Surgical Strategies in Endocrine Tumors

Jennifer M.J. Schreinemakers

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Surgical Strategies in Endocrine Tumors

Chirurgische strategieën bij endocriene tumoren

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof.dr.J.C. Stoof, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op dinsdag 18 mei 2010 des middags te 4.15 uur

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The surgical investigator must be a bridge-tender, channeling knowledge from basic science to the patient's bedside and back again. He (she) traces his origin from both sides of the bridge. He is thus a bastard and is called this by everybody. Those at the end of the bridge say that he is not a very good scientist, and those at the other say that he does not perform enough operations. It is much harder to stay in the middle of the bridge than it is in the retreat to one end or the other. But all of the fundamentals advances in surgery from Vasalius to Halsted to Cushing have been made by those willing to maintain this uncomfortable posture – the bridge- builder.

Francis D. Moore

aan mama en papa

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Chapter 1

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General introduction and outline of this thesis

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Endocrine surgery has become more custom-made throughout the years. Endocrine tumors can be sporadic or develop as part of familial syndromes. Several familial syndromes are known to cause endocrine tumors. The most common are multiple endocrine neoplasia (MEN) syndromes type 1, 2A and 2B.
MEN1 is an autosomal dominant inherited disorder caused by a germline mutation on chromosome 11 in the MEN1 gene. Patients with MEN 1 are classically prone to developing tumors in the parathyroid glands, anterior pituitary gland, and pancreatic islet cells. Many of these patients also develop gastrinomas in the duodenum.[1]
MEN2 is subdivided into two distinct clinical syndromes, MEN2A and MEN2B. As MEN1, both syndromes inherit in an autosomal dominant fashion. Virtually all MEN2A and 2B patients develop medullary thyroid carcinoma (MTC). Additionally, patients with MEN2A are predisposed to pheochromocytoma, and primary parathyroid hyperplasia. MEN2B is characterized by pheochromocytomas as well, but not by hyperparathyroidism. Other features of MEN2B are mucosal neuromas, intestinal ganglioneuromas and developmental disorders.
For many endocrine tumors associated with MEN1, MEN2A ad 2B, there is no conclusive surgical treatment. Due to the rarity of the syndrome, it has been difficult to conduct randomized controlled trials or prospective controlled trials to answer questions regarding the surgical management. This thesis addresses the currently available surgical strategies in the management of endocrine tumors.

Thyroid Gland

Thyroid Cancer

Thyroid cancer is the most common endocrine cancer. There are several types of thyroid cancer; papillary, follicular, Hürthle cell, medullary and anaplastic carcinoma. Thyroid cancer derives from follicular and parafollicular thyroid cells. The incidence of thyroid cancer is rising. In the Netherlands the incidence of thyroid cancer is 2-3 per 100.000 per year.[2, 3] This increase is mainly caused by the diagnosis of micropapillary thyroid cancer.
Surgery is the most important treatment for thyroid cancer. In 1791, DeSault performed the first partial thyroidectomy. Mortality was very high in those days (40%). Theodor Kocher (1841-1917) is considered to be the father of modern thyroid surgery. His mortality rate was only 12% during his first 100 thyroidectomies and decreased to 0.2% during the first 500 procedures. He was awarded the Nobel Price for Medicine in 1909 for his work on physiology, pathology and surgery of the thyroid. The classical neck incision most commonly used to perform a thyroidectomy is still named after him. By 1920 thyroid surgery had become a common operation. Recent developments in thyroid surgery include endoscopic thyroid surgery and robotic-assisted thyroid surgery.[4, 5]

Papillary Thyroid Carcinoma

Papillary thyroid cancer (PTC) is the most common thyroid cancer accounting for 80-85% of all thyroid cancers. The incidence of PTC has risen to 7.7 per 100.000 in

the United States.[2]
PTC has an excellent prognosis. Even young patients with multifocal disease and lymph node metastases do well. However, 20 to 30% of these patients develop recurrent disease. About 7% of patients with PTC die within 10 years of diagnosis because of the disease.[6] Patients between ages 20 and 45 have the best prognosis. The presence of lymph node metastases in these young patients has no effect on survival. Patients older than 45 years have a worse outcome. Furthermore, the presence of lymph node metastases, large tumors, or tumors that locally invade the surrounding tissues, indicate a worse prognosis.
Next to clinical prognostic factors, there is increasing evidence that biomolecular factors play a role in the prognosis of papillary thyroid cancer. For example, the BRAF V600 mutation is the most common mutation in papillary thyroid cancer.[7] Patients with the BRAF V600 mutation have a higher risk of recurrent and persistent PTC. In Chapter 2 we give an overview of what is currently known on the prognostic factors for PTC, clinical staging systems and biomolecular markers. The increasing information on biomolecular markers in PTC may have future treatment implications.

In addition to the BRAF mutation status, matrix metalloproteinases (MMPs) may have prognostic value. MMPs degrade components of the extracellular matrix and the basement membrane. MMPs and their inhibitors, tissue inhibitor metallopeptidases (TIMPs) are critical to numerous physiological and pathophysiological processes, e.g. wound healing, angiogenesis, tumor growth, invasion, and metastasis.[8-10] MMPs are associated with the development and aggressiveness of certain cancers. Although some MMPs have been shown to correlate with increased risk for papillary thyroid cancer metastases, the prognostic value of MMPs in thyroid cancer is still not clear. In *Chapter 3* we report on gene-expression levels of several MMP genes and TIMPs in different stages of papillary thyroid cancer in an attempt to determine their prognostic value in PTC.

Another predictor of a worse prognosis in papillary thyroid cancer appears to be the presence of metastatic lesions that are fluorodeoxyglucose-positron emission tomography (FDG-PET) positive.[11] These lesions often lose the ability to take up radioactive iodine. This loss is also associated with a decreased survival.[12] FDG-PET and FDG-PET/CT scans are valuable to identify these lesions.[13] One could argue that the more aggressive nature of these FDG-PET positive lesions in the neck warrants more aggressive surgical therapy. To address this matter, we compared the outcome of two different groups of patients with recurrent papillary thyroid cancer according to their FDG-PET scan status: FDG-PET/FDG-PET-CT scan positive lesions, versus FDG-PET/FDG-PET-CT negative lesions in *Chapter* 4. Additionally, we studied the impact of the FDG-PET or FDG-PET/CT scan on the surgical management of these patients to see if an aggressive surgical management leads to an improved outcome.

Medullary Thyroid Carcinoma in MEN2A and MEN2B
Medullary thyroid carcinoma (MTC) arises from the parafollicular C-cells. MTC accounts for 5-10% of all thyroid cancers. C-cell hyperplasia is often found in

association with MTC and is generally considered a precursor of MTC.[14]
MTC can be sporadic or familial. In sporadic cases the tumors are generally single and confined to one lobe. Familial cases of MTC are related to MEN2A, MEN2B and familial non-MEN (FMTC). In MEN2 related MTC, the tumors are generally multifocal and bilateral. These patients tend to present at a younger age than sporadic patients. MTC in MEN2B develops at an earlier age and is more aggressive than in MEN2A.[15] Patients with FMTC do not develop other endocrinopathies and present later on in life with MTC.

Surgical treatment of MTC in MEN2A and MEN2B
Surgery is the only curative treatment for MTC. MTC used to be the main cause of death in patients with MEN2 syndrome. Nowadays, genetic testing is available and the surgical treatment of MTC can be customized for family members at risk. These patients now undergo total, a "prophylactic" thyroidectomy at an earlier age as recommended by the international guidelines.[1] In *Chapter* 5, we set out to study the differences in treatment for patients operated on at the age as recommended by the current guidelines and those operated on at an older age. The second purpose of this study was to assess any differences in treatment and outcome in patients treated in the era before and after genetic testing became available. Our final aim was to analyze prognostic risk factors for recurrent MTC in these patients.

Parathyroid Glands

Primary Hyperparathyroidism
Primary hyperparathyroidism (pHPT) is a relatively common disease with ~ new 100.000 cases reported each year in the United States.[16] Patients with pHPT have an increased plasma calcium, an increased or unsuppressed parathyroid hormone (PTH) level, a low or normal phosphorus, high plasma chloride and low plasma bicarbonate.[17] Most cases are sporadic and women are affected more often than men (3:1). Primary hyperparathyroidism can be caused by parathyroid adenomas (80-90%) and hyperplasia. In most cases adenomas develop in a single gland. Hyperplasia of the parathyroid glands develops in multiple glands without a known stimulus for PTH secretion.[17, 18]
Primary hyperparathyroidism is the most prevalent endocrinopathy in MEN1 with a prevalence of 90-100%[19] and is often the first presentation of the syndrome.[20] These patients tend to be younger (2nd and 3rd decade) than patients with sporadic pHPT and the disease has a more aggressive course.[20, 21] Usually, it manifests as multiglandular disease.[22] In MEN2A, pHPT tends to be less aggressive and less common with a prevalence of 25-30% than pHPT in MEN1.

Surgical Treatment of Primary Hyperparathyroidism in MEN1 and MEN2A
Surgery is the only cure for patients with primary hyperparathyroidism. The first successful parathyroidectomy was performed by Olch in 1928.[23] Soon surgeons realized that not only solitary adenomas exist, but also double adenomas and

multiglandular disease and a bilateral neck exploration became the standard surgical approach for this disease.[23] In the 1980s less invasive procedures became more popular. Nowadays a selective, “focused”, “direct”, or “minimally invasive” parathyroidectomy is commonly performed for single sporadic parathyroid adenomas with a high success rate of 95%.[24 25]
In familial cases of MEN1 success rates are much lower. Controversy exists regarding the optimal surgical strategy for primary hyperparathyroidism in MEN1 and MEN2. There is a high risk of recurrence in MEN1 after surgical intervention, even after extensive surgery.[26, 27] At the same time, while extensive surgery may offer instant cure, this comes at the cost of a higher risk of permanent hypoparathyroidism (hypocalcaemia) and recurrent laryngeal nerve injury.[26, 27] MEN2A related PHPT is less common than in MEN1 and generally less aggressive. Yet, controversy remains on its optimal surgical management as well. In *Chapters* 6 and 7, we aimed to determine the optimal surgical strategy for pHPT in MEN1 and MEN2A. For MEN1 we performed a cohort study and a meta-analysis to determine the optimal surgical therapy. For MEN2A we performed a relatively large cohort study.

Pancreatic islet cells (endocrine pancreas)

Pancreatic Endocrine Tumors in MEN1
Patients with MEN1 also carry an increased risk of developing multiple pancreatic endocrine tumors (PETs). Common PETs include insulinomas, gastrinomas and non-functioning PETs (NF-PETs).

Insulinomas
Insulinomas are the most common functional PETs. Ninety percent of sporadic insulinomas are benign, solitary and smaller than 2 cm.[17] However, in patients with MEN1, insulinomas are often multiple and evenly distributed throughout the head, body and tail of the pancreas.
Gastrinomas
Gastrinomas are endocrine tumors that produce gastrin. In 1955 Zollinger and Ellison reported two patients with primary peptic ulceration of the jejunum associated with islet cell tumors of the pancreas. The clinical syndrome was called Zollinger-Ellison syndrome (ZES). Gastrinomas in MEN1 are often located in the duodenum as well as in the pancreas.
Other Functioning Pancreatic Endocrine Tumors
Other functioning tumors of the pancreatic islet cells (PETs) are somatostatinomas, vasoactive intestinal polypeptide tumors (VIPomas), and glucoganomas. These tumors are rare and often large and malignant at the time of diagnosis.
Non-Functioning Pancreatic Endocrine Tumors (NF-PETs)
Since these tumors do not cause a clinical syndrome, at diagnosis, these tumors are often large. These tumors become clinically apparent when they have become large and cause symptoms due to local compression. Frequently, NF-PETs in MEN1 are multiple and distributed throughout the pancreas like insulinomas.[17] NF-PETs show

invasive growth and lymph node metastasis in many cases.

Surgical Treatment of Pancreatic Endocrine Tumors in MEN 1
Surgical strategies for PETs in MEN are remarkably controversial, ranging from aggressive and extensive surgery to more conservative approaches including watchful waiting and sparing resections. In addition, the type or extent of pancreatic resection depends on the type of tumor. Because of the existing controversies, we conducted a systematic literature review (*Chapter* 8) with the objective of investigating the indications for surgery and the preferred operative procedures for PETs in MEN1.

