70 mg for 2 weeks	100
Lee, 2006[37]	23	Not specified	Pamidronate 60-1600 mg/day for 1-17 days or clodronate 300 iv for 1-15 days with or without 1600 mg/day p.o. for 3-7 days vs. no bisphosphonate	0 vs. 53
Hisham, 2000[43]	1	Extensive metabolic bone changes	Pamidronate once 60 mg iv	100
Graal, 1998[38]	1	Low BMD, vertebral fractures	Pamidronate 15 mg/day iv for 5 days, followed by twice daily 150 mg orally	100
Kumar, 1996[51]	1	Extensive metabolic bone changes	Pamidronate twice 30 mg iv	0
Brossard, 1993[7]	1	Osteopenia, subperiosteal bone resorption, multiple osteolytic lesions	Pamidronate once 60 mg iv	0

HBS: hungry bone syndrome, BMD: bone mineral density

Table 3. Details of post-operative treatment of patients who developed hungry bone syndrome after parathyroidectomy

Author	Age/ Sex	Radiological signs of PTH-induced bone disease	Pre-operative treatment	Post-PTx calcium (min)	Post-operative treatment (maximum dose per day)
Silaghi, 2011[25]	48/F	Osteolytic lesions, resorption of distal phalanx of fingers			Calcium, vitamin D and bisphosphonates
Kim, 2011[24]	29/F			1.78 mmol/l	Calcium iv and orally
Corsello, 2010[45]	64/F		Zoledronate twice 4mg/day, calcitonin 400 IU/day	1.0 mmol/l	Calcium gluconate 6-8 g/day iv, calcitriol 3 ug/day (transient 1.5 ug/day iv)
Yong, 2010[44]	23/M	Fracture, extensive osteitis fibrosa cystica, lytic lesions, osteoporosis	Pamidronate twice 90 mg iv	2.0 mmol/l	Calcium iv and orally total 11.4 g/day, calcitriol 2 ug/day, magnesium iv and orally
Ajmi, 2010[16]	48/F	Diffusely increased scintigraphic uptake, radiolucent lesions		1.5 mmol/l	Calcium iv, vitamin D orally
Hussain, 2008[21]	25/F	General loss of bone density, old fractures, healed with malunion		1.9 mmol/l	10% Calcium gluconate 10 ml iv, calcitriol 3 ug orally
Rathi, 2008[17]	45/F	Subperiostal erosions, brown tumors, chondrocalcinosis		1.83 mmol/l	Elemental calcium 2.5 g iv and 8 g orally, calcitriol 4 ug orally, magnesium orally
Demirci, 2007[23]	53/F	Subperiostal erosions, salt and peper skull, lytic lesions	Alendronate 70mg/week, calcitriol 0.5 ug/day	1.4 mmol/l	Elemental calcium 720 mg iv and 14 g orally, and calcitriol 2 ug orally
Meydan, 2006[42]	52/F	Multiple lytic lesions, foci of increased scintigraphic uptake			Calcium and vitamin D
Kuzucu, 2002[18]	50/F	Multiple foci of intense scintigraphic uptake	Bisphosphonates	1.6 mmol/l	Calcium iv and orally, calcitriol
Hisham, 2000[43]	36/M	Florid skeletal changes	Calcitonin 600 mg for 2	1.8 mmol/l	Calcium iv and 4 g orally, calcitriol 2 ug

			weeks, calcitriol 1 ug pamidronate 60 mg iv		
Graal, 1998[38]	34/F	Osteoporosis, vertebral fractures	Pamidronate 75 mg iv twice daily 150 mg orally	1.79 mmol/l	Calcium iv 1.6 g/day, calcitriol 2 ug/day, magnesium iv and orally
Chen, 1996[19]	20/F	Generalized osteoporotic changes, subperiosteal erosions, multiple brown tumors		1.17 mmol/l	Calcium, phosphorus and magnesium supplementation
Varthakavi, 1985[29]	44/F	Focal osteolytic lesions, periosteal erosions, osteosclerosis, fracture		1.17 mmol/l	Calcium gluconate 144 ml iv
Laitinen, 1977[41]	69/F			1.6 mmol/l	Calcium 10 g iv
Scott, 1976[22]	35/M	Widespread demineralization		1.3 mmol/l	10% Calcium gluconate 20 ml iv, calcium 12 g, orally magnesium chloride iv, calciferol 2.5 ug orally
Falko, 1976[15]	19/M	Deossification of skull, Subperiosteal erosions, and demineralization		1.3 mmol/l	Calcium gluseptate 30 g iv, 50% magnesium sulfate 5 ml im, vitamin D 150.000 Units/day
Jones, 1973[26]	67/F			1.2 mmol/l	Calcium iv and orally, 200 mg magnesium sulphate iv
Ahuja, 1968[27]	38/F	Multiple fractures, cystic changes and mottling, subperiostal erosions			Calcium 4 Gm/day iv and 12 Gm/day orally vitamin D 1.200.000 Units/day

PTH: parathyroid, PTx: parathyroidectomy, iv: intravenous

Bone mineral density increased post-operatively by a remarkable 332% within 1 year in Indian patients with overt skeletal disease and/or osteitis fibrosa cystica (31). Follow-up radiographs show recovery of subperiosteal resorption and remineralisation of "brown tumours", osteolytic lesions and fracture sites (7,16,27,31). Scintigraphy shows an increased uptake 1 month after parathyroidectomy, known as "flare phenomenon", which reflects a healing response due to a significant increase in bone formation with a high influx of calcium into the skeleton (16,19). A moderately increased uptake can still be seen 8 months after parathyroidectomy (18) and a decrease in the number of lesions and a normalization of uptake in the remaining lesions one year after parathyroidectomy (16).

Management of hungry bone syndrome

The treatment of a hungry bone syndrome is aimed in the short term primarily at replenishing the depleted skeletal calcium stores following withdrawal of the effect of high circulating levels of PTH on the skeleton. The first case reports of a hungry bone syndrome, which appeared in the late 70's, described the difficulties encountered in the management of this severe complication of parathyroidectomy before active metabolites of vitamin D and their synthetic analogues became available for use in the clinic (15,22,26,27). Severely decreased serum calcium levels of $\leq$1.3 mmol/l were reported despite treatment with very high doses of calcium, magnesium and cholecalciferol (15,26,27). However, difficulties in the management of hungry bone syndrome are still being reported, also after active metabolites of vitamin D and their synthetic analogues became available for use in the clinic (23).

The amount of elemental calcium supplementation required to treat the severe hypocalcaemia varies between 8 and 12 grams per day (17,21,23,29,41,44,45). Initially calcium is supplemented intravenously, followed by oral preparations, with lower doses of calcium being possible because of concomitant use of active metabolites of vitamin D, in the form of calcitriol or alfacalcidol (2 to 4 ug per day) (17,21-23,38,43-45), and after replenishing magnesium stores as required. The

amount of magnesium required to correct hypomagnesaemia is not always reported and supplementations has been variably given in the form of intravenous magnesium chloride or sulphate or oral magnesium sulphate (15,17,19,22,26,38,44). In 11 of 18 cases serum magnesium level was not mentioned and in 1 of 18 cases serum magnesium levels were within the normal range (16,18,21,23-25,27,29,41-43,45).

Treatment options to prevent hungry bone syndrome

Pre-operative treatment with vitamin D

Depleted vitamin D status has been postulated to be a risk factor for the development of hungry bone syndrome and it has generally been recommended to supplement vitamin D to normalize 25(OH) vitamin D levels, although there are so far no available data to support the premise that this would contribute to the prevention of hungry bone syndrome (1,38).

Pre-operative treatment with bisphosphonates

Two case reports of patients with a short history of PHPT, but with extensive hyperparathyroid-related bone disease, who were treated with either pamidronate i.v. 30 mg on 2 consecutive days or with a single infusion of 60 mg once, demonstrated that serum calcium decreased pre-operatively and that patients needed much less (1500 mg of elemental calcium orally per day) or no calcium supplementation post-operatively (7,51). One case report showed that a patient with a history of severe PHPT for > 8 years who received alendronate for 6 years, in addition to a total of 180 mg pamidronate i.v. pre-operatively, did not develop a hungry bone syndrome post-operatively (52).

In a retrospective study, Lee *et al.* (37) also demonstrated that none of 6 patients who had received bisphosphonates pre-operatively (either clodronate 300-1600mg/day or pamidronate 60mg/day) developed hungry bone syndrome post-operatively, compared to 9 of 17 patients who had not received bisphosphonates pre-operatively (Table 2). There were no significant differences in pre-operative mean serum calcium (3.00 ± 0.15 vs 3.01 ± 0.04 mmol/l), PTH (34.8 ± 11 vs 33.4 ±

10 pmol/l) or alkaline phosphatase (224 ± 50 vs. 174 ± 60 U/l) levels between groups. A retrospective case serie of 46 patients with severe bone disease, who were treated with intravenous zolidronate pre-operatively also reported a low frequency of hungry bone syndrome of only 4% (35). Another retrospective case serie of 6 patients with radiological characteristics of osteitis fibrosa cystica, who were pre-operatively treated with bisphosphonates (alendronate 20-30mg/day for 4-6 weeks or a single dose of pamidronate 90 mg i.v. or ibandronate 150 mg i.v.) reported that none of the patients needed intravenous supplementation of calcium post-operatively (50).

In contrast, a case report of a patient with severe, prolonged and extensive bone involvement (florid metabolic bone changes on X-ray), has shown that a single dose of 60 mg pamidronate i.v. combined with calcitriol 1-2 ug/day was able to significantly decrease (but not normalize) serum alkaline phosphatase levels (1600 U/l to 420 U/l), but not able to completely prevent a hungry bone syndrome (43). Four others cases have also shown that treatment of severe hyperparathyroidism with alendronate (70 mg/week), pamidronate (twice 90 mg i.v. or 5 times 15 mg i.v.) or zolendronate (twice 4 mg i.v.) was unable to completely prevent a hungry bone syndrome (23,38,44,45).

Pre-operative treatment with active metabolites of vitamin D

Boyle *et al.* (53) showed that pre-operative treatment of severe hyperparathyroidism with 2 µg/day of $1,25(OH)_2D$ for 1-10 weeks significantly decreased pre-operative alkaline phosphatase levels in 3 of 7 patients with prominent radiologically apparent bone cysts, and 3 other patients required intravenous calcium to a total of less than 1 g in the first 12 post-operative days. In contrast, Heath *et al.* (4) showed that 6 patients with PHPT and radiological evidence of bone involvement, who were treated pre-operatively with 2 µg/day of $1,25(OH)_2D$ for 1 week, were as likely to develop hungry bone disease as patients with PHPT and radiological evidence of bone involvement who did not receive active vitamin D pre-operative (2 of 6 vs. 1 of 6, respectively).

DISCUSSION

Hungry bone syndrome is characterized by a rapid, profound and persistent hypocalcaemia associated with functional hypoparathyroidism, which follows parathyroidectomy in patients with severe primary hyperparathyroidism and preoperative high bone turnover. The duration of the hungry bone syndrome is the time taken to remineralize the skeleton, which is also mirrored by normalization of bone turnover markers, healing of radiological features of osteitis fibrosa cystica and brown tumours and by significant gains in bone mass.

Hungry bone syndrome should be distinguished from the relatively common short- or longer-term hypocalcaemia encountered after thyroid surgery due to temporary or permanent hypoparathyroidism and from the temporary hypocalcaemia often observed after parathyroidectomy for primary hyperparathyroidism with no or marginally increased bone turnover pre-operatively, which resolves spontaneously or readily after supplementation of active vitamin D until recovery of parathyroid function. In the context of primary hyperparathyroidism, a prerequisite for developing a hungry bone syndrome is a severely increased bone turnover state prior to surgery. A period of functional hypoparathyroidism may be followed by a period of increased PTH levels, more often encountered and exacerbated by associated vitamin D deficiency. These increased PTH levels are however unable to correct the hypocalcaemia, which will only resolve after adequate mineralization of the skeleton and normalization of bone turnover, often requiring huge doses of active metabolites or analogues of vitamin D and of calcium supplements, for periods sometimes exceeding 12 months after successful surgery.

Our literature search suggests that over the last 2 decades the prevalence of hungry bone syndrome has decreased in the Western World, most likely due to the early detection of still asymptomatic primary hyperparathyroidism by routine calcium screening before the advent of clinically evident bone disease, such as osteitis fibrosa cystica (38,49), although exact numbers are missing.

One of the identified risk factors for a post-operative hungry bone syndrome is the older age at the time of surgery, with age being associated with vitamin D deficiency, a decrease in 1α-hydroxylase activity, and lower dietary calcium intakes (1), all 3 factors contributing to a negative calcium balance and clinical bone disease (4). It has indeed been shown that patients with osteitis fibrosa cystica, have lower levels of $1,25(OH)_2D$ than expected, due to high levels of serum calcium which directly inhibit renal 1α-hydroxylase production, but may also result in renal impairment and further decrease in 1α-hydroxylase activity (54). A testable hypothesis for the development of bone disease, and for the development of a hungry bone syndrome, relates to the possibility that low circulating levels of $1,25(OH)_2D$ with resultant decreased fractional absorption of calcium, leads to undermineralization of the skeleton (1,4). Low levels of $1,25(OH)_2D$ may thus represent a measurable risk factor for the development of a hungry bone syndrome, independently of age, although 25(OH)D deficiency has been proposed to be the more significant risk factor (38).

Pre-operative serum alkaline phosphatase levels reflect the state of bone turnover and, therefore, the degree of osteoclast activity and of bone resorption. It has been suggested that pre-operative serum alkaline phosphatase concentrations may predict the degree and duration of hypocalcaemia after successful parathyroidectomy (55). Radiological evidence of bone disease, in the form of osteitis fibrosa cystica, subperiostal bone erosions or resorption or bone cysts, has also been stated to be a risk factor for the development of hungry bone disease (4,15-17,26,27,31,40). Pathological risk factors include the volume and weight of the removed adenoma(s), which have been found to be significantly greater in patients who developed a hungry bone syndrome compared to patients who had an uncomplicated post-operative course (1,3).

Treatment of the hungry bone syndrome is aimed in the short term primarily at replenishing the circulating calcium deficit, resulting from the increased utilisation of calcium arising from the abrupt cessation of bone resorption by the removal of the stimulatory effect of PTH in the face of continuing bone formation to refill the multiple resorption cavities. In the longer term, treatment is aimed at restoring

calcium homeostasis by substituting the temporarily missing stimulatory effect of PTH on the 1α-hydroxylase enzyme by providing the active form of vitamin D to ensure adequate intestinal absorption of calcium. Doses and duration of treatment are guided by serum calcium levels and achievement of normalisation of bone turnover (3,15,20,26-28,49), which can sometimes exceed 12 months after successful surgery.

It is estimated that 10 grams of calcium daily is on average necessary to restore and maintain serum calcium within the normal range (15,17,21-23,29,41). Lower doses of calcium are possible because of concomitant use of active metabolites of vitamin D, in the form of calcitriol or alfacalcidol (2 to 4 ug per day) (17,21-23,38,43-45), or very occasionally higher doses (unpublished personal observations). The reason for this is post-operative low PTH levels which can last for up to 6 months after parathyroidectomy, resulting in lack of stimulation of the 1α-hydroxylase enzyme.

When calcium-containing solutions are given intravenously, administration into large veins or via a central venous catheter is recommended to minimize the risk of local irritation or tissue necrosis, by accidental extravasation in surrounding tissues. Parenteral calcium could be initially administered intermittently, for example in the form of one or two 10 ml ampoules of 10% calcium gluconate diluted in 50-100 ml of 5% dextrose infused slowly over 10 minutes. Continuous administration of a diluted solution of ten 10 ml ampoules of 10% calcium gluconate in 1 litre of 5% dextrose or 0.9% saline is often subsequently required to prevent recurrence of hypocalcaemia. This is given at an initial infusion rate of 50 ml/hour, slowing down as required, aiming at maintaining serum calcium at least at the lower end of the reference range. Electrocardiographic monitoring is recommended as dysrhythmias may occur in case of too rapid correction of the hypocalcaemia (56)

Of the available oral calcium preparations calcium carbonate has the highest % of elemental calcium (40%), followed by citrate salts (20%). If a patient cannot tolerate these calcium supplements, other calcium preparations are available, although they do not contain sufficient elemental calcium per tablet and compliance may be affected by the large number of tablets required to be taken orally to achieve

the same calcium level: calcium lactate (13%), calcium gluconate (9%) and calcium glubionate (6.6%) (20). Oral calcium therapy should be initiated as soon as practical, but should always be combined with active vitamin D metabolites or analogues such as calcitriol or alfacalcidol to ensure adequate intestinal absorption of the administered oral preparation.

Treatment of hypomagnesaemia depends on the degree of magnesium deficiency. When high doses of magnesium are required, this should only be given intravenously in adequate dilutions of magnesium sulphate, and not orally or intramuscularly. Lower doses of magnesium can be supplemented as magnesium oxide orally or magnesium sulphate intramuscularly. Hypocalcaemia does not resolve until the magnesium deficiency has been corrected (3,15,26-28,49).

The severity of a hungry bone syndrome is dictated by the degree of bone resorption pre-operatively, the highest pre-operative bone turnover being associated with the most severe and prolonged hungry bone syndrome. Preventive measures for a hungry bone syndrome should thus aim at reducing bone turnover pre-operatively. This should be first achieved by correcting any prevailing vitamin D deficiency. Depleted vitamin D status has been postulated to be associated with an increased risk of developing postoperative hypocalcaemia and hungry bone syndrome (57-60). Preliminary data suggest that pre-operative correction of vitamin D deficiency may decrease levels of PTH and bone turnover, without exacerbating hypercalcaemia (57,60,61). Although the effect of preoperative vitamin D treatment on postoperative hypocalcaemia has not been evaluated by randomized controlled intervention studies in primary hyperparathyroidism, it is our experience that a preoperative replete vitamin D status is associated with a decreased likelihood of a severe or prolonged hungry bone syndrome.

Bisphosphonates are potent antiresorptive agents widely used in the management of bone disorders associated with increased bone turnover, such as Paget's disease of bone or metastatic bone disease. These agents inhibit osteoclastic bone resorption, decrease activation frequency of remodeling sites, resulting in refilling of remodeling space and increased mineralization of bone. Bisphosphonates have been shown to reduce bone turnover in severe

hyperparathyroid bone disease (38,62,63). In this context preoperative bisphosphonate treatment would have a potential beneficial effect on the severity and duration of a hungry bone syndrome by significantly decreasing or normalising bone turnover before surgery is attempted (38,64). In contrast, short-term preoperative treatment may exacerbate postoperative hypocalcaemia by just reducing bone resorption, without allowing time for a coupled decrease in bone formation. There are as yet no prospective studies or randomized control trials addressing the use of bisphosphonates in the prevention or limitation of duration of a hungry bone syndrome. There are, however, case reports and small case series of patients with extensive hyperparathyroid-related bone disease (35,37,54,64) or with longstanding severe PHPT (52) who received pamidronate pre-operatively and did not develop a hungry bone syndrome post-operatively (23,27,35-37). Other case reports have shown that pamidronate combined with calcitriol decreased serum alkaline phosphatase levels, but was unable to completely prevent a hungry bone syndrome in patients with severe, prolonged and extensive bone involvement (23,38,43-45). However, in these case reports, the duration of pre-operative treatment with bisphosphonates and of active vitamin D was deemed to be too short and the dosage to low, since serum alkaline phosphatase levels had not normalized before surgery (23,38,43-45).

Because low levels of $1,25(OH)_2D$ are a risk factor for the development of post-operative hungry bone syndrome (1,39), it has also been hypothesized that pre-operative supplementation of $1,25(OH)_2D$ could shorten symptomatic hypocalcaemia and hospital course (1,53,54). Data on the results of $1,25(OH)_2D$ supplementation are, however, conflicting (4,53). A major limitation of the studies looking at prevention of hungry bone syndrome, using either bisphosphonates or $1,25(OH)_2D$ supplementation or both, is the lack of patient randomization.

CONCLUSION

Hungry bone syndrome is a relatively uncommon, but serious complication of parathyroidectomy for severe primary hyperparathyroidism associated with high

bone turnover. There are no clear guidelines for the management of the hungry bone syndrome but treatment is aimed at replenishing the severe calcium deficit and at correcting the effects of the functional hypoparathyroidism by using high doses of calcium and active metabolites of vitamin D. Adequate correction of magnesium deficiency and normalization of bone turnover are required for resolution of the hypocalcaemia which may last for a number of months after successful surgery. Adequate pre-operative treatment with bisphosphonates may reduce the severity and duration of postoperative hypocalcaemia. Further prospective studies are needed to optimize pre- and postoperative treatment strategies in patients with primary hyperparathyroidism and skeletal manifestations, at high risk for a hungry bone syndrome.

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6

Patients with primary hyperparathyroidism have lower circulating sclerostin levels than euparathyroid controls

Antoon H. van Lierop, Janneke E. Witteveen, Neveen A.T. Hamdy, Socrates E. Papapoulos

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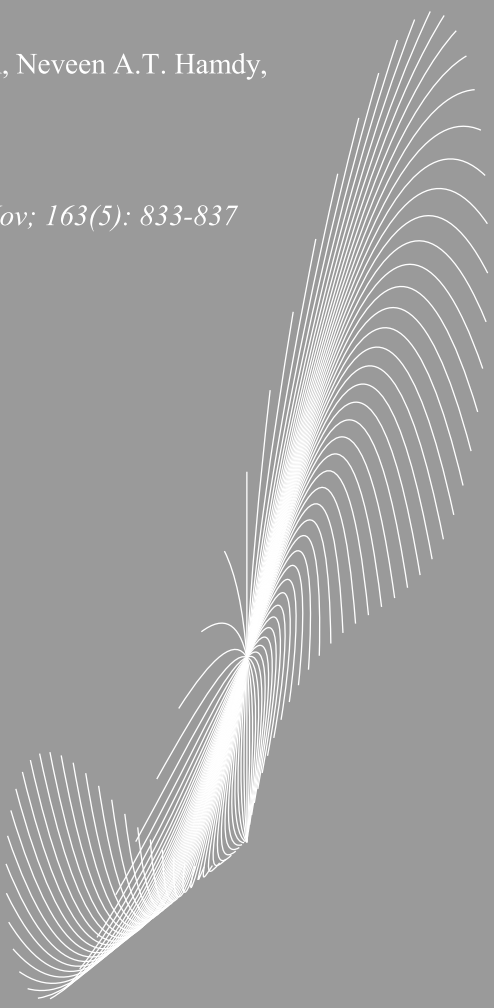

ABSTRACT

Objective: *In vitro* and *in vivo* studies in animal models have shown that parathyroid hormone (PTH) inhibits the expression of the *SOST* gene, which encodes sclerostin, an osteocyte-derived negative regulator of bone formation. We tested the hypothesis that chronic PTH excess decreases circulating sclerostin in humans.

Design: We studied 25 patients with elevated serum PTH concentrations due to primary hyperparathyroidism (PHPT) and 49 patients cured from PHPT after successful parathyroidectomy (PTx; euparathyroid controls (EuPTH)).

Methods: We measured plasma PTH and serum sclerostin levels and the serum markers of bone turnover alkaline phosphatase, P1NP, and β-CTX.

Results: As expected by the design of the study, mean plasma PTH was significantly higher (P<0.001) in PHPT patients (15.3 pmol/l; 95% confidence interval (CI): 11.1-19.5) compared with that of EuPTH controls (4.1 pmol/l; 95% CI: 3.6-4.5). PHPT patients had significantly lower serum sclerostin values compared with those in EuPTH subjects (30.5 pg/ml; 95% CI: 26.0-35.1 vs 45.4 pg/ml; 95% CI: 40.5-50.2: P<0.001) and healthy controls (40.0 pg/ml; 95% CI: 37.1-42.9: P=0.01). Plasma PTH concentrations were negatively correlated with serum sclerostin values (r=-0.44: P<0.001). Bone turnover markers were significantly correlated with PTH, but not with sclerostin.

Conclusion: Patients with primary hyperparathyroidism have significantly lower serum sclerostin values compared with PTx controls with normal PTH concentrations. The negative correlation between PTH and sclerostin strongly suggests that *SOST* is downregulated by PTH in humans.

INTRODUCTION

Parathyroid hormone (PTH) exerts its calciotropic action by acting directly on bone and kidney and indirectly on the intestine to increase the transport of calcium to the circulation. The skeletal effect of PTH is to increase the rate of remodeling (1), and it is generally believed that this effect is achieved by the binding of PTH to its specific receptor (PTHR1) on stromal/osteoblastic cells of the bone marrow. This in turn stimulates the production of RANK ligand and decreases that of its decoy receptor osteoprotegerin (OPG) (2-5). However, recent *in vitro* and animal studies suggest that some of the effects of PTH on bone are also exerted by specific binding of the hormone to PTHR1 in osteocytes, resulting in inhibition of the expression of the *SOST* gene (6-9). This gene encodes sclerostin, a protein exclusively expressed in osteocytes in the skeleton (10), which decreases bone formation by binding to LRP5/6, resulting in inhibition of the Wnt signaling pathway in osteoblasts (11,12).

Whether chronic PTH excess has similar effects on sclerostin secretion in humans as in animal models has not so far been investigated. In this study, we tested the hypothesis that chronic hypersecretion of PTH, as seen in patients with hyperparathyroidism, may decrease sclerostin secretion, and that PTH may thus represent a potential regulator of sclerostin production in humans. To this effect, we measured sclerostin in serum of patients with untreated primary hyperparathyroidism (PHPT) and in a control group of patients with PHPT after establishment of cure following parathyroidectomy (PTx).

PATIENTS AND METHODS

Patients

Thirty-four consecutive patients with PHPT that was untreated, persistent, or recurrent following PTx, and 54 patients cured after successful PTx (euparathyroid controls, EuPTH) were studied. Inclusion criteria included willingness to participate in the study, no impairment in renal function (serum creatinine levels <120 μmol/l), adequate vitamin D status (25-hydroxy vitamin D (25-OHD) levels >50 nmol/l),

and no use of bone and mineral metabolism modifying agents such as bisphosphonates, calcimimetics, or glucocorticoids.

We defined PHPT as plasma PTH concentrations above the upper limit of the normal laboratory reference range (>8 pmol/l) in the presence of increased serum calcium concentrations (>2.55 mmol/l).

Patients with EuPTH were included when cure was confirmed by post-operative normalization of serum PTH and calcium concentrations, which was sustained for at least 6 months after PTx.

As per inclusion criteria, nine patients were excluded from the PHPT group, three because of impaired renal function and six because of the use of bisphosphonates, and six were excluded from the EuPTH group because of the use of bisphosphonates.

The study was approved by the Medical Ethics Committee of the Leiden University Medical Center, and informed consent was obtained from all patients.

Serum biochemistry

Serum calcium was adjusted for albumin binding; phosphate and creatinine were measured by semi-automated techniques. Serum alkaline phosphatase (ALP) activity was measured using a fully automated P800 modulator system (Roche BV). P1NP (a marker of bone formation) and β-CTX (a marker of bone resorption) were determined using the E-170 system (Roche BV). Plasma PTH was measured using the Immulite 2500 (Siemens diagnostics, Breda, The Netherlands) and serum 25-OHD was measured using the LIAISON 25-OHD TOTAL assay (DiaSorin S.A./N.V., Bruxelles, Belgium)

Sclerostin measurement

Sclerostin was measured in serum by an electrochemiluminescence assay (MSD 96-well MULTI-ARRAY Human Sclerostin Assay, Gaithersburg, MD, USA) in which two polyclonal antibodies were raised against the whole sclerostin molecule. The sclerostin standard for the assay was produced in a NS0-derived myeloma cell line, and the purity was checked by SDS-PAGE gel with silver stain. In our hands**,** the

intra- and inter-assay coefficient of variation values were 4.9 and 7% respectively, the detection limit was ± 1 pg/ml, and the detection range was 1-10.000 pg/ml.

Sclerostin was measured in the serum of 77 healthy subjects (30 male and 47 female, aged 20-77 years). All had normal serum calcium concentration, renal function, and bone turnover and none were using bisphosphonates, the calcimimetic cinacalcet, or glucocorticoids. Sclerostin was detected in serum of all healthy subjects: mean 40.0 pg/ml (95% CI = 37.1-42.9 pg/ml), range 12.4-68.19 pg/ml, while it was undetectable in serum of three patients with sclerosteosis.

Statistical analysis

Data were analyzed using SPSS 16.0 (SPSS Inc. Chicago, IL, USA). Between-group differences in baseline characteristics and serum biochemistry were assessed by Student's *t*-test. Pearson correlation coefficients were calculated to assess correlations between PTH (after logarithmic transformation), sclerostin, and biochemical markers of bone turnover. A probability level of random difference of 0.05 was considered significant.