Adrenal Glands
The adrenal glands were first described in 1552 by Bartolomaeus Eustachius as glandulae renis incumbents.[28] In 1856 Brown-Séquard showed that the adrenal glands are essential to life after he had performed the first adrenalectomies on animals.[29]

Adrenal Tumors
Pheochromocytomas
Pheochromocytomas are rare tumors that derive from catecholamine producing chromaffin-cells.[30, 31] The incidence is estimated at 2-8 per million.[32, 33] Eighty to ninety percent of pheochromocytomas are located in the adrenal gland. Extra-adrenal pheochromocytomas are spread throughout the sympathic neuroendocrine system along the paravertebral and para-aortic axis.[33, 34]
Historically, pheochromocytoma has been named the 10% tumor, indicating that 10% are familial, malignant, multifocal and bilateral. Recently, it has been shown that up to 25% of pheochromocytomas occur as part of a familial syndrome.[31, 33, 35, 36]
Patients with pheochromocytoma typically present with episodic hypertension, headaches, diaphoresis, and flushing.[32] Some patients may even develop hypertensive crisis resulting in cardiovascular shock.[32] Forty percent of patients with pheochromocytomas are asymptomatic.[32, 37] Individual pheochromocytomas vary in size, catecholamine production and urinary metabolites excretions.[38] Yet, it is unknown whether a correlation exists between pheochromocytoma size, hormone levels and clinical presentation.[38, 39] Therefore, we investigated the relationship between tumor size and hormone levels upon diagnosis in *Chapter* 11.

Adrenal cortical adenomas
Adrenal cortical adenomas that secrete cortisol can cause Cushing's syndrome. These functional adenomas can be treated adequately with unilateral adrenalectomy. Adenomas that produce aldosterone are generally solitary and small (<2cm). They cause primary hyperaldosteronism (Conn's syndrome), characterized by moderate to severe hypertension and hypokaliemia.

Surgical Treatment of Adrenal Gland Tumors

The adrenals glands may be approached via different routes: transabdominal, lateral transabdominal, lateral retroperitoneal and posterior retroperitoneal. In 1889 Thornton performed the first transabdominal adrenalectomy.[40] Each approach to the adrenal glands has its own advantages and disadvantages. Depending on the specific indication (as well as on personal preference), surgeons have to decide which approach is most appropriate.

Until 1992 adrenalectomy was done by conventional open surgery. At that time, Gagner introduced the laparoscopic adrenalectomy via the lateral transabdominal approach.[41] [42] Since its introduction, laparoscopic adrenalectomy has become the procedure of choice for adrenal glands tumors smaller than 6cm.[43-45] Most open procedures require large incisions to create exposure. The advantages of laparoscopic compared to conventional open adrenalectomy include less blood loss and postoperative pain, fewer complications and shorter hospital stay.[43, 44, 46] Although the transabdominal lateral approach is favored by most surgeons, others prefer the transabdominal anterior[43] and posterior retroperitoneal approach.[43, 47, 48] In *Chapter* 9, we conducted a cost-effectiveness analysis to compare endoscopic to open adrenalectomy. In *Chapter* 10, we describe our experience with the posterior retroperitoneoscopic adrenalectomy.

Taken together, the studies described in this thesis offer more insight in the prognostic implications of several factors in endocrine cancers and the surgical treatment of endocrine cancer.

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PART 1
Thyroid Gland

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Chapter 2

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Diagnostic markers and prognostic factors in thyroid cancer

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Introduction

Thyroid cancer is the most common endocrine malignancy and over the last three decades the incidence increased more than 2-fold in the US.[1] Medical surveillance and more sensitive diagnostic tools contribute to that (improved detection of subclinical tumors). Thyroid cancer occurs three times more often in women compared to men and is now the sixth most common cancer amongst women. There is also a difference in race and ethnicity. Thyroid cancer rates are twice as high in White as Black and notably elevated among people from Southeast Asia.[2] Thyroid cancer arises from follicular (papillary, follicular, follicular variant of papillary, Hürthle cell and anaplastic cancer) or parafolllicular cells (medullary cancer). Ninety-five percent of thyroid cancers derive from follicular cells and have excellent 5-year and 10-year survival rates, except from anaplastic thyroid cancer. Over the years, various prognostic staging systems have been developed. More recently, the understanding of the molecular mechanism of thyroid carcinogenesis has improved, leading to new insights in and potential usefulness of somatic genetic mutations and gene expression profiling.[3-5] The main goal of recent studies is to improve the accuracy of fine-needle aspiration (FNA) biopsy (up-to-date the best diagnostic tool) and to predict the outcome of disease. In this review we address the issues related to both potential clinical and molecular biomarkers and their potential usefulness, based on the most recent literature.

Calcitonin and thyroglobulin

Calcitonin is a tumor marker that may be used to help early cancer diagnosis. Calcitonin is elevated in the serum of patients with medullary thyroid cancer and may with further clinical evaluations, be useful in screening for this cancer. Calcitonin is a hormone produced by the parafollicular cells in the thyroid gland that helps to regulate blood calcium levels. Since medullary carcinoma of the thyroid is often inherited, serum calcitonin can be measured to detect the cancer in its earliest stages in family members who are at risk.[6, 7] Thyroglobulin is a critical prohormone produced in thyroid follicular cells. Thyroglobulin covalently binds to iodine and is stored in the follicular lumen, until TSH stimulation leads to the intracellular reuptake of thyroglobulin and its subsequent proteolytic degradation into the active forms of thyroid hormone, T3 and T4. Although T3 and T4 are actively secreted into the bloodstream and are detectable using assays on peripheral blood, small amounts of “physiological” thyroglobulin also make it into the peripheral circulation and are detectable at low levels in healthy euthyroid people. Because thyroglobulin is exclusively produced in thyroid follicular cells, its measurement in peripheral blood after total thyroidectomy for thyroid cancer has become an established method of detecting recurrent or persistent disease. In a retrospective review of 366 consecutive patients from 2000-2008 who underwent a thyroidectomy for FNA-diagnosed follicular and Hürthle-cell neoplasms in our institute, serum thyroglobulin levels were not predictive. Its use should primarily remain as a postoperative biomarker of thyroid cancer recurrence, as it has limited utility in the preoperative setting (unpublished data).

Staging systems

In a recent review,[8] 17 staging systems were found to predict cancer-specific survival of thyroid cancer (Table 1). Fourteen staging systems significantly predicted cancer specific survival in this study. The 3 highest ranked staging systems by proportion of variation were the Metastases, Age, Completeness of Resection, Invasion, Size (MACIS), followed by the new AJCC/UICC 6th edition tumor, node, metastases (TNM) and the European Organization for Research and Treatment of Cancer (EORTC).

Table 1. Staging Systems to predict cancer-specific survival of thyroid cancer[8]

Staging System
-European Organization for Research and Treatment of Cancer (EORTC)
-Mayo Clinic (Age, Grade, Extent, Size or AGES)
-Lahey Clinic (Age, Metastases, Extent, Size or AMES)
-University of Chicago (Clinical Class, DeGroot classification)
-Karolinska Hospital and Institute (DNA ploidy, Age, Metastases, Extent, Size or DAMES)
-Mayo Clinic (Metastases, Age, Complete resection, Invasion, Size or MACIS)
-University of Bergen (Sex, Age, Grade or SAG)
-Ohio State University (OSU)
-Noguchi Thyroid Clinic (Noguchi)
-Memorial Sloan Kettering (Grade, Age, Metastases, Extent, Size or GAMES)
-University of Münster (Münster)
-National Thyroid Cancer Treatment Cooperative Study (NTCTCS)
-University of Alabama and M.D. Anderson (UAB &MDA)
-Virgen de la Arrixaca University at Murcia (Murcia)
-AJCC/UICC 6th edition TNM (TNM)
-Cancer Institute Hospital in Tokyo (CIH)
-Oncology Training and Research Hospital in Turkey (Ankara)

MACIS

This system was developed as an update of the AGES staging system, introduced at the Mayo Clinic in 1987. It was based solely on patients with papillary thyroid cancer (PTC). The following prognostic factors form the basis for this system: distant metastases (metastases), age, completeness of surgery, tumor invasion, and size. A scoring system allocates patients into four risk groups. The total score = 3.1 (if aged ≤ 39 yr), or 0.08 x age (if aged ≥ 40 yr), + 0.3 x tumor size in cm, + 1 (if not completely resected), + 1 (if locally invasive), + 3 (if distant metastases) Four risk groups: Group 1 = < 6.0, Group 2 = 6.0– 6.99, Group 3 = 7.0–7.99 Group 4 = > 8.0, with relevant 5- and 10-year carcinoma specific survival rates as high as 100% and 97.5% for group 1, 85.6% and 83.3% for group 2, 81.4% and 80.3% for group 3, and 55.5% and 30.2% for group 4.[9]

AJCC/UICC (6th edition) TNM Staging System (TNM)

The TNM staging system was first described in the 1940s, and the 6th edition came into use in January 2003. Like its previous editions, the TNM is a system that

describes the anatomic extent of the primary tumor (T), the involvement of regional lymph nodes (N), and distant metastasis (M). Although the system is applicable to all histologies of thyroid carcinoma, the stage grouping varies with different histologic types. PTC and FTC are being staged in the same way. It is the only staging system that regularly undergoes revision to keep up with prevailing changes in the field of thyroid carcinoma (Table 2).

Table 2. AJCC/UICC (6th edition) TNM Staging System (TNM)

TNM		
T1, tumor ≤ 2 cm in greatest dimension limited to the thyroid		
T2, tumor > 2 cm, but _4 cm, in greatest dimension limited to the thyroid		
T3, tumor > 4 cm in greatest dimension limited to the thyroid or any tumor with minimal extrathyroidal extension (eg, extension to sternothyroid muscle or perithyroid soft tissues)		
T4a, tumor of any size extending beyond the thyroid capsule to invade subcutaneous soft tissues, larynx, trachea, esophagus, or recurrent laryngeal nerve		
T4b, tumor invades prevertebral fascia or encases carotid artery or mediastinal vessels		
N1a, metastasis to level VI (pretracheal, paratracheal, and prelaryngeal/Delphian lymph nodes)		
N1b, metastasis to unilateral, bilateral, or contralateral cervical or superior mediastinal lymph nodes		
M1, distant metastases		
Under 45 yr		
	Stage I	Any T Any N M0
	Stage II	Any T Any N M1
45 yr and older		
	Stage I	T1 N0 M0
	Stage II	T2 N0 M0
	Stage III	T3 N0 M0
		T1 N1a M0
		T2 N1a M0
		T3 N1a M0
	Stage IVA	T4a N0 M0
		T4a N1a M0
		T1 N1b M0
		T2 N1b M0
		T3 N1b M0
		T4a N1b M0
	Stage IVB	T4b Any N M0
	Stage IVC	Any T Any N M1

European Organization for Research and Treatment of Cancer (EORTC)

The EORTC system was the first staging system for thyroid carcinoma, developed in 1979.[10] The system was developed from a multivariate analysis of 507 patients from 23 European hospitals with a median follow-up of 40 months. Patients are divided into 5 risk groups on the basis of a prognostic scoring system; age, gender, histology, differentiation, tumor invasion, and distant metastases are the risk factors taken into account.[8, 9]

Fine needle aspiration (FNA)

FNA biopsy is currently the best diagnostic test for differentiating between benign and malignant thyroid tumors.[5] Together with ultrasound it is of great use for the triage of patients with thyroid nodules into operative or nonoperative candidates.[11] The overall accuracy of FNA biopsy is greater than 95% for papillary, medullary and anaplastic thyroid cancers. For patients with familial non-medullary thyroid cancer (FNMTC), however, the reliability of FNA appears to be less accurate than it is for other patients because of the high incidence of multifocal thyroid cancer and coexistence of benign nodules.[12] Furthermore, FNA is not able to distinguish between follicular adenoma and carcinoma, or between Hürthle cell adenoma and carcinoma. The follicular variants of papillary thyroid cancer are often difficult to diagnose cytologically.[5]

Most recently, it has been proposed to subdivide the general category of indeterminate cytology into three subcategories: (i) follicular lesion of undetermined significance (FLUS), (ii) follicular or oncocytic (Hürthle cell) neoplasm, and (iii) suspicious for malignancy, with a predicted probability of malignancy of 5-10%, 20-30%, and 50-75%, respectively.[13, 14]

For patients with suspicious or indeterminate FNA cytology findings, none of the preoperative clinical (age, sex, solitary vs multiple nodules), imaging (tumor size, nodule ultrasound characteristics), and cytologic (atypia, mitotic index) factors studied so far are accurate enough to determine who should undergo thyroidectomy.[15] Since FNA cytology cannot discriminate between benign and malignant thyroid nodules in up to 30% of thyroid nodules, and because false negative and discordant FNA cytology results do occur, additional diagnostic tests that would improve the preoperative accuracy of distinguishing benign from malignant thyroid neoplasms are necessary to improve patient outcome.[5]

Somatic mutations

Recent studies have suggested that differences in common genetic changes found in thyroid cancer of follicular origin can be of clinical use. Activating genetic alterations in signal transduction pathway are common in these thyroid cancers and involve tyrosine kinase receptors (RET/PTC, NTRK), signaling proteins (BRAF, RAS) and nuclear proteins (PAX8-PPARγ). Figure 1 shows these genetic changes, which are usually mutually exclusive.[5] The incidence of RET/PTC rearrangements in papillary thyroid cancer ranges from 20 to 40%. Most patients with RET/PTC rearrangements in their primary PTC are younger than 45 years. A total of 15 different RET/PTC rearrangements have been reported; the most widely studied are RET/PTC1 (RET kinase fused with the *H4* gene) and RET/PTC3 (RET kinase fused with the RFG gene). The NTRK1 rearrangement occurs rarely and exclusively in PTC (5–13%).[16] BRAF mutations occur most commonly (about 40%) in PTC. The most common type of BRAF mutation found in primary PTC is a T to A substitution at nucleotide 1799 in exon 15, which results in conversion of a valine to glutamic acid at codon 600 (V600E) of the BRAF protein.[17-19] BRAF mutation has been associated with poor prognosis in patients with PTC. The four classical RAS proto-oncogenes encoding

H-RAS, K-RAS A, K-RAS B and N-RAS belong to an extended family of small G proteins. RAS proteins are plasma membrane GTPases activated by growth factor receptors, non-receptor tyrosine kinases, and to lesser extent G-protein-coupled receptors, leading to activation of downstream effector pathways.[20] The PAX8-PPARγ fushion protein results from a balanced translocation of the paired box gene 8 (PAX8) and peroxisome proliferator-activated receptor γ (PPARγ) genes.[21] RAS or PAX8-PPARγ mutations are identified in ~80% of follicular carcinomas.[22]

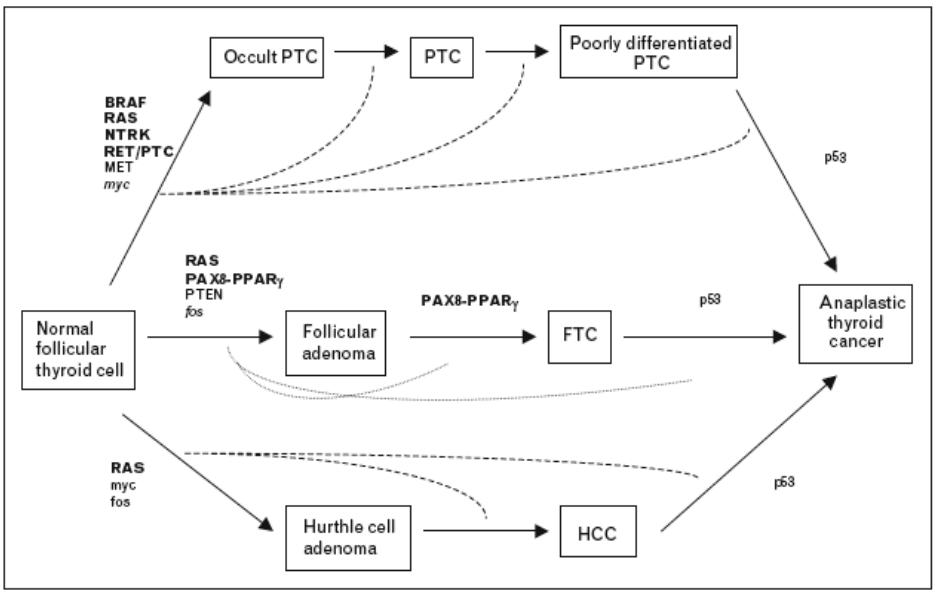

Figure 1. Multistep model of thyroid carcinogenesis and the common genetic changes associated with the different histologic cancer of follicular cell origin.[5, 23]

BRAF mutation is the most common and its detection has a 100% positive predictive value for papillary carcinoma.[24] BRAF mutation is the most common and its detection has a 100% positive predictive value for papillary carcinoma. BRAF V600E mutation is primarily present in conventional papillary thyroid cancer. It is associated with an aggressive tumor phenotype and higher risk of recurrent and persistent disease in patients with conventional papillary thyroid cancer. Testing for this mutation may be useful for selecting initial therapy and for follow-up monitoring.[15] RAS mutations were the second most common finding and also appeared to be of high diagnostic value. These mutations are found in thyroid carcinomas and also in benign follicular adenomas and some hyperplastic nodules. RAS mutations are found in 20–50% of follicular thyroid cancers with the following relative frequency: NRAS > HRAS > KRAS. The presence of RAS mutations in both follicular adenomas and carcinomas has been taken as evidence that RAS activation may be an early step in thyroid carcinogenesis, although this has not been conclusively proved.[20] A current study by Nikiforov et al, which tested samples from a series of consecutive thyroid

nodules followed prospectively, indicates that finding a RAS mutation in an FNA sample confers a 87.5% probability of malignancy, including a 62.5% probability of a papillary carcinoma and a 25% probability of a follicular carcinoma. The high risk of malignancy provides justification for recommending surgery to all patients with RAS-positive nodules. One out of 8 (12.5%) RAS- positive nodules was a benign follicular adenoma, and therefore in this case the molecular test was false positive. However, RAS-positive "benign" follicular adenomas may be precursor lesions for RAS-positive follicular carcinomas. Moreover, if the transition to carcinoma occurs, the presence of RAS mutations is associated with worse prognosis and propensity for further conversion to undifferentiated carcinoma. Therefore, surgical removal of RAS-positive follicular adenomas may be justifiable to prevent this putative progression. Chromosomal alterations of PPAR*γ*, resulting in the expression of the fusion protein PPFP, may be an early event in the development or progression of follicular thyroid cancer and perhaps the follicular variant of papillary cancer. The detection of these alterations in follicular adenomas may support a stepwise adenoma to carcinoma sequence, or indicate the presence of "carcinoma in situ." However, the PAX8/PPAR*γ* rearrangement in itself may not be sufficient for the development of a malignant phenotype: additional genetic or epigenetic events may be required to enable the full phenotypic expression of follicular thyroid carcinoma.[25] Sapio et al reported similar findings in a prospective study for mutation analysis for RET/PTC1, RET/ PTC3, BRAF, and NTRK, and found that a thyroid cancer diagnosis could be confirmed in 25% of the 16 suspicious for papillary thyroid cancer FNA biopsy results.[5] The diagnostic predictive value of testing for RAS and PAX8-PPARγ is unclear because both these changes may occur in benign thyroid tumors. Because the common genetic changes are mutually exclusive, BRAF, RET/ PTC, NTRK, RAS, and PAX8-PPARγ mutations will be present in approximately 90% of all thyroid cancer of follicular cell origin. Therefore, the combined testing for these genetic changes will result in higher rates of improving the accuracy of FNA biopsy.[5] In a study by Henderson recurrent papillary thyroid cancer is significantly associated with a predominant BRAF mutation. RET/PTC rearrangements, although commonly associated with primary papillary thyroid cancer in younger patients, are uncommonly found in recurrent papillary thyroid cancer patients. In addition, the incidence of dual mutations was higher in patients with recurrent papillary thyroid cancer than in those primary PTC.

Gene expression profiling

Several investigators have used microarray technology to study the expression profiles and to identify molecular markers for thyroid malignancy.[3, 26-29] A variety of potential molecular markers have been studied to help distinguish benign from malignant thyroid neoplasms.[30] Our group found four independent novel diagnostic markers (ECM1, TMPRSS4, ANGPT2, and TIMP1) of malignant thyroid neoplasms that were confirmed by real time RT-PCR. ECM1 is extracellular matrix protein 1; TMPRSS4 is transmembrane protease, serine 4; ANGPT2 angiopoietin 2 and TIMP a metallopeptidase inhibitor 1. These four genes in combination had a sensitivity of 100%, a specificity of 94.6%, a positive predictive value of 96.5%, and a negative

predictive value of 100% in 95 thyroid tumor samples that would be indeterminate or suspicious on FNA biopsy.[30] In 31 thyroid FNA biopsy samples, these four genes in combination had a sensitivity of 91.0%, a specificity of 95.0%, a positive predictive value of 92.9%, and a negative predictive value of 92.3%.[5, 30] Moreover, RT-PCR validation done on a subset of 12 of the 75 genes further confirmed the differential expression in malignant versus benign tumors. In a recent study by Prasad et al, RT-PCR confirmed the differential expression of 12 genes; nine were overexpressed (HMGA2, LRRK2, PLAG1, DPP4, CDH3, CEACAM6, PRSS3, SPOCK1, and PDE5A) and three were underexpressed (RAG2, AGTR1, and TPO5) in malignant tumors compared with benign tumors (Table 3).[3] A recent prospective study by Franco et al confirms galectin-3 and HMBE-1 to be good molecular markers too; the combined use of HBME-1 and galectin-3 in indeterminate FNABs, achieved a 10% increase in sensitivity. These markers showed excellent sensitivity and specificity and may improve patient's selection for surgery.[31-34] Galectin-3 (Gal-3) is a beta-galactoside-binding protein with anti-apoptotic activity, HBME-1 alone or in combination with CK 19 is highly discriminatory in the diagnostic workup for papillary thyroid carcinoma.[35] Umbricht et al, showed telomerase reverse transcriptase (TERT), to be a marker for thyroid cancer with an overall sensitivity of 91% and specificity was 79%.[36] In a retrospective study by Yossie et al, lactoferrin immunoreactivity was strongly associated with neoplastic proliferation and seemed to be a useful auxiliary marker to distinguish malignant from benign thyroid lesions in cytologic smears and biopsy samples.[37]

Matrix metalloproteinases

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases that degrade components of the extracellular matrix and the basement membrane. MMPs are critical to numerous physiologic and pathophysiologic processes, including wound healing, angiogenesis, tumor growth, invasion, and metastasis.[38-40] MMPs can be inhibited by tissue inhibitor metallopeptidases (TIMPs). MMPs are associated with the development and aggressiveness of certain cancers. In normal thyroid cell lines MMP-1, MMP-2, MMP-10, MMP-14, TIMP-1, TIMP-2, TIMP-3 and TIMP-4 are expressed.[38] This pattern of expression changes in thyroid cancer cell lines. Expression of MMP-1, MMP-2, MMP-9, MMP-11, MMP13 and MMP-14 are increased or de novo in thyroid cancer cell lines.[38] In well-differentiated thyroid cancer cell lines TIMP-1 and TIMP-2 are still expressed. However, in anaplastic cell lines, expression of TIMP-3 and TIMP-4 is often lost.[38] In thyroid cancer tissue, MMP-2 expression is significantly higher in PTC than in normal thyroid tissue.[41] In tissue of patients with PTC and lymph node metastasis this MMP-2 expression is even higher.[41, 42] MMP-9 expression is also increased in PTC with an aggressive presentation (larger tumor size, nodal metastases and vascular invasion). MMP-7 and MMP-11 are not or faintly expressed in normal thyroid tissue. In PTC, on the contrary, both MMP-7 and MMP-11 are expressed. However, there appears to be an inverse relationship between tumor size, T-stage, and differentiation grade on one side and the expression of MMP-7 and MMP-11 on the other side.

With increased tumor size, T stage and differentiation the levels of MMP-7 and

MMP-11 expression are decreased.[39] In patients with medullary thyroid cancer (MTC), levels of MMP-2 in the initial thyroid cancer specimen are associated with prognosis.[43] Increased levels of MMP-3 and MMP-9 have been found in patients with MTC.[44] Although gene expression of specific MMPs has been shown to correlate with increased risk for papillary thyroid cancer metastases, the prognostic value of MMPs in thyroid cancer is still not clear.

Table 3. Molecular markers useful in the distinction of malignant from benign thyroid tumors.

Description	Gene Symbol
High mobility group AT-hook 2, transcript variant 1 b	HMGA2
Leucine-rich repeat kinase 2 b	LRRK2
Pleiomorphic adenoma gene 1 b	PLAG1
Dipeptidyl-peptidase 4 (CD26, adenosine deaminase complexing protein 2)	DPP4
Cadherin 3, type 1, P-cadherin (placental)	CDH3
Carcinoembryonic antigen-related cell adhesion molecule 6 (nonspecific cross-reacting antigen)	CEACAM6
Protease, serine, 3 (mesotrypsin)	PRSS3
Sparc/osteonectin, cwcv and kazal-like domains proteoglycan (testican) 1	SPOCK1
Phosphodiesterase 5 A, GMP-specific, transcript variant 3	PDE5A
Recombination activating gene 2	RAG2
Angiotensin II receptor, type 1, transcript variant 5	AGTR1
Thyroid peroxidase, transcript variant 5	TPO5
Extracellular matrix protein 1	ECM1
Transmembrane protease, serine 4	TMPRSS4
angiopoietin 2	ANGPT2
Metallopeptidase inhibitor 1	TIMP
Galactin-3	GAL-3
Mouse anti-human antigen mesothelial cell	HBME-1
Telomerase reverse transcriptase	TERT
Cytokeratin 19	CK 19

[3, 30, 32-36]

MicroRNA

MicroRNAs (miRNAs) constitute a recently identified class of small endogenous noncoding RNAs that act as negative regulators of the protein-coding gene expression and may impact cell growth, differentiation, apoptosis and adhesion, i.e., all fundamental cellular processes implicated in carcinogenesis. MiRNA expression is deregulated in many types of human cancers, including thyroid cancer.[45, 46] It is approximated that one miRNA can potentially regulate more than 200 different genes. A study by the Croce group revealed that approximately 50% of all annoted human miRNA are located in areas of the genome associated with cancer or 'fragile sites' and thus miRNAs might have a crucial function in cancer progression.[46, 47] Specific subsets of overexpressed and downregulated miRNAs have been identified

in various tumor types, suggesting that aberration in miRNA expression is important in tumor development and progression. Overexpression of a miRNAs could result in downregulation of tumor suppressor genes (oncogenic miRNAs or oncomiRs), and under expression of miRNAs could lead to upregulation of oncogenes (suppressor miRNAs) with subsequent effects on cell proliferation, apoptosis, angioinvasion, and other carcinogenic actions.