RESULTS

Baseline characteristics

There were no differences in age, gender, weight, or body mass index between patients with PHPT and EuPTH controls (Table 1).

Table 1. Subject characteristics

	PHPT	EuPTH	*P* value*
Male : Female	10:15	13:36	0.41
Age (years)	59.6 ± 16.7	62.4 ± 10.9	0.44
Weight (kg)	80.0 ± 18.2	81.1 ± 15.3	0.78
BMI (kg/m²)	27.0 ± 6.0	28.1 ± 4.6	0.42

Values are given as mean ± standard deviation, BMI = body mass index, * PHPT vs EuPTH

As expected by the inclusion criteria, mean serum calcium and PTH concentrations were significantly higher and those of phosphate significantly lower

in the PHPT group compared with the EuPTH group in which PTH concentrations were detectable in all patients. There were no differences in serum 25-OHD or creatinine concentrations between the two groups.

Patients with PHPT had significantly higher levels of biochemical markers of bone formation (P1NP) and bone resorption (β-CTX) compared with EuPTH controls. Combining all patients, there was a significant positive correlation between plasma PTH concentrations and the concentrations of all three measured biochemical markers of bone turnover (ALP: r=0.23, P=0.047; P1NP: r=0.45, P<0.001; β-CTX: r=0.54, P<0.001). There was also a significant correlation between PTH and P1NP (r=0.51, P=0.009) in the PHPT group, but not between PTH and ALP (r=0.35, P=0.085), or PTH and β-CTX (r=0.31, P=0.13). In the EuPTH group alone, PTH was not correlated with any of the biochemical markers of bone turnover (Table 2).

Table 2. Biochemical measurements

	PHPT	EuPTH	Reference range	*P* value*
Calcium homeostasis				
PTH (pmol/l)	15.3 ± 10.7	4.1 ± 1.6	1.5 - 8.0	<0.001
Calcium (mmol/l)	2.61 ± 0.13	2.26 ± 0.12	2.15 - 2.55	<0.001
Phosphate (mmol/l)	0.92 ± 0.14	1.14 ± 0.24	0.90 - 1.50	<0.001
25 (OH) D (nmol/l)	53.1 ± 34.3	53.9 ± 20.2	30 -120	0.90
Creatinine (μmol/l)	80.2 ± 18.1	76.8 ± 14.7	44 - 80	0.39
Bone turnover				
ALP (U/l)	87.0 ± 23.0	75.7 ± 23.6	40 - 120	0.055
P1NP (ng/ml)	45.9 ± 16.9	34.0 ± 15.5	16 - 80	0.004
β-CTX (ng/ml)	0.32 ± 0.15	0.17 ± 0.11	0.01– 0.66	<0.001

* PHPT vs EuPTH

Serum sclerostin

Mean serum sclerostin level of patients with PHPT (30.5 pg/ml, 95% CI: 26.0-35.1) was significantly lower than that of patients with EuPTH and healthy controls (45.4 pg/ml, 95% CI: 40.5-50.2; P<0.001, and 40.0 pg/ml, 95% CI: 37.1-42.9; P=0.01, respectively; Figure 1). There was no significant difference in mean sclerostin values between EuPTH and healthy subjects (P=0.13).

There was no significant correlation between PTH and sclerostin concentrations within each individual group of patients but there was a significant negative correlation between sclerostin and PTH when all patients were pooled together (r= -0.44, P<0.001: Figure 2).

There was no significant relationship between serum sclerostin and biochemical markers of bone turnover in patients with PHPT or in all patients combined.

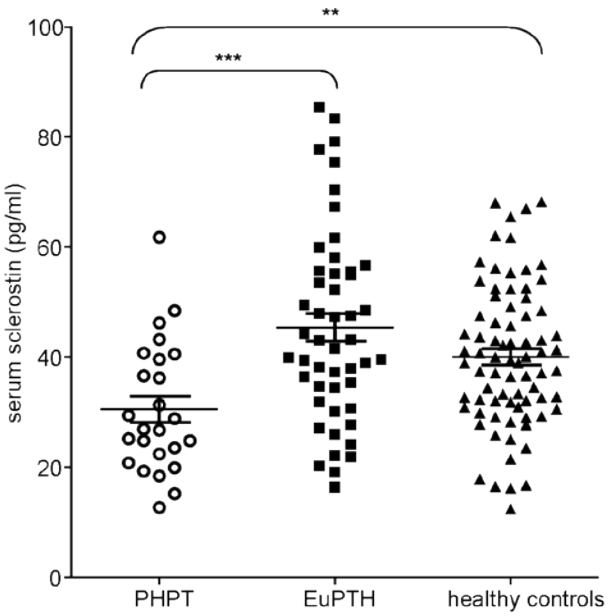

Figure 1. Serum sclerostin levels in PHPT, EuPTH and healthy subjects. *** P <0.001, ** P =0.01 (Student's *t*-test).

DISCUSSION

Data from our study demonstrates that in humans, chronic PTH excess, as observed in patients with PHPT, is associated with a significant decrease in circulating sclerostin and that there is a significant negative correlation between PTH and serum sclerostin levels. Taken together, these results suggest that, similar to animal models (6-9), PTH has a regulatory role on sclerostin production in humans also.

Sclerostin, a glycoprotein expressed by osteocytes in bone and encoded by the *SOST* gene, has emerged in recent years as an important regulator of bone formation in humans as well as in animals (13,14). Inactivating mutations of the *SOST* gene leading to sclerostin deficiency have been shown to be associated with the rare skeletal disorder sclerosteosis, which is characterized by a marked increase in bone mass (13). Deletion of the *Sost* gene in mice has also been shown to increase bone formation, bone mass, and bone strength (14) demonstrating an evolutionary conservation of the function of sclerostin in the regulation of bone formation. Moreover, inhibition of sclerostin secretion by an MAB to sclerostin has been shown to increase bone formation and bone mass in rodents, primates, and humans (14-16). Conversely, transgenic mice overexpressing *Sost* have low bone mass and impaired biomechanical competence (17). The mechanism of action of sclerostin to decrease bone formation involves inhibition of the Wnt signaling pathway (11,12), although its precise molecular mechanism and factors controlling its secretion are yet to be determined. Recent animal studies have shown that mechanical loading and high PTH levels downregulate the expression of *Sost* in osteocytes and decrease the production of sclerostin resulting in stimulation of bone formation (8,18).

Human studies of sclerostin regulation have lagged behind due to lack of noninvasive techniques to determine sclerostin production. A number of assays have been recently developed for the measurement of sclerostin in blood. In our study, we used a sclerostin assay that proved to have excellent performance characteristics in our hands with a wide detection range. Sclerostin was detectable in serum in all healthy subjects studied, suggesting that the protein is secreted and enters the circulation, while it was undetectable in three patients with sclerosteosis in whom it was measured. The difference in absolute values with other commercial assays is not clear and may be related to different methodologies. We chose to study patients with PHPT in order to mimic as closely as possible the effect of chronic PTH excess on sclerostin, as previously studied in animals (6,8). In addition, we chose to use as controls, patients with PHPT cured after PTx to exclude potential confounding factors, other than PTH excess.

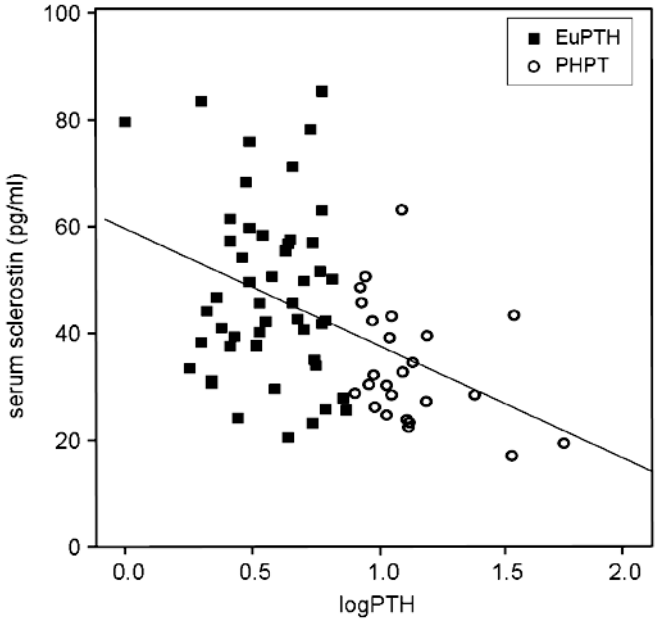

Figure 2. Relationship between circulating sclerostin and PTH levels. r=-0.44 , P<0.001 (Pearson's correlation). PTH levels were log transformed because of skewness.

We show here that serum sclerostin levels are significantly decreased in patients with chronic PTH excess due to PHPT compared to EuPTH and healthy subjects although there was an overlap of values between groups. PTH has been shown to decrease *SOST* transcription *in vitro* (6,7), and continuous and intermittent chronic administration of PTH to rodents is associated with decreased *Sost* mRNA and sclerostin expression in osteocytes (6,7,9). Moreover, transgenic mice expressing a constitutively active PTH receptor in osteocytes exhibit decreased expression of sclerostin and increased Wnt signaling associated with increased bone mass (8). Additional evidence for an interaction between PTH and *Sost*/sclerostin was recently provided by a study showing that the anabolic actions of PTH on bone was blunted in *Sost*-overexpressing mice (19). In keeping with the notion of a regulatory role of PTH for sclerostin production, our data show a significant correlation between circulating PTH and sclerostin. These data extend those of Mirza *et al.* (20), who recently reported a negative relationship between serum PTH and

sclerostin in healthy postmenopausal women, and those of Drake *et al.* (21), who showed that intermittent PTH treatment decreased serum sclerostin levels in postmenopausal women. Put together, these data suggest that *SOST* regulation by PTH is also conserved between humans and rodents.

As expected in the presence of chronic PTH excess, patients with PHPT had increased bone turnover as indicated by increased biochemical parameters of bone formation and resorption. There was a significant relationship between circulating PTH concentrations and serum P1NP and β-CTX. However, we did not find a significant relationship between biochemical parameters of bone turnover and serum sclerostin, either in patients with PHPT or the combined group of PHPT and EuPTH subjects. This lack of correlation between sclerostin and bone turnover markers was previously reported in healthy postmenopausal women (20) but not in elderly women (22). The functional significance of circulating sclerostin is as yet not established.

The actions of PTH on bone are complex and involve a variety of signaling pathways in bone marrow stroma cells, osteoblasts, and osteocytes (23,24). Despite the significant progress in our understanding of the actions of PTH on bone, it should be appreciated that the cellular and molecular actions of PTH, which determine the action of the hormone on bone remodeling and bone balance, have only been partially unraveled and studies are needed to further elucidate these actions.

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Increased circulating levels of FGF23: an adaptive response in primary hyperparathyroidism?

Janneke E. Witteveen, Antoon H. van Lierop, Socrates E. Papapoulos, Neveen A.T. Hamdy

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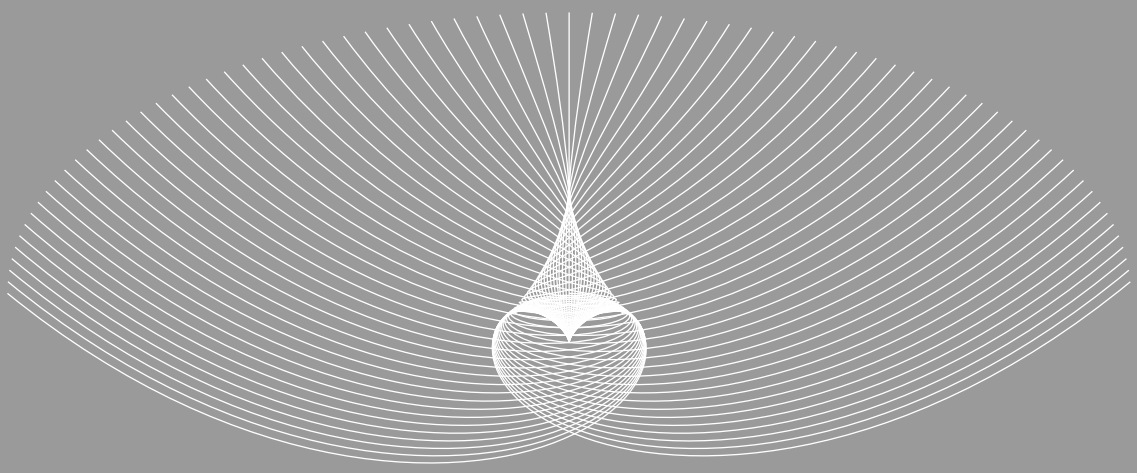

ABSTRACT

Introduction: Fibroblast growth factor 23 (FGF23) and parathyroid hormone (PTH) are major players in the bone-parathyroid-kidney axis controlling phosphate homeostasis. In patients with primary hyperparathyroidism (PHPT) data on the relationship between PTH and FGF23 are scarce and not always concordant.

Objective: The aim of our study was to evaluate the relationship between PTH and FGF23 in patients with PHPT and in euparathyroid patients cured after successful parathyroidectomy (PTx).

Patients & Methods: Twenty-one patients with PHPT and 24 patients in long-term cure after successful PTx (EuPTH) were studied. All patients underwent biochemical evaluation of renal function, parathyroid status, vitamin D status, bone turnover markers, and serum intact FGF23 levels.

Results: Mean serum FGF23 concentration was significantly higher in PHPT than in EuPTH patients (50.8 ± 6.1 pg/mL vs. 33.1 ± 2.6 pg/mL, P=0.01). FGF23 levels significantly correlated with PTH levels (r=0.361, P=0.02), also after correction for $1,25(OH)_2D$ levels (r=0.419, P=0.01). FGF23 levels showed a significant negative correlation with $1,25(OH)_2D$, which was more pronounced in PHPT than in EuPTH patients (r= −0.674, P=0.001, vs. r= −0.509, P=0.01).

Conclusion: Our findings suggest that in PHPT, FGF23 levels are increased independent of $1,25(OH)_2D$ levels. The more pronounced negative relationship between FGF23 and $1,25(OH)_2D$ in the presence of high circulating PTH levels suggest that the increase in FGF23 levels may be an adaptive mechanism to counteract the PTH-induced increase in $1,25(OH)_2D$ levels, although not completely overriding it.

INTRODUCTION

Parathyroid hormone (PTH) and the active metabolite of vitamin D ($1,25(OH)_2D$) are prime regulators of calcium homeostasis but also have significant effects on phosphate homeostasis by respectively downregulating or upregulating the sodium phosphate co-transporters in the proximal tubules of the kidneys and in enterocytes of the intestinal tract (1-8). However, the major player of the bone-kidney axis controlling phosphate homeostasis has been shown to be fibroblast growth factor 23 (FGF23). FGF23 acts as a phosphaturic factor by the same mechanism of action as PTH, downregulating the cotransporters NaPi2a and NaPi2c in the kidney after binding to its receptor, FGFR-1, in the presence of Klotho (9-11). FGF23 also decreases $1,25(OH)_2D$ synthesis in the proximal tubules by direct inhibition of the 1α-hydroxylase enzyme (9,10,12).

FGF23 is predominantly produced and secreted by osteocytes in bone (9,10). This process is positively regulated by $1,25(OH)_2D$, via a vitamin D response element (VDRE) in the *fgf23* promoter (9,13-15). The process is also regulated by serum phosphate, although the exact mechanism by which this is achieved remains unclear. Extracellular phosphate does not appear to directly stimulate *FGF23* mRNA levels or *fgf23* promoter activity in osteoblastic cultures (9,14). Data on the effect of changes in phosphate intake on FGF23 concentrations are inconsistent, with different responses observed with short-term or long-term alterations in phosphate intake (16-20). It has also been shown that early and rapid changes in renal phosphate excretion occur following a high-phosphorus meal, independent of FGF23, PTH, secreted frizzled-related protein (sFRP-4), or $1,25(OH)_2D$, suggesting the presence of an intestinal "phosphate sensor", although its exact biochemical nature is not known (21-25).

The PTH/PTHrP receptor (PTHR1) is present on osteocytes (26) and constitutive activation of this receptor has been shown to upregulate *FGF23* mRNA expression *in vitro* (27,28). Administration of PTH (1-34) in mice and in healthy individuals is associated with an increase in $1,25(OH)_2D$ and in serum FGF23 levels and with a decrease in serum phosphate levels (13,28,29). In contrast, although intermittent administration of PTH to postmenopausal women

with osteoporosis induced an increase in $1,25(OH)_2D$ and in FGF23 levels, this was not associated with a decrease in serum phosphate levels (30). Taken together, these data suggest that PTH is a regulator of FGF23 synthesis and that this is likely to be independent of serum phosphate concentrations.

In patients with primary hyperparathyroidism (PHPT), data on the relationship between PTH and FGF23 are scarce and not always concordant. Compared to healthy controls, circulating FGF23 levels have been found to be elevated in patients with PHPT before parathyroidectomy (31,32) and to decrease immediately post-operatively (32), supporting the notion that PTH stimulates FGF23 secretion. However, this post-operative normalization of FGF23 levels was not observed in all studies (31,33), or was observed only transiently post-parathyroidectomy, with FGF23 levels returning to the originally high pre-operative values 7 days after surgery (32). The latter data suggest a possible alteration in FGF23 regulation, independent of PTH levels, in patients with PHPT. The aim of our study was to address the relationship between PTH and FGF23 in patients with primary hyperparathyroidism and in those with this disorder after cure following successful parathyroidectomy.

PATIENTS AND METHODS

Study population

Twenty-one consecutive patients with primary hyperparathyroidism, which was untreated, persistent or recurrent after PTx, and 24 consecutive euparathyroid patients who had a successful PTx for sporadic PHPT at the Leiden University Medical Center (LUMC) were invited and agreed to take part in the study of a 18 months period. All patients were under regular follow-up at the Outpatient Clinic of the Department of Endocrinology and Metabolic Diseases of the LUMC, with patients with persistent hyperparathyroidism being followed more closely than those cured after PTx, who were mostly seen at 1- or 2-year intervals.

The diagnosis of PHPT was established on the basis of a serum PTH concentration above the upper limit of the normal laboratory reference range (>8 pmol/L) in the presence of a high or inappropriately normal serum calcium

concentration (>2.55 mmol/L). Eight of these latter patients had PTH concentrations of 13.6 ± 2.2 pmol/L, (range 8.4-27.4 pmol/L) in the presence of a normal serum calcium (serum calcium 2.46 ± 0.02, range 2.38-2.52 mmol/L), in the absence of vitamin D deficiency ($25(OH)D_3$ 55.6 ± 7.2, range 35-93 nmol/L). Four of these eight patients had a genetically confirmed MEN-1 mutation, the other four patients had evidence for a parathyroid adenoma on localization studies and became hypercalcemic under vitamin D supplementation.

A diagnosis of cure was based on sustained normal serum calcium and PTH concentrations more than 6 months after PTx.

All patients and controls had to have a creatinine clearance >60 ml/min to be included in the study to preclude the confounding effect of renal impairment on FGF23 levels. All patients and controls had a 25(OH) vitamin D_3 level of >30 nmol/L except for 5 patients who had levels between 25 and 28 nmol/L. These five patients were, however, hypercalcaemic (2.72 ± 0.02, range 2.67-2.80 mmol/L) with increased PTH levels (serum PTH 23.5 ± 9.0, range 8.6-54.3 pmol/L) and high normal $1,25(OH)D_2$ levels (serum $1,25(OH)D_2$ 142 ± 20, range 87-205 pmol/L), which was the reason to withhold the vitamin D supplementation.

Serum Biochemistry

Serum concentrations of calcium (reference range 2.15-2.55 mmol/L), albumin (reference range 34-48 g/L), phosphate (reference range 0.90-1.50 mmol/L), and creatinine (reference range 44-80 μmol/l) were measured using semi-automated techniques. Creatinine clearance was calculated using Modification of Diet in Renal Disease (MDRD) formula. Serum alkaline phosphatase (ALP; reference range 40-120 U/L) was measured using a fully automated P800 modulator system (Roche BV). Serum P1NP (a marker of bone formation) and β-CTX (a marker of bone resorption) were determined using the E-170 system (Roche BV). Serum concentrations of intact PTH (reference range 1.5-8 pmol/L) were measured using the Immulite 2500 (Siemens diagnostics, Breda, Holland). Serum 25-hydroxycholecalciferol ($25(OH)D_3$; reference range 30-120 nmol/L) was measured using the LIAISON® 25-OH Vitamin D TOTAL assay (DiaSorin S.A./N.V., Bruxelles, Belgium) and $1,25(OH)_2$ vitamin D was measured using LIAISON®

1,25-OH2 Vitamin D TOTAL assay (DiaSorin S.A./N.V.). .Serum intact FGF23 (reference range 18-50 pg/mL (34)) was measured using an immunometric assay (Kainos Laboratories, Inc., Tokyo, Japan; intra-assay coefficient of variation (CV) 6% and inter-assay CV 10%).

Statistical analysis

Statistical analysis was performed using the SPSS 16.0 software (SPSS, Inc., Chicago, IL, USA). Results are expressed as mean ± S.E.M. unless otherwise stated. Chi-square test and Student's *t*-test were used as appropriate for categorical variables and continuous variables. Pearson correlation coefficients were calculated to assess correlations between FGF23, PTH, $1,25(OH)_2D$, creatinine clearance, phosphate and calcium. Serum PTH, FGF23, and $1,25(OH)_2D$ levels are shown in Table 1 in absolute values, but were log transformed before statistical correlation and regression analysis to correct for skewness. The relationship between several biochemical variables and FGF23 was investigated by backward regression analysis. A probability level of random difference of $P<0.05$ was considered significant.

The study was approved by the local ethics committee and informed consent was obtained from all patients prior to inclusion in the study.

RESULTS

Patients with PHPT did not differ significantly in age, gender, weight, body mass index (BMI) and renal function from those in long-term cure after successful PTx (EuPTH; Table 1).

Mean serum calcium and PTH concentrations were significantly higher and mean serum phosphate and 25(OH) vitamin D_3 concentrations were significantly lower in the PHPT group compared with the EuPTH group. However, serum $1,25(OH)_2D$ concentrations and the bone turnover markers, ALP, P1NP and CTX, were significantly increased in the PHPT group compared with the EuPTH group (Table 1).

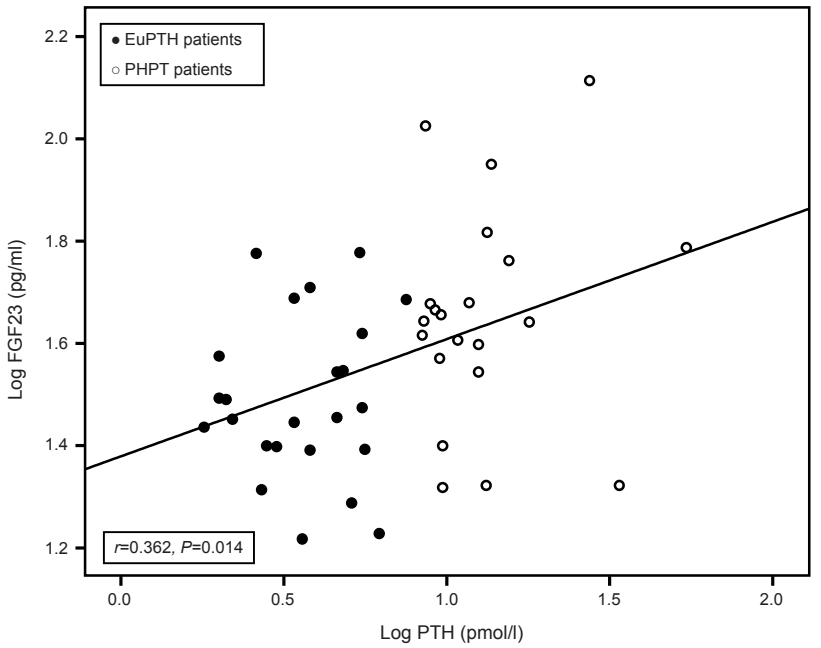

Figure 1: Relationship between serum FGF23 and PTH levels (Pearson's correlation). PTH and FGF23 levels were log transformed because of skewness.

Mean serum FGF23 concentration was significantly higher in patients with PHPT than in EuPTH patients (50.8 ± 6.1 pg/mL vs. 33.1 ± 2.6 pg/mL, P=0.01; Table 1). There was a significant positive relationship between PTH and FGF23 levels when PHPT and EuPTH were analyzed together (r=0.361, P=0.02; Figure 1), and this relationship was sustained and more pronounced after correction for 1,25$(OH)_2$D levels (r=0.419, P=0.01). There was no significant relationship between PTH and FGF23 when PHPT and EuPTH patients were analyzed separately (r=0.187, P=0.4, vs. r=0.114, P=0.6, respectively).

There was also no significant relationship between PTH and 1,25$(OH)_2$D levels in either PHPT patients (r= −0.269, P=0.3) or EuPTH patients (r=0.016, P=0.9) or when both groups were analyzed together (r=0.061, P=0.7).

In patients with PHPT, there was a significant negative correlation between FGF23 and 1,25$(OH)_2$D levels (r= −0.674, P=0.001; Figure 2). This relationship remained significant, albeit less marked, in EuPTH patients (r= −0.509, P=0.01; Figure 2). The negative relationship between FGF23 and 1,25$(OH)_2$D remained

significant when all patients were pooled together (r= −0.393, P<0.01). Using backward stepwise regression analysis, we also demonstrate that FGF23 levels exhibit significant and independent associations with PTH and 1,25$(OH)_2$D levels (β=0.372, P=0.015, and β= −0.429, P=0.003 respectively; Table 2).

Table 1. Demographic and laboratory data in 21 patients with PHPT and 24 patients in sustained cure after successful parathyroidectomy.

	PHPT (*n=21*)	EuPTH (*n=24*)	Ref. range	*P* value
Gender (men:women)	6:15	8:16		0.738
Age (years)	57 ± 3	63 ± 2		0.144
Height (cm)	172 ± 2	170 ± 1		0.279
Weight (kg)	79 ± 6	74 ± 2		0.425
BMI (kg/m^2)	27 ± 2	26 ± 1		0.664
Serum biochemistry				
MDRD (ml/min per 1.73 m^2)	90 ± 5	84 ± 3	>60	0.376
Corrected calcium (mmol/l)	2.59 ± 0.03	2.27 ± 0.02	2.15-2.55	0.000
Phosphate (mmol/l)	0.89 ± 0.04	1.10 ± 0.04	0.9-1.5	0.000
PTH (pmol/l)[a]	15.2 ± 2.4	3.9 ± 0.3	1.5-8.0	0.000
PTH (median (IQR)	11.7 (9.4-14.6)	3.7 (2.6-5.5)	1.5-8.0	0.000
25$(OH)D_3$ (nmol/L)	48 ± 4	60 ± 4	30-120	0.030
1.25$(OH)_2$D (pmol/l)[a]	163 ± 14	125 ± 7	40-140	0.020
1,25$(OH)_2$D (median (IQR))	150 (119-203)	125 (95-144)	40-140	0.020
FGF23 (pg/mL) [a]	50.8 ± 6.1	33.1 ± 2.6	18-50	0.012
FGF23 (median (IQR))	44.0 (36.1-59.6)	29.2 (24.8-40.6)	18-50	0.006
ALP (U/l)	93 ± 5	71 ± 4	40-120	0.002
P1NP (ng/ml)	41.4 ± 4.4	27.4 ± 2.4	16-80	0.010
β-CTX (ng/ml)	0.31 ± 0.04	0.12 ± 0.01	0.01-0.66	0.000

PHPT: primary hyperparathyroidism, EuPTH: euprathyroid controls, MDRD: glomerular filtration rate, IQR: interquartile ranger, [a] Log transformedbefore correlation analysis

There was no significant relationship between FGF23 concentrations and creatinine clearance or serum phosphate concentrations in either PHPT patients (r=0.085, P=0.7, and r=0.349, P=0.09, respectively) or EuPTH patients (r= −0.398, *P=0.06* and r= −0.247, P=0.3, respectively). Also using backward stepwise regression analysis, creatinine clearance and serum phosphate levels failed to emerge as significant modulating factors for FGF23 levels in this model (β= −0.033, P=0.811 and β= −0.068, P=0.642 respectively; Table 2).

There was also no significant relationship between FGF23 levels and all three markers of bone turnover, serum ALP activity, P1NP, or CTX concentrations, in either PHPT or EuPTH patients after correction for PTH and $1,25(OH)_2D$ levels.