Our current understanding of miRNA deregulation in thyroid carcinomas is based on several independent studies that collectively analyzed more than 200 thyroid tumors. Snap-frozen tissue was the most commonly used miRNA source; however, the utility of formalin-fixed paraffin-embedded tissue for miRNA analysis was validated in a variety of human tissues including thyroid.[48] Analysis of miRNA expression in normal thyroid tissue and in major types of thyroid tumors revealed that majority of known miRNAs were expressed in normal thyroid tissues, whereas in thyroid neoplasms 32% of miRNAs were found to be consistently upregulated, and 38% were downregulated with more than a 2-fold change as compared to normal tissue.[48] Our group investigated 100 thyroid tissue samples (7 normal thyroid, 14 multinodular goiter, 12 follicular variant of papillary thyroid cancer, 8 papillary thyroid cancer, 15 follicular adenoma, 12 follicular carcinoma, 12 Hurthle cell adenoma, and 20 Hurthle cell carcinoma) and 125 indeterminate FNA samples using a miRNA array to identify differentially expressed genes (5-fold higher or lower) in normal, malignant and benign thyroid neoplasms. Nine miRNAs showed > 5-fold expression difference between benign and malignant thyroid neoplasm on miRNA array analysis. Four of 9 miRNA were validated to be significantly differential expressed between benign and malignant thyroid neoplasm by quantitative PCR. miRNA-100, -125b, -138 and -768-3p were significantly overexpressed in malignant samples. All four miRNA were also significantly overexpressed in Hürthle cell carcinoma samples and only miRNA-125b was significantly overexpressed in follicular carcinoma samples. The overall accuracy for distinguishing benign from malignant thyroid neoplasm was 79%, 98% for Hürthle cell neoplasms, and 71% for follicular neoplasms. MiRNA-138 was significantly overexpressed in the FNA samples ($p=0.04$) that were cancers of follicular origin on final pathology with an accuracy of 75%. Other miRs that were shown to be either up- or downregulated in different thyroid tumors in recent studies, are summarized in Table 4.

A study of miRNA expression in papillary thyroid tumors with known mutations revealed a strong correlation between the miRNA profile and mutational status.[48] Papillary carcinomas positive for BRAF, RET/PTC, and RAS mutations, and those with no known mutations demonstrated significant differences in the expression of certain miRNAs. For example, miRNA-187 was expressed at higher levels in papillary thyroid tumors harboring RET/ PTC rearrangements; miRNA-221 and miRNA-222 were found at the highest levels in BRAF- and RAS-positive tumors and tumors with no known mutations and the highest expression of miRNA-146b in papillary thyroid tumors carrying RAS mutations.[48] Further improvement of diagnostic precision is achieved by supplementary testing of FNA material for somatic mutations known to occur in thyroid tumors. However, the sensitivity of such testing is limited because a

significant proportion of papillary and follicular carcinomas (25–30%) do not harbor any known mutations. These cases would benefit most from additional diagnostic modalities, such as miRNA profiling.[48, 54, 55]

Table 4. miRNA expression in different thyroid tumors in recent literature

Tissue	up/downregulation	miRNAs
Papillary carcinoma	UP	-21, -31, -34a, -146, -146b, -155, -172, -181a, -181b, -187, -213, -220, -221,-222,-223, -224
Papillary carcinoma	DOWN	-26a-1, -30c, -138, -218, -292, -300-319, -345
Follicular carcinoma UP		-155, -187, -221, -222, -224
Follicular adenoma	UP	-190, -205, -210, -224, -328, -339, -342
Hürthle cell carcinoma	UP	-31, -183, -203, -221, -224, -339
Hürthle cell adenoma	UP	-31, -183, -203, -221, -224, -339
Poorly diff. carcinoma	UP	-129, -146b, -183 -187, -221, -222, -339
Anaplastic carcinoma	UP	-137, -155, -187, -205, -214, -221, -222, -224, -302c
Anaplastic carcinoma	DOWN	-30d, 125b, 26a, 30a-5p
Medullary carcinoma	UP	-9, -10a, -124a, -127, -129, -137, -154, -224, -323, -370

[45, 48-53]

Conclusions and future perspective

There has been significant progress toward identifying biomarkers that would improve the accuracy of FNA biopsy in the evaluation of patients with thyroid nodules and predicting disease aggressiveness. Molecular testing for common somatic genetic mutation testing is most promising because false positive results are unlikely and unnecessary diagnostic surgery can be avoid in these cases. Gene expression profiling studies have identified many possible biomarkers with high accuracy and although the clinical application is still unclear, analyses based on combined multigene RT-PCR assays of candidate markers seem to have the future as diagnostic tools. Expression analysis of differentially expressed miRNA is a promising diagnostic approach for distinguishing benign from malignant thyroid neoplasm that is indeterminate on thyroid FNA biopsy. The available data provide a basis for larger prospective trials to evaluate in which way these emerging biomarkers can be useful in the management of thyroid tumors.

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Chapter 3

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Expression of matrix metalloproteinases in different stages of papillary thyroid cancer; a case-control study

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Abstract

Goal

The goal of this study was to determine gene-expression of several matrix metalloproteinases (MMP) and tissue-inhibitor metalloproteinases (TIMP) genes in patients with different stages of papillary thyroid cancer (PTC) using Real-Time PCR. Furthermore, we sought to determine if the level of gene-expression of these MMPs and TIMPs can be used as a prognostic factor for PTC.

Methods

Clinical data and tumor tissue from 56 patients were selected from the UCSF PTC database. RNA was extracted from the tissues and gene-expression of the target MMPs and TIMPs was determined using Real-Time PCR. Gene-expression of MMP 2, 7, 9, 11, 13, 14 and TIMP 1, 2 ,3, and 4 were determined in patients with PTC confined to the thyroid gland, patients with central lymph node metastases (LNM), lateral LNM, central and lateral LNM, recurrent PTC and patients who died of the disease and compared between different stages. We also compared gene-expression between different T-stages.

Results

All MMPs and TIMPs showed expression in all samples with PTC. First we compared MMPs and TIMPs between different stages of disease. Between patients with lateral lymph node metastasis and their controls there was a significant difference for MMP 14 (lower in index patients than matched controls) and a trend for TIMP 3 and TIMP 4. Between other index groups and their controls we found trends for 2, MMP 13, TIMP 2, and TIMP 3 and 4 of which MMP 13 had a higher expression in index groups most frequently.

Secondly, we compared patients who had stage T1 to those who had T2, T3 and or T4. We found that MMP 7, MMP 13 and MMP 14 were significantly different between patients. The gene-expression of MMP 14 was higher in patients with stage T1 than in those with T2 +T3 +T4. The levels of gene-expression of both MMP 7 and MMP 13 were lower.

Conclusion

We conclude that multiple MMPs and TIMPs are differently expressed in different stages of PTC of which MMP 13 is of specific interest. MMP and TIMPs are potential prognostic factors for PTC.

Introduction

Thyroid carcinoma is the most common endocrine cancer. The incidence of thyroid cancer is 8.7 per 100.000 and rising. Papillary thyroid cancer (PTC) is the most common form of thyroid cancer, accounting for 70-80% of all cases.[1]

The prognosis of PTC is generally good, but ten percent of the patients dies.[2] Several prognostic factors are known to be associated with a worse outcome: older age, male sex, the presence of lymph node metastasis, larger tumor size and extrathyroidal tumor extension. More recently, outcome has been associated with several genetic factors, including the BRAF and RET/PTC genes[3, 4] and the matrix metalloproteinases (MMPs) family.

Matrix metalloproteinases are a group of enzymes associated with the development and aggressiveness of certain cancers. They promote tumor progression by degradation of the extracelluar matrix (ECM),[5, 6] and can be inhibited by tissue inhibitor metalloproteinases (TIMPs). Several MMPs are expressed in certain cancers, including PTC. MMP 2 overexpression has been noted in PTC with lymph node metastasis.[5-7] Furthermore, the intensity of MMP 2 immunohistochemical staining has been correlated with clinical outcome of patients and if lymph node metastases were present at initial surgery.[8, 9] MMP 7 expression is associated with aggressiveness of several types of cancer including PTC.[5] Other MMPs (MMP 9, MMP 11, MMP 13 and MMP 14) appear to be involved as well.

In several cancers, the number of different MMPs that are expressed increases with the progression of the tumor.[10] It is unknown if this is also true for PTC. It is also unknown what the role of TIMPs are in tumor progression. Most studies have used immunohistochemistry to study the use of MMPs and TIMPs as a prognostic factor or thyroid cancer cell lines.

The goal of this study was to determine gene-expression of several MMP and TIMP genes in patients with different stages of PTC using Real-Time PCR. Furthermore, we sought to determine if the level of gene-expression of these MMPs and TIMPs can be used prognostic factor for PTC.

Methods

We conducted a case-control study among patients with papillary thyroid cancer (PTC) in different stages with a long-term follow-up. From the UCSF PTC database patients were selected whose tissue had been stored in the databank at -80°C. Clinical data were collected from the database as well. Patients were categorized into the following groups, based on the clinical stage of their PTC: PTC alone (confined to the thyroid gland), PTC and central lymph node metastases, PTC and lateral lymph node metastases, PTC and central & lateral lymph node metastases, PTC and recurrent disease and patients who died of PTC. Patients with a stage more than PTC confined to the thyroid gland were classified as index patients. We collected patients with PTC confined to the thyroid gland who matched the index patients on the basis of T stage (TNM classification), age and gender.

RNA Extraction

First, frozen PTC tissue was sectioned for RNA isolation. Total RNA was extracted from homogenized frozen tissue using TRIzol reagent (Invitrogen, Carlsbad, California, USA), according to the manufacturer's protocol. RNA quantity was assessed with Nanodrop®.

Real-Time Quantitative RT-PCR (Taqman®)

Gene-expression of the target MMPs and TIMPs was determined using a Real-Time PCR machine from Applied Biosystems (Carlsbad, California, USA). Total RNA was reverse transcribed into cDNA using the iScript c-DNA synthesis kit (Bio-rad, Hercules, California, USA) by following manufacturer's instructions.

For the amplication of cDNA, TaqMan® MasterMix (AppliedBiosystems) was used. All the PCR experiments were performed in a final volume of 10 μl (ABI PRISM 7900 Sequence Detection System; Applied Biosystems). The PCR thermal cycler condition was 95°C for 12 minutes, followed by 40 cylces at 95°C for 15 seconds and 60°C for 1 minute.

Gene-expression was measured by the Ct-level. This is the cycle treshold required for the fluorescent signal of the dye labeled to the cDNA to exceed the background. The lower the Ct levels the higher the gene-expression of the target gene. To determine gene-expression of several MMP family members, the following primers and probes of Applied Biosystems were used: MMP 2 (MMP2 Hs00234422_m1), MMP 7 (Hs00159163_m1), MMP 9 (Hs 00957562_m1), MMP 11 (Hs00171829_m1), MMP 13 (Hs00942589_m1), MMP 14 (Hs00237119_m1), TIMP 1 (Hs99999139_m1), TIMP 2 (Hs 00234278_m1), TIMP 3 (Hs00165949_m1) and TIMP 4 Hs (00162784_m1). As a control, GUS-B (Hs99999908_m1) was used. Of each tissue sample, the gene-expression was determined in triplicate. As a control for each MMP and TIMP we also performed an experiment in triplicate with a human reference.

Statistical analysis

We calculated the average Ct (mean) and standard deviation (SD) of the gene-expression of the three experiments of each sample. We then calculated delta Ct and percent expression normalized to GUS-B. The gene expression level was normalized to GUS-B mRNA expression as follows: Normalized gene expression = 2½Ctgene of interest_CtforU18_ · 100%, where Ct = the quantitative PCR cycle threshold. If gene-expression was lower than 29, there was an abundant of target nucleic material in the sample, if it was between 30 to 37, there was a moderate amount of target nucleic material and if 38 to 40 there was minimal amount of target nucleic material. The total number of runs was 40, if the Ct-level treshold was beyond 40, the gene-expression was judged to be undetermined. First, we compared samples of index patients with different stages of disease to their controls. Secondly, we compared patients with PTC stage T1 to those with stage T2 or higher. The T stage was based on the TNM classification.

We used the Kruskal-Wallis test to determine differences in Ct, delta Ct and percent expression normalized to GUS-B. We considered a p-value of 0.05 to be significant.

Results

The percentage of successful Taqman® experiments per plate (of 392 wells) varied between 67 and 97% (Table 1).