Table 2. Result of multiple regression analysis, demonstrating a significant association between FGF23, PTH and $1,25(OH)_2D$

Predictor	*B*	S.E.M.	*β*	*t*	*P* value
PTH	0.895	0.353	0.372	2.534	0.015
$1.25(OH)_2D$	−0.192	0.061	−0.429	−3.124	0.003
Phosphate	−7.9595	16.996	−0.068	−0.468	0.642
MDRD	−0.039	0.160	−0.033	−0.241	0.811

DISCUSSION

Data from our study show that patients with primary hyperparathyroidism have higher levels of FGF23 than cured controls, and that this increase is independent of $1,25(OH)_2D$ levels. We further demonstrate a significant negative relationship between FGF23 and $1,25(OH)_2D$ levels, that is more pronounced in patients with PHPT, suggesting that FGF23 at least partially antagonizes the stimulatory effects of PTH on the 1α-hydroxylase enzyme, although not totally overriding it.

Data on FGF23 levels in PHPT and in the euparathyroid state following successful PTx are scarce and not always concordant. Two studies (31,33) demonstrated no significant difference in pre- and post-PTx FGF23 levels, but a further study (32) showed a return of FGF23 levels to high pre-operative levels several days after PTx. The authors of this latter paper (32) suggested that one of the reasons for these discrepant results may be the post-operative use of active vitamin D metabolites or analogues in their patients, which had not been taken into consideration in the interpretation of their results. To our knowledge, FGF23 levels have never been previously evaluated in long-term euparathyroid patients after successful PTx. Our findings from this study suggest that the increase in FGF23 levels observed in PHPT is reversible when the euparathyroid state is achieved by cure after successful PTx, providing that renal function is not impaired.

Although the cross-sectional design of our study does not allow the definitive determination of a causal relationship between PTH and FGF23, our data are in keeping with recently published data in parathyroidectomized rats, in which a direct relationship between PTH and FGF23 independent of $1,25(OH)_2D$ is demonstrated in the presence of high but not low levels of PTH (35).

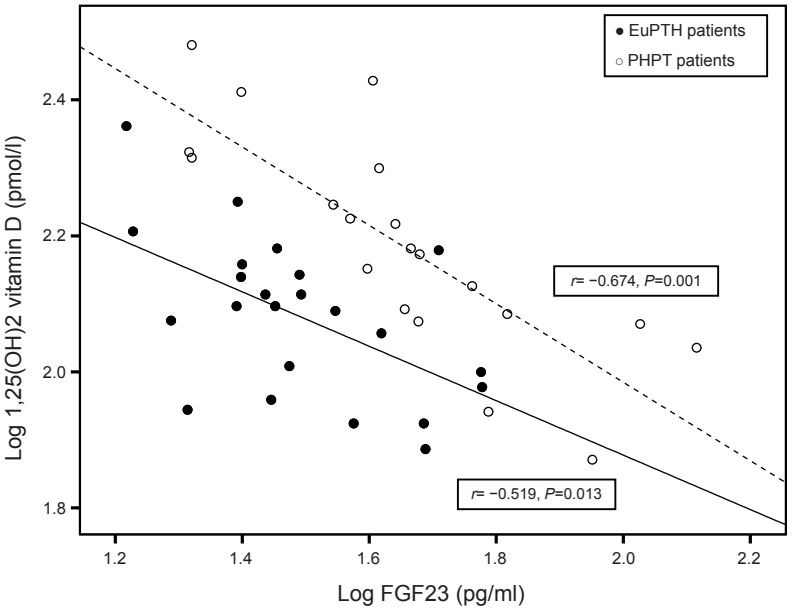

Figure 2: Relationship between serum FGF23 and $1,25(OH)_2D$ levels in 21 patients with primary hyperparathyroidism (PHPT: white dots) and 24 patients in long-term cure after parathyroidectomy for PHPT (EuPTH: black dots). FGF23 and $1,25(OH)_2D$ levels were log transformed because of skewness.

In the presence of high PTH and FGF23 levels in patients with PHPT, it is intruiging that a significant number of these patients do not develop hypophosphatemia despite chronic exposure to the two phosphaturic hormones, PTH and FGF23. In keeping with previous observations (13,30), indeed only 11 of our 22 patients with PHPT (50%) had phosphate levels below the lower limit of normal (<0.90 mmol/l). This suggests that in PHPT, factors other than PTH, FGF23 or their combined effect may play a role in phosphate homeostasis. A clear contender is $1,25(OH)_2D$. The net effect of $1,25(OH)_2D$ on gut, kidney, bone and parathyroids is to increase serum phosphate levels, by upregulating NaPi2b co-

transporter expression in the intestinal tract (1,7) and NaPi2 co-transporter (*NaPi3*) gene in the kidney, and by directly reducing PTH synthesis and secretion by the parathyroid (29).

In our study, patients with PHPT had significantly increased $1,25(OH)_2D$ levels compared with euparathyroid patients, but also demonstrated significantly increased FGF23 levels. A new hypothesis has been recently proposed to explain the need for two phosphaturic hormones, PTH and FGF23, with the former repressed and the latter induced by $1,25(OH)_2D$ (36). The suggested negative feedback loop includes FGF23-induced inhibition of $1,25(OH)_2D$ synthesis. It has been proposed that these counter-regulatory effects of FGF23 on the bone-kidney axis have the physiological task of securing the maintenance of serum phosphate levels, thus providing protection against the hyperphosphatemia-related soft tissue and vascular calcifications (37-40). A possible explanation for the antagonizing effect of FGF23 on 1α-hydroxylase enzyme may be the shorter half-life of PTH compared with longer half-life of FGF23 (41).

Our findings from this study extend our insight into the role of FGF23 in pathological states by showing that in primary hyperparathyroidism, FGF23 production is increased in the presence of high circulating PTH levels and that this increase is reversible after the euparathyroid state is achieved following successful PTx. The more pronounced negative relationship between FGF23 and $1,25(OH)_2$ vitamin D in patients with PHPT suggests that in these patients the increase in FGF23 levels may be an adaptive mechanism to counteract the PTH-induced $1,25(OH)_2D$ levels, although not completely overriding it.

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8

Challenges and pitfalls in the management of parathyroid carcinoma, 17 years follow-up of a case and review of the literature

Janneke E. Witteveen, Harm R. Haak, Job Kievit, Hans Morreau, Johannes A. Romijn, Neveen A.T. Hamdy

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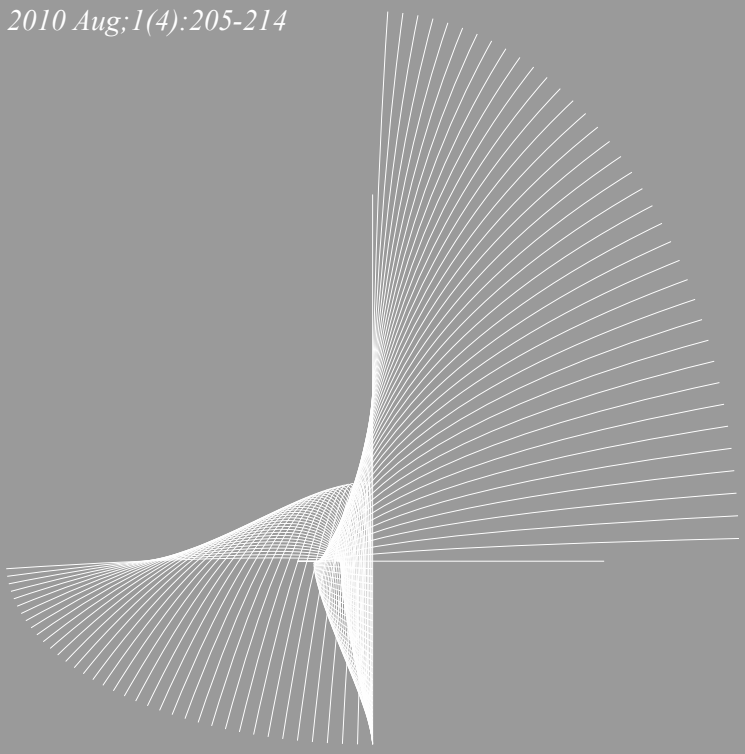

ABSTRACT

A 29-year-old men presented to his primary-care physician with nausea, severe weight loss and muscle weakness. He had a hard, fixed neck swelling. He was severely hypercalcaemic with 10-fold increased parathyroid hormone (PTH) concentrations. A diagnosis of primary hyperparathyroidism was established and the patient was referred for parathyroidectomy. At neck exploration, an enlarged parathyroid gland with invasive growth into the thyroid gland was found and removed, lymph nodes were cleared and hemithyroidectomy was performed. A suspected diagnosis of parathyroid carcinoma was confirmed histologically. Serum calcium and PTH levels normalized post-operatively, but hyperparathyroidism recurred within 3 years of surgery. Over the following 17 years, control of hypercalcaemia represented the most difficult challenge despite variable success achieved with repeated surgical interventions, embolisations, radiofrequency ablation of metastases and treatment with calcimimetics, bisphosphonates and haemodialysis using low-dialysate calcium. In this paper, we report the challenges and pitfalls we encountered in the management of our patient over nearly two decades of follow-up and review recent literature on the topic.

INTRODUCTION

Parathyroid carcinoma is a rare disorder, accounting for 0.1 to 5.0% of all cases of primary hyperparathyroidism (1-5). The disease presents at a mean age of 50 years and is equally likely to occur in males as in females (2-6) (Table 1). Clinical and biochemical manifestations are those of severe primary hyperparathyroidism often with renal and skeletal complications (2-5,7). A palpable neck mass can be felt in 30-76% of patients (3,5,8,9). Parathyroid carcinoma is associated with germline and somatic mutations in the *HRPT2* gene (3,10) and with irradiation of the neck for other pathologies (3,11).

A diagnosis of parathyroid carcinoma is suggested by the presence of intra-operative features of local invasion and the diagnosis is confirmed by the World Health Organization histopathological criteria for parathyroid carcinoma. These include the presence of vascular invasion, perineural space invasion, capsular penetration with growth into adjacent tissues and/or metastasis (12).

Although parathyroid carcinoma is a slow growing tumor, it inevitably metastasises and is invariably fatal due to the eventual inability to control hypercalcaemia. Survival is estimated to range from 50% to 86% at 5 years and 35% to 70% at 10 years (1-6,13,14). The most effective treatment for parathyroid carcinoma is radical surgery with en bloc removal of the lesion together with the ipsilateral thyroid, thyroid isthmus and lymph nodes (2,3,5,13,15). Surgery is also the treatment of choice in case of local recurrence or development of metastases (5,7,16), although radiotherapy (2-5,8,9), chemotherapy (3,5,9,17,18), embolisation (19) or radio frequency (RF) ablation (19-21) may also be attempted.

We report here the nearly two decades' odyssey of a male patient with multiple metastases from parathyroid carcinoma, describing in the process the challenges and pitfalls we encountered in the management of this difficult malignancy. We also review all original case reports and case series on parathyroid carcinoma, fulfilling the WHO criteria and with a follow-up of at least 6 months after initial parathyroidectomy, which were published in the English literature since the last large review on this topic in 2001 (3). The literature search was conducted in PubMed using the keywords ("Parathyroid Neoplasms" OR "Parathyroid

carcinoma" OR "Parathyroid cancer" OR "Parathyroid cancers") AND ("Case Reports" OR "case report" OR “case” OR “cases”). The last search was conducted on June 2, 2010. Patients with changes suggestive of carcinoma in the context of autonomous (tertiary) hyperparathyroidism were excluded because of the controversy in diagnosing malignancy in this case. Of an initial 872 publications, only 45 fulfilled the WHO criteria for parathyroid carcinoma and follow-up as per our search criteria, and were selected for further analysis.

CASE REPORT

A 29 year old man presented to his primary care physician with a 2-month history of severe weight loss (20kg) associated with polyuria, polydipsia, nausea, muscle weakness, difficulty in concentrating and debilitating tiredness. On physical examination, he was clinically dehydrated and had a palpable hard, fixed swelling in the left side of the neck. He was using no medication and had no significant past or family history. Laboratory investigations revealed a severe hypercalcaemia of 4.9 mmol/l (normal range 2.15-2.55 mmol/l), a decreased creatinine clearance of 45 ml/min (normal >120ml/min) and a 10-fold increased serum intact parathyroid hormone (PTH) concentration: 88 pmol/l (normal <8 pmol/l). These findings suggested a diagnosis of severe primary hyperparathyroidism probably due to parathyroid carcinoma. No further investigations were requested; the patient was intensively rehydrated and referred for parathyroidectomy. At neck exploration, an enlarged parathyroid gland (3.5x3x3.5 cm) invading the ipsilateral thyroid lobe was found and removed from the left side of the neck (Figure 1). A left hemithyroidectomy was also performed. The diagnosis of parathyroid carcinoma was confirmed histologically by the finding of capsular and vascular invasion. Serum calcium and PTH concentrations normalized within a few days of surgery associated with a significant improvement in renal function. A second surgery was performed within 1 month of the first surgery to ensure complete removal of all malignant tissue and provide a negative surgical margin. The patient was started on a course of radiotherapy, which had to be discontinued before completion because of severe pharyngitis and esophagitis.

Table 1: Overview of cases of parathyroid carcinoma published between 2001-2010

Author	Age/ Sex	No. of operations	Radio-therapy	Chemo-therapy	Emboli-sation	RF ablation	PTH Immu-nissation	Calci-tonin	Cina-calcet	Bisphos-phonates	Development of local recurrence or distant metastasis	Outcome	Follow-up (years)
Savli (22)	47/F	1									No	A/FOD	1
Tyler (23)	44/M	2	Yes								Yes	A/FOD	5.5
Dotzenrath (24)	43/M	4	Yes								Yes	DOD	12
	43/F	12								Yes	Yes	A/WD	8
	62/F	2									Yes	D/other	9.5
Yamashita (25)	43/M	2									Yes	A/WD	12
Eurelings (26)	45/F	1	Yes	Yes							Yes	DOD	4
Brown (27)	51/M	2									No	A/FOD	2
Bhansali (28)	40/M	2		Yes				Yes		Yes	No	DOD	0.5
Hundley (29)	33/F	5									Yes	A/FOD	20
Schmidt (30)	76/F	1									No	A/FOD	1
Schoretsanitis (31)	55/F	1									No	A/FOD	2
Dionisi (32)	35/M	2								Yes	Yes	DOD	4
Rufener (33)	67/M	3									Yes	A/FOD	4
Munson (34)	41/F	1	Yes								No	A/FOD	5.5
	46/F	1	Yes								No	A/FOD	4.5
	49/F	1	Yes								No	A/FOD	5.5
	57/F	1	Yes								No	A/FOD	4.5
Dogan (35)	50/F	1									No	A/FOD	1.5
Betea (36)	50/F	2					Yes	Yes		Yes	Yes	A/WD	11
Kern (37)	54/F	2	Yes								Yes	DOD	2.5
Kirkby-Bott (38)	44/M	2	Yes	Yes							Yes	DOD	3.5
	70/M	1	Yes								No	A/FOD	7
	36/F	1	Yes								No	A/FOD	5
	66/M	2	Yes								Yes	DOD	8
	46/M	1	Yes	Yes							Yes	A/WD	1

	25/F	1								No	A/FOD	1
	44/M	1								No	A/FOD	1
Lin (39)	38/M	1								No	A/FOD	0.5
Ahmad (40)	42/F	1			Yes					Yes	A/FOD	1
Chiofalo (41)	66/M	1							Yes	No	A/FOD	1
Cheah (16)	32/M	2								No	À/FOD	0.5
	42/F	1								No	A/FOD	0.5
	52/F	2								Yes	A/FOD	5.5
Yoshida (42)	61/F	2							Yes	Yes	A/WD	18.5
Ikeda (43)	48/M	4	Yes							Yes	A/WD	6
Pahlavan (44)	21/M	2							Yes	No	A/FOD	2
Agarwal (45)	22/F	2								Yes	A/FOD	9
Mezhir (46)	64/F	7	Yes					Yes	Yes	Yes	A/FOD	8
Placzkowski (47)	51/F	4	Yes				Yes	Yes	Yes	Yes	A/FOD	12
Sahasranam (48)	53/M	1								No	A/FOD	2
Tkaczyk (49)	55/M	2	Yes							No	A/FOD	1
Tan (50)	55/F	1								No	A/FOD	0.5
Iwata (51)	61/M	1								No	A/FOD	3
Montenegro (52)	50/M	2	Yes						Yes	Yes	DOD	5
Rathi (53)	45/F	1								No	A/FOD	0.5
Artinyan (19)	71/M	2		Yes	Yes			Yes	Yes	Yes	A/FOD	0.5
Cetani (54)	53/M	3						Yes		Yes	A/FOD	2
Temmim (55)	63/F	1								No	A/FOD	2
Tamura (56)	70/M	1								Yes	A/WD	0.5
Schoretsanitis (57)	72/M	1								No	A/FOD	4
	78/F	1								No	A/FOD	4
	55/F	1								No	A/FOD	6
	71/M	1								Yes	DOD	2
	75/M	1								No	A/FOD	4
Kung (58)	72/F	1								No	A/FOD	2
Marcy (59)	42/F	1								No	A/FOD	1
Yuan (60)	38/F	2								No	A/FOD	4
Rock (61)	60/M	1							Yes	No	A/FOD	0.5
Yong (62)	23/M	1						Yes	Yes	No	A/FOD	2
Chaychi (63)	79/F	1								No	A/FOD	0.5

Tochio (21)	42/M	2				Yes				Yes	Yes	A/WD	8.5
	51 ± 2/	1.9 ± 0.2	18/62	4/62	1/62	3/62	1/62	3/62	5/62	14/62	27/62		4.3 ± 0.5
	30M:32F		(29%)	(6%)	(2%)	(5%)	(2%)	(5%)	(8%)	(23%)	(44%)		

Results are expressed as mean ± S.E., DOD: Dead of disease, D/other: Dead of other cause, A/WD: alive with disease, A/FOD: alive free of disease

A recurrence of hyperparathyroidism was documented on routine laboratory control 3 years after parathyroidectomy. This was mild, with a serum calcium concentration of 2.74 mmol/l, a PTH concentration of 8.4 pmol/l and a stable creatinine clearance of 75 ml/min. The patient was completely asymptomatic. Tc99m-MIBI-SPECT and ultrasonography identified a lesion in the left side of the neck, which was found to be a local recurrence of the primary tumor at extensive bilateral neck exploration. The recurrent tumor had invaded the esophagus, part of which was excised and reconstructed using tissue from the sternocleidomastoid muscle. The edges of the excised specimen were tumor free and resected draining lymph nodes were clear of tumor tissue. DNA analysis of the resected recurrent tumor demonstrated a somatic *HRPT2* mutation, c.165delC located on exon 2 (64). Despite the radical nature of the surgery, mild hyperparathyroidism persisted post-operatively, suggesting the presence of residual probably metastatic tumor tissue. No further localization studies or surgery were planned principally because of the patient's reluctance to undergo further surgery but also because of the mild nature of the hyperparathyroidism. Closer clinical monitoring was arranged.

Over the following 4 years, serum calcium and PTH concentrations and bone turnover markers slowly increased, and periods of dehydration were associated with transient worsening of renal function, which remained, however, on the whole stable despite persistent hypercalciuria and development of nephrocalcinosis. Progressive bone loss, predominantly cortical, was also documented and treatment with the non-nitrogen-containing bisphophonate clodronate was started, resulting in normalisation of bone turnover and stabilisation of BMD measurements. Rehydration and treatment with bisphosphonates eventually failed in controlling hypercalcaemia and bone loss. Localisation studies were undertaken to identify the source of PTH production. Ultrasound (US), selective venous sampling for PTH (SVS) and CT scan of the neck succeeded in localizing a small dense lesion in the left supraclavicular region. CT scan of the thorax also revealed a small solitary lesion of 1 cm radius in the inferior lobe of the right lung (Figure 2A). The patient refused to undergo a thoracotomy, but did agree to a fourth neck exploration at which a lymph node metastasis was excised from the left supraclavicular region

(Figure 1). Although less severe, hyperparathyroidism persisted post-operatively, as expected due to the unresected lung metastasis. Two attempts at embolisation of the lung metastasis failed to reduce tumor load. In contrast radiofrequency ablation (RFA) of the lesion, although complicated by an episode of severe pleuritis, was very successful in achieving biochemical remission, with serum PTH concentrations decreasing from 265 pmol/l to <1 pmol/l (Figure 2), suggesting that the metastasis targeted with RFA was the only source of PTH secretion. However, remission was not permanent and PTH levels started to rise within 9 months of RFA. The patient finally agreed to undergo a right inferior lobectomy (Figure 3), which was complicated by multiple small cerebral infarcts resulting in right hemianopia, dysphasia, acalculia and transient epilepsy. Disappointedly, there was also only partial improvement in serum calcium and PTH concentrations, which continued to increase within 2 months of lobectomy (Figure 3).

Further localisation studies, in the form of Tc99m-MIBI-SPECT and a CT scan of the neck and thorax, were unable to localise any residual pathological parathyroid tissue suggesting the presence of (micro) metastasis. The now available calcimimetic cinacalcet was prescribed eventually at a maximum dose of 90 mg twice daily, which decreased PTH concentrations from 120 pmol/l to 85 pmol/l, stabilized calcium concentrations to about 2.80 mmol/l and stabilized creatinine clearance at 51 ml/min. In the course of the following year serum calcium and PTH concentrations further increased, however, despite maximal doses of cinacalcet and intermittent intravenous bisphosphonates.

Repeat Tc99m-MIBI-SPECT and CT scan of the thorax, now demonstrated a new large (4x3x2cm) subcarinal mediastinal metastasis, which was successfully excised using a transpericardial approach (Figure 4). The elimination of the source of PTH secretion was associated with a very severe hungry bone syndrome for which the patient needed intensive care for over 2 months, requiring very large doses of iv administered calcium (up to 14 g/day) and maximal doses of active vitamin D metabolites. Remission lasted for more than 1 year, after which PTH levels started to rise again and a new metastatic lesion was localised pretracheally in

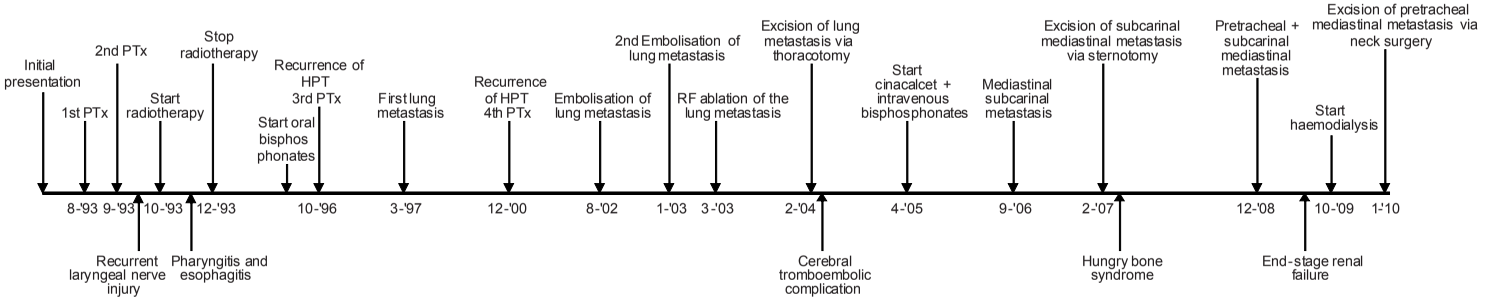

Figure 1. Disease course of our patient spanning over 17 years.

the upper mediastinum on CT scan of the thorax. There was also suggestion of a second subcarinal lesion at the site of the previously excised metastasis.

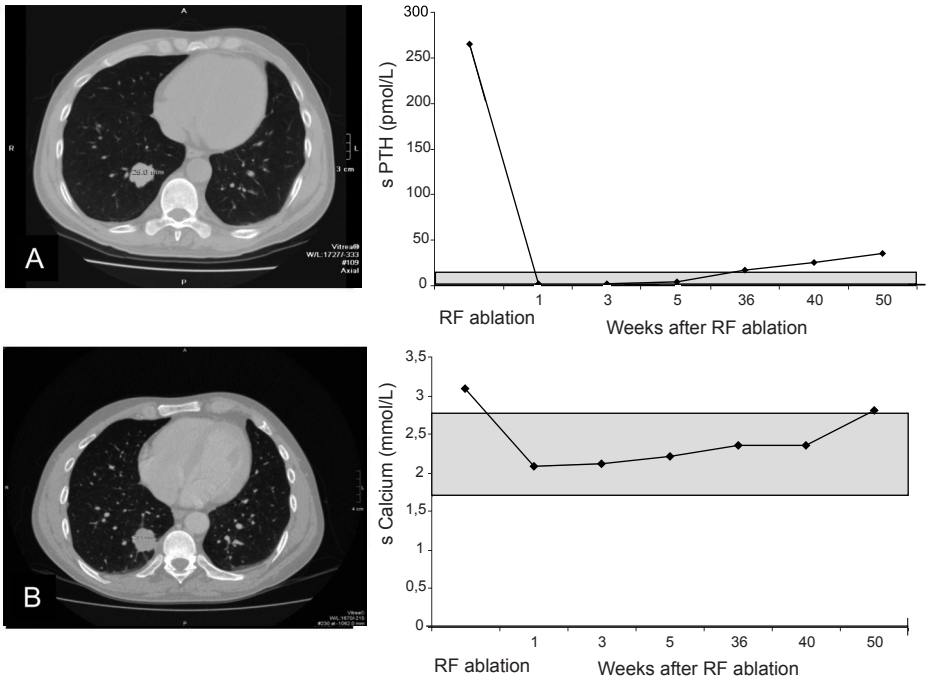

Figure 2. Radiological (A. pre-RF ablation, B. after RF ablation) and biochemical changes after RF ablation of the lung metastasis, demonstrating a severe drop in serum PTH concentrations, which persisted for almost a year.

In view of the complications associated with his previous surgeries, the patient was at that stage again reluctant to undergo any further surgery. Within the next few months PTH steadily increased associated with uncontrollable hypercalcaemia and deteriorating renal function eventually requiring haemodialysis using a very-low-dialysate calcium concentration. Localisation studies confirmed the 2 previously identified pretracheal and subcarinal mediastinal lesions. The pretracheal lesion was accessible from the neck and was radically removed by a cervical approach (Figure 1). There were no post-operative complications and serum PTH concentration decreased from 590 pmol/l to 155 pmol/l, as expected due to the remaining

subcarinal mediastinal metastasis. Surgical removal of this metastasis would have required a third thoracotomy, which would be associated with too high an operative risk, especially in view of the patient's poor clinical condition. Excision of the metastasis by mediastinoscopy was also not considered to be an option because of multiple scarring due to previous surgeries. The patient remains on haemodialysis and is still treated with cinacalcet, with intermittent courses of intravenous bisphosphonates. The patient was not considered to be a candidate for PTH immunisation (36,65), because of surgical clearance of cervical and mediastinal lymph nodes, poor general immune status and recurring infections.

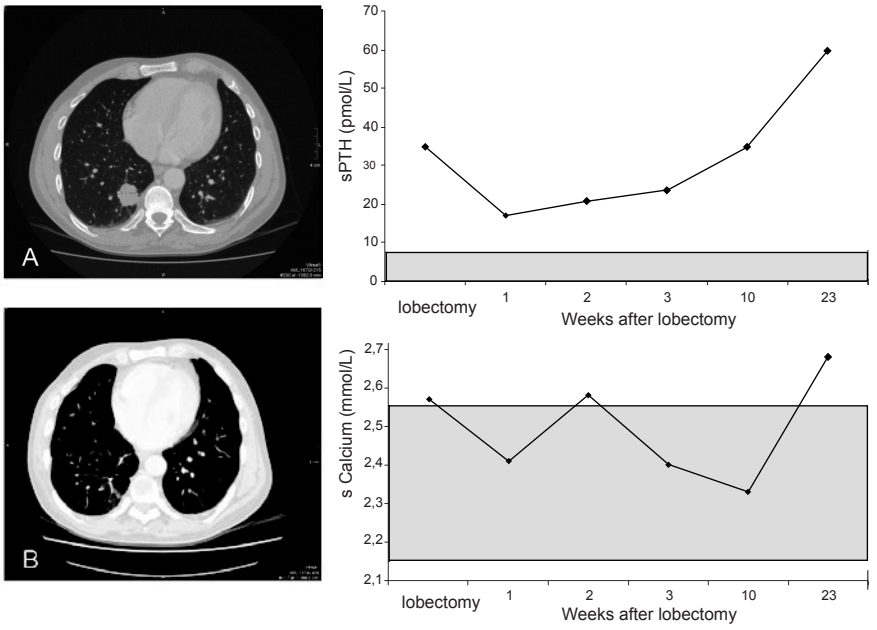

Figure 3: Radiological and biochemical changes following lobectomy of the lung metastasis, which resulted in only partial improvement in serum calcium and PTH concentrations.

REVIEW OF THE LITERATURE 2001-2010

Sixty-two new cases have been reported in the literature since the last published review in 2001 (3), and data of these cases are summarised in Table 1. The median age of the reported patients was 50 years (21-79) and there was an equal number of

men and women reported. Data on initial presentation were available in 43 of the 62 patients (69%), 37 of which were symptomatic (86%). Polyuria and polydipsia were reported in 9 patients (21%), constipation in 7 (16%), tiredness in 13 (30%), bone and joint pain in 17 (40%), muscle weakness in 7 (16%), nausea and vomiting in 10 (23%) and weight loss in 7 patients (16%). Seventeen patients (40%) had renal stones and 6 patients (14%) had sustained a documented fracture. A palpable neck mass was reported in 22 of 27 patients in whom this was checked (81%).

Similar to the review of 2001, surgery is still the most frequently chosen therapy for the initial treatment of parathyroid carcinoma, but also for the treatment of recurrent or metastatic parathyroid carcinoma (Table 1). It is of note that the use of both chemotherapy and radiotherapy has decreased over the last 10 years, likely due to several reports and reviews which showed disappointing results (2-5). The last case report on the use of chemotherapy and radiotherapy dates from 2005 and 2008, respectively (Table 1). In contrast to the review of 2001, reports on the use of the newer techniques, embolisation (n=1), radiofrequency ablation (n=3) and PTH immunisation (n=1), have recently been published, although the number of cases reported is still very limited. Use of the calcimimetic, cinacalcet, has increased over the last few years after the first report in 2007, while the use of bisphosphonates has remained stable.

The median follow-up reported in the 62 cases is relatively short at 3 years (range 0.5-20 years), considering that the average time to a first recurrence is also approximately 3 years (1,3). This could explain why only 27 of the 62 patients (44%) developed a local recurrence and/or distant metastasis and 9 patients (15%) died as a result of parathyroid carcinoma during the reported follow-up period.

DISCUSSION

To our knowledge, we report here one of the longest survival and follow-up in one center of a patient with recurrent metastatic parathyroid carcinoma. Although parathyroid carcinoma is a slow growing tumor, it is associated with a relatively high incidence of local recurrence (40-82% within 5 years) (1-3,5,6,13). Metastasis

tend to occur later in the course of the disease with spread to cervical nodes (30%), lung (40%) and liver (10%), via both lymphatic and hematogenous routes (3). Survival is estimated to range from 35% to 70% at 10 years (1-6,13,14), with an average survival of 6 years after local recurrence, and of 4,5 years after the development of distant metastases (2).

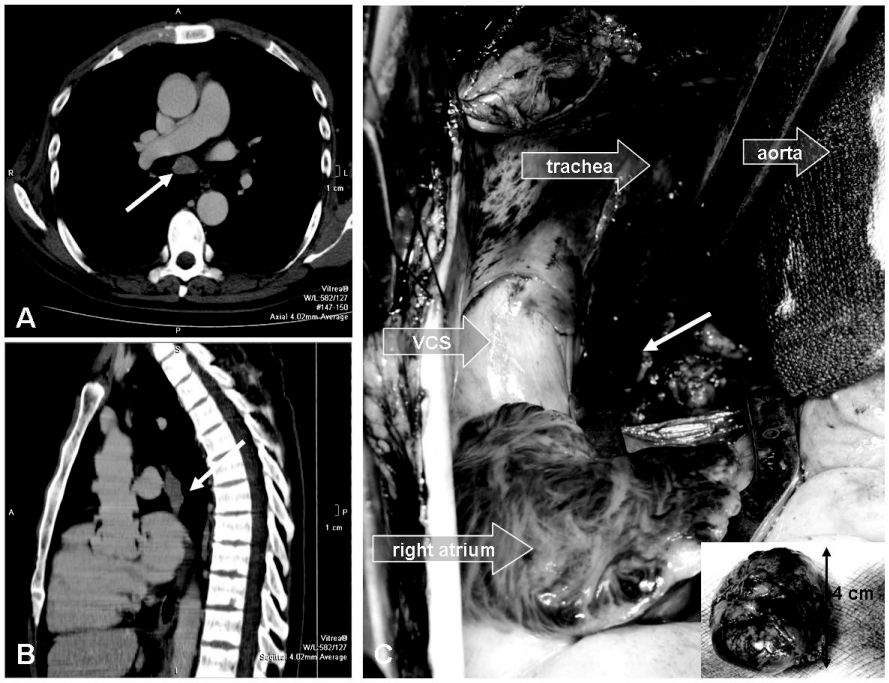

Figure 4: CT scan of the thorax demonstrating a large (4x3x2 cm) subcarinal mediastinal lymph nodded metastasis (white arrow) (A+B), which was successfully excised using a transpericardial approach (C).

In patients with parathyroid carcinoma, determinants of survival include delay in diagnosis, the presence of locally invasive features, distant metastasis at diagnosis and the radical nature of initial surgery (3,6,13,14). A further important determinant of survival and disease-free survival is the due care taken at initial surgery to avoid rupturing the capsule of the gland to prevent local seeding of tumor tissue (3). Initial surgery has been reported not to be radical in up to 48% of patients because of failure to establish the diagnosis of parathyroid carcinoma pre- or intra-

operatively (6,14). This was also the case in our patient at initial surgery, despite very suggestive clinical features of parathyroid carcinoma, in the form of severe hyperparathyroidism in a young male patient, palpable neck swelling and intra-operative features of local invasion. A second surgery had to be performed within one month of the first to ensure complete removal of all malignant tissue and to provide a negative surgical margin.

The main determinant of survival in all patients remains, however, the eventual inability to control hypercalcaemia (2,5), and its association with fatal renal and cardiovascular complications (1,2,5,7). End-stage renal failure has been reported in up to 84% of patients due the deleterious effects of severe persistent hypercalcaemia on kidney function (2,5,7). A persistently elevated circulating PTH level is also associated with increased bone turnover and bone loss predominantly at cortical sites and with increased risk of fractures. In parathyroid carcinoma, the main goal of treatment is control of hyperparathyroidism by eradicating the source of PTH secretion (7,16).

Although the morbidity associated with re-exploration of the neck is estimated to be 6-17% (7), that of re-exploration of the mediastinum or lungs is clearly much higher. In our patient, the recurrent laryngeal nerve had to be unavoidably sacrificed during the second surgical intervention due to invasive growth of remnants of the primary tumor, resulting in transient loss of voice and permanent hoarseness. Subsequent lung surgery was associated with severe trombo-embolic complications.

The increasing morbidity attached to repeated surgical interventions has led to the search for other treatment options, such as radiotherapy, chemotherapy, RF ablation, embolisation, use of the calcimimetic cinacalcet and PTH immunisation. Although parathyroid tumors are relatively resistant to radiotherapy (2-5), this management approach showed some promise as adjuvant treatment for microscopic residual disease (8,9). In our patient, the prescribed course of radiotherapy could not be completed due to the development of severe side effects in the form of pharyngitis and esophagitis.

Embolisation of localized and/or ectopic parathyroid adenomas has been reported to have only limited success (19,66-68), and in metastatic parathyroid

carcinoma, embolisation has only been described in combination with RF ablation in one patient (19). In our patient, the outcome of twice attempted embolisation of a lung metastasis was disappointing, largely because it was not technically possible to selectively embolise the arterial branch that fed the lung metastasis.

The outcome of combined embolization and RF ablation in a patient with liver metastasis due to parathyroid carcinoma (19), and the effectiveness of RF ablation in various types of cancer (69) has led us to consider RF ablation in our patient. This approach was indeed very successful in eradicating PTH production by the metastasis, as has also been subsequently reported in 2 patients with parathyroid carcinoma and lung metastases (20,21). The beneficial effect was unfortunately transient, lasting only 9 months after the procedure, when PTH production was documented to resume from the same site.

In parathyroid carcinoma, attempts at reducing tumor load are not always successful, in which case control of serum calcium by other means becomes central to prevent the deleterious effects of hypercalcaemia on various organ systems. Intensive rehydration, use of medications such as bisphosphonates and calcimimetics and use of dialysis represent diverse means to control hypercalcaemia and are often used in combination. Bisphosphonates decrease the skeletal efflux of calcium from bone by suppressing osteoclast-mediated bone resorption and overall bone turnover (3). These agents, however, have no effect on renal tubular reabsorption of calcium so that they improve hypercalcaemia but do not normalise serum calcium (3,70). Cinacalcet is a calcimimetic, which reduces parathyroid hormone secretion by binding to the calcium-sensing receptor on parathyroid cells, increasing the sensitivity of these cells to extracellular calcium concentrations (15,71,72). These agents are widely used in the management of secondary and tertiary hyperparathyroidism in renal failure and have been also recently registered for the management of primary hyperparathyroidism, including that due to parathyroid carcinoma. In our patient, combined treatment with bisphosphonates and cinacalcet resulted in reasonable control of the hypercalcaemia initially, but eventually failed to do so, even when used at maximal doses. Dialysing against low-dialysate calcium became necessary in order to better control the hypercalcaemia.

The very high operative risks associated with a third thoracotomy, the patient's poor lung function and current metabolic status despite regular dialysis, preclude the option of further surgery to remove the identified source of PTH secretion. PTH immunisation is being explored as a potential non-invasive option, although experience with this approach is still limited and the patient's general condition may not permit its use (36,65,73).

Although we are rapidly running out of options in the management of our patient, we believe that we have nonetheless managed to secure for him a longer albeit not disease-free survival. This clinical case with a follow up spanning over 17 years illustrates that the long-term management of patients with metastatic parathyroid carcinoma remains indeed a daunting task, despite all recent imaging, surgical and medical advances.

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9

Downregulation of CASR expression and global loss of parafibromin staining are strong negative determinants of prognosis in parathyroid carcinoma

Janneke E. Witteveen, Neveen A.T. Hamdy, Olaf M. Dekkers, Job Kievit, Tom van Wezel, Bin T. Teh, Johannes A. Romijn, Hans Morreau

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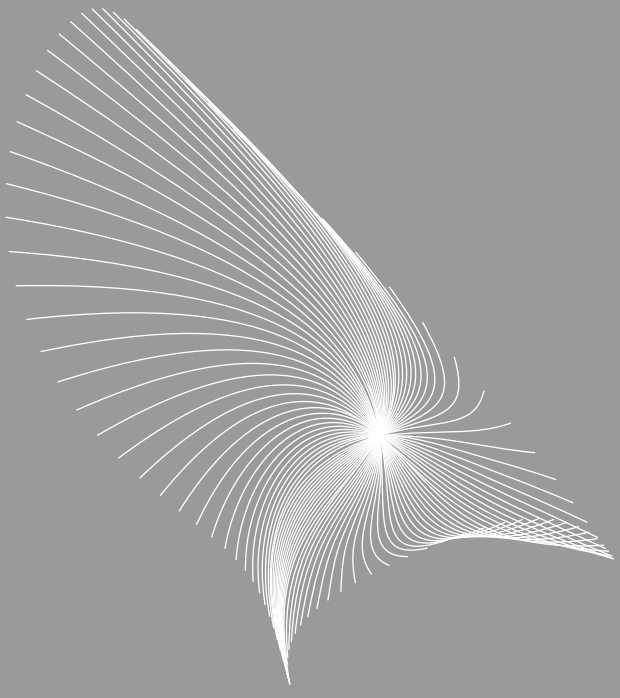

ABSTRACT

Introduction:

Parathyroid carcinoma is associated with mutations in the *HRPT2/CDC73* gene and with decreased parafibromin and calcium-sensing receptor (CASR) expression, but in some cases establishing an unequivocal diagnosis remains a challenge. The aim of our study was to evaluate the prognostic value of CASR and parafibromin expression and of *HRPT2/CDC73* mutations in patients with an established diagnosis of parathyroid carcinoma.

Patients & Methods:

Data on survival and disease-free survival were obtained from hospital records of 23 patients with an established diagnosis of parathyroid carcinoma in whom CASR and parafibromin expression and *HRPT2/CDC73* mutation analysis were available from paraffin-embedded pathological specimens. Kaplan-Meier curves were used for survival analysis.

Results:

Downregulation of CASR expression, global loss of parafibromin staining and a *HRPT2/CDC73* mutation were respectively found in 7 (30%), 13 (59%) and 4 (17%) patients, and were associated with, respectively, 16-fold, 4-fold and 7-fold increased risk of developing local or distant metastasis.

Conclusion:

These findings suggest that, although downregulation of CASR expression, global loss of parafibromin staining and mutations in the *HRPT2/CDC73* gene are tools of proven value to assist in establishing a diagnosis of parathyroid carcinoma, their absence does not exclude it. Notwithstanding, we demonstrate a significant added value of these markers as strong determinants of increased malignant potential and thus as negative prognostic markers in this malignancy.

INTRODUCTION

Parathyroid carcinoma is a rare disorder, accounting for 0.1-5.0% of all cases of primary hyperparathyroidism (PHPT) (1-6). The disease presents at a mean age of 50 years and, contrary to sporadic hyperparathyroidism, is equally likely to occur in men and women (1,3-5,7). Clinical manifestations are those of severe PHPT, often associated with renal and skeletal complications (1,3-5,8). A neck mass is palpable in 30-76% of patients (1,5,9,10). The diagnosis of parathyroid carcinoma is suspected by intra-operative features of local invasion and confirmed by the World Health Organization (WHO) histopathological criteria for parathyroid carcinoma. These include vascular invasion, perineural space invasion, capsular penetration with growth into adjacent tissues and/or metastasis (11). Despite the availability of these histopathological criteria, the diagnosis of parathyroid carcinoma remains a challenge in some cases, and markers such as mutations in the tumor-suppressor gene *HRPT2/CDC73* (12-15), and global loss of expression of its protein, parafibromin (16,17), are proven valuable tools frequently used to assist in establishing a diagnosis of this malignancy.

Mutations in the *HRPT2/CDC73* gene, which encodes for the protein parafibromin (18), are associated with the hyperparathyroidism-jaw tumor syndrome (HPT-JT; OMIM 607393). Patients with a HPT-JT syndrome have a 15-24% chance of developing parathyroid carcinoma, and loss of heterozygosity of chromosome 1q, the location of the *HRPT2/CDC73* gene, is reported to be found in 55% of parathyroid carcinomas (1,17,19-21).

The calcium-sensing receptor (CASR) regulates not only the synthesis and secretion of PTH, but also the proliferation of the parathyroid glands. We have previously reported that 31% of parathyroid carcinomas had irregular or absent staining for CASR compared to only 1% of sporadic adenomas (21). We have also demonstrated, using gene expression analysis, a significant downregulation of the CASR in patients with sporadic carcinoma or familial HPT-JT syndrome compared with patients with sporadic PHPT, PHPT due to *MEN-1* or *MEN-2* mutations or PHPT due to lithium use (22). Downregulation of CASR has been previously

shown to be a negative prognostic factor in colon carcinoma (23-25), but there are no available data on the prognostic value of downregulation of CASR in parathyroid carcinoma. There are also no available data on the prognostic value of loss of parafibromin staining in the presence of a *HRPT2/CDC73* mutation.

The aim of our study was to evaluate the prognostic value of downregulation of CASR expression, loss of parafibromin staining and the presence of mutations in the *HRPT2/CDC73* gene in patients with a WHO criteria-based diagnosis of parathyroid carcinoma.

PATIENTS AND METHODS

Patients

We identified 29 patients who had initial surgery for parathyroid carcinoma in various hospitals in the Netherlands in the period 1985-2000. All resected tumors were screened for somatic and/or germline mutations in the *HRPT2/CDC73* and the *MEN-1* genes, and immunohistochemical staining for parafibromin and CASR was performed on all 29 pathological specimens in the Department of Pathology of the Leiden University Medical Center (LUMC) (21). Four patients were lost to follow-up, one patient died (normocalcaemic) of a myocardial infarction 4 months after initial parathyroidectomy and one patient did not meet the histopathological WHO criteria for parathyroid carcinoma and were excluded from the final analysis. A total of 23 patients were thus included in the study. The study was approved by the local Ethics Committee of the LUMC.

Methods

Clinical data were obtained from the hospital records of the 23 patients included in the study, with special emphasis on time to development of local recurrence and/or distant metastasis and when applicable time and cause of death. Data on initial clinical presentation were available in 16 of the 23 patients (70%) including physical examination of the neck in 14 of the 23 patients (61%). Serum biochemistry before initial surgery was available in 20 of the 23 patients (87%) and

hypercalcemia was documented in all patients, although exact concentrations were not available in 3 patients. Operative and pathology data were available in all 23 patients.

End points of the study

The primary end point of the study was the development of local recurrence and/or distant metastasis. Recurrence was defined as recurrence of an increase in serum calcium and parathyroid hormone (PTH) concentrations after a period of normalization of at least 6 months after initial surgery. Local recurrence was defined on the basis of a surgically removed lesion from a site adjacent to the primary tumor that was histologically confirmed to be parathyroid carcinoma. Distant metastasis was defined as radiologically and/or surgically and histologically proven distant metastasis diagnosed at least 6 months after successful excision of a primary tumor.

The secondary end point of the study was death related to parathyroid carcinoma, which was defined as death due to PTH-related uncontrollable hypercalcemia. Patients who died from other causes in the setting of normocalcemia were censored at the time of death.

Histopathological diagnosis of parathyroid carcinoma

The histopathological diagnosis of parathyroid carcinoma was established using the WHO criteria for this malignancy. These include vascular invasion, perineural space invasion, capsular penetration with growth into adjacent tissues and/or metastasis (11). The presence of a trabecular pattern, fibrous bands and/or multiple mitoses were considered to be minor criteria. All pathological specimens were re-evaluated in our Department of Pathology using the WHO criteria and only those fulfilling these criteria were included in the analysis.

Pathological analysis

Pathological analysis of the primary tumor has been reported in detail in previous publications (14,21). In brief, formalin-fixed, paraffin-embedded (FFPE) specimens

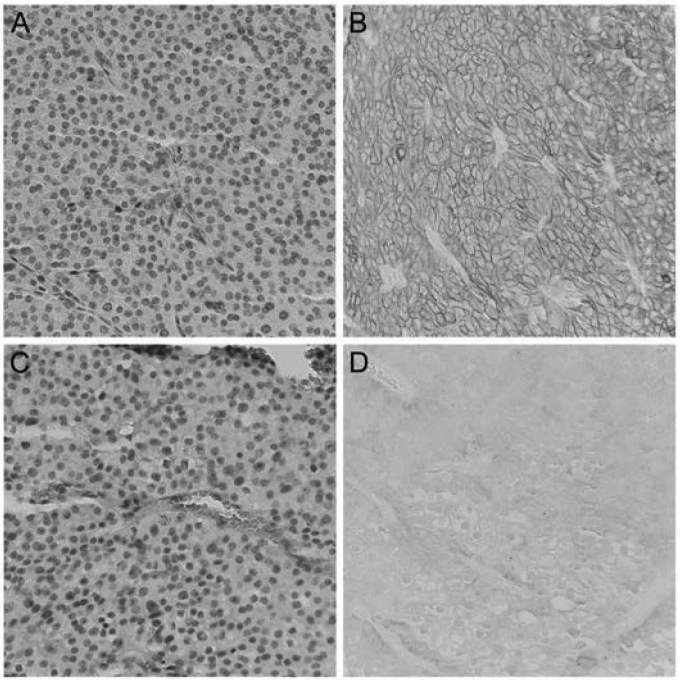

Figure 1: Staining with hematoxylin-eosin for CASR expression (A/C) and immunohistochemical (IHC) staining of CASR (B/D) in 2 patients with parathyroid carcinoma (patient 7 (A/B) and patient 1 (C/D)) showing maintained membranous CASR expression in patient 7 (A/B) and loss of membranous CASR expression in Patient 1 (C/D). Magnification x 25.

of the primary parathyroid carcinomas of all 23 patients were screened for *HRPT2/CDC73* and *MEN-1* gene mutations (14), and all were immunohistochemically stained for parafibromin and CASR (Figures 1 and 2) (17,21). Downregulation of CASR expression was defined as irregular or absent staining pattern for CASR on the membrane of parathyroid tumor cells. Focal loss of parafibromin staining was previously defined as the absence of nuclear staining in variably sized regions. Global loss of parafibromin staining was defined as the absence of nuclear staining in all tumor tissue. The three identified germline *HRPT2/CDC73* mutations were positioned on exon 2 (c.176C>T, pSer59Phe, patient no. 1), exon 7 (c.687_688delAG, p.Arg229fsX, patient no. 23) and exon 7 (c.691_692insT, p.Trp231fsX35, patient no. 21), respectively (14). The two

identified somatic *HRPT2/CDC73* mutations were positioned on exon 1 (c128G>A, pTrp43fsX, patient no. 21) and exon 2 (c.165delC, pTyr55fsX, patient no. 6), respectively (14). The three identified somatic *MEN-1* mutations were positioned on exon 2 (c.168_171delCAAC, pAns57fsX61, patient no. 16), exon 3 (c.631G>T, pVall211Phe, patient no. 5) and exon 9 (c.1256delG, pGly419fsX26, patient no.14), respectively (14). The mutation specifics have been partly curated, that is, updated according to the latest published literature, in the Cosmic Database of the Sanger Institute (www.sanger.ac.uk/genetics/CGP/cosmic).

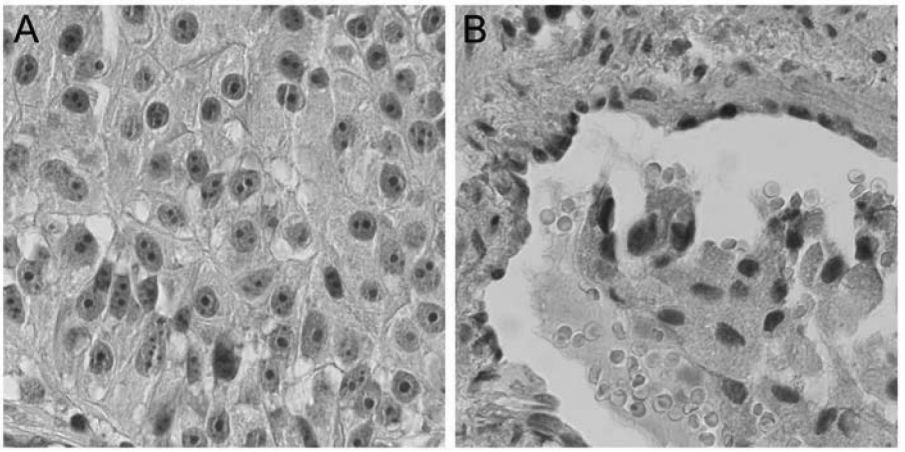

Figure 2: Immunohistochemical staining of parafibromin in a patient with parathyroid carcinoma (patient 23) showing global loss of parafibromin staining in the nuclei of parathyroid carcinoma cells (A) and in the nuclei of parathyroid carcinoma cells located intravascular (B). In contrast, the nuclei of the endothelial cells of the vascular wall stained positive for parafibromin (B). Magnification x 100.

Statistical analysis

Statistical analysis was performed using the SPSS 16 (SPSS inc., Chicago, IL, USA) software. Results are expressed as mean ± s.d. unless otherwise stated. The chi-square test and Student's *t*-test were used for categorical variables and continuous variables. Disease-free survival (time to first local recurrence and/or distant metastasis) and overall survival (time to death related to parathyroid carcinoma) was determined by the Kaplan-Meier method and *P*-values were

calculated by the log-rank test. Cox regression analysis was used to determine hazard ratios (HRs).

RESULTS

Demographic data

A total of 12 women and 11 men, with a mean age of 51 ± 16 years at the time of diagnosis, were studied (Table 1). At initial presentation, polyuria and polydipsia were present in 50%, tiredness in 31%, muscle- or bone pain in 25%, constipation in 13% of patients and a neck swelling was palpable in 11 of 14 patients (79%). Mean serum calcium concentration was 3.28 ± 0.55 mmol/l (reference range 2.15-2.55 mmol/l), mean PTH level was 75 ± 57 pmol/l (reference range 1.5-8 pmol/l), mean alkaline phosphatase concentration was 386 ± 297 U/l (reference range 40-120 U/l) and creatinine clearance was <60 ml/min in 59% of patients.

Surgical data

Of the 23 patients, 17 (74%) had bilateral neck exploration and the remainder unilateral neck exploration. Parathyroidectomy was combined with hemithyroidectomy in 12 patients (52%) because of thyroid invasion (n=11) or pre-operatively identified thyroid pathology (n=1). Invasive growth of the tumor was observed intra-operatively in 14 of 21 patients (67%), including invasion of the thyroid (n=8), the recurrent laryngeal nerve (n=2), the esophagus (*n*=1), both the thyroid and esophagus (n=1) and unspecified tissue (n=2) (Table 1).

Histopathological data

On pathological evaluation, cystic features were observed in 6 of the 23 tumors (26%) and fibrotic bands were present in 19 of the 23 tumors (83%). There was no mitotic activity in 7 of the 23 tumors (30%), sporadic mitotic activity was observed in 10 (44%) and >1/10 high-power field (HPF; more than 1 mitotic figure per HPF) was observed in 6 patients (26%). Invasion of blood vessels was documented in 21 primary tumors (91%). Four patients had local lymph node metastases.

Disease-free survival and survival

Median duration of follow-up after initial parathyroidectomy was 10 years, (range 1-20 years). Of the 23 patients, 7 (30%) developed local recurrence of the tumor after a median duration of 3 years (range 1-7 years) after initial parathyroidectomy. One or more distant metastasis developed in 11 patients (48%) after a median duration of 2 years (range 0.5-13 years): lung metastasis (n=10), lymph node metastasis (n=5), bone metastasis (n=2), liver metastasis (n=1) and brain metastasis (*n*=1). Disease-free survival was 96, 60 and 40%, respectively, at 1, 5 and 10 years after initial parathyroidectomy.

Of the 23 patients, 13 (57%) had died at the time of the study. In 11 of these patients (85%), death was related to severe uncontrollable hypercalcemia. Death was unrelated to parathyroid carcinoma in the other two patients, who died respectively of septic shock and of a cerebrovascular accident, in the setting of normocalcemia. Overall survival rates were 91, 74 and 65%, respectively, at 1, 5 and 10 years after initial parathyroidectomy.

There was no significant difference in survival and disease-free survival between patients who had a hemithyroidectomy and those who did not (61 *vs.* 58%, *P*=0.7 and 81 *vs.* 67%, *P*=0.8, respectively).

Downregulation of CASR expression

Of the 23 patients, 7 (30%) demonstrated downregulation of CASR expression in the resected primary tumor (Figure 3). Of these 7 patients, 5 also had somatic and/or germline *HRPT2/CDC73* or *MEN-1* mutations. Patients with downregulation of CASR expression demonstrated more global than focal parafibromin loss compared to patients with normal CASR expression (6/6 *vs.* 7/16, *P*=0.02). At the time of initial surgery, positive lymph nodes were also more frequently found in patients with downregulation of CASR expression compared to patients with normal CASR expression (3/5 vs. 1/16, *P*<0.01).