Table 1. Percentage of Succesful experiments per MMP/TIMP

MMP	Experiments/Wells	Undetermined	Succesful percentage
MMP 2	369	22	94%
MMP 7	310	25	92%
MMP 9	330	29	91%
MMP 11	252	39	85%
MMP 13	296	99	67%
MMP 14	266	14	95%
TIMP 1	242	10	96%
TIMP 2	232	8	97%
TIMP 3	214	9	96%
TIMP 4	210	41	80%

Overall, we found that in patients with PTC the following MMPs and TIMPs had a high gene-expression (abundant of target nucleic acid present in sample): MMP 14 (mean Ct=29) and TIMP 1 (mean Ct =27). There was a moderate expression of MMP 2 (mean Ct=30), MMP 7 (mean Ct=32), MMP 9 (mean Ct=30), MMP 11 (mean Ct=33), MMP 13 (mean Ct=37), TIMP 2 (mean Ct=30), TIMP 3 (mean Ct=30) and TIMP 4 (mean Ct=37).

In Figure 1, gene-expression of MMPs and TIMPs that were significantly different between index patients and their matched controls or that showed a trend are shown. We found that there was no significant difference between patients who had central lymph node metastases (index) and their matched controls. There was a trend that MMP 2, MMP 13, TIMP 2, and TIMP 3 were different in this group. We found that the gene-expression of TIMP 3, MMP 13 and MMP 2 was higher in the index patients (PTC and central lymph node metastases) than in their matched controls. The gene-expression of TIMP 2 was lower in the index patients. Between patients with lateral lymph node metastasis and their controls there was a significant difference for MMP 14 and a trend for TIMP 3 and TIMP 4. We found a higher gene-expression of TIMP 3 and TIMP 4 and a lower expression of MMP 14 in index patients compared to their matched-controls. For patients who had central and lateral lymph node metastasis and their controls we found a trend towards higher gene-expression of MMP 13, TIMP 2, and TIMP 4 and lower gene-expression of TIMP 3 in index patients compared to control patients, albeit not significant. For patients who had developed recurrent disease, gene-expression of MMP 13 was significantly higher and there was a trend for higher gene-expression of TIMP 3. When we combined all patients with central lymph node metastasis and/or lateral lymph node metastasis, those who developed recurrent disease and those who died of disease, we did not found significantly different expressions of MMPs or TIMPs and only a weak trend for higher gene-expression of MMP 13 and TIMP 3.

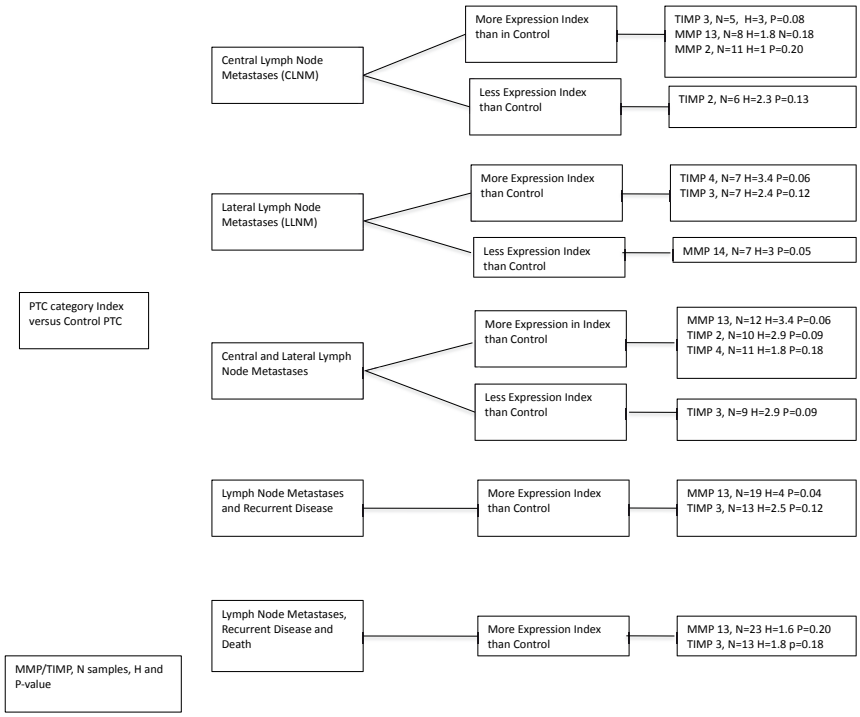

Figure 1. Comparisson of expression of MMPs and TIMPs between different stages of PTC in index patients and their controls.

Secondly, we compared patients who had stage T1 to those who had T2, T3 and or T4 (Figure 2). We found that MMP 7, MMP 13 and MMP 14 were significantly different between patients who had T1 stage tumors and those had who T2, T3 or T4 stage tumors. The gene-expression of MMP 14 was higher in patients with stage T1 than in those with T2 +T3 +T4. The levels of gene-expression of both MMP 7 and MMP 13 were lower. The same significant differences were seen for MMP 7 and MMP 13 when only T1 was compared to T2. When comparing T1 to T3, only MMP 13 remained significant. The rest showed the same differences in MMPs and TIMPs, but these were trends. There was a trend of TIMP 3 and TIMP 4 having a higher expression in T1 compared to T2. MMP 14 showed a trend of higher expression in T1 than in T3.

Discussion

MMPs are involved in multiple aspects of the neoplastic development tumor growth, neoangiogenesis, intravasation, extravasation, and almost all metastatic steps. We found that the with Real-Time Quantification PCR, MMP 2, MMP 7, MMP 9, MMP 11, MMP 13, MMP 14, TIMP 1, TIMP 2, TIMP 3 and TIMP 4 were all highly or moderately

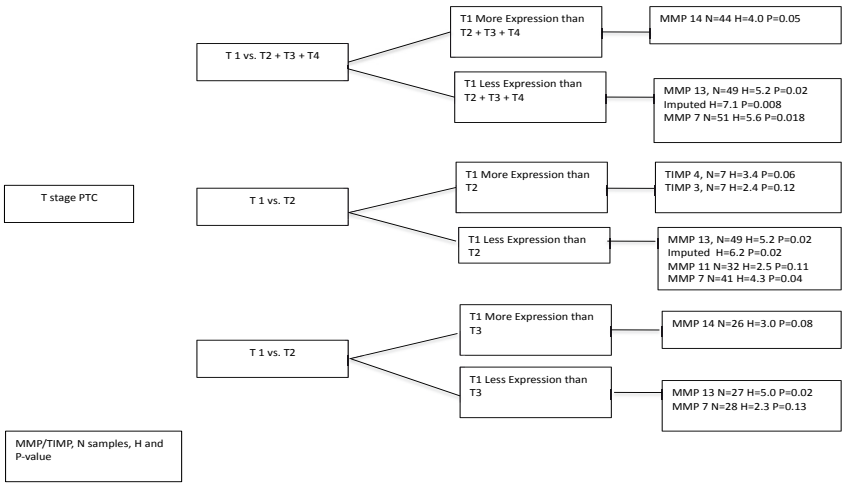

Figure 2. Comparisson of expression of MMPs and TIMPs between different T stages

expressed in the PTC tissue samples in our study. Others have also found several MMPs and TIMPs to be involved in PTC in different studies using different techniques.[10] [5, 6, 8, 9]

When we compared the gene-expression in different stages of PTC and different T-stages, we found differences in expression in several MMPs and TIMPs. In the comparison between different stages of PTC with or without lymph node metastases, recurrent disease and death of disease, MMP 13 had a significantly higher expression in higher stages. Other MMPs and TIMPs that were significantly different or that showed trends between different stages of disease or T stage were MMP 2, MMP 7, TIMP 2, TIMP 3, TIMP 4. In lower tumor stages MMP 14 was overexpressed compared to higher T stages. In higher T stages MMP 7 and MMP 13 showed more expression compared to the lower T stage. MMP 13 showed the most significant difference in expression. Therefore, especially, MMP 13 appears to be of specific interest

In a study on expression of MMPs and TIMPs in cell lines, it was found that in normal thyroid cell lines, MMP 1, MMP 2, MMP 10, MMP 14, TIMP 1, TIMP 2, TIMP 3 and TIMP 4 are expressed. In PTC cell lines, the expression of MMP 1, MMP 2, MMP 14 and TIMP 4 was increased compared to normal cells. MMP 9, MMP 11 and MMP 13 were newly induced compared to normal cells.[10] The finding of significant differences in different stages of MMP 13 in our study may indicate that with an increased stage of PTC, the expression increases even more.

MMP 13 appears to be expressed in areas where rapid extracellular matrix remodeling is required as in invasive tumor growth.[11] MMP 7 is mainly expressed by tumor cells and contributes to tumor invasion.[5] It has been shown that there appears to be an inverse relationship between tumor stage and expression of MMP 7 and 11.[5] The

higher the tumor stage, the lower the expression of these MMPs. We did not confirm that with our real time PCR analysis. MMP 7 was often more expressed in tumor tissue of higher stages. The same applies to MMP 11, which showed a trend that the expression was higher in T stage 2 than T stage 1.
In one study that studied MMP 2 expression with immunohistochemical staining in PTC tissue, a clear relationship between the intensity (index of) of MMP 2 staining and clinical outcome was observed.[8]
It has been reported that the number of different MMPs family members and the level of expression increases with progression of the tumor, and that the relative level if any individual family member increases with tumor stage.[10] This is interesting because different expression patterns may also provide useful prognostic information. In this study, we did not study expression patterns of several MMPs and TIMPs.
Limitations of our study were small sample size and insufficient amount of cDNA to test all MMPs of interest. Additionally, we did not perform immunohistochemical testing of all tissue. We only included tissues samples of PTC and did not include normal tissue. This might explain why we did not find large differences in the gene-expression of different stages of PTC. It could be that with more aggressive disease or recurrent disease, the expression of MMPs and TIMPs changes.
From this feasibility study and other studies, several MMPs and TIMPs have been shown to be involved in PTC. Additionally, different MMPs and TIMPs are differently expressed in different stages of the disease. Our preliminary finding are interesting and have to be explored more extensively, since MMPs and TIMPs may be prognostic markers for PTC. Immunohistochemistry studies and studies using fresh tumor tissue will help us to elucidate their expression patterns even more. MMP gene expression is thus a great factor of interest which can be adopted in the future to determine surgical strategy.

Conclusion
We conclude that multiple MMPs and TIMPs are differently expressed in different stages of PTC of which MMP 13 is of specific interest. MMP and TIMPs are potential prognostic factors for PTC.

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Chapter 4

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Fluorodeoxyglucose-positron emission tomography scan positive recurrent papillary thyroid cancer and the prognosis and implications for surgical management

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Submitted for Publication

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Abstract

Aims

To compare outcomes for patients with recurrent or persistent papillary thyroid cancer (PTC) who had metastatic tumors that were fluorodeoxyglucose-positron emission tomography (FDG-PET) positive or negative, and to determine whether the FDG-PET scan findings changed the outcome of medical and surgical management.

Methods

From a prospective thyroid cancer database, we retrospectively identified patients with recurrent or persistent PTC and reviewed data on demographics, initial stage, location and extent of persistent or recurrent disease, clinical management, disease-free survival and outcome. We further identified subsets of patients who had an FDG-PET scan or an FDG-PET/CT scan and whole-body radioactive iodine scans and categorized them by whether they had one or more FDG-PET avid (PET positive) lesions or PET negative lesions. The medical and surgical treatments and outcome of these patients were compared.

Results

Between 1984-2008, 40 of 144 patients who had recurrent or persistent PTC underwent FDG-PET (n=10) or FDG-PET/CT scans (n=30); 23 patients(55%) had one or more PET-positive lesion(s), fifteen (40%) had PET-negative lesions, and two had indeterminate lesions. Most PET-positive lesions were located in the neck (16/23). Patients who had a PET-positive lesion had significantly larger tumors (34 vs. 21mm, P=0.039) and a higher TNM stage (P=0.025). Only patients who had PET-positive lesions died (6/23 vs. 0/15 for PET-negative lesions; p=0.030). Two of the seven patients who underwent surgical resection of their PET- positive lesions were cured.

Conclusion Patients with recurrent or persistent PTC and FDG-PET positive lesions have a worse prognosis. Some patients benefit and others may be cured by re-operation if the lesion is localized in the neck or mediastinum.