Of the 7 patients with downregulation of CASR expression, 6 developed local recurrence and/or distant metastasis compared with only 8 of the 16 patients with

Table 1: Operative, pathological and follow-up data of patients with parathyroid carcinoma

Patient number	Age/ Sex	Peroperative invasive growth	Vaso-invasion	CASR expression	Para-fibromin loss	Mutation screening	Development of local recurrence	Development of lymph node metastasis	Development of distant metastasis	Follow-up (years)	Outcome
1	30/F	Yes	Yes	Downregulation	Global	germline *HRPT2*	Yes	Yes	Yes	5	DOD
2	71/F	Yes	Yes	Normal	Focal		Yes	No	No	15	DOD
3	74/F	Yes	Yes	Normal	Global		No	No	Yes	13	A/WD
4	72/F	No	Yes	Normal	Focal		No	No	No	10	A/FOD
5	77/M	Yes	Yes	Downregulation	Global	somatic *MEN-1*	No	Yes	Yes	1	DOD
6	29/M	Yes	Yes	Downregulation	Global	somatic *HRPT2*	Yes	Yes	Yes	16	A/WD
7	57/M	Yes	No	Normal	Global		No	No	No	13	A/FOD
8	48/M	Yes	Yes	Normal	Focal		No	No	No	16	A/FOD
9	32/M	No	Yes	Normal	Global		Yes	No	No	10	A/WD
10	75/M	No	Yes	Normal	Focal		No	No	No	6.5	D/other
11	66/F	No	Yes	Normal	Focal		Yes	No	Yes	8	DOD
12	66/F	Yes	Yes	Normal	Global		No	Yes	Yes	13.5	DOD
13	34/M	NA	Yes	Normal	Global		Yes	Yes	Yes	7	DOD
14	50/F	Yes	Yes	Normal	Global	somatic *MEN-1*	No	No	Yes	3.5	DOD
15	29/F	No	Yes	Downregulation	Global		No	No	No	1.5	DOD
16	51/F	Yes	Yes	Normal	Focal	somatic *MEN-1*	No	No	No	9	A/FOD
17	54/M	No	Yes	Normal	Focal		No	No	Yes	13	DOD
18	36/F	No	Yes	Normal	Global		No	No	No	18	A/FOD
19	62/F	Yes	Yes	Downregulation	Global		No	No	Yes	1	DOD
20	39/M	Yes	No	Normal	Focal		No	No	No	10	A/FOD
21	41/M	Yes	Yes	Downregulation	Global	germline+somatic *HRPT2*	No	No	Yes	3	DOD
22	62/M	Yes	Yes	Normal	Focal		No	No	No	11	A/FOD
23	36/F	NA	Yes	Downregulation	Global	germline *HRPT2*	Yes	No	No	20	D/other

NA: not available, ND: not determined, DOD: Dead of disease, D/other: Dead of other cause, A/WD: alive with disease, A/FOD: alive, free of disease

normal CASR expression (log-rank test, P=0.1). The time to development of a local recurrence and/or distant metastasis was significantly shorter in patients with downregulation of CASR expression compared with patients with normal CASR expression (1±1 years vs. 9±5 years, P<0.01). In addition, 5 of the 7 patients with downregulation of CASR expression and 6 of 16 patients with normal CASR expression had parathyroid carcinoma-related death a mean of 2±2 years and 10±5 years, respectively, after initial surgery.

Both the 5-year disease-free survival and the 5-year overall survival were lower in patients with downregulation of CASR expression compared to patients with normal CASR expression (81% vs. 0% and 94% vs. 29%, respectively, Figure 3). Downregulation of CASR expression was associated with a 16-fold increased risk of developing a local or distant metastasis (HR 16; 95% CI 3-87; P<0.01; Table 2) and a 3-fold increased risk of death (HR 2.9; 95% CI 0.8-9.7; P=0.09).

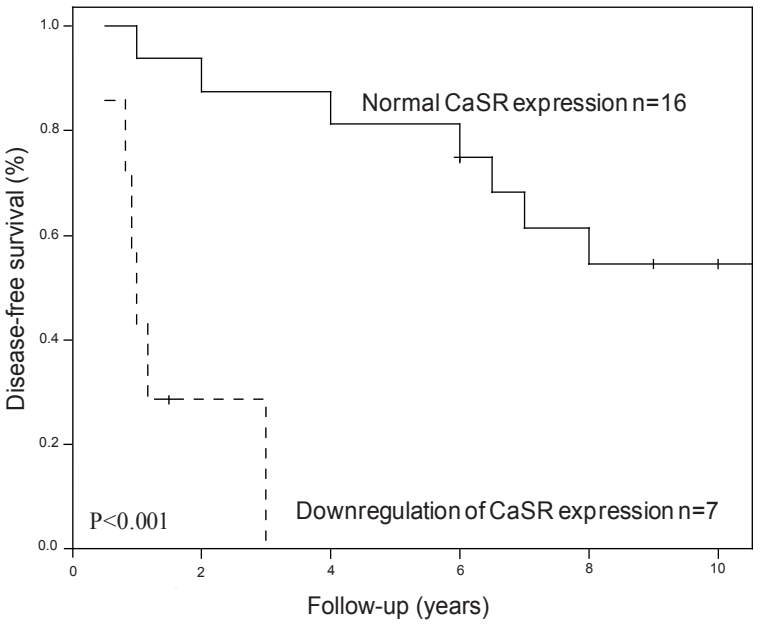

Figure 3. Significant decrease in 5-year disease-free survival in patients with downregulation of CASR expression compared with patients with normal CASR expression (81% vs. 0%).

Loss of parafibromin

Parafibromin loss was determined in the resected primary tumor in all 23 patients. A total of 14 specimens showed global loss (Figure 2) and 9 showed focal loss of parafibromin staining. Of the 14 patients with global parafibromin loss, 11 developed a local recurrence and/or distant metastasis compared with 3 of 9 patients with focal parafibromin loss (log-rank test *P*=0.03). Also, 8 of 14 patients with global parafibromin loss and 3 of 9 patients with focal parafibromin loss had a parathyroid carcinoma-related death (log-rank test *P*=0.27). Both the 5-year disease-free survival (89 vs. 41%) and the 5-year overall survival (100 vs. 57%) were lower in patients with global parafibromin loss compared with patients with focal parafibromin loss (Figure 4). Global parafibromin loss was associated with a fourfold increased risk of developing local recurrence and/or distant metastasis (HR 4.3; 95% CI 1.2-15.9; *P*=0.03; Table 2) and with a twofold increased risk of death (HR 2.0; 95% CI 0.5-7.8; *P*=0.3).

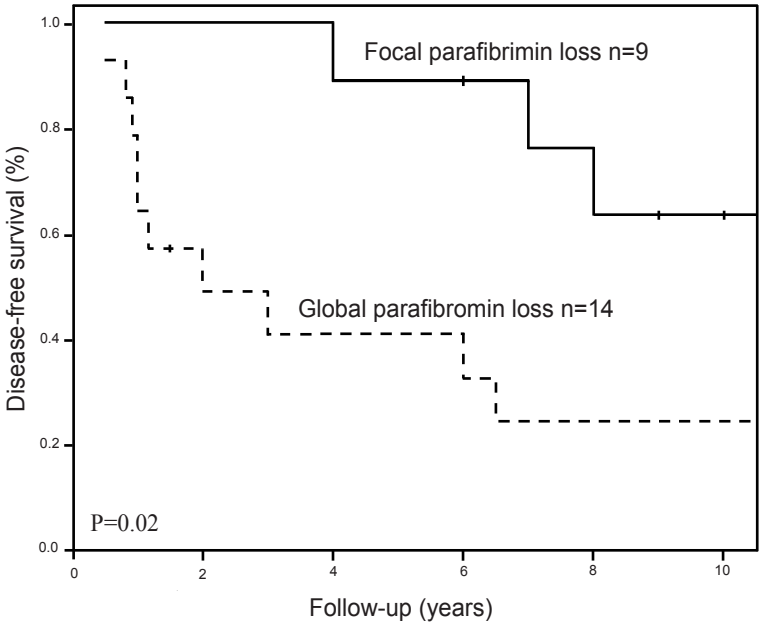

Figure 4: Significant decrease in 5-year disease-free survival in patients with global loss of parafibromin staining compared with patients with focal loss of this protein (89 vs. 40%).

HRPT2/CDC73 mutation

Of the 23 patients with parathyroid carcinoma, 2 had a germline *HRPT2/CDC73* mutation, one had a somatic *HRPT2/CDC73* mutation and one patient had both a germline and a somatic *HRPT2/CDC73* mutation (Table 1). All 4 patients with an *HRPT2/CDC73* mutation developed a local recurrence and/or distant metastases compared to only 10 of the 19 patients without this mutation (log-rank test: P=0.08). Time to development of recurrence/metastasis was shorter in patients with an *HRPT2/CDC73* mutations compared with patients without this mutation (1±1 year vs. 8±5 years, P<0.01). Two of the 4 patients with an *HRPT2/CDC73* mutation and 9 of 19 patients without this mutation had a parathyroid carcinoma-related death. Both the 5-year disease-free survival (73 vs. 0%) and the 5-year overall survival (79 vs. 50%) were lower in patients with an *HRPT2/CDC73* mutation compared with patients without this mutation (Figure 5). *HRPT2/CDC73* mutations were associated with a 7-fold increased risk of developing local recurrence and/or distant metastasis (HR 7.4; 95% CI 1.8-30.6, P<0.01; Table 2).

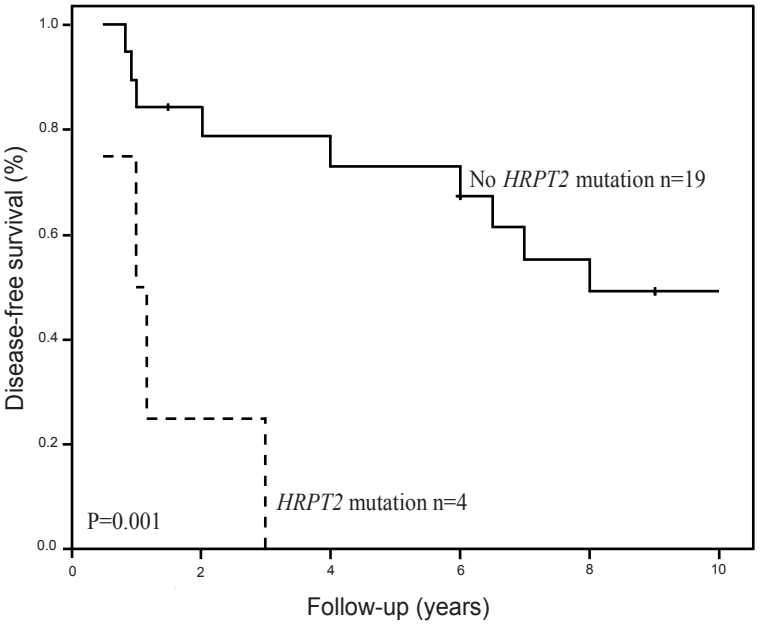

Figure 5: Significant decrease in 5-year disease-free survival in patients with germline and/or somatic *HRPT2/CDC73* mutation compared with patients without a mutation in the *HRPT2/CDC73* gene (73 vs. 0%).

MEN-1 mutation

Of the 23 patients with parathyroid carcinoma, 3 had a somatic *MEN-1* mutation (Table 1). Patient 16 had intra-operative findings of invasion of the thyroid gland and pathological features of vaso-invasion, but did not develop local or distant metastases during 9 years of follow-up. Patient 14 had a palpable neck swelling at presentation, intra-operative findings of invasion, pathological features of vaso-invasion and developed lymph node, lung and bone metastases 11 months after initial parathyroidectomy. There was no response to chemotherapy and the patient died of uncontrollable hypercalcemia soon after the diagnosis of metastases. Patient 5 also had a palpable swelling in the neck, intra-operative findings of invasion, pathological features of vaso-invasion, a positive lymph node and developed lung and brain metastases 18 months after initial parathyroidectomy. The patient died of uncontrollable hypercalcemia 24 months later. In these 3 patients with somatic *MEN-1* mutations the 5-year disease-free survival and the 5-year overall survival were lower compared with patients without this mutation (respectively, 64 vs. 33% and 80 vs. 33%). Somatic *MEN-1* mutations were associated with a 2-fold increased risk of developing a local recurrence and/or distant metastasis (HR 1.7; 95% CI 0.4-7.8, P=0.5; Table 2) and a 3-fold increased risk of death (HR 3.3; 95% CI 0.7-16.6, P=0.15).

Combined effect of negative prognostic factors

Of 23 patients, 14 had one or more negative prognostic factors, but only 4 of the patients had a combination of an *HRPT2/CDC73* mutation, global loss of parafibromin staining as well as downregulation of CASR expression. Both the 5-year disease-free survival and the 5-year overall survival were lower in patients with one or more negative prognostic factors compared with patients without one of these factors (89 vs. 41% and 100 vs. 57%, respectively; Figure 6). Patients with one or more negative prognostic factors had a 4-fold increased risk of developing local recurrence and/or distant metastasis (HR 4.3; 95% CI 1.2-15.9, P=0.03; Table 2) and a 2-fold increased risk of death (HR 2.0; 95% CI 0.5-7.8, P=0.3).

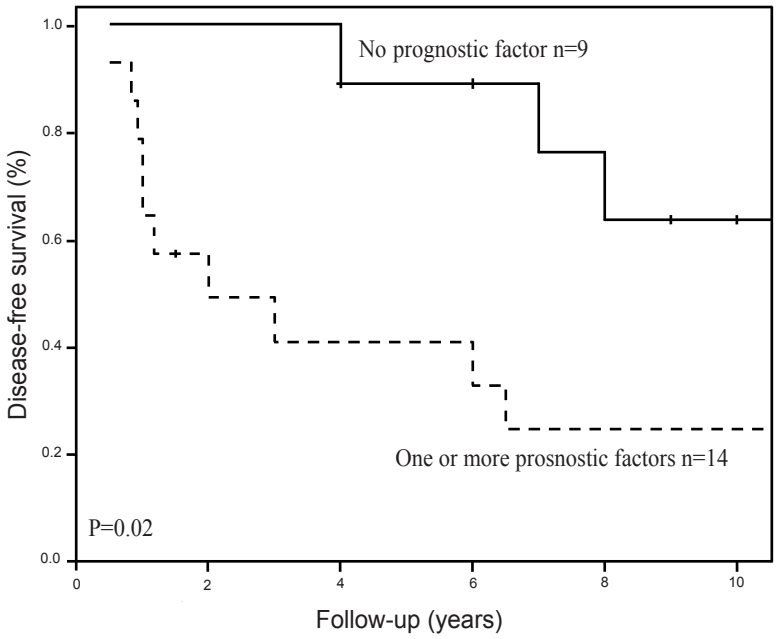

Figure 6: Significant decrease in 5-year disease-free survival in patients with one or more of the studied prognostic factors (germline and/or somatic *HRPT2/CDC73* mutation, global loss of parafibromin staining or downregulation of CASR expression) compared with patients without one of these prognostic factors (89 vs. 41%).

DISCUSSION

Data from our study in patients with a WHO-based diagnosis of parathyroid carcinoma suggest that downregulation of CASR expression, global loss of parafibromin staining and the presence of germline and/or somatic *HRPT2/CDC73* mutations are strong negative determinants of prognosis in patients with this malignancy, with clear associations with decreased disease-free survival and overall survival.

Although downregulation of CASR expression was present in only 7 of 23 patients, this molecular alteration was associated with a 16-fold increased risk of developing local recurrence and/or distant metastasis. This suggests that this tumor marker represents a significant determinant of prognosis in parathyroid carcinoma. The CASR holds an important function in the control of calcium homeostasis by the parathyroid gland. Activation of the CASR by its binding with calcium ions leads to

inhibition of PTH secretion, PTH gene expression and thus PTH synthesis, and of parathyroid cell proliferation (23). Mutations in the *CASR* gene that result in a loss of function of the CASR, such as seen in familial benign hypercalcemia, increase the calcium-dependent set-point for inhibition of PTH release from the parathyroid cell (26). However, no mutations in the *CASR* gene have been so far identified in patients with parathyroid carcinomas (27). It also remains unclear whether increased parathyroid cell proliferation leads to decreased CASR expression as observed in autonomous hyperparathyroidism in chronic renal failure, or whether decreased CASR expression leads to increased parathyroid cell proliferation and increased malignant potential in patients with parathyroid carcinoma (21,23). Notwithstanding, evaluating CASR expression may not only be used as a prognostic tool in the management of patients with parathyroid carcinoma, but may also serve the purpose of determining whether calcimimetics may play a role in the therapeutic management of these patients. In one patient in our series (patient no. 6) demonstrating loss of CASR expression in his primary tumor, the calcimimetic cinacalcet, prescribed at a maximum dose of 90 mg twice daily, was indeed unable to decrease PTH or calcium levels to any extent (personal observation).

Table 2: Association between prognostic factors and the development of local recurrence and/or distant metastasis

	HR (95% CI)	*P* value
Downregulation of CASR expression	16.3 (3.0-87.3)	0.000
HRPT2/CDC73 mutation	7.4 (1.8-30.6)	0.005
Global loss of parafibromin	4.3 (1.2-15.9)	0.028
One or more of these prognostic factors	4.3 (1.2-15.9)	0.028

HRPT2/CDC73 mutations are known to be associated with the HPT-JT syndrome. These mutations have also been found in 67-100% of patients with parathyroid carcinoma selected on the basis of local recurrence or distant metastasis (12,13,15). In contrast, in our study, in which patients were selected on the basis of the WHO histopathological criteria for parathyroid carcinoma, rather than on the basis of the presence of a local recurrence and/or of distant metastasis, we could only demonstrate a germline and/or somatic *HRPT2/CDC73* mutation in 4 of the 23

patients (17%). These were 4 of 14 patients who developed a local recurrence and/or distant metastasis during follow-up (29%), suggesting that factors other than mutations in the *HRPT2/CDC73* gene may also play a role in the tumorigenesis of parathyroid carcinoma. One of the limitations of our study is that we may not have identified all mutations in the HRPT2/CDC73 gene. The low mutation frequency in our population may thus be due to technical reasons, because of the nature of the paraffin-embedded tissue studied, leading to the inability to completely screen all DNA sequences of the *HRPT2/CDC73* gene or screen the DNA for large somatic or germline genomic deletions (14). However, exons 1, 2 and 7, which harbor up to 85% of all somatic *HRPT2/CDC73* mutations (12,15,18,28,29), were completely screened in all patients. Silencing of *HRPT2/CDC73* due to promoter hypermethylation was also not studied, although this may not be relevant, as it was recently documented that hypermethylation does not play a role in loss of this gene (30). Although the numbers are small, our data do suggest that the presence of mutations in the *HRPT2/CDC73* gene are associated with a significant decrease in disease-free survival and also survival, albeit the latter not significantly. These data suggest that mutations in this gene determine the malignant potential of a parathyroid carcinoma and may thus be used as a prognostic tool in this malignancy.

In patients with parathyroid carcinoma, most of the identified *HRPT2/CDC73* mutations are predicted to prematurely truncate the parafibromin protein (12,13,16,28), which often lead to loss of parafibromin staining. Parafibromin is known to be involved in cell transcription, proliferation, differentiation and apoptosis, although, the exact mechanism by which this protein increases the malignant potential of parathyroid carcinoma remains to be established (28). In our population, all patients with germline and/or somatic *HRPT2/CDC73* mutations had global loss of parafibromin staining. We also found global loss of parafibromin staining in 10 patients in whom no mutations were identified, possibly pointing at the difficulties in capturing all mutations in our study population. In keeping with previous studies, which showed that loss of parafibromin staining was more frequently observed in patients who developed a local recurrence and/or distant metastasis compared with patients who did not (62-96% vs. 22-50%) (16,17,28), we

indeed observed that patients with global loss of parafibromin staining had a short disease-free survival and survival. In contrast, focal loss of parafibromin staining did not appear to influence the clinical outcome. Moreover, in our experience scoring of focal loss of parafibromin staining is difficult to use in daily practice, because of commonly encountered regional differences in immunohistochemical staining. In this case, it is the general staining pattern that is taken in consideration for decision making. Our data support the premise that focal loss of parafibromin staining might not be as useful as global loss of parafibromin staining as a diagnostic or prognostic tool in parathyroid carcinoma. Gill *et al.* (31) indeed proposed a scoring system in which focal loss would be considered positive, and concluded that complete absence of nuclear staining for parafibromin is diagnostic of parathyroid carcinoma or HPT-JT syndrome-related (benign) tumor.

MEN-1 mutations are frequently found in familial (95%) and also in sporadic adenomas (20-30%), but so far only 4 cases of somatic *MEN-1* mutations and 3 clinically suspected germline *MEN-1* mutations have been reported in patients with parathyroid carcinoma (14,32-34). In our population, 3 patients demonstrated a somatic *MEN-1* mutation. In all three patients, a diagnosis of parathyroid carcinoma was established on the basis of intra-operative findings of invasive growth (100%), vaso-invasion (100%) and distant metastasis (67%). These findings suggest that patients with a somatic *MEN-1* mutation are at risk of developing parathyroid carcinoma and that somatic *MEN-1* mutations could have a role in parathyroid carcinogenesis.

Among many other markers, downregulation of CASR expression, global loss of parafibromin staining and mutation in the *HRPT2/CDC73* gene are tools to assist in establishing a diagnosis of parathyroid carcinoma. Our data demonstrate a significant added value of these tumor markers as strong negative determinants of the malignant potential of parathyroid carcinomas. We therefore advocate the use of these prognostic tools in the management of patients with this malignancy. Findings from our study strengthen the link between molecular alterations and clinical course of patients with parathyroid carcinoma.

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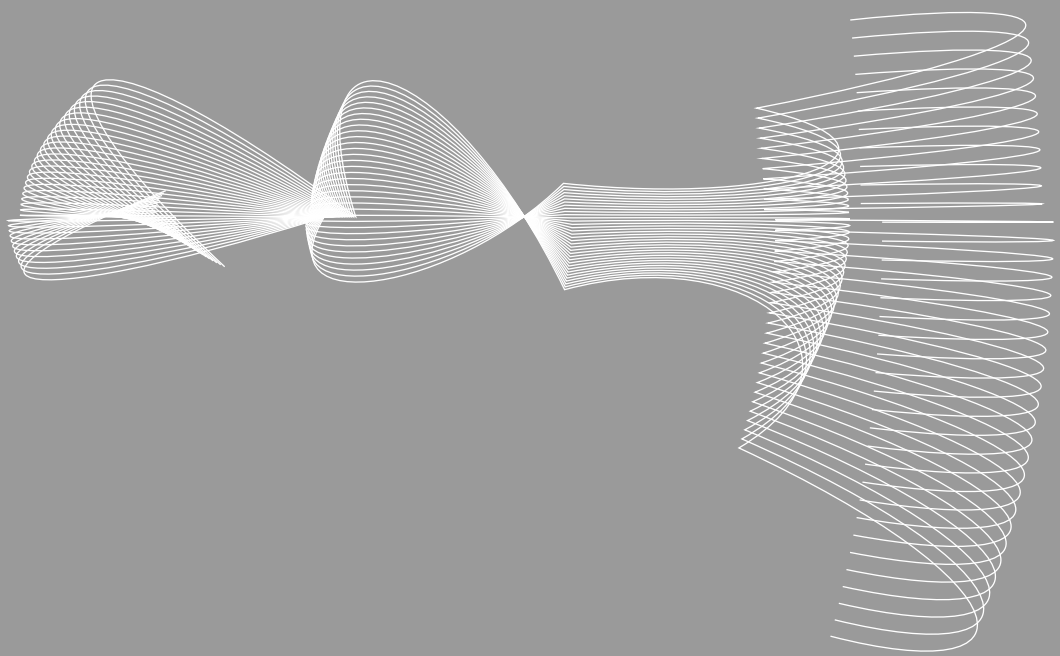
10

Challenges and pitfalls in the management of persistent primary hyperparathyroidism, a case series

Janneke E. Witteveen, Job Kievit, Hans Morreau, Johannes A. Romijn, Neveen A.T. Hamdy

Submitted



ABSTRACT

Background: Persistent hyperparathyroidism is rare after surgery for sporadic hyperparathyroidism, occurring in only 0-6% of cases. The management of these patients is complex, with a generally poor cure rate despite repeated surgery. The aim of our study was to identify the challenges and pitfalls encountered in the complex management of patients with persistent hyperparathyroidism after initial parathyroidectomy.

Methods: Using Leiden University Medical Centre hospital records, we identified 20 patients with sporadic primary hyperparathyroidism who had undergone 33 revision surgeries for persistent hyperparathyroidism. Patients with a *MEN-1, MEN-2* or *CASR* mutation or carcinoma were excluded. Clinical, operative and histology data were collected for the period covering the course of the disease.

Results: The most common causes of persistent hyperparathyroidism were a missed ectopic gland at initial surgery (33%) and missed multiglandular disease (15%). Parathyromatosis was documented in 9% of patients. Pre-operative localisation studies had poor sensitivity: ultrasound 18%, Tc99m-MIBI-SPECT 25%, CT 30%, MRI 20%. In contrast, selective venous sampling for PTH had a sensitivity of 50% and a specificity of 89%. The decrease in intraoperative PTH was significantly less marked in patients in whom hyperparathyroidism persisted compared to those who achieved cure (63 ± 26 vs. 89 ± 11%, P=0.003). The risk of complications increased with each subsequent surgery: 20% after first, 50% after 2nd and 67% after 3rd surgery.

Conclusion: Persistent PHPT represents a significant management challenge largely due to the poor predictive value of pre-operative localisation studies and to the risk of complications, which increases with each subsequent revision surgery.

INTRODUCTION

In primary hyperparathyroidism (PHPT) cure is reported in 93 to 100% of cases after parathyroidectomy, also when a minimally invasive approach is opted for (1-4). Removal of all hyperactive parathyroid tissue is of significant clinical relevance, since patients who achieve cure show significant post-operative increase in bone mass (5,6), no recurrence of nephrolithiasis (5,6) and improvement in various parameters of quality of life (7,8).

Persistent hyperparathyroidism is defined as residual biochemical features of hyperparathyroidism in the form of increased serum calcium and PTH concentrations documented directly post-operatively or within the first 6 months after removal of one or more hyperactive parathyroid glands at initial surgery and persisting thereafter (4). Persistent PHPT is most commonly due to a pathological gland missed at initial surgery, which is often small (<1.5 cm) and hyperplastic in nature (9,10), or rarely due to parathyromatosis from gland spillage during previous surgery (11). It is of note that the diagnosis of persistent hyperparathyroidism may be overlooked or delayed after parathyroidectomy, particularly in the case of multiple gland disease due to transient normalization of serum calcium and PTH concentrations due to suppression of the activity of smaller glands by the dominant first resected large gland. Pathological small parathyroid glands may indeed take up to 6 months to recover and become hyperactive in their own right (4).

Initial bilateral neck exploration or the more limited surgical approaches are associated with very few complications in the hands of experienced surgeons (12,13). In contrast, re-exploration for persistent hyperparathyroidism is associated with a 3-fold increase in morbidity due to scarring and disruption of the normal patterns of drainage due to previous surgery (14,15). In this case, pre-operative localisation studies are imperative in order to reduce operating time and the risk of complications. However, the value of standard localisation studies has been shown to be significantly curtailed in patients with persistent PHPT, predominantly because of the high frequency of hyperplastic, ectopically located and small sized

parathyroid glands (16-18). Management of patients with persistent hyperparathyroidism represents therefore a recurring challenge.

The aim of our study was to evaluate the demographic, clinical, biochemical, operative and pathology data of a series of patients with persistent hyperparathyroidism who had had one or more revision surgeries after initial parathyroidectomy, identifying in the process the challenges and pitfalls encountered in their complex management.

PATIENTS AND METHODS

Study population

Using our hospital records, we identified 20 patients who had undergone a total of 33 revision surgeries for persistent PHPT in the Leiden University Medical Center. Eighteen of the 20 patients (90%) had had their initial parathyroidectomy at another hospital. All revision surgeries were undertaken by two surgeons with considerable experience in endocrine surgery. Patients with a *MEN-1*, *MEN-2* or *CASR* mutation were not included in the study.

Methods

All available demographic data, clinical data, data on pre-operative localisation studies, operative data, including data on intra-operative PTH measurements, pre- and post-operative laboratory data and histopathological data were obtained from the patients' hospital records.

All pre-operative localisation studies, including Tc99m-MIBI-SPECT and selective venous sampling for PTH (SVS), followed by CT-scan to confirm localisations suggested by Tc99m-MIBI-SPECT or SVS, were reviewed and analysed as previously reported (19,20).

Bilateral neck exploration consisted of visualization of all four parathyroid glands, leading to either excision of a single enlarged parathyroid gland, or to subtotal or total parathyroidectomy with auto-transplantation if more than one parathyroid gland was found to be enlarged. If no parathyroid glands could be

visualized or no enlarged gland was found, the surgeon proceeded to dissection of the ipsilateral anterior compartment extending from the level of the hyoid bone superiorly to the suprasternal notch inferiorly and to a hemithyroidectomy on the side of the missing parathyroid. Less invasive neck exploration was only undertaken in patients with positive preoperative localisation studies and was guided by intra-operative PTH (IOPTH) monitoring in the majority op patients. IOPTH monitoring consisted of two initial baseline PTH measurements with an interval of 15-20 minutes, followed by 5 measurements at 3-minute intervals after excision of the pathological parathyroid(s). Surgery was considered successful if IOPTH decreased by more than 50% within 7 minutes of excision of a pathological parathyroid gland(s).

Histological preparations obtained at surgery conducted in our hospital (including all last revision surgeries) were independently reviewed by an experienced pathologist. A diagnosis of sporadic parathyroid adenoma was based on the presence of a benign encapsulated neoplasm mostly consisting of one cell type with an adjacent rim of normal glandular tissue, usually involving a single gland (21). A diagnosis of parathyroid hyperplasia was based on an absolute increase in parathyroid parenchymal cell mass resulting from proliferation of a mixture of the different parathyroid cells, present in multiple parathyroid glands in the absence of a known stimulus for PTH hypersecretion such as renal failure or vitamin D deficiency (21).

Laboratory data on serum calcium and PTH concentrations as measured more than 6 months post-operatively were used to indicate cure or persistence of hyperparathyroidism.

Statistical analysis

Statistical analysis was performed using the SPSS 16 software (SPSS inc., Chicago, IL, USA). Results are expressed as mean ± SD unless otherwise stated. Chi-square test and Student's *t*-test were used as appropriate for categorical variables and continuous variables. A probability level of random difference of $P<0.05$ was considered to be significant.

Ethical consideration

The methods used in this study were part of the clinical routine work-up of patients undergoing revision surgery in our hospital. The study was approved by the Local Ethics Committee of the Leiden University Medical Center and all patients consented to the use of their data.

RESULTS

The study population consisted of 20 patients, 4 male and 16 female, with an established diagnosis of PHPT, which persisted for a mean of 5 ± 7 years (range 0-24) after initial parathyroidectomy (PTx). Duration since first presentation with clinical and biochemical features of hyperparathyroidism was 12 ± 9 years (range 2-35) and all patients had had at least 1 revision surgery (range 1-5).

Findings before initial surgery

Nine of the 20 patients (45%) presented with nephrolithiasis, two with height loss because of documented vertebral fractures, two with generalized bone and muscle pain, one with polyuria and polydipsia, one with severe weight loss and in one patient the diagnosis was made during a hospital admission for acute pancreatitis. In the last 4 cases, the diagnosis was established by the incidental finding of hypercalcemia on general medical screening in 3 cases, and in the process of an endocrine work-up for thyroid disease in the last case.

At the time of diagnosis, only 1 of the 20 patients was asymptomatic. Although symptoms related to hypercalcemia such as polyuria, polydipsia and constipation were present in only 5 patients (25%), 47% had non-specific symptoms such as muscle or bone pain and tiredness was reported by 41% of patients (Table 1).

Mean preoperative serum calcium concentration was 2.86 ± 0.21 mmol/L and mean PTH level was 13 ± 6 pmol/L. Renal impairment (creatinine clearance <60 ml/min) was documented in 25% of patients.

At presentation, renal stones were documented on ultrasound of the kidneys in 58% of patients and 29% had osteoporosis on Dual energy X-ray absorptiometry

(DXA). Six of the 20 patients (30%) had parathyroid localisation studies before initial PTx, using a Tc99m-MIBI-SPECT scan and/or an ultrasound scan of the neck.

Eleven of the 20 patients (55%) had initially undergone bilateral neck exploration, 25% had unilateral neck exploration and the type of surgery was not specified in 4 cases. Initial surgery was guided by intra-operative PTH (IOPTH) monitoring in only 2 patients (13%). In 3 of 20 patients (15%) surgery was combined with a hemithyroidectomy, because of pre-operatively identified thyroid pathology in 1 patient and because of a negative bilateral neck exploration which raised suspicion of an intrathyroidal parathyroid gland in the other 2 patients. In both cases an intrathyroidal pathological parathyroid gland was indeed found and removed. In one of the 20 patients (5%) surgery was combined with a total thymectomy, because of a negative bilateral neck exploration with suspicion of an intrathymic parathyroid gland, although no pathological gland was found at this location (Table 1).

A single adenoma was removed at surgery in 9 of 20 patients (45%), an ectopic pathological parathyroid gland in 2 cases, one or more hyperplastic glands were removed in one case and no pathological glands were found in 8 cases.

Surgery was complicated by recurrent laryngeal nerve paralysis in 2 cases, in one of which it was transient, and by cellulitis of the neck in one case.

Findings at revision surgery

Prior to first revision surgery, 6 of the 20 patients (30%) had symptoms of hypercalcemia such as polyuria, polydipsia and constipation, 30% had symptoms of muscle or bone pain and 40% complained of tiredness. Prior to second revision surgery, polyuria and polydipsia was reported by 1 of 8 patients, constipation by 1 of 8 patients, muscle or bone pain by 1 of 8 patients and tiredness by 3 of 8 patients. Renal impairment (creatinine clearance of <60ml/min) was documented in 3 of 15 patients (20%) prior to first revision surgery and in 1 of 7 patients (14%) prior to second revision surgery. Ultrasound of the kidneys was performed in 18 of 20

Table 1. Demographic, operative and pathology data in 20 patients with persistent PHPT following surgery

	Prior to initial PTx (n=20)	At second PTx (n=20)	At third PTx (n=8)
Gender (Men:Women)	4:16	4:16	2:6
Age (years)	52 ± 12 (35-70)	57 ± 11 (36-71)	57 ± 11 (36-72)
Biochemistry prior to initial surgery			
s-calcium (mmol/L)	2.86 ± 0.21	2.73 ± 0.16	2.94 ± 0.29
PTH (pmol/L)	13 ± 6	17 ± 6	18 ± 13
phosphate (mmol/L)	0.77 ± 0.14	0.84 ± 0.14	0.90 ± 0.26
alkaline phosphatase	181 ± 176	98 ± 43	140 ± 79
creatinine clearance (ml/min)	66 ± 15	68 ± 16	75 ± 18
u-calcium (mmol/24 hours)	11.0 ± 6.5	11.2 ± 7.2	14.3 ± 8.1
Clinical presentation			
Polyuria & polydipsia	4/17 (24%)	3/18 (17%)	1/7 (14%)
Constipation	1/17 (6%)	3/18 (17%)	1/7 (14%)
Tiredness	7/17 (41%)	8/18 (44%)	3/7 (43%)
Muscle or bone pain	8/17 (47%)	6/18 (33%)	1/7 (14%)
Complications			
Renal impairment	3/12 (25%)	3/15 (20%)	1/7(14%)
Nephrolithiasis/Nephrocalcinosis	11/19 (58%)	8/18 (44%)	4/7 (57%)
Osteoporosis	2/7 (29%)	5/9 (56%)	3/6 (50%)
Fractures	0/20	1/18 (6%)	0/7
Type of surgery			
Bilateral neck exploration	11/16 (69%)	12/18 (67%)	5/8 (63%)
Unilateral neck exploration	5/16 (31%)	4/18 (22%)	3/8 (38%)
Sternotomy alone or combined	0/16	2/18 (11%)	1/8 (13%)
Combined with thyroidectomy	3/16 (19%)	5/18 (28%)	1/8 (13%)
Combined with thymectomy	1/16 (6%)	2/18 (11%)	3/8 (38%)
Use of IOPTH monitoring	2/16 (13%)	8/18 (44%)	6/8 (75%)
No data available	4//20	2/20	0/8
Pathology			
Adenoma	10/19 (53%)	8/20 (40%)	2/8 (25%)
Hyperplasia	2/19 (11%)	5/20 (25%)	5/8 (63%)
No pathological glands found	7/19 (37%)	7/20 (35%)	1/8(12%)
Outcome of surgery			
Cure	0/20	8/20 (40%)	4/8 (50%)
Persistence	20/20 (100%)	12/20 (60%)	4/8 (50%)

s: serum, u: urine, IOPTH: Intra-operative PTH measurement

patients (90%) before first revision surgery and nephrolithiasis was demonstrated in 8 (44%), all of whom also had evidence for nephrolithiasis before initial surgery. Ultrasound data were available in 7 of 8 patients (88%) before second revision surgery and demonstrated persistent nephrolithiasis in 4 patients (57%). One patient had developed this complication de novo after persistence of PHPT for 30 years after failed revision surgery.

BMD measurements were available in 9 of 20 patients (45%) who underwent first revision surgery, and demonstrated osteoporosis in 5 (55%), osteopenia in 3 (33%) and a normal BMD in 1 case. Prior to second revision surgery BMD was measured in 6 of 8 patients (75%) and demonstrated osteoporosis in 3 (50%) and osteopenia in the other 3 (50%). Only one postmenopausal patient sustained a fracture of the wrist, prior to first revision surgery. None of the patients had sustained a clinical vertebral fracture at any time since diagnosis.

The 20 patients underwent a total of 33 revision surgeries. Twenty of the 33 revision surgeries were bilateral neck explorations (61%), 9 (27%) were unilateral neck explorations, a sternotomy was performed in 2 cases and the type of surgery was not specified in the last 2 cases. Surgery was combined with a hemithyroidectomy in 8 of the 33 cases (24%) and in 6 of 33 cases (18%) with a thymectomy. The decision to perform a hemithyroidectomy was based on the intra-operative finding of an intrathyroidal node in 3 cases, negative bilateral neck exploration with suspicion of an intrathyroidal parathyroid gland in 4 cases, and the incidental finding of a small papillary thyroid carcinoma in one case.

Nineteen of the 33 revision surgeries (58%) were guided by IOPTH measurements. A decrease in PTH levels of more than 50% was observed in all 10 patients who achieved cure after revision surgery, but also in 7 of the 9 patients who had persistence of PHPT after revision surgery. However, the decrease in PTH levels was significantly greater in patients who achieved cure after revision surgery compared to patients with persistence after revision surgery (89 ± 11% *vs.* 63 ± 26%, P=0.003; Figure 1). A decrease in PTH concentration of more than 50% was also reached faster, albeit not significantly, in patients who achieved cure after

revision surgery compared to patients with persistence of HPTH after revision surgery (6 ± 4 minute vs. 10 ± 7 minutes, *P*=0.09).

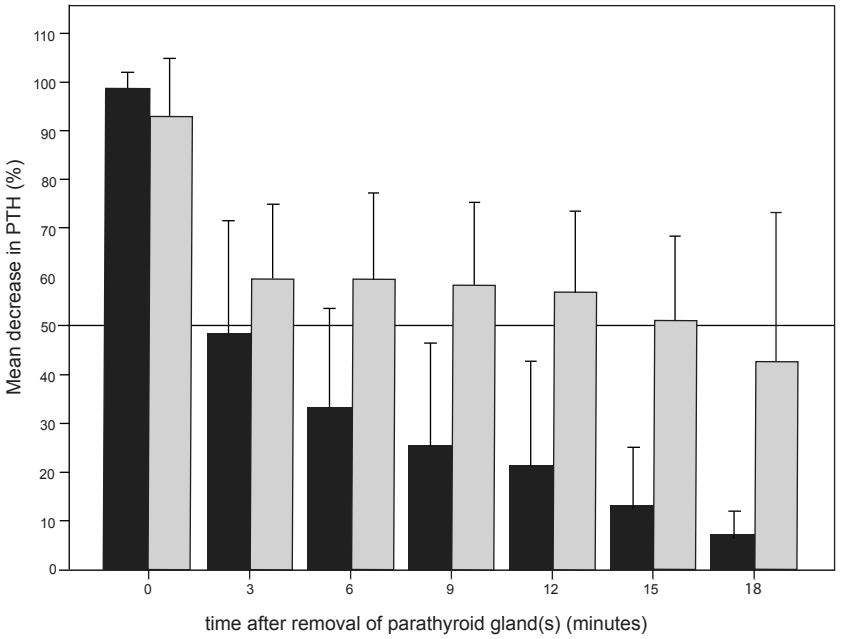


Figure 1: IOPTH measurement during revision surgery in patients with persistent PHPT who were cured post-operatively (black bars) compared to those in whom hyperparathyroidism persisted post-operatively (gray bars).

Four of the 20 first revision surgeries (20%) were associated with complications: fever (n=1), hypoparathyroidism (n=1), infected hematoma (n=1) and recurrent laryngeal nerve paralysis (n=1) (Figure 2). Four of the 8 second revision surgeries (50%) were associated with one or more complications; recurrent laryngeal nerve paralysis (n=1), transient recurrent laryngeal nerve paralysis (n=1), hungry bone syndrome (n=1) and hypoparathyroidism (n=1). Two of 3 third revision surgeries (67%) were complicated by a wound infection. The patient who also underwent fourth and fifth revision surgery had an uncomplicated fourth revision surgery, but the fifth revision surgery was complicated by permanent unilateral recurrent laryngeal nerve paralysis.

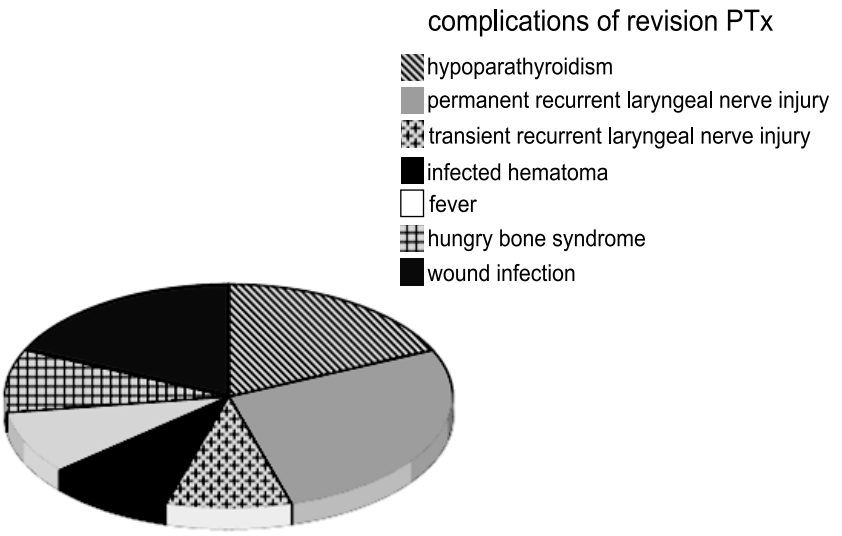


Figure 2: Complications documented after 33 revision surgeries in patients with persistent PHPT.

At first revision surgery a single and first adenoma was removed in 5 cases (25%), a second adenoma in 3 cases (15%), one or more hyperplastic parathyroid glands in 5 cases (25%) and no pathological parathyroid tissue was found in 7 cases (35%) (Figure 3). At second revision surgery a single and first adenoma was removed in 1 case (13%), one or more hyperplastic glands in 5 cases (63%), and seeded cell aggregations from parathyromatosis in 2 cases (25%). At third revision surgery one or more hyperplastic glands were removed in 1 case and cell aggregations from parathyromatosis in 2 cases. One patient also underwent a fourth and fifth revision surgery, during which remnants of hyperplastic autotransplantated parathyroid tissue and cell aggregations from parathyromatosis were respectively excised.

After first revision surgery 8 of 20 patients (40%) were cured and 12 of 20 patients (60%) had persistence of PHPT (Figure 3). Eight of the 12 patients who

were not cured by first revision surgery underwent a second revision surgery. Surgery was not undertaken in the other 4 patients, because of mild asymptomatic hyperparathyroidism in 2 cases, reluctance of 1 patient to undergo further surgery because of recurrent laryngeal nerve paralysis at previous surgery, and in the last patient surgery was not advocated because of 2 negative bilateral neck explorations and persistently negative localisation studies except for SVS, which suggested a mediastinal localisation of hyperactive parathyroid tissue, although this was not confirmed by CT-scan of the region. After second revision surgery 4 of 8 patients were cured (50%) and 4 of 8 patients (50%) had persistence of PHPT. Three of the 4 patients with persistence of PHPT went on to have a third revision surgery exclusively on the basis of a positive PTH sampling, with otherwise negative localisation studies, which failed to result in cure in all 3 cases. Only 1 of these 3 patients underwent a fourth and fifth revision surgery, neither of which again resulted in cure. These last three patients are currently being treated with the calcimimetic cinacalcet and an oral bisphosphonate with reasonable control of serum calcium and PTH concentrations and no further bone loss.

Pathology of excised hyperactive parathyroid tissue in persistent PHPT

At revision surgery, an adenoma was found in 9 of 33 revision surgeries (27%), which was a single adenoma in 6 cases (ectopic n=4, intrathyroidal n=1, normal anatomical location n=1), and a second adenoma in 3 cases (normal anatomical location n=2, intrathyroidal n=1).

A hyperplastic gland was found in 10 of 33 revision surgeries (30%), in the context of a missed multiglandular disease (MGD) in 5 cases, an ectopically located gland in 3 cases and an intrathyroidally located gland in 2 cases. In one patient the autotransplanted parathyroid tissue that was implanted in the sternocleidomastoid muscle had become hyperplastic, also showing invasive growth in the muscle, for which the patient had to undergo 2 explorations to remove all identifiable hyperplastic parathyroid tissue.

Ectopic parathyroid glands (n=7) were found in the thymus (n=3), mediastinum (n=1), caudally in the neck ventral to the trachea (n=1), caudally in the neck near the

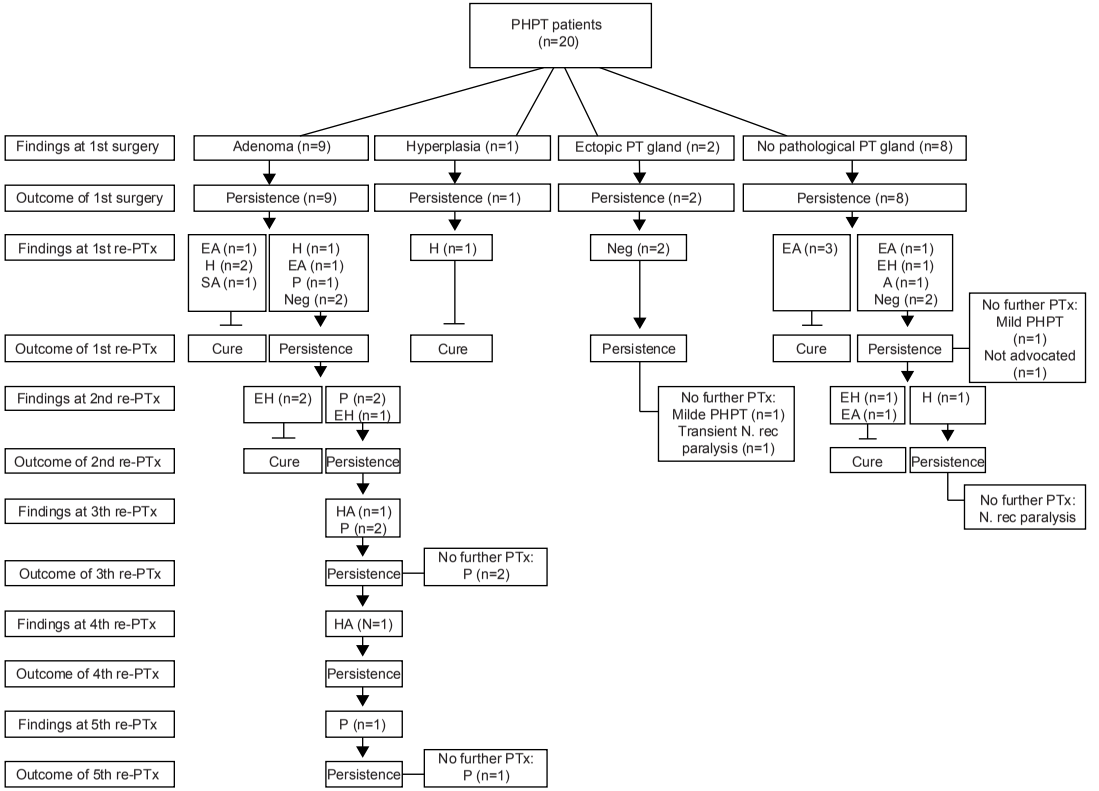


Figure 3: Flowchart of findings at revision surgery and outcome of the surgical intervention in the 20 patients with persistent PHPT after initial surgery.

aortic arch (n=1), and high on the left side of the neck on the prevertebral fascia (n=1).

Spillage of hyperactive parathyroid tissue leading to parathyromatosis occurred during initial surgery in 1 patient, during first revision surgery in 1 patient and during fourth revision surgery in 1 patient. Hyperparathyroidism persisted in these 3 patients despite a total of 6 revision surgeries.

Table 2. Predictive value of localisation studies prior to revision surgery

	Sensitivity	Specificity	PPV	NPV
Tc99m-MIBI-SPECT (*n*=35)	25%	96%	60%	83%
Ultrasound of the neck (*n*=16)	18%		29%	
CT-scan (*n*=11)	30	81%	30%	81%
MRI-scan (*n*=7)	20%	100%	50%	80%
Selective venous sampling for PTH (n=14)	50%	89%	50%	85%

PPV: Positive predictive value, NPV: Negative predictive value

Predictive value of pre-operative localisation studies in persistent PHPT

One or more localisation studies were performed prior to 27 of 33 revision surgeries compared to only 6 of 20 performed prior to initial surgeries. An ultrasound of the neck (US) was performed prior to 6 of 20 initial surgeries (30%) and prior to 10 of 33 revision surgeries (30%). US had a sensitivity of 67% prior to initial surgery and of 18% prior to revision surgery. Tc99m-MIBI-SPECT was performed prior to 5 of 20 initial surgeries (25%) and prior to 26 of 33 revision surgeries (79%). Tc99m-MIBI-SPECT failed to localise hyperactive parathyroid glands in the 5 patients in whom it was performed prior to initial surgery, and had a localising sensitivity of only 25% prior to revision surgery. A CT scan was performed in only 1 of the 20 patients before initial surgery and prior to 10 of 33 revision surgeries (30%), in which case it was mainly performed to confirm localisation suggested by Tc99m-MIBI-SPECT, SVS or both. CT scan had a general sensitivity of 30% (Table 2). An MRI scan was performed before 1 of the 20 initial surgeries and prior to 6 of 33 revision surgeries. MRI scan had a general sensitivity of 20%. Selective venous sampling (SVS) for PTH was not performed before initial surgery but SVS data

were available prior to 15 of the 33 revision surgeries and had a localisation sensitivity of 50% (Table 2).

DISCUSSION

Data from our case series of 20 patients with persistent PHPT suggest that their management is complex and associated with a number of pitfalls. The most common causes of persistent HPTH were a missed ectopic gland at initial surgery (33%) and missed multiglandular disease (15%). The more challenging surgical complication of parathyromatosis was observed in 9% of patients. Pre-operative localisation studies had poor sensitivity: US 18%, Tc99m-MIBI-SPECT 25%, CT 30%, MRI 20%. In contrast, selective venous sampling for PTH had a sensitivity of 50% and a specificity of 89%, although it failed to accurately localise hyperactive tissue in 43% of patients. The decrease in intraoperative PTH was significantly less marked in patients in whom hyperparathyroidism persisted compared to those who achieved cure (63 ± 26 vs. 89 ± 11%, P=0.003). The risk of complications, particularly that of recurrent laryngeal nerve palsy, increased with each subsequent surgery: 20% after first, 50% after 2nd and 67% after 3rd surgery.

In primary hyperparathyroidism, surgery is the treatment of choice resulting in cure in the vast majority of patients. We have previously demonstrated that the most commonly encountered sporadic hyperparathyroidism does not recur if cure is established 6 months after parathyroidectomy (4). The prevalence of persistent hyperparathyroidism is thus low, with only a small number of patients demonstrating persistence of clinical and biochemical features of hyperparathyroidism after initial surgery.

The main challenge in the management of persistent PHPT lies in the decreasing value of localisation studies after initial and each subsequent revision surgery. Tc99m-MIBI-SPECT has a lower predictive value in patients with persistent PHPT, likely to be due to disturbance in local vascular supply by previous surgery, but also due to differences in gland pathology and size in persistent hyperparathyroidism, ultimately affecting radiopharmaceutical uptake. It is of interest that Tc99m-MIBI-

SPECT performed prior to initial surgery failed to identify hyperactive parathyroid glands in 5 patients in whom hyperparathyroidism subsequently persisted, although this observation could not be confirmed in the remaining 15 patients with persistent PHPT, who did not have this localisation study before initial surgery. Although SVS is positive in all patients with persistent PHPT, this localisation study cannot accurately localise hyperactive parathyroid glands, as it reflects drainage of the hyperactive parathyroid gland rather than its actual anatomical localisation, and drainage pattern is further confounded by disturbance in local vascular supply as a result of previous surgery.

Although pre-operative localisation studies are important to guide the surgeon and decrease operating time, knowledge of anatomy and embryology of the parathyroid glands is also important to successfully locate previously missed hyperactive parathyroid glands. This is particularly so as ectopic parathyroid glands are found in 23-77% of cases of persistent hyperparathyroidism (14,22-27). In keeping with previous observations (22-27), our case series confirms that the thymus is one of the most frequent sites of ectopic parathyroid glands (43%), followed by the mediastinum (14%), ventral to the trachea (14%) and on the prevertebral fascia (14%). Other locations of hyperactive parathyroid glands are the tracheoesophageal groove (2.6-33%), para- or retroesophageal area (1.8-21.4%), carotid sheath (0.6-14.3%), carotid bifurcation (8.3%), aortopulmonary window (1.3-6.3%), and parapharyngeal area (0.6-8.4%) (22-27).

Data on the use of IOPTH measurement during revision surgery for persistent PHPT are somewhat conflicting. Some studies thus report a sensitivity of 94-100% (24,26,28), whereas data have also been reported on misleading decreases in PTH levels of 50-60% during IOPTH measurements (25). Although we show that 78% of patients with persistent PHPT have a positive IOPTH measurement at revision surgery according to the “Miami criteria” (a drop in PTH levels of more than 50%) (29,30), we and others (25,28) demonstrate that patients who achieve cure after revision surgery have a greater and more rapid decrease in PTH levels during IOPTH measurement compared to patients with persistent PHPT after revision surgery. These findings suggest that in patients with persistent PHPT, IOPTH

measurements may be more valuable in predicting cure when stricter cut-off values than the ones suggested by the "Miami criteria" are used.

In keeping with previous reports (22-24,26-28), the overall complication rate at first revision surgery was 20% in our case series. As expected, the overall complication rate significantly increased at second and third revision surgery (50 and 67%, respectively). The most common complications reported by us and others are hypoparathyroidism (2.6-12.9%) and recurrent laryngeal nerve injury leading to transient (2.3-13.5%) or permanent paralysis (0.08-25%) (22-28).

A rare but challenging complication of parathyroid surgery is the development of parathyromatosis, which is characterized by intraoperative rupture of a hyperactive parathyroid gland which leads to seeding of parathyroid tissue in and around the site from which the ruptured gland is removed. In contrast to previously published data (11), we observe in our case series that parathyromatosis leading to persistent hyperparathyroidism occurred in only 1 case during initial surgery and in 2 cases during revision surgery. In these cases, cure could not be achieved by surgery, despite repeated removal of small aggregates of hyperactive parathyroid tissue. In these cases patients have been shown to benefit from treatment with the calcimimetic cinacalcet, with reasonable control of the biochemical features and complications of hyperparathyroidism. Long-term studies are required to establish the efficacy and safety of these agents in recalcitrant persistent hyperparathyroidism.

Our findings from this case series indicate that the management of patients with persistent PHPT is complex and challenging. When initial surgery fails to achieve cure, each reoperation jeopardizes the ability of localisation studies to accurately localise residual hyperactive parathyroid glands and increases the risk of complications in its own right because of distortion and scarring of surgical planes as a result of previous interventions. In these patients, re-operation should be carefully planned and attempts at surgery strongly discouraged if a clear localisation of hyperactive tissue is not secured pre-operatively. The availability of calcimimetics allows a more conservative approach in the management of these patients. This is particularly if parathyromatosis is strongly suspected, and at least

until such time as the hyperactive tissue may have grown sufficiently to be adequately localised, which may take several years, as parathyroid cell growth and proliferation is slow. Whether calcimimetic agents, which have been shown to suppress proliferation of parathyroid cells *in vitro* and in animal studies (31-34), may have a similar effect in patients with persistent PHPT remains to be established. Further studies using cinacalcet in the long-term are required in patients with persistent hyperparathyroidism to explore this issue.