Introduction

Papillary thyroid cancer (PTC) accounts for 80-85% of all thyroid cancers. Although most patients with PTC have a good prognosis, recurrent disease develops in about 20-30%, and about 7% of patients die from progressive disease within 10 years of diagnosis.[1] After initial surgery for PTC, follow-up consists of regular ultrasound examination of the neck, measurements of serum thyroglobulin (Tg) and Tg antibodies, and whole-body iodine scans (WBS).[2] Patients who have recurrent or persistent disease usually have increased serum Tg levels. Recurrent PTC can be identified best by ultrasound imaging of the neck, increased basal or stimulated serum Tg levels, and whole-body iodine scans. Metastatic PTC tumors can be identified by radioactive iodine scans, because well-differentiated thyroid cells often take up and concentrate iodine.[3]

The loss of iodine uptake by metastatic PTC is associated with worse survival.[4] The sites where iodine uptake is negative in patients with recurrent or persistent PTC may be localized with fluorodeoxyglucose-positron emission tomography (FDG-PET) or FDG-PET-Computed Tomography (FDG-PET/CT). Both FDG-PET scans and FDG-PET/CT scans are especially useful for identifying the site of recurrent or persistent disease in patients who have increased serum Tg levels and negative whole-body iodine scans.[5] The sensitivity of FDG-PET scans (63-95%) and FDG-PET/CT scans (66-98%) is similar in patients with well-differentiated thyroid cancer, increased Tg levels and negative whole body scans,[6] but FDG-PET scans (0-25%[7]) have much lower specificity than do FDG-PET/CT scans (81%[6]). The positive predictive value of FDG-PET/CT ranges between 92-100%[8, 9] and the negative predictive value is about 27%.[9]

Metastatic PTCs that are FDG-PET-positive, but radioactive-iodine negative, often have a higher malignant histological grade than the primary tumor, a pattern that is less common for FDG-PET-negative PTCs.[10] Furthermore, PTCs that are FDG-PET positive and have a higher maximum standard uptake value (SUV max) are associated with a worse prognosis.[11] Whether the more aggressive nature of these FDG-PET-positive PTCs in the neck warrants more aggressive surgical therapy is not unknown. To address this question, we compared the outcome of patients with recurrent or persistent PTC according to whether the cancer was positive or negative on FDG-PET or FDG-PET/CT scans. We also determined the outcome and surgical management of lesions that were FDG-PET or FDG-PET/CT positive.

Methods

Data collection

After obtaining approval from our institutional review board, we searched the database of the California Cancer Registry to identify all patients with PTC treated at the University of California San Francisco (UCSF) between 1984 and 2008. Some of these patients were referred to UCSF because of recurrent or persistent disease after having their initial treatment at other medical centers. For all patients with recurrent or persistent PTC, we recorded data on demographics, initial stage of PTC, location and number of recurrences, disease-free survival and outcome.

We further identified the subset of patients who had either an FDG-PET scan or an FDG-PET/CT scan and a whole-body radioactive iodine (^{131}I) scan, and categorized them by whether they had one or more FDG-PET avid (PET-positive) lesions or PET-negative lesions. The medical and surgical treatment and outcome of these patients were then compared. A common indication for a FDG-PET scan was an elevated blood Tg level and a negative ^{131}I scan. We reviewed all reports of the FDG-PET and FDG-PET/CT scans and documented the number of FDG-PET positive lesions and the standard uptake value of these FDG-PET avid lesions. The sites of the metastases were also recorded.

Statistical Analysis

Data are presented as mean ± standard deviation (SD) or median and interquartile range based on the distribution of data. T-tests, Mann-Whitney U tests and chi-square tests were used as appropriate to compare groups. A p-value <0.05 was considered statistically significant. All analyses were done using SPSS 16.0 (SPSS Inc. Chicago Illinois).

Results

For the 24-year period, 1052 patients with PTC were identified, 144 of whom had recurrent or persistent PTC. There were nearly twice as many women as men, and the mean age was 46 years (Table 1). Forty patients (28%) underwent an FDG-PET scan, of which 30 were FDG-PET/CT scans. Twenty-three patients (55%) had one or more lesions that were FDG-PET positive, 15 (40%) had one FDG-PET negative lesion, and two (5%) had a lesion with uncertain uptake (Figure 1). FDG-PET positive lesions were most often seen in the neck, followed by neck and lung, lung, multiple sites and lungs. The median standard uptake value was 4.20 g/ml (range 1.5-29).

The clinical characteristics of patients with FDG-PET positive and FDG-PET negative tumors are listed in Table 1. Patients with positive lesions had significantly larger tumors and a higher TNM tumor stage. Their mean serum thyroglobulin level was also higher at the time of evaluation (34 μl/L versus 3.1), but this difference was not statistically significant (P=0.062). Despite the slightly lower ^{131}I uptake in patients with PET-positive lesions, patients whose lesions were ^{131}I-negative were treated surprisingly more frequently with ^{131}I (Table 2). Disease-free survival was similar between the two groups, but only patients with PET-positive lesions died of recurrent or persistent disease (6 out of 23 versus none out of 15, p=0.030) (Table 2). Four of these patients died of metastatic PTC. One additional patient who died of an unknown cause and another who died of endocarditis had extensive metastatic disease.

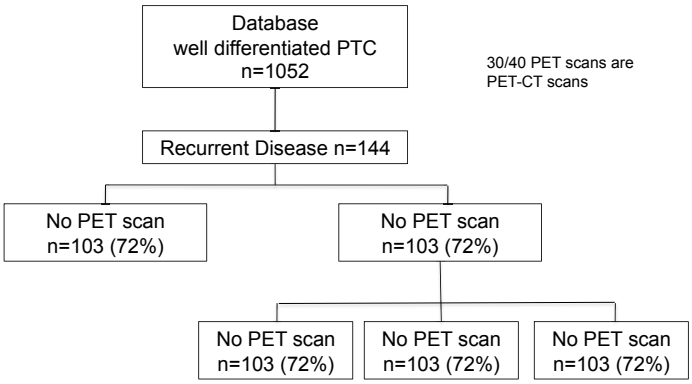

Figure 1. Flow Chart Showing Patient Selection and Outcome for Patients with PTC Treated at the University of California San Francisco between 1984 and 2008.

Table 1. Characteristics of patients with FDG-PET-positive and FDG-PET-negative Recurrent Papillary Thyroid Cancer

Characteristic	FDG-PET Positive N=23	FDG-PET Negative N=15	P-value
Mean age years ± SD*	54 ± 17	42 ± 20	0.07
Gender Male : Female	10:12	5:11	0.483
Initial TNM Stage			0.025
Stage 1	4	8	
Stage 2	0	1	
Stage 3	12	1	
Stage 4	5	2	
Mean tumor size (mm) ± SD	34 ± 19	21± 13	0.039
Extrathyroidal extension of the tumor at diagnosis (yes/no)	10 (46%)	7 (44%)	0.634
Lymph node metastases at diagnosis (yes/no), n (%)	12 (55%)	9 (56%)	0.985
Median thyroglobulin level at recurrence µg/L(range)	34 (0.9-164380)	3.1 (0.3-695)	0.062

* Average age of the total 147 patients with recurrent PTC is 46 ± 20 years. SD = standard deviation.

Table 2. Comparison of Outcomes for Patients with FDG-PET Positive and FDG-PET Negative Recurrent Papillary Thyroid Cancer

Characteristic	FDG-PET Positive (N=23)	FDG-PET Negative (N=15)	P-value
Disease-free survival, (months), median (range)	17 (1-200)	41 (4-67)	0.809
Surgical Treatment, (n)	7	8	0.291
^{131}I Uptake (yes/no), n (%)	5 (23%)	8 (53%)	0.281
^{131}I Therapy (yes/no), n (%)	10 (59%)	6 (38%)	0.517
Outcome, n (%)			0.240
Stable disease	4 (23%)	5 (9%)	
Cure*	1 (5%)	3 (18%)	
Progressive Disease	8 (36%)	9 (57%)	
Death†	6 (27%)†	0 (0%)†	

† There was a significant difference for 'death' between the PET + and PET – group in the Fisher's exact test (P=0.030)

Seven patients underwent surgical resection of a recurrent FDG-PET positive lesion(s) (Table 3). One of these patients was cured after a mediastinal lesion (Tg 0.2μg/L) was resected and all scanning tests were negative seven years after reoperation. Another patient had no evidence of residual disease on an ultrasound of the neck, whole-body iodine scan and PET scan; the Tg level was 3.8μg/L four years after PET-positive metastases were removed. One patient who had a PET-positive lesion in the neck who underwent surgical resection died of metastatic thyroid cancer. Five other patients who died did not undergo surgical resection of their PET-positive lesions, primarily because of the presence of diffuse pulmonary, hepatic or bone metastasis or sites in the head and neck region that were less accessible for removal. In the PET-negative group, two patients appeared to be cured, one after a removal of a metastatic cervical lymph node from the neck, and another with radioactive iodine therapy. The remainder of patients in both groups had stable or progressive disease (Table 3).

Discussion

FDG-PET and FDG-PET/CT can help identify metastatic thyroid cancer in patients with recurrent or persistent PTC who have increased serum thyroglobulin levels and negative or equivocal whole-body iodine scans.[3, 8, 12] Metastatic PTCs that lose the ability to concentrate radioactive iodine are believed to be clinically more aggressive and have a worse outcome, probably because they are less well differentiated and cannot be treated successfully with radioactive iodine therapy. Papillary thyroid cancers that are radioactive- iodine negative take up FDG-PET more readily because of their higher metabolic activity.[3]

Table 3. Surgical Treatment and Outcome of Patients with FDG-PET Avid Recurrent Papillary Thyroid Cancer

Patient	Location of FDG-PET Avid lesions	Surgical Indication	Operative Details	Outcome After Surgical Treatment of FDG-PET Avid Lesion
1	Neck lesions only, levels 2 and 3	Palpable mass, level 2/3 FNA: metastatic PTC	Incision, upper neck, left side	Persistent disease; Tg 56.5 μg/L, no more PET positive lesions; ultrasound: multiple small nodes <1cm
2	2 neck lesions, right side; jugular node; SUVmax 7.1	Patient qualified for surgery in spite of the result of the PET scan Palpable lymph node, right side of the neck	Right sided neck dissection	Cure after surgical resection of both lesions. Low Tg levels (3.8μg/L), ultrasound without evidence of suspicious lymph nodes in the neck, negative PET scan and a negative whole body iodine scan after 4 years of follow-up.
3	Neck lesion only	Lesion detected on ultrasound: size 1.2 cm FNA: recurrent tumor	Lymph node resection in the central neck	Progressive disease. PET positive lesions in the neck and lungs at Patient is scheduled for another neck dissection.
4	Neck lesion only	Palpable lesion near right clavicle. Tg level was 63.8 μg/L.	Right neck dissection	Died
5	Neck and lung metastases; SUVmax 2	Lesion detected on ultrasound: size 1cm. Tg level was 8.6 μg/L.	Central neck dissection	Progressive disease. Tg level increased from 7.9 to 17 μg/L. PET scan showed lung metastasis but no residual disease in the neck.
6	Neck lesion; PET scan initially interpreted as negative, but later interpreted as positive.	Palpable mass of 2.3cm near the left mandible, also shown by ultrasound. The patient was operated on in spite of PET scan results. FNA: recurrent tumor	Several small nodes were resected and the large node.	FU only months, outcome yet unclear

When we compared the outcome of patients with recurrent or persistent PTC who had FDG-PET positive or FDG-PET negative metastatic tumors, we found that patients who had positive lesions had larger tumors at the time of initial treatment, and that their initial TNM stage was higher than patients who had a negative lesion. These patients also had higher serum thyroglobulin levels at the time of the FDG-PET scan. In our cohort study, only patients with FDG-PET positive lesions died. Although our

sample size is relatively small, this observation supports previous reports that PET-positive lesions are associated with a worse prognosis.[3] One of those previous studies showed that patients with PET-positive lesions were also older, had a higher initial cancer stage and higher thyroglobulin levels.[11] These risk factors indicate that PET- positive patients have extensive and more aggressive PTC and are more likely to have either recurrent or persistent thyroid cancer. Another study found that most patients with PET-positive metastases also had high-risk clinical characteristics like extensive extrathyroidal extension of the primary tumor, and that 70% of patients with PTC had less well differentiated metastases than found in their primary tumors.[10]

In our study, only two of seven patients with PET-positive neck lesions who underwent reoperation were cured. One of these patients had a mediastinal lymph node metastasis resected. The other patient had two lymph nodes resected and had no evidence of residual disease on imaging studies four years after surgery, but the thyroglobulin level remained slightly elevated. Although one might question why palliative reoperations were done in patients with distant metastasis, we believe that surgical resection of PET-positive lesions in the neck is indicated in to control locoregional disease. One might anticipate that PET-positive lesions would grow faster and be more likely to cause local morbidity. Although we failed to show a clear benefit of surgical resection in this study, we believe that resection of recurrent or persistent neck lesions should usually be performed especially for recurrent tumors that are growing or are larger than 1cm.

In our study, all FDG-PET-positive lesions that were resected were either palpable or seen on ultrasound of the neck or MRI of the mediastinum. Ultrasound is generally more sensitive for identifying nodal metastases in the neck than FDG-PET and FDG-PET/CT.[13] Ultrasound-guided needle biopsy can provide a definitive diagnosis. In two patients, the decision to operate was made regardless of the FDG-PET scan result. In Table 4 we suggest possible indications and contraindications for resection of PET-positive cervical or mediastinal metastatic disease in patients with or without distant metastases.

In a previous study, the impact of FDG-PET/CT for recurrent PTC on the clinical management changed the treatment plan in 40% of 33 patients, supported the treatment plan made before the PET-scan in 27%, and failed to contribute to the management in 33%.[9] FDG-PET/CT scanning was most useful when the thyroglobulin levels were greater than 10ng/ml. In that study, 22 out of 30 patients were treated surgically. However, they did not show the outcome of these patients. Another study that evaluated the clinical value of FDG-PET scans in 37 patients who had an increased thyrogloblin level and a negative whole-body iodine scan found that 28 patients had a positive PET scan. In 29 patients, the management of these patients appears to have changed from the initial treatment plan (23 underwent surgery, 14 of whom were disease-free after a mean of 6 months.[14] FDG-PET/CT scan may contribute to the surgical strategy in patients with persistent or recurrent PTC.

Table 4. Suggested Indications and Contraindications for Surgical Resection of FDG-PET Positive Lesions in Patients with Persistent or Recurrent Papillary Thyroid Cancer

Indications	Contraindications
Solitary or enlarged neck lesion(s) on ultrasound,or palpable lesions	Solitary neck lesion(s) < 1cm on ultrasound in the presence of distant metastases.
Cervical metastases > 1cm on ultrasound or palpable lesions, despite the presence of distant metastases in otherwise healthy patients	Diffuse distant metastases without evidence of neck lesions
Upper mediastinal lesion(s) or solitary pulmonary or bone lesion(s)	PET positive lesion in neck which is not palpable or can not be identified by ultrasound examination or other localization studies

In our study, 27% of patients with PET-positive lesions had metastatic lesions that took up radioactive iodine. This is a lower percentage than the 37.5% reported previously.[11] In that study, prognosis was surprisingly worse in patients who had both PET-positive lesions and radioactive iodine uptake, and survival was similar, whether their lesions did or did not take up radioactive iodine.[11] We were surprised to find that among our patients who had PET- positive lesions that were less likely to take up radioactive iodine, yet more were treated with radioactive iodine than patients with PET-negative lesions. In a report of the Memorial Sloan Kettering Cancer Center experience, patients with PET positive lesions were treated with higher doses of radioactive iodine than those with PET-negative lesions. Despite this higher dose of radioactive iodine, subsequent FDG-PET uptake was similar after one year, the PET volume of the lesions was larger, and the thyroglobulin level was also higher after one year of follow-up.[15]

The results of reoperation of patients with PET-positive lesions have not been studied previously. Nevertheless, our study had several limitations: its retrospective design, relatively small sample size, and inability to obtain histological confirmation for all FDG-PET and FDG-PET/CT lesions. Moreover, using FDG-PET scanning to localize recurrent or persistent PTC has some established disadvantages. First, it has relatively low sensitivity, specificity and positive predictive value. Second, it is expensive. Third, the decision to operate does not always depend on the scan results, which are certainly of most value in patients who have elevated thyroglobulin levels, but radioactive-iodine negative and also have other negative scans. However, FDG-PET scanning can document patients with multiple distant metastases who are not candidates for reoperation.

In conclusion, patients with recurrent or persistent PTC that is FDG-PET scan positive have a worse prognosis, although some can be cured by re-operation after resection of metastatic tumors in the neck or mediastinum. We believe that localized neck lesions that are FDG-PET scan positive should be resected.

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Chapter 5

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Factors predicting outcome of total thyroidectomy in young patients with multiple endocrine neoplasia type 2. a nationwide long-term follow up study

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Abstract

Background

MEN 2 is caused by a *RET*-mutation in chromosome 10. All MEN 2 patients develop medullary thyroid carcinoma (MTC). The age-related risk of MTC is associated with the type of *RET*-mutation. Our aim was to identify prognostic factors associated with recurrent MTC in MEN 2 patients.

Methods

In a nationwide case-control study all patients who underwent total thyroidectomy in the Netherlands under the age of 20 years were classified into standard (1), high (2) or very high risk (3) for MTC based on *RET* mutation type. Disease-free patients were compared with those with recurrent disease.

Results

A total of 93 patients were included. Sixty-six percent had MTC on histology, the youngest being one year old. Codon 634 was most affected. Sixteen (18%) patients had persistent or recurrent disease, one of whom died. Significantly associated determinants of outcome in univariate analysis were higher age at surgery, no age-appropriate prophylactic surgery according to risk level, elevated preoperative calcitonin levels, affected codon, and the presence of lymph node metastases at surgery. On multivariate analysis only age of surgery was the single independent factor associated with persistent disease.

Conclusions

Prophylactic thyroidectomy beyond the recommended age is associated with persistent/recurrent disease. In addition, codon 634 mutation is associated with a high risk of recurrence requiring early surgery for all these patients.

Introduction

Multiple endocrine neoplasia type 2 (MEN 2) syndrome is a rare autosomal inherited complex of endocrine tumors caused by a mutation in the REarranged during Transfection (*RET*) proto-oncogene located on chromosome 10. According to the mutation type, MEN 2 can be divided into MEN type 2A and 2B. Almost all MEN 2A and 2B patients develop medullary thyroid carcinoma (MTC). MTC is a neuroendocrine cancer of the calcitonin-producing parafollicular C cells. In MEN 2A, pheochromocytomas and primary hyperparathyroidism can also present in respectively 40 and 20-30%.[1, 2] In MEN 2B primary hyperparathyroidism is absent but this disease is also characterized by the development of mucosal neuromas, intestinal ganglioneuromas, and in some instances, a Marfanoid habitus. Familial medullary thyroid carcinoma (FMTC) is an inherited disorder caused by *RET* leading only to MTC. The progression from C-cell hyperplasia, the precursor lesion of MTC, into intrathyroidal MTC, and eventually lymphatic and distant metastatic spread is age related. [3-5]

In 1993 and 1994, the relationship between germline *RET* proto-oncogene mutations and MEN type 2A and type 2B syndrome became apparent.[6-8] Since then, DNA analysis is available to identify patients with hereditary MTC and to perform predictive testing in asymptomatic family members of mutation carriers. A genotype-phenotype relationship exists for different *RET*-proto-oncogene mutations.[9, 10] The expression of MTC in terms of aggressiveness varies between the different codon mutations. Based on this genotype-phenotype relationship patients are classified into different risk levels. [11]

In earlier years, patients underwent surgery when they presented with a clinically apparent thyroid nodule or had elevated plasma calcitonin levels. By then, many had already progressed into late stage MTC with lymph node or distant metastases. The availability of presymptomatic DNA analysis by direct detection of germline *RET* proto-oncogene mutations provided a rationale to perform prophylactic surgery because in 95% of patients with MEN 2 and FMTC, C-cell hyperplasia develops into MTC.[4, 9, 10, 12, 13]

In recent years, timing of prophylactic thyroidectomy in asymptomatic carriers of germline *RET* gene mutations has been customized based on the age and type of mutation.[9, 10] In an effort to further optimize the management of these children, we conducted a nationwide study including all Dutch patients who underwent total thyroidectomy in childhood in the past 25 years. We set out to study the differences in treatment for patients operated on at the age as recommended by the current guidelines and those operated on at an older age. The second purpose of this study was to assess any differences in treatment and outcome in patients treated in the era before and after genetic testing became available. Finally, our aim was to analyze prognostic risk factors for recurrent MTC in these patients.

Methods

This case-control study was performed in the cohort of all Dutch patients with a germline *RET* mutation, who were operated in one of the eight university hospitals in the Netherlands from 1980 up until 2006. Inclusion criteria were: MEN 2 syndrome, an age of 19 years or younger at the time of surgery and having undergone a total thyroidectomy.

The participating centers were the Free University Medical Center Amsterdam (n=4), Leiden University Medical Center (n=5), Academic Hospital Maastricht (n=8), Radboud University Medical Center Nijmegen (n=8), Academic Medical Center Amsterdam (n=9), Erasmus Medical Center Rotterdam (n=12), University Medical Center Groningen (n=19) and University Medical Center Utrecht (n=28).

In total, 93 patients were identified and data were collected retrospectively from hospital records in the participating centers by two authors (J.M.J.S. and M.R.V). Patients were divided into those who were operated until 1994 when predictive genetic testing became available ("pre-*RET* era") and those after 1994 ("*RET* era"). The follow-up of the patients included in this study was part of regular medical care. The approaches described in this paper did not involve any randomization, experimental intervention or questionnaire and the anonymity of patients was not breached. Therefore this article meets the conditions required under Dutch Law (WGBO) for making medical and/or personal data available for statistical or other scientific research.

Surgery

Almost all 93 patients were operated on by a team consisting of a pediatric surgeon and an endocrine surgeon. All patients underwent total thyroidectomy. An additional prophylactic lymph node dissection of the central zone of the neck was not performed routinely. A central or lateral neck dissection was performed in case a suspicious lymph node was present during the surgery or on preoperative imaging. In the "pre-*RET*" era (until 1994) patients were operated on based on family history and/or elevated basal and/or stimulated calcitonin levels. In the "*RET* era" (after 1994), most patients were operated on based on the mutation analysis. Whenever possible, the parathyroid glands were left in situ.

Laboratory tests

Calcitonin and pentagastrin-stimulated calcitonin levels were measured to determine the level of tumor activity. We could not retrieve all calcitonin levels, especially from the "pre-RET era". It was however often noted if calcitonin levels were elevated or not. Furthermore, as each center used its own test methodology with according reference values (which differed between the participating hospitals and changed during the study period) we indicated whether or not these levels were elevated according to the local reference values.[14] Patients were considered cured when postoperative (pentagastrin-stimulated) calcitonin was in the lower limits of the reference values.

Genetic testing

In the Netherlands, screening for mutations in a *RET* proto-oncogene is carried out in one central genetics laboratory at the University Medical Center Utrecht. Mutation analysis was performed by PCR-amplification followed by direct sequence analysis of exons 10, 11, 14, 13, 15 and 16 of the *RET* proto-oncogene. Sporadic cases of MTC were identified by mutation analysis of *RET* exons 15 and 16 in DNA isolated from tumor samples, following a normal *RET* mutation screening result on DNA from peripheral blood. All results of *RET* mutation screening were confirmed on a duplicate DNA sample. PCR conditions and oligonucleotide primer sequences are available upon request from R.B.L. Based on the affected codon patients were stratified into three risk levels of developing MTC according to the 2001 guidelines;[11] standard risk (level 1; codons 768, 790, 791, 804 and 891), high risk (level 2; codons 630, 634, 609, 611, 618 and 620) and very high risk (level 3; codons 918, 883, 922) (Table 1).[15]

Table 1. Risk level MTC based on codon mutation

Risk level		Codon	Recommended age of surgery
Risk Level 1	Standard Risk	768, 790, 791, 804 and 891	5-10 years
Risk Level 2	High Risk	630, 634, 609, 611, 618 and 620	<5 years
Risk Level 3	Very High Risk	918, 883, 922	< 6 months

Risk level of MTC based on the 1999 consensus statement from the Seventh International Workshop on Multiple Endocrine Neoplasia [11, 15]

Pathological examination and tumor staging

Pathological examination of the thyroid specimens was performed separately at each hospital. Generally, the thyroid specimen was fixed and transversally laminated in thin sections, which was then prepared for light microscopy. The transition zone between the upper and middle third of the thyroid lobes was the focus of attention, as C-cells are normally situated in this area. The sections were investigated for the presence of C-cells, characterization of cytoplasmic levels of calcitonin, chromogranin and carcinoembryonic antigen (CEA), destructions of follicles, amyloidal formation and invasion of vessels and or nerves by these cells. C-cell hyperplasia was diagnosed when more than 7 C-cells per follicle were present, clusters of C-cells or over 20 C-cells were observed per field. MTC was diagnosed in case of atypia of C-cells and their nuclei in combination with follicular basal membrane destruction as a sign of infiltrative C-cell proliferation. [5]

Statistical analysis

Statistical analysis was performed using SPSS 16 (SPSS Inc, Chicago, Illinois). In this case-control study, children with persistent or recurrent MTC were identified. Their exposure to possible prognostic factors was compared to those who were cured. Patients with C-cell hyperplasia were excluded from the analysis. Associations between categorical variables were evaluated with univariate analysis by Chi-square analysis and in case of a sample size 5 or less by the Fischer' s exact test. The student's T-test was used to analyze differences in mean values, the Mann-Whitney U test for

differences in median values, and the ANOVA test for comparison of multiple groups. The correlation between factors was studied. A binary logistic regression analysis was performed to determine factors associated with recurrent disease. A p-value of <0.05 was considered to be significant. Factors included into the multivariate analysis were age at surgery, the affected codon and risk level 1,2 or 3, presence of lymph node metastasis and preoperative calcitonin levels. Furthermore, for each patient it was determined whether surgery had been performed at an appropriate age as recommended by the guidelines (age-appropriate prophylactic surgery) and if the surgery had been performed in the pre-*RET*-era (before 1994) or in the *RET*-era (after 1994)[11] This factor was included in the analysis as well.

Results

Of the 93 patients identified, 38 (41%) were male and 55 (59%) female. Demographic, surgical en follow-up data are summarized in Table 2.

The majority of cases had MEN 2A syndrome (n=87, 94%). The remaining patients had MEN 2B syndrome (n=6, 6%). Mutation analysis revealed that codon 634 (62 patients) was most frequently affected, followed by codon 618 (10 patients), codon 611 (9 patients), codon 918 (6 patients), codon 620 (3 patients), and codon 804 (1 patient). In two patients, who underwent surgery in the early eighties, the genetic mutation could not be retrieved from the national database. In Table 2, patient characteristics are also given for patients operated in the "pre-*RET* era" and "*RET* era".