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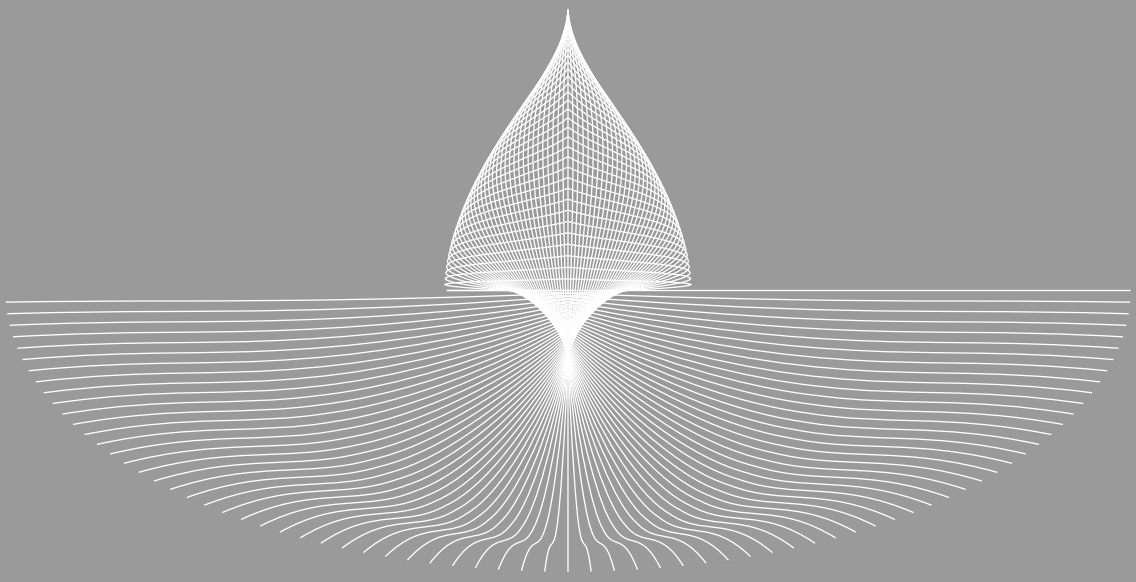
11

General Discussion



CONTENTS

I. Introduction

Primary hyperparathyroidism (PHTP) is the most common cause of hypercalcaemia in the outpatient setting. Over the past few decades, the clinical profile of PHPT has drastically changed from “bones, stones, groans and psychic moans" to an almost asymptomatic disease. Progress in pre-operative localisation studies, surgical techniques and intra-operative PTH (IOPTH) monitoring has increased cure rates following initial surgery for PHPT to 93-100% (1-3). Despite the progress in initial surgery for PHPT, management of persistent and recurrent primary hyperparathyroidism remains a challenge.

In this thesis we report the incidence of persistent PHPT after initial surgery for primary hyperparathyroidism in our hospital over a period of 24 year. We describe the demographic, clinical, biochemical, surgical and pathological characteristics of patients with persistent PHPT after initial parathyroidectomy for sporadic PHPT. In addition, we evaluate the value of pre-operative localisation studies prior to revision surgery and the skeletal and post-operative complications of chronic high circulating levels of PTH. Furthermore we discuss the management of recurrent hyperparathyroidism in parathyroid carcinoma, and the effect of molecular alterations in the *HRPT2* and *CASR* gene on prognosis in these patients.

II. Cure after parathyroidectomy for primary hyperparathyroidism

In primary hyperparathyroidism, surgical removal of all pathological parathyroid tissue is the only approach that provides a definitive and durable cure. Patients who are cured after surgery show a significant postoperative increase in bone mass (4-6), no recurrence of renal stones (4,7) and improvement in various parameters of quality of life compared to patients who had no surgery or unsuccessful surgery (8-12). Cure rate has been reported to be in the range of 94-100% for bilateral neck, as well as less invasive surgery, more than 6 months after parathyroidectomy (PTx) (13-22). Based on these findings, it has become common practice to limit the post-operative follow-up to 6 months. However, in a 5 year follow-up of 91 patients who were successfully operated for PHPT, Westerdahl *et al.* reported a rather high recurrence rate of 4%, which was largely due to previously undiagnosed germline mutations in the *MEN-1* gene and consequent multiple gland pathology (23). In ***Chapter 2*** we set out to evaluate cure rate and its maintenance in the long-term after initial parathyroidectomy in patients with sporadic PHPT who underwent surgery in our hospital in the period between 1984 and 2008. We evaluated the cure at short-term (3 and 6 months) and long-term follow-up (range 1-24 years) in 73 patients with sporadic PHPT. In the process, we also assessed the value of various factors in the prediction of cure in patients with sporadic PHPT.

At the time of first follow-up (within 3 months of surgery), 70 of the 73 patients (96%) had evidence for biochemical cure of hyperparathyroidism (HPTH). At the time of the second post-operative follow-up (6 months after PTx) and the long-term follow-up (range 1-24 years), 68 of the 73 patients (93%) had evidence for biochemical cure. There were no significant differences in gender, age at presentation, clinical presentation, biochemical and pathological findings between patients who achieved and maintained cure and patients with persistence of HPTH after initial PTx. It was of note, that all 5 patients with persistent HPTH had undergone surgery before 2001 and that with the implementation of pre-operative localisation studies and intra-operative PTH (IOPTH) monitoring in the standard care of patients with PHPT, cure rate has increased to 100% since 2001.

Our study provides evidence that 'recurrent' hyperparathyroidism does not occur in sporadic PHPT when cure is established 6 months after parathyroidectomy. This strongly suggests that long-term follow-up of these patients is not necessary. In contrast, close follow-up is advocated within the first 6 months after PTx to definitively establish cure and to enable the identification of those with residual gland pathology and thus persistent hyperparathyroidism.

III. Localisation studies in persistent primary hyperparathyroidism

In ***Chapter 2*** we demonstrated that the use of pre-operative localisation studies increased the cure rate in patients with sporadic PHPT who underwent initial PTx. Before initial bilateral neck surgery preoperative localisation studies are not deemed necessary, because experienced surgeons have, on average, a 98% chance of excising all pathological tissue (22,24). However, localisation studies become mandatory when the surgeon opts for a more focused unilateral or minimally invasive approach and before reoperative parathyroidectomy for persistent hyperparathyroidism, as a second (or more) neck exploration is technically more challenging than initial surgery and may be associated with as much as a threefold increase in morbidity (22,25-27). Limited data suggest that the localizing value of Tc99m-MIBI-SPECT is much lower for the preoperative localisation of residual active parathyroid glands before reoperative parathyroidectomy (28-30).

In ***Chapter 3*** we evaluated the predictive value of Tc99m-MIBI-SPECT in patients with persistent PHPT prior to revision surgery and compared this to the predictive value of this localisation study in patients with sporadic PHPT prior to initial surgery.

Tc99m-MIBI-SPECT was able to detect and accurately localize 61% of the pathological glands removed at initial surgery in patients with sporadic PHPT and 33% of the pathological glands removed at reoperative parathyroidectomy in patients with persistent PHPT. Compared to patients with sporadic PHPT cured after initial surgery, patients with persistent PHPT had a higher proportion of

hyperplastic glands (55% vs. 26%), more ectopically located glands (44% vs. 22%), and smaller pathological gland size (1.21 cm vs. 2.03 cm). We demonstrate that the ability of Tc99m-MIBI-SPECT to detect and accurately localize pathological parathyroid glands was decreased in the case of hyperplasia and a gland diameter <1.5 cm, explaining the decreased diagnostic value of Tc99m-MIBI-SPECT observed in patients with persistent PHPT.

The combined effect of disturbance in the local vascular supply by previous surgery, as well as differences in gland pathology and size ultimately affecting radiopharmaceutical uptake, are likely to lead to the limited ability of the widely used Tc99m-MIBI-SPECT to detect and accurately localize pathological parathyroid glands in patients with persistent hyperparathyroidism before reoperative parathyroidectomy.

Because of the limited diagnostic value of Tc99m-MIBI-SPECT prior to revision surgery for persistent PHPT, we assessed in ***Chapter 4*** whether selective venous sampling (SVS) of parathyroid hormone (PTH) could contribute to a more accurate preoperative localisation of residual hyperactive parathyroid tissue in patients with persistent hyperparathyroidism. The diagnostic value of SVS is based on the assumption that regional drainage of each one of the four parathyroid glands is into the adjacent superior, middle and inferior thyroid veins, respectively (31). Despite potential anatomical variations, SVS is successful in predicting the side of a pathological parathyroid gland in 39-93% of patients with PHPT (31-41) and, more importantly, in 66-75% of patients with negative noninvasive studies (36,37,42). However, the major limitation of SVS is that it pinpoints the area of venous drainage of a hyperactive gland rather than its exact anatomical location.

We evaluated the results of Tc99m-MIBI-SPECT and SVS performed prior to 20 revision surgeries in 18 patients with persistent or recurrent PHPT or with autonomous tertiary hyperparathyroidism (THPT) due to end-stage renal failure. Tc99m-MIBI-SPECT was found to have a sensitivity of 30% compared to a sensitivity of 75% with SVS. SVS was found to accurately localize 10 of the 14 pathological glands (71%) which had been inaccurately localized or completely

missed by Tc99m-MIBI-SPECT, while Tc99m-MIBI-SPECT was only able to localise 1 of the 6 pathological glands (17%) inaccurately localised by SVS. Localising sensitivity was 100% when concordance was achieved between SVS and Tc99m-MIBI-SPECT. In keeping with Tc99m-MIBI-SPECT, the diagnostic value of SVS was also decreased, although to a lesser extent, in the case of hyperplastic parathyroid glands and glands with a diameter of less than 1.5 cm. In contrast to Tc99m-MIBI-SPECT, SVS had a sensitivity of 83% for ectopic parathyroid glands, which are a frequent cause of persistent PHPT. This study also demonstrates that the ability of selective venous sampling for PTH to accurately localize residual hyperactive parathyroid glands in patients with persistent PHPT is significantly higher than that of the non-invasive Tc99m-MIBI-SPECT imaging technique.

These data hold implications for the management of patients with persistent PHPT. From a practical point of view, and in keeping with recent guidelines of the European Association of Nuclear Medicine (43), our data suggest that it is always worth to perform a Tc99m-MIBI-SPECT scan as a first preoperative localisation study in patients with persistent hyperparathyroidism, followed by the invasive, but more reliable, selective venous sampling for PTH technique. Concordance of both techniques leads to a reassuring sensitivity of 100%. Our data clearly suggest that SVS for PTH should be reinstated as a valuable tool in the armamentarium of localisation studies in the pre-operative work-up of patients with persistent hyperparathyroidism.

IV. Complications of the surgical management of primary hyperparathyroidism

High circulating levels of PTH are associated with increased bone turnover in favour of bone resorption leading to mineral depletion and decreased bone mass (44). This effect is achieved by the binding of PTH to its specific receptor (PTHR1) on stromal/osteoblastic cells of the bone marrow, which stimulates the production of RANK ligand and decreases that of its decoy receptor osteoprotegerin (OPG) (45-59). Following successful surgery, the rapid decrease in serum PTH levels and

the pre-operative high bone turnover state, lead to a transient hypocalcaemia, due to increased influx of calcium, phosphate and magnesium into bone. Post-operative, severe and persistent hypocalcaemia is usually due to a "hungry bone syndrome", a syndrome seldom seen in patients with sporadic PHPT. The syndrome is predominantly observed in patients with severe hyperparathyroidism-associated increase in bone turnover, particularly in patients with parathyroid carcinoma. In ***Chapter 5***, we performed a systemic review of the literature on hungry bone syndrome, summarizing its main features and suggestions for its prevention and management.

Although exact numbers are missing, the number of patients who develop hungry bone syndrome is presumed to have largely decreased due to a considerable decrease in the number of patients with clinically evident bone disease, such as osteitis fibrosa cystica, due to the earlier detection of mostly asymptomatic PHPT by routine calcium screening (60,61). An older age at diagnosis, radiological evidence of PHPT-related bone disease, and greater weight/volume of the resected parathyroid glands have been reported to be risk factors associated with the development of a post-operative hungry bone syndrome. There are scarce data on the best means to treat, minimize or prevent this severe complication of parathyroidectomy. Treatment of the hungry bone syndrome is aimed in the short term primarily at replenishing the circulating calcium deficit caused by the increased calcium utilisation to refill the multiple resorption cavities. In the longer term, treatment is aimed at restoring calcium homeostasis by substituting the temporarily missing stimulatory effect of PTH on the 1α-hydroxylase enzyme by providing the active form of vitamin D to ensure adequate intestinal absorption of calcium (61-66). Pre-operative normalisation of bone turnover with the use of bisphosphonates and pre-operative $1,25(OH)_2D$ supplementation are useful options to prevent the hungry bone syndrome, but there are no available prospective data to confirm this premise (60,67-72).

V. Aspects of osteocyte function in primary hyperparathyroidism

The PTH/PTHrP receptor (PTHR1) is also present on osteocytes (73). Recent *in vitro* and animal studies suggest that binding of PTH to the PTHR1 on osteocytes leads to inhibition of the expression of the *SOST* gene (74-77). This gene encodes sclerostin, a protein exclusively expressed in osteocytes in the skeleton (78), which decreases bone formation by binding to LRP5/6, resulting in inhibition of the Wnt signaling pathway in osteoblasts (79,80). To evaluate whether chronic PTH excess has similar effects on sclerostin secretion in humans as in animal models, we tested the hypothesis that chronic hypersecretion of PTH, as seen in PHPT, may decrease sclerostin secretion, and that PTH may thus represent a potential regulator of sclerostin production in humans in ***Chapter 6***.

To this effect, we measured sclerostin in serum of 34 patients with untreated, persistent, or recurrent PHPT and in 54 patients cured after successful parathyroidectomy for PHPT (EuPTH). Mean serum sclerostin level of patients with PHPT (30.5 pg/ml, 95% CI: 26.0-35.1) was significantly lower than that of patients with EuPTH (45.4 pg/ml, 95% CI: 40.5-50.2; $P<0.001$). There was a negative correlation between serum sclerostin and PTH concentrations when all patients were pooled together ($r= -0.44$, $P<0.001$).

Data from our study demonstrate that in humans, chronic PTH excess, as observed in patients with PHPT, is associated with a significant decrease in circulating sclerostin levels and that there is a significant negative correlation between PTH and serum sclerostin levels. Taken together, these results suggest that, similar to the case in animal models (74-77), PTH has a regulatory role on sclerostin production also in humans. The functional significance of circulating sclerostin is as yet to be established.

Recent *in vitro* studies have also shown that binding of PTH to the PTHR1 on osteocytes upregulates Fibroblast Growth Factor 23 (FGF23) mRNA expression (81,82). FGF23 is believed to be the major player in the bone-kidney axis controlling phosphate homeostasis. FGF23 acts as a phosphaturic factor by the

same mechanism of action as PTH, by down regulating the cotransporters NaPi2a and NaPi2c in the proximal tubules of the kidney after binding to its receptor, FGFR-1, in the presence of Klotho (83,84). FGF23 also decreases $1,25(OH)_2D$ synthesis in the proximal tubules by direct inhibition of the 1α-hydroxylase enzyme (83-85). In patients with PHPT, data on the relationship between PTH and FGF23 are scarce and not always concordant. Compared to healthy controls, circulating FGF23 levels have been found to be elevated in patients with PHPT before parathyroidectomy (86,87) and to decrease immediately post-operatively (87), supporting the notion that PTH stimulates FGF23 secretion. The aim of the study described in ***Chapter 7*** was to address the relationship between PTH and FGF23 in patients with PHPT and in patients cured after successful parathyroidectomy for PHPT.

We measured serum intact FGF23 in 22 patients with untreated, persistent, or recurrent PHPT and in 24 patients cured after successful parathyroidectomy for PHPT (EuPTH). Mean serum FGF23 concentration was significantly higher in patients with PHPT than in EuPTH patients (50.4 ± 27.2 pg/ml vs. 33.1 ± 12.5 pg/ml, P=0.01). There was a positive correlation between PTH and FGF23 levels (r=0.362, P=0.01), and this relationship was sustained and more pronounced after correction for $1,25(OH)_2D$ levels (r=0.422, P=0.01). In patients with PHPT, there was a significant negative correlation between FGF23 and $1,25(OH)_2D$ (r= −0.780, P<0.01). This relationship remained significant albeit less marked in EuPTH patients (r= −0.519, P=0.02).

In conclusion, we demonstrate that FGF23 production is increased in the presence of high circulating PTH levels and that this increase is reversible after the euparathyroid state is achieved following successful PTx. We further demonstrate a significant negative relationship between FGF23 and $1,25(OH)_2D$ levels, which is more pronounced in patients with PHPT. Based on the more pronounced negative relationship between FGF23 and $1,25(OH)_2D$ in PHPT patients, we propose that the PTH-induced increase in FGF23 levels may be an adaptive mechanism to maintain serum phosphate levels within normal limits by counteracting the potentially deleterious $1,25(OH)_2D$-induced phosphate retention.

The findings that PTH inhibits sclerostin production and stimulates FGF23 production demonstrate that the actions of PTH on bone are more complex than previously suggested, involving a variety of signaling pathways within bone itself, but also in the parathyroid-bone-kidney axis. Despite the significant progress in our understanding of the actions of PTH on bone, it should be appreciated that the cellular and molecular actions of PTH have only been partially unraveled and studies are needed to further elucidate these actions.

VI. Clinical and molecular aspects of parathyroid carcinoma

Surgical and medical advances have secured longer survival in patients with parathyroid carcinoma. To illustrate this point, we report in ***Chapter 8*** the case of a patient with metastatic parathyroid carcinoma with a follow up spanning 17 years. We also review all original case reports and case series on patients with parathyroid carcinoma, who fulfill the WHO criteria for this malignancy who had been followed-up for at least 6 months after initial parathyroidectomy, which were published in the English literature since the last large review on this topic in 2001 (88).

Data from our case are in keeping with those obtained from our review of the literature on this topic, showing that radical initial surgery is of paramount importance to prevent local seeding of tumor tissue, thereby not only increasing disease-free survival and survival, but also decreasing the chance of having to undergo re-operative surgery. Invasive growth of tumor tissue in adjacent structures, such as the recurrent laryngeal nerve or esophagus, increases the morbidity associated with re-operative surgery in patients with parathyroid carcinoma. The increasing morbidity associated with repeated surgical interventions has led to the search for other treatment options for this malignancy, such as radiotherapy, chemotherapy, radiofrequency (RF) ablation, embolization, use of the calcimimetic Cinacalcet and that of PTH immunisation. Parathyroid tumors are relatively resistant to chemotherapy and radiotherapy (88-93) and the use of both therapies has significantly decreased over the last 10 years. Successful embolization

has only been described in combination with RF ablation (94) and was unsuccessful in our patient, while RF ablation has been shown to be successful in 3 cases (95-97) as well as in our patient. The eventual inability to control hypercalcaemia is associated with increased bone turnover with an increased risk of fractures and with fatal renal and cardiovascular complications (89,90,98,99). When attempts at reducing tumor load are not successful, intensive rehydration, use of medications such as bisphosphonates and calcimimetics and use of dialysis are used with variable results. A potential non-invasive option is PTH immunisation, although experience with this approach is still limited (100-102).

In conclusion, the long-term management of patients with metastatic parathyroid carcinoma remains indeed a daunting task, despite all recent imaging, surgical and medical advances.

Several genes have been discovered to play a role in the etiology of PHPT among which are the *MEN-1*, *HRPT2/CDC73* and *CASR* genes. Mutations in the *HRPT2/CDC73* gene, which encodes for the protein parafibromin (103), are associated with the hyperparathyroidism-jaw tumor syndrome (HPT-JT; OMIM 607393). Patients with a HPT-JT syndrome have a 15-24% chance of developing parathyroid carcinoma (88,104-106). In patients with the HPT-JT syndrome, as well as patients with sporadic carcinoma, downregulation of the calcium sensing receptor (CASR) was also discovered (107). Moreover, downregulation of CASR expression, mutations in the *HRPT2/CDC73* gene and loss of its protein, parafibromin, have been shown to be valuable markers to assist in establishing a diagnosis of parathyroid carcinoma. There are, however, no available data on the prognostic value of these markers in parathyroid carcinoma. In ***Chapter 9*** we evaluated the prognostic value of downregulation of CASR expression, loss of parafibromin staining and the presence of mutations in the *HRPT2/CDC73* gene in patients with a WHO criteria based diagnosis of parathyroid carcinoma.

We determined the disease-free survival and overall survival in 23 patients who had initial surgery for parathyroid carcinoma in various hospitals in the Netherlands in the period 1985-2000. Resected primary parathyroid carcinomas of all 23 patients

had previously undergone *HRPT2/CDC73* and *MEN-1* gene mutations analysis (108) and parafibromin and CASR staining analysis (104,109).

The 5-year disease-free survival and the 5-year overall survival were lower in patients with downregulation of CASR expression compared to patients with normal CASR expression (81% vs. 0% and 94% vs. 29%, respectively). Both the 5-year disease-free survival (73% vs. zero) and the 5-year overall survival (79% vs. 50%) were lower in patients with a *HRPT2/CDC73* mutation compared to patients without this mutation. Both the 5-year disease-free survival (89% vs. 41%) and the 5-year overall survival (100% vs. 57%*)* were lower in patients with global parafibromin loss compared to patients with focal parafibromin loss. The risk of developing a local or distant metastasis was 16-fold increased in the case of downregulation of CASR expression, 7-fold increased in the case of *HRPT2/CDC73* mutations and also 7-fold increased in the case of global parafibromin loss. The 5-year disease-free survival and the 5-year overall survival were lower in patients with somatic *MEN-1* mutations compared to patients without this mutation (respectively 64% vs. 33% and 80% vs. 33%), but somatic *MEN-1* mutations were not associated with a significant increased risk of developing a local recurrence and/or distant metastasis.

Findings from this study hold several clinical implications. Firstly, downregulation of CASR, *HRPT2/CDC73* mutations and global loss of parafibromin are strong negative determinants of the malignant potential of parathyroid carcinomas. Secondly, the finding of somatic *MEN-1* mutations in patients with parathyroid carcinoma, suggests that these mutations could play a role in parathyroid carcinogenesis, although they do not influence the prognosis of parathyroid carcinoma. Thirdly, evaluation of CASR expression may also serve to determine whether calcimimetics may play a role in the therapeutic management of patients with parathyroid carcinoma.

On the basis of the findings described in ***Chapter 9***, we advocate the use of the assessment of downregulation of CASR, *HRPT2/CDC73* mutations and global loss of parafibromin not only as diagnostic tools but also as prognostic tools in the management of patients with parathyroid carcinoma.

VII. Management of patients with persistent primary hyperparathyroidism

The management of patients with persistent sporadic PHPT represents a significant clinical challenge, which requires a multidisciplinary approach. In ***Chapter 10*** we report in a case serie, the demographic, clinical, biochemical, surgical and pathological characteristics of patients with persistent PHPT after initial parathyroidectomy for sporadic PHPT.

Our data suggest that the most common causes of persistent hyperparathyroidism are a missed ectopic gland at initial surgery (33%) and missed multiglandular disease (15%). We documented parathyromatosis in 9% of patients. Pre-operative localisation studies had poor sensitivity: ultrasound 18%, Tc99m-MIBI-SPECT 25%, CT 30%, MRI 20%. In contrast, selective venous sampling for PTH had a sensitivity of 50% and a specificity of 89%. The decrease in intraoperative PTH was significantly less marked in patients in whom hyperparathyroidism persisted compared to those who achieved cure (63 ± 26 vs. 89 ± 11%, P=0.003). The risk of complications increased with each subsequent surgery: 20% after first, 50% after 2nd and 67% after 3rd surgery.

Our findings from this case series indicate that the management of patients with persistent PHPT is complex and challenging. When initial surgery fails to achieve cure, each reoperation jeopardizes the ability of localisation studies to accurately localise residual hyperactive parathyroid glands and increases the risk of complications in its own right because of distortion and scarring of surgical planes as a result of previous interventions. In these patients, re-operation should be carefully planned and attempts at surgery strongly discouraged if a clear localisation of hyperactive tissue is not secured pre-operatively.

VIII. Summary

- In patients with sporadic PHPT, the rate of persistence after initial PTx is 7% and that of recurrence none.

- In the case of persistent PHPT, the sensitivity of the widely used, non-invasive Tc99m-MIBI-SPECT imaging technique is decreased and is significantly lower than that of the invasive technique of selective venous sampling for PTH.
- Chronic excess of PTH has a catabolic effect on bone, leading to mineral depletion of bone. Patients with pre-operative radiological signs of severe PTH-associated bone disease are at risk of developing hungry bone syndrome after surgery, which may be prevented by pre-operative treatment with bisphosphonates and 1,25(OH)2D.
- PTH inhibits sclerostin production and stimulates FGF23 production, presumably to counterbalance its own actions on bone and on 1,25(OH)2D, resulting in novel feedback loops.
- In contrast to sporadic PHPT, recurrent PHPT does occur in patients with parathyroid carcinoma. In these patients, downregulation of CASR, *HRPT2/CDC73* mutations and global loss of parafibromin are strong negative determinants of the disease-free survival and overall survival.
- Recent progresses in surgical and medical treatment of patients with parathyroid carcinoma have made it possible to secure longer survival, even in patients with tumors demonstrating 2 of the 3 identified molecular negative prognostic factors.

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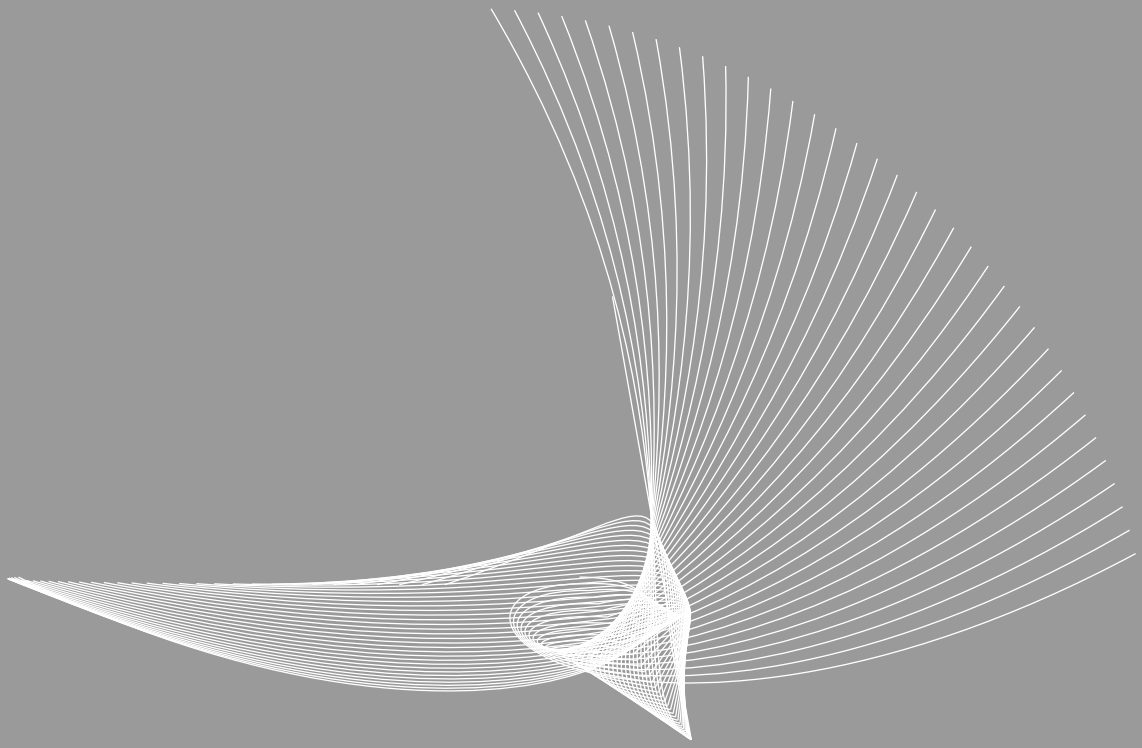
12

Nederlandse samenvatting



INHOUDSOPGAVE

I. Introductie

Primaire hyperparathyreoïdie (PHPT) is de meest voorkomende oorzaak van hypercalciëmie in de algemene bevolking. De klinische presentatie van PHPT is over de laatste decennia drastisch veranderd, van een symptomatische ziekte met verminderde botdichtheid, verhoogde kans op fracturen, nierstenen, en psychische symptomen, naar een bijna asymptomatische ziekte. Vooruitgang in pre-operatieve lokalisatie studies, chirurgische technieken, en intra-operatieve PTH (IOPTH) metingen hebben er voor gezorgd dat de genezingspercentages voor initiële operatie voor PHPT gestegen zijn naar 93-100% (1-3). Ondanks deze vorderingen in initiële behandeling van PHPT, blijft de behandeling van persisterende en recidiverende primaire hyperparathyreoïdie een uitdagende taak.

In dit proefschrift rapporteren we de incidentie van persisterende PHPT na initiële operatie voor PHPT in ons ziekenhuis over een periode van 24 jaar. We beschrijven de demografische, klinische, biochemische, chirurgische en pathologische karakteristieken van patiënten met persisterende PHPT na initiële operatie voor sporadische PHPT. Tevens evalueren we de waarde van pre-operatieve lokalisatie studies voor revisie chirurgie en de skelet en post-operatieve complicaties van chronisch verhoogde concentraties van serum PTH. Verder bespreken we de behandeling van recidiverende hyperparathyreoïdie in patiënten

met bijschildklier carcinoom, en het effect van moleculaire veranderingen in het *HRPT2* en *CASR* gen op de prognose van deze patiënten.