The median age of all patients at surgery was 8.9 years (IQR 5.7-12.25). Patients who were operated in the "pre-*RET* era" were older than patients operated in the "*RET* era" (10.6 versus 7.8 years). A preoperatively elevated calcitonin level was found in 43 patients (46%). A central compartment neck dissection was performed in 26 patients (28%) and a modified lateral neck dissection in 8 (9%), in most cases because of evident intra-operative lymph node metastases (Table 2). Seven patients (7%) developed definitive hypoparathyroidism (15% of patients in the "pre-*RET* era" and 4% in "*RET* era") and two (2%) patients suffered from permanent laryngeal nerve damage (none in the "*RET*-era"). No association between the age at surgery, a central neck dissection or the complication rate was found. None of the patients operated on below the age of 5 years (n=14) developed a complication. Five patients were not operated on prophylactically as four already had a clinically apparent tumor and another a palpable lymph node at the time of surgical intervention.

Table 2. Patient characteristics

	Overall	Pre-*RET* era n=25 (27%)	*RET* era n=68 (73%)	P-value of comparison for pre-*RET* era and *RET* era
Sex M : F (n ; %)	38 (41%) : 55 (59%)			
Median age (IQR)	8.9 years (5.7 – 12.6)	10.6 years (7.4-13.2)	7.8 (5.3-12.2)	0.022
Median follow-up (IQR)	7 years (3-11)			
Codon (n ; %)				
634	62 (67%)			
Cys→Arg	37			
Cys→Gly	9			
Cys→Tyr	8			
Cys→Trp	8			
620	3 (3%)			
Cys→Arg	3			
611	9 (10%)			
Cys→Tyr	9			
618	10 (11%)			
Cys→Ser	8			
Cys→Tyr	1			
Cys→Arg	1			
918	6 (6%)			
Met→Thr	6			
804				
Val→Leu	1 (1%)			
Unknown	2 (2%)			
Risk level (n)				
1 standard risk	1			
2 high risk	84			
3 very high risk	6			
Unknown	2			
Preoperative calcitonin levels*				Not significant
Normal	47 (51%)	14	33	
Elevated	43 (46%)	10	33	
Unknown	3 (3%)			
Age appropriate surgery				0.004
Yes	22 (24%)	1	21	
No	71 (76%)	24	47	
Histology				Not significant
C-cell hyperplasia	30 (32%)	7	22	
Medullary thyroid carcinoma	61 (66%)	18	43	
Normal histology	2 (2%)		2	
Lymph node metastasis	8 (9%)	5	3	0.02

Disease status at follow-up		75 (81%)
	Disease free	16 (17%)
	Recurrent disease	1
	Disease related death	2
	Unknown	

* The mean age of patients with normal calcitonin levels was 8.8 years versus a mean age of 9.0 years for patients with elevated calcitonin levels (not significantly different).
† Eleven of the 14 patients with normal calcitonin levels in the "pre-RET era" were operated in the transition period between 1993 and 1994. Genetic testing had become available in the UMCU at that time. Two of the other three patients had C-cell hyperplasia.

Univariate analysis showed that a higher age at surgery, no age-appropriate prophylactic surgery, affected codon and risk level, preoperative calcitonin level and lymph node metastasis at operation were associated significantly with persistent or recurrent disease (Table 3). No association was seen with gender and different variants of codon 634 mutations. In multivariate analysis, only surgery at a higher age was significantly associated with persistent or recurrent disease. In both the "pre-*RET* era" and the "*RET* era" the majority of patients were not operated on at an age-appropriate age, but this percentage was significantly higher in the "pre-*RET* era" (96% versus 69%, P=0.004).

On histological examination 61 patients had already developed MTC (66%), whereas 30 (32%) patients displayed C-cell hyperplasia. In only two patients histological examination showed no abnormalities. One of these patients had a Cys634Gly mutation and was operated at the age of one. The other had a Cys620Ser mutation and was 5 years of age at the time of surgery. There was no significant difference in median age between patients with C-cell hyperplasia and MTC (8.0 versus 9.7 years). Lymph node metastases were observed in 8 patients and were only found in patients with the Met918Thr mutation associated with MEN 2B syndrome and the Cys634Arg mutation, which is associated with MEN 2A syndrome. The youngest patient with lymph nodes metastases was 7 years old and had a Cys634Arg mutation. The median age at surgery of patients with histologically lymph node metastases was 13 years (IQR 10-14). We found more lymph node metastases in patients operated in the "pre-*RET* era" than in the "*RET* era" (Table 2). None of the patients who had lymph node metastases had a normal preoperative calcitonin level and none had calcitonin levels lower than 40pg/ml. An overview of the earliest age at which MTC was demonstrated per codon in our series compared to literature is shown in Table 4.

Table 3. Factors associated with residual or recurrent disease in patients with MTC; univariate analysis

Factor	Residual/recurrent disease n=16 (27%) (n=, %)	No evidence of residual/recurrent disease n=44 (73%) (n=,%)	Likelihood-ratio + P-value
Preoperative Calcitonin levels elevated	N=13 (81%)	N=20 (46%)	6.5, P=0.011
Codon*	634, n = 11 918, n = 4 unknown, n=1	634, n 35 618, n= 3 620, n=1 611, n=3 918, n=1 Unknown, n=1	10.7, P=0.03
Risk level†			7.1, P=0.007
I	n=0	n=0	
II	n=11	n=42	
III	n=4	n=1	
Unknown	n=1	n=1	
Lymph node metastases at total thyroidectomy Total n=8	Lymph nodes present, n=8	No lymph node metastases, n=44	26.0, P<0.0001
Surgery at an appropriate age based on risk level	No, n=16 Yes, n=0	No, n=31 Yes, n=13	9.3, P=0.002
Surgery in the pre-*RET*-era or in the *RET*-era	Pre-RET, n=7 RET-era, n= 9	Pre-RET, n= 11 RET-era, n= 33	No significant difference P=0.14 *T-test or Mann-Whitney U-test, P value and Odds-ratio*
Mean Age at surgery‡ in years (range)	12.1 (range 1-19)	8.6 (range 1-19)	P=0.002 95% CI 1.2-5.7
Mean Follow-up in years (+/-SD)**	12.3 (+/-7.1)	7.4 (+/- 4.7)	P=0.004 95% CI 1.6-8.0

CI= confidence interval

* We calculated the percentage of patients who had MTC who were cured per codon. Seventy-six percent of patients with codon 634 was cured, 100% of patients with a 618, 620 and 611 mutation and only 20% of those with a 918 mutation.

† When we analyzed this for risk level, 79% of the patients with risk level 2 and 20% with risk level 3 were cured.

‡ Single factor also significantly associated in multivariate analysis

**The duration of follow-up, however, was not statistically different between both groups after excluding patients from the “pre-*RET* era” (8.2 versus 6.2 years).

Table 4. Earliest presentation of Medullary Thyroid Carcinoma in patients with a *RET* proto-oncogene mutation

Codon with *RET* mutation and Risk level	Earliest age at presentation of MTC*	Earliest age at presentation of MTC	Earliest age of LN§metastases	Earliest age at presentation of MTC with LN metastases
	Current series	Literature	Current series	Literature
918 RL 3	1.13 yrs	0.75 yrs[9]	11 yr	2.7 yrs[15]
634 RL 2	1.25 yrs	1.1 yrs [15]	7 yrs	5 yrs[15]
611 RL 2	6 yrs	3 yrs[9]	-	28 yrs[16]
620 RL 2	6 yrs	5 yrs [10]	-	22 yrs[15]
618 RL 2	12 yrs	7 yrs[9]	-	11 yrs[10 17, 18]
804 RL 1	-	6 yrs [19]	-	6 yrs [19]

*MTC is medullary thyroid carcinoma
§ LN are lymph node metastasis
- not observed
yrs years
RL; Risk level of MTC based on the 1999 consensus statement from the Seventh International Workshop on Multiple Endocrine Neoplasia [11, 15]

Follow-up

The median follow-up period was 7 years (IQR 3-11). At the time of the last visit, 75 (81%) patients were disease free according to the postoperative calcitonin levels. The duration of follow-up was significantly different between patients who had persistent or recurrent disease and those who were cured. Thirteen of the 16 patients with persistent or recurrent disease at follow-up had elevated basal calcitonin or pentagastrin-stimulated calcitonin levels preoperatively. None of these 16 patients have been operated at an appropriate age as recommended by the guidelines. Three of the 27 patients who had MTC and normal preoperative calcitonin levels developed persistent or recurrent disease (Table 3). All patients with lymph node metastases at operation developed persistent or recurrent disease. All 8 patients underwent a central neck dissection and 5 of them a lateral neck dissection as well. One MEN 2B patient died at the age of 18 years (i.e. 6 years post-operatively) because of metastatic disease. No differences in presentation could be found between the different codon 634 mutations in terms of age at surgery and recurrent disease.
If we excluded patients who were operated on in the “pre-*RET* era”, patients who had persistent or recurrent disease were still older than those who were cured (12.3 versus 7.9 years, P=0.01) and were less frequently operated at an appropriate age (p=0.034). These patients more often had an elevated preoperative calcitonin level (P=0.04) and lymph node metastasis (P=0.001) compared to those who were cured. The duration of follow-up, however, was not statistically different for both groups after excluding patients from the “pre-*RET* era” (8.2 versus 6.2 year).

Discussion

Due to the well organized infrastructure of a relatively small country as the Netherlands we were able to establish a unique nationwide overview of all Dutch patients with hereditary MTC who underwent a total thyroidectomy based on their *RET* proto-oncogene mutation since 1980. In the Netherlands all patients with medullary thyroid carcinoma, especially those at young age, are operated on in a university hospital. We included all patients that underwent surgery in those hospitals in our study and it is therefore unlikely that our results are biased because of selection of the population.

We evaluated potential prognostic factors responsible for the outcome of prophylactic thyroidectomy in MEN 2 patients. The presence of lymph node metastasis, elevated preoperative calcitonin levels, codon/risk level, no age-appropriate prophylactic surgery and a higher age at surgery were associated with persistent/recurrent disease in univariate analysis. Higher age at surgery was the only independent prognostic variable for persistent or recurrent disease in multivariate analysis. This could be an indication that operating at an earlier age might lead to a better outcome, thus preventing recurrent disease. It appears that progression from C-cell hyperplasia into MTC is age-related.[4, 5, 9]

Twenty-three percent of the patients who were operated on at a higher age than is currently recommended by the guidelines, developed recurrent disease. These data, once again underline the importance of early, prophylactic surgery. However, the majority of patients who had C-cell hyperplasia were not operated on at the recommended age either. Based on the latter observation, the argument could also be reversed because many patients are cured despite the fact that they are operated on at an older age than is recommended. We do, however, want to keep in mind that almost a quarter of patients with MTC operated on at an older age, developed persistent or recurrent disease.

In our series, all patients with nodal metastases had persistent or recurrent disease. Lymph node metastases only appeared in patients who were operated at a relatively high age. One patient with persistent disease (elevated calcitonin levels) was only one-year old (codon 918 mutation). Therefore, we believe that prophylactic thyroidectomy should not be delayed until after the recommended age in literature.

In literature, three risk levels have been identified. Patients in the highest risk level (level 3: codon 918, 883, 922) tend to have an aggressive presentation of MTC. In our cohort, as expected, these patients presented with MTC at an early age. They are therefore in need of a more aggressive approach.[9, 15]

Persistent and recurrent disease only occurred in children with mutations in codon 918 and 634. Furthermore, we found MTC in a one-year old child with a codon 634 mutation. Since no differences in biological behavior in follow-up could be observed between the different codon 634 mutations in our series, we feel that all patients with a codon 634 mutation should be managed equally.

Surgery at a younger age is believed to be associated with a higher complication rate. We found no evidence for this statement in our study. The complication rate is more or less comparable with other series, even in the very young.[20] Additionally, in our study performing a central neck dissection was not associated with a higher complication

rate. More complications occurred in the "pre-*RET* era". Although numbers are too small to draw any firm conclusions, we think this difference in complication rates between the "pre-RET era" and "RET era" may have been caused by improvements in operation technique. Since endocrine surgeons are more experienced in thyroid surgery than pediatric surgeons, it is common practice in the Netherlands to perform surgery together to decrease the complication risk.
The youngest patient in our series with lymph node metastasis was 7 years of age and carried a codon 634 mutation. Another report describes a five-year old patient with a nodal metastasis.[21] This is in contrast with other large series in which lymph node metastases under the age of 11 and 14 years were not observed.[9, 10] It is controversial if a central neck dissection should be performed. We think that it is not necessary to perform a routine prophylactic central neck dissection before the age of 5 or if preoperative calcitonin levels are normal in patients who do not have MEN 2B syndrome. None of our patients with lymph node metastases had a normal preoperative elevated calcitonin. Machens has suggested to perform a central neck dissection if preoperative calcitonin levels are elevated regardless