II. Genezing na parathyreoïdectomie voor primaire hyperparathyreoïdie

Chirurgische verwijdering van alle pathologische bijschildklieren is de enige behandeling om patiënten met primaire hyperparathyreoïdie definitief te genezen. Na een succesvolle operatie is er een significante post-operatieve stijging in botmassa (4-6), ontstaan er geen nieuwe nierstenen (4,7) en verbeteren verschillende parameters van kwaliteit van leven (8-12). Genezingspercentages van 93-100% zijn gerapporteerd voor bilaterale halsoperaties en minder invasieve operaties, meer dan 6 maanden na parathyreoïdectomie (PTx) (13-22). Op basis van deze bevindingen is het gangbaar geworden om patiënten te controleren tot 6 maanden na de operatie. Echter, tijdens een 5 jarige controle periode van 91 patiënten die succesvol geopereerd waren voor PHPT, rapporteerde Westerdahl *et al*. een recidief percentage van 4%, wat voornamelijk bleek te berusten op niet eerder gediagnosticeerde kiembaan mutaties in het *MEN-1* gen (23). In ***Hoofdstuk 2*** hebben we daarom gekeken naar het genezingspercentage na PTx op de korte (3 en 6 maanden) en lange termijn (1-24 jaar) in 73 patiënten met sporadische PHPT die een initiële operatie ondergingen tussen 1984 en 2008. Tevens hebben we gekeken naar voorspellende factoren voor genezing in patiënten met sporadische PHPT.

Bij de eerste controle (binnen 3 maanden na operatie) waren 70 van de 73 patiënten (96%) genezen. Bij de 2e controle (6 maanden na operatie) en bij de lange termijncontrole (1-24 jaar) waren 68 van de 73 patiënten (93%) genezen. Er waren geen significante verschillen in geslacht, leeftijd bij presentatie, klinische presentatie, biochemische en pathologische bevindingen tussen patiënten die genezen zijn en diegene met persisterende hyperparathyreoïdie (HPTH) na een initiële operatie. Het was opmerkelijk dat alle 5 patiënten met persisterende HPTH een operatie hadden ondergaan voor 2001 en dat na de implementatie van pre-operatieve lokalisatie studies en intra-operatieve PTH (IOPTH) bepaling als standaard zorg in 2001 het genezingspercentage gestegen is naar 100%.

Onze studie laat zien dat 'recidiverende' hyperparathyreoïdie niet voorkomt in sporadische PHPT indien genezing vastgesteld is 6 maanden na PTx. Dit suggereert dat lange termijncontrole niet nodig is in deze patiënten groep. De eerste 6 maanden na PTx is nauwlettende controle wel sterk aanbevolen om definitieve genezing vast te stellen en diegene met achtergebleven klier pathologie in een vroeg stadium te kunnen identificeren.

III. Lokalisatie studies in persisterende primaire hyperparathyreoïdie

In ***Hoofdstuk 2*** hebben we laten zien dat het genezingspercentage in patiënten met sporadische PHPT die PTx ondergingen, steeg door het gebruik van pre-operatieve lokalisatie studies. Pre-operatieve lokalisatie studies worden niet nodig geacht voor initiële bilaterale nek operaties, omdat een ervaren chirurg in gemiddeld 98% van de gevallen al het pathologische weefsel verwijdert (22,24). Lokalisatie studies zijn wel nodig wanneer de chirurg een unilaterale of minimaal invasieve aanpak kiest en voorafgaand aan re-operatieve PTx voor persisterende HPTH, aangezien een tweede (of volgende) nek exploratie technisch ingewikkelder is dan een initiële en geassocieerd is met een soms drievoudige stijging in morbiditeit (22,25-27). Beperkte data suggereren dat de lokalisatie waarde van Tc99m-MIBI-SPECT lager is voorafgaand aan re-operatieve PTx dan voorafgaand aan initiële PTx (28-30).

In ***Hoofdstuk 3*** hebben we de voorspellende waarde van Tc99m-MIBI-SPECT in patiënten met persisterende PHPT voorafgaand aan revisie operatie beoordeeld en vergeleken met de voorspellende waarde in patiënten met sporadische PHPT voorafgaand aan initiële operatie.

Tc99m-MIBI-SPECT was in staat 61% van de pathologische bijschildklieren die verwijderd werden tijdens initiële operatie in patiënten met sporadische PHPT accuraat te lokaliseren en 33% van de pathologische bijschildklieren die verwijderd werden tijdens re-operatieve PTx in patiënten met persisterende PHPT. Patiënten met persisterende PHPT hadden vaker hyperplastische klieren (55% vs. 26%), meer ectopisch gelokaliseerde klieren (44% vs. 22%), en kleinere pathologische klieren

(1.21 cm vs. 2.03 cm) dan patiënten met sporadische PHPT die genezen waren na initiële operatie. Tc99m-MIBI-SPECT heeft een lagere sensitiviteit voor het lokaliseren van hyperplastische klieren en klieren met diameter <1.5 cm, wat de lagere diagnostische waarde in patiënten met persisterende PHPT kan verklaren.

De beperkte sensitiviteit van Tc99m-MIBI-SPECT in patiënten met persisterende PHPT voorafgaand aan re-operatieve PTx, is waarschijnlijk het resultaat van verminderde opname van het radiofarmacon door een combinatie van een verstoring in de lokale bloed toevoer door voorafgaande operaties en verschillen in klier pathologie en grootte.

Vanwege de beperkte diagnostische waarde van Tc99m-MIBI-SPECT voorafgaand aan revisie chirurgie voor persisterende PHPT, hebben we in ***Hoofdstuk 4*** onderzocht of selectieve veneuze sampling (SVS) van bijschildklierhormoon (PTH) kan bijdragen aan een betere pre-operatieve lokalisatie van achtergebleven hyperactief bijschildklierweefsel in patiënten met persisterende HPTH. De diagnostische waarde van SVS is gebaseerd op de aanname dat regionale drainage van elk van de 4 bijschildklieren geschiedt via, respectievelijk, de naastgelegen bovenste, middelste of onderste schildkliervenen (31). Ondanks potentiële anatomische variaties, kan een SVS in 39-93% van de patiënten met PHPT de kant van een pathologische bijschildklier aanwijzen (31-41) en, nog belangrijker, in 66-75% van de patiënten met negatieve non-invasieve lokalisatie studies (36,37,42). De grootste beperking van SVS is dat het gebied van veneuze drainage van een hyperactieve klier wordt aangewezen en niet de exacte anatomische lokalisatie.

We hebben de resultaten geëvalueerd van Tc99m-MIBI-SPECT en SVS, welke verricht waren voorafgaande aan 20 revisie operatie in 18 patiënten met persisterende of recidiverende PHPT of autonome tertiaire hyperparathyreoïdie (THPT) ten gevolge van eind stadium nierfalen. Tc99m-MIBI-SPECT had een sensitiviteit van 30% en SVS had een sensitiviteit van 75% in deze patiënten groep. SVS kon accuraat 10 van de 14 pathologische klieren (71%) lokaliseren die door Tc99m-MIBI-SPECT niet goed gelokaliseerd of compleet gemist waren, terwijl Tc99m-MIBI-SPECT alleen 1 van de 6 pathologische klieren (17%) accuraat kon

lokaliseren die niet goed gelokaliseerd waren door SVS. De lokalisatie sensitiviteit was 100% indien er overeenstemming was tussen SVS en Tc99m-MIBI-SPECT. In overeenstemming met Tc99m-MIBI-SPECT, was de diagnostische waarde van SVS ook verlaagd, hoewel in mindere mate, in het geval van hyperplastische bijschildklieren en klieren met een diameter <1.5 cm. In tegenstelling tot Tc99m-MIBI-SPECT, had SVS een sensitiviteit van 83% voor ectopische bijschildklieren, een frequente oorzaak van persisterende PHPT.

Deze bevindingen hebben aanzienlijke betekenis voor de zorg van patiënten met persisterende PHPT. Vanuit praktisch oogpunt en in overeenstemming met de recente richtlijnen van de Europese Associatie van Nucleaire Geneeskunde (43), laten onze data zien dat het altijd de moeite waard is om een Tc99m-MIBI-SPECT scan te maken als een eerste pre-operatieve lokalisatie studie in patiënten met persisterende PHPT, gevolgd door de invasieve maar betrouwbaardere SVS. Overeenstemming tussen deze beiden technieken leidt tot een geruststellende sensitiviteit van 100%. Onze data laten duidelijk zien dat SVS voor PTH geherintroduceerd zou moeten worden als diagnosticum in de pre-operatieve zorg van patiënten met persisterende PHPT.

IV. Complicaties van chirurgische behandeling van primaire hyperparathyreoïdie

Een chronisch verhoogde waarde van het serum PTH is geassocieerd met verhoogde bot omzetting ten gunste van bot resorptie, wat leidt tot mineraal onttrekking en verminderde botdichtheid (44). Binding van PTH aan zijn specifieke receptor (PTHR1), op stromale/osteoblastische cellen van het beenmerg, stimuleert de productie van RANK ligand en vermindert dat van zijn oplosbare ‘lokaasreceptor’ osteoprotegerine (OPG) (45-59). Na succesvolle chirurgie zorgt de snelle daling in serum PTH en de pre-operatieve hoge bot omzetting, voor een tijdelijke hypocalciëmie, door instroom van calcium, fosfaat en magnesium in het bot. Ernstige post-operatieve hypocalciëmie, wordt meestal veroorzaakt door het "hungry bone" syndroom, een syndroom dat zelden gezien wordt in patiënten met

sporadische PHPT. Het syndroom wordt met name gezien bij patiënten met ernstige hyperparathyreoïdie geassocieerde bot omzetting, met name in patiënten met bijschildkliercarcinoom. In ***Hoofdstuk 5*** vatten we de belangrijkste kenmerken en suggesties voor preventie en behandeling samen die voortgekomen zijn uit een systemische review van de literatuur over "hungry bone" syndroom.

De exacte incidentie van patiënten die post-operatief een "hungry bone" syndroom ontwikkelen is onbekend, maar er wordt verondersteld dat het aantal patiënten met deze post-operatieve complicatie gedaald is door een daling in het aantal patiënten met klinische evidente botaandoeningen, zoals osteitis fibrosa cystica, vanwege de eerdere opsporing van veelal asymptomatische PHPT door routinematig screenen van serum calcium (60,61). Risico factoren voor het krijgen van een "hungry bone" syndroom zijn oudere leeftijd bij diagnose, radiologische tekenen van hyperparathyreoïdie geassocieerde bot ziekten, en groter gewicht/volume van de verwijderde bijschildklier(en). Er zijn weinig data over de beste manier om deze ernstige complicatie van PTx te behandelen, minimaliseren of te voorkomen. De behandeling van het "hungry bone" syndroom is in eerste instantie gericht op het aanvullen van het calcium tekort, wat veroorzaakt wordt door het toegenomen calcium verbruik bij het vullen van de velen resorptie holtes in het bot. Op de langere termijn is behandeling gericht op herstel van de calcium homeostase, door tijdelijk het ontbrekende stimulerende effect van PTH op het 1α-hydroxylase enzym te vervangen, door het geven van de actieve vorm van vitamine D, en zodoende de intestinale calcium absorptie te garanderen (61-66). Pre-operatieve normalisatie van bot omzetting door middel van bisfosfonaten en pre-operatieve suppletie van actief vitamine D worden gezien als waardevolle opties om "hungry bone" syndroom te voorkomen, maar er zijn geen prospectieve data om deze aanname te bevestigen (60,67-72).

V. Aspecten van osteocyt functie in primaire hyperparathyreoïdie

De PTH/PTHrP receptor (PTHR1) is ook aangetoond op osteocyten (73). Recente *in vitro* en dierstudies suggereren dat binding van PTH aan de PTHR1 op

osteocyten leidt tot remming van de expressie van het *SOST* gen (74-77). Dit gen codeert voor sclerostine, een eiwit dat alleen in de osteocyten van het skelet gevonden wordt (78), en de botformatie remt door binding aan LRP5/6, met als resultaat remming van Wnt signaal routes in osteoblasten (79,80). Om te bepalen of chronisch verhoogde serum PTH concentraties hetzelfde effect heeft op sclerostine secretie in mensen als in diermodellen, hebben we in ***Hoofdstuk 6*** de hypothese getest dat chronisch verhoogde serum PTH concentraties, zoals in PHPT, sclerostine secretie verlagen, en dat PTH dus een potentiële regulator van sclerostine productie in mensen is.

Hiervoor hebben we sclerostine gemeten in het serum van 34 patiënten met onbehandelde, persisterende of recidiverende PHPT en in 54 patiënten die genezen waren na PTx voor PHPT (EuPTH). De gemiddelde serum sclerostine concentratie was lager in patiënten met PHPT dan in patiënten met EuPTH (30.5 pg/ml, 95% CI: 26.0-35.1 vs. 45.4 pg/ml, 95% CI: 40.5-50.2; $P<0.001$). Er was ook een negatieve correlatie tussen sclerostine en PTH wanneer alle patiënten samen genomen werden ($r= -0.44$, $P<0.001$).

Deze resultaten laten zien dat in mensen, chronisch verhoogde serum PTH concentraties, zoals in patiënten met PHPT, is geassocieerd met een daling in circulerende sclerostine waarden en dat er een negatieve correlatie is tussen PTH en sclerostine spiegels. Samenvattend, suggereren deze bevindingen dat PTH ook een remmend effect heeft op sclerostine productie in mensen, net als in diermodellen. De functionele betekenis van serum sclerostine dient nog verder uitgezocht te worden.

Recente *in vitro* studies hebben ook laten zien dat binding van PTH aan de PTHR1 op osteocyten *fibroblast growth factor 23* (FGF23) mRNA expressie stimuleert (81,82). Het wordt verondersteld dat FGF23 de grootste speler is in de bot-nier as en de fosfaat homeostase reguleert. FGF23 werkt als een fosfaat excretie bevorderende factor via hetzelfde mechanisme als PTH, door het verminderen van NaPi2a en NaPi2c co-transporters in de proximale tubuli van de nieren na het binden aan zijn receptor, FGFR-1, in de aanwezigheid van Klotho (83,84). FGF23

vermindert ook 1,25 $(OH)_2D$ synthese in de proximale tubuli door directe remming van het 1α-hydroxylase enzym (83-85). Gegevens over de relatie tussen PTH en FGF23 in patiënten met PHPT zijn schaars en vaak tegenstrijdig. De circulerende FGF23 waarden zijn hoger in patiënten met PHPT dan in gezonde vrijwilligers (86,87) en dalen direct na PTx (87), hetgeen het idee ondersteunt dat PTH de FGF23 secretie stimuleert. Het doel van de studie die beschreven wordt in ***Hoofdstuk 7*** was het bepalen van de relatie tussen PTH en FGF23 in patiënten met PHPT en in patiënten genezen na succesvolle PTx voor PHPT.

We hebben intact FGF23 spiegels gemeten in 22 patiënten met onbehandelde, persisterende, of recidiverende PHPT en in 24 patiënten genezen na succesvolle PTx voor PHPT (EuPTH). De gemiddelde serum FGF23 concentratie was significant hoger in patiënten met PHPT dan in EuPTH patiënten (50.4 ± 27.2 pg/mL vs. 33.1 ± 12.5 pg/mL, P=0.01). Er was een positieve correlatie tussen PTH en FGF23 concentraties (r=0.362, P=0.01), en deze correlatie was behouden en zelfs uitgesprokener na correctie voor 1,25$(OH)_2D$ concentraties (r=0.422, P=0.01). Er was een negatieve correlatie tussen FGF23 and 1,25$(OH)_2D$ gevonden (r=-0.780, P<0.01) in patiënten met PHPT. Deze correlatie was ook significant, maar minder uitgesproken in EuPTH patiënten (r= −0.519, P=0.02).

De data van onze studie laten zien dat FGF23 productie verhoogd is bij patiënten met verhoogde serum PTH waarden en dat deze stijging in FGF23 reversibel is nadat verhoogde serum PTH waarden genormaliseerd zijn na succesvolle PTx. We laten verder zien dat er een negatieve correlatie is tussen FGF23 en 1,25$(OH)_2D$ concentraties, die sterker is in patiënten met PHPT. Door deze sterkere negatieve correlatie tussen FGF23 en 1,25$(OH)_2D$ bij patiënten met PHPT, veronderstellen wij dat de PTH-geïnduceerde stijging in FGF23 concentraties een aanpassingsmechanisme is om serum fosfaat concentraties binnen de normale grenzen te houden, door de potentieel schadelijke 1,25$(OH)_2D$-geinduceerde fosfaat retentie te compenseren.

De bevinding dat PTH sclerostin productie remt en FGF23 productie stimuleert laat zien dat de effecten van PTH op bot complexer zijn dan voorheen gesuggereerd

werd en betrekking hebben op verschillende signaal routes in het bot zelf, maar ook in de bijschildklier-bot-nier as. Ondanks de significante voortgang van onze kennis van de effecten van PTH op bot, moeten we toch erkennen dat de cellulaire en moleculaire effecten van PTH nog maar deels ontrafeld zijn en verdere studies zijn nodig om de effecten verder verklaren.

VI. Klinische en moleculaire aspecten in primaire hyperparathyreoïdie

Chirurgische en medicinale ontwikkelingen hebben gezorgd voor een langere overleving in patiënten met een bijschildkliercarcinoom. Om dit te illustreren beschrijven we in ***Hoofdstuk 8*** de casus van een patiënt met gemetastaseerd bijschildkliercarcinoom met een follow-up van 17 jaar. Tevens hebben we alle originele casus beschrijvingen en case series over patiënten met bijschildkliercarcinoom bekeken, die voldeden aan de WHO criteria, een follow-up van minimaal 6 maanden na PTx hadden en die werden gepubliceerd in de Engelse literatuur, sinds het verschijnen van het laatste grootte overzicht over dit onderwerp in 2001 (88).

De gepresenteerde casus en de in de literatuur beschreven casussen laten zien dat radicale initiële operatie extreem belangrijk is om locale verspreiding van tumor weefsel te voorkomen en daardoor niet alleen de ziektevrije overleving en overleving te verhogen, maar ook de kans op re-operatie te verkleinen. Invasieve groei van tumor weefsel in naastliggende structuren, zoals de nervus recurrens of de oesofagus, verhoogt het risico op morbiditeit tijdens re-operatie. De gestegen morbiditeit die verbonden is aan re-operaties heeft ervoor gezorgd dat er andere behandelingsopties zijn ontwikkeld, zoals radiotherapie, chemotherapie, radiofrequentie ablatie, embolisatie, het calciumimeticum cinacalcet en PTH immunisatie. Bijschildkliercarcinomen zijn relatief resistent voor chemotherapie en radiotherapie (88-93), waardoor het gebruik van deze technieken gedaald is gedurende de afgelopen 10 jaar. Succesvolle embolisatie is alleen beschreven in combinatie met RF ablatie (94) en was niet succesvol in onze patiënt, terwijl RF ablatie wel succesvol was in 3 casussen (95-97) net als in onze patiënt. Het ontstaan

van oncontroleerbare hypercalciëmie is geassocieerd met dodelijke nier en cardiovasculaire complicaties (89,90,98,99) en verhoogde botomzetting met een verhoogd risico op fracturen. Wanneer pogingen om het tumor volume te verminderen niet slagen, worden intensieve rehydratie, medicatie zoals bisfosfonaten en calciumimetica en dialyse vaak gebruikt met wisselend resultaat. Een nieuwe potentiële niet-invasieve optie is PTH immunisatie, ondanks dat ervaringen met deze aanpak nog beperkt zijn (100-102).

Concluderend blijft de lange termijn zorg van patiënten met een gemetastaseerd bijschildkliercarcinoom een uitdagende taak, ondanks recente diagnostische, chirurgische en medicinale vooruitgang.

Er zijn verschillende genen ontdekt die een rol spelen bij de ontwikkeling van PHPT, onder andere het *MEN-1*, *HRPT2* en *CASR* gen. Mutaties in het *HRPT2/CDC73* gen, dat codeert voor het eiwit parafibromine (103), zijn geassocieerd met het hyperparathyroidism-jaw tumor syndroom (HPT-JT; OMIM 607393). Patiënten met een HPT-JT syndroom hebben 15-24% kans op het ontwikkelen van een bijschildkliercarcinoom (88,104-106). In patiënten met het HPT-JT syndroom en patiënten met sporadische bijschildkliercarcinomen is een verminderde expressie van het calcium sensing receptor (CASR) aangetoond (107). Bovendien, is het bekend dat verminderde CASR expressie, mutaties in het *HRPT2/CDC73* gen en verlies van zijn eiwit, parafibromine, waardevolle hulpmiddelen zijn bij het vast stellen van de diagnose bijschildkliercarcinoom. Er zijn echter geen gegevens bekend over de prognostische waarde van deze markers in bijschildkliercarcinomen. In ***Hoofdstuk 9*** hebben we de prognostische waarde van verminderde CASR expressie, verlies van parafibromine kleuring en de aanwezigheid van mutaties in het *HRPT2/CDC73* gen in patiënten met een op WHO criteria gebaseerde diagnose van bijschildkliercarcinoom vastgesteld.

We hebben de ziektevrije overleving en overleving bepaald van 23 patiënten die een initiële operatie voor bijschildkliercarcinoom ondergingen in verschillende ziekenhuizen in Nederland gedurende de periode 1985 en 2000. De verwijderde bijschildkliercarcinomen van alle 23 patiënten hadden tijdens een eerdere studie

HRPT2/CDC73 en *MEN-1* gen mutatie analyse (108) en parafibromine en CASR kleuring analyse (104,109) ondergaan.

De 5-jaars ziektevrije overleving en de 5-jaars overleving waren lager in patiënten met verminderde CASR expressie dan in patiënten met normale CASR expressie (81% vs. 0% en 94% vs. 29%, respectievelijk). Zowel de 5-jaars ziektevrije overleving (73% vs nul) als de 5-jaars overleving (79% vs. 50%) waren lager in patiënten met een *HRPT2/CDC73* mutatie dan in patiënten zonder deze mutatie. Zowel de 5-jaars ziektevrije overleving (89% vs. 41%) als de 5-jaars overleving (100% vs. 57%) waren lager bij patiënten met globaal verlies van parafibromine vergeleken met patiënten met focaal parafibromine verlies. Het risico op het ontwikkelen van metastasen lokaal en/of op afstand was 16 keer verhoogd in het geval van verminderde CASR expressie, 7 keer verhoogd in het geval van *HRPT2/CDC73* mutaties en ook 7 keer verhoogd in het geval van globaal parafibromine verlies. De 5-jaars ziektevrije overleving en 5-jaars overleving waren lager in patiënten met somatische *MEN-1* mutaties vergeleken met patiënten zonder deze mutaties (respectievelijk 64% vs. 33% en 80% vs. 33%), maar somatische *MEN-1* mutaties waren niet geassocieerd met een significante stijging in het risico op het ontwikkelen van metastasen lokaal en/of op afstand.

De bevindingen van deze studie hebben verschillende klinische implicaties. Ten eerste, zijn verminderde CASR expressie, *HRPT2/CDC73* mutaties en globaal verlies van parafibromine sterke negatieve determinanten van de maligne potentie van bijschildkliercarcinomen. Ten tweede, de observatie dat somatische *MEN-1* mutaties gevonden worden bij patiënten met bijschildkliercarcinomen, suggereert dat deze mutaties een rol kunnen spelen in het ontstaan van bijschildkliercarcinomen, hoewel ze de prognose niet beïnvloeden. Ten derde, kan de bepaling van CASR expressie ook gebruikt worden om te bepalen of patiënten met bijschildkliercarcinoom behandeld kunnen worden met een calciumimetica.

Op basis van de resultaten beschreven in ***Hoofdstuk 9***, bevelen we de bepaling van verminderde CASR expressie, *HRPT2/CDC73* mutaties en globaal verlies van parafibromine aan, niet alleen als diagnostisch hulpmiddel, maar vooral als prognostisch hulpmiddel in de zorg voor patiënten met bijschildkliercarcinomen.

VII. Zorg voor patiënten met persisterende primaire hyperparathyreoïdie

De zorg voor patiënten met persisterende sporadische PHPT vormt een grote klinische uitdaging, die vraagt om een multidisciplinaire aanpak. In ***Hoofdstuk 10*** beschrijven we de demografische, klinische, biochemische, chirurgische en pathologische karakteristieken van patiënten met persisterende PHPT na initiële parathyreoïdectomie voor sporadische PHPT.

Data van onze studie suggereren dat een gemiste ectopische bijschildklier bij initiële operatie (33%) en gemiste hyperplasie in meerdere bijschildklieren (15%) de meest voorkomende oorzaken van persisterende hyperparathyreoïdie zijn. We hebben parathyromatose gedocumenteerd in 9% van de patiënten. Pre-operatieve studies hebben een slechte sensitiviteit: echografie 18%, Tc99m-MIBI-SPECT 25%, CT 30%, MRI 20%. Selectieve veneuze sampling van PTH heeft echter een sensitiviteit van 50% en een specificiteit van 89%. De daling in intra-operatief PTH was significant minder uitgesproken in patiënten bij wie hyperparathyreoïdie persisteerde in vergelijking tot patiënten die genezen waren na operatie (63 ± 26 vs 89 ± 11%, P=0.003). Het risico op complicaties nam toe met elke volgende operatie: 20% na de eerste, 50% na de 2e en 67% na de 3e operatie.

Onze bevindingen van deze serie patiënten laten zien dat de behandeling van patiënten met persisterende hyperparathyreoïdie complex en uitdagend is. Elke heroperatie verlaagt de sensitiviteit van de lokalisatie studies om resterende hyperactieve bijschildklieren accuraat te lokaliseren en verhoogt het risico op complicaties, door verstoring en verlittekening van het chirurgische veld bij eerdere chirurgische interventies. In deze patiënten dient heroperatie zorgvuldig gepland te worden en chirurgische pogingen uitgesteld te worden totdat hyperactief weefsel duidelijk gelokaliseerd kan worden pre-operatief.

VIII. Conclusie

- De kans op persisteren van hyperparathyreoïdie is 7% en dat van recidiveren van hyperparathyreoïdie nul, na initiële operatie voor sporadische PHPT.
- De veel gebruikte, niet-invasieve Tc99m-MIBI-SPECT scan techniek heeft een lagere sensitiviteit in het geval van persisterende PHPT, en deze sensitiviteit is significant lager dan de sensitiviteit van de invasieve SVS techniek.
- Chronische hyperparathyreoïdie heeft een catabool effect op bot, wat zorgt voor mineraal onttrekking van bot. Patiënten met pre-operatieve radiologische tekenen van ernstig hyperparathyreoïdie geassocieerde botziekten lopen post-operatief het risico op het ontwikkelen van "hungry bone" syndroom, wat voorkomen zou kunnen worden door pre-operatieve behandeling met bisfosfonaten en actief vitamine D.
- PTH remt de productie van sclerostine en stimuleert de productie van FGF23, waarschijnlijk om het eigen effect van PTH en dat van $1,25(OH)_2D$ op bot tegen te gaan, waardoor nieuwe feedback mechanismen ontstaan.
- Patiënten met bijschildkliercarcinoom hebben in tegenstelling tot patiënten met sporadische PHPT wel een kans op recidiverende PHPT. In deze patiënten, zijn verminderde CASR expressie, *HRPT2/CDC73* mutaties en globaal verlies van parafibromine expressie sterke negatieve determinanten van de ziektevrije overleving en overleving.
- Recente ontwikkelingen in de chirurgische en medicinale behandeling van patiënten met bijschildkliercarcinoom hebben het mogelijk gemaakt een langere overleving te verzekeren voor deze patiënten, zelfs in patiënten met tumoren waarin 2 van de 3 negatieve prognostische factoren gevonden worden.

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CURRICULUM VITAE

Janneke E. Witteveen werd geboren op 10 januari 1982 te Eindhoven. Zij behaalde in 2001 haar VWO diploma aan het Eckart College te Eindhoven, waarna zij begon met de studie geneeskunde aan de Universiteit Leiden. Na het behalen van haar artsexamen in maart 2008, werkte ze als arts-onderzoeker bij de afdeling Endocrinologie en Metabole ziekten van het Leids Universitair Medisch Centrum, onder leiding van Prof. Dr. J.A. Romijn en Dr. N.A.T. Hamdy. In deze periode werden zowel klinische studies bij patiënten met persisterende of recidiverende primaire hyperparathyreoïdie verricht als basale studies naar genetische afwijkingen bij patiënten bij Sterno-Costo-Claviculaire Hyperostosis (SCCH) verricht. Per 1 januari 2011 is zij begonnen met de opleiding tot internist in het Medisch Centrum Haaglanden, te Den Haag.

LIST OF PUBLICATIONS

J.E. Witteveen, J. Kievit, H. Morreau, J.A. Romijn, N.A.T. Hamdy, No recurrence of sporadic primary hyperparathyroidism when cure is established 6 months after parathyroidectomy, European Journal of Endocrinology, 2010 Feb;162(2):399-406.

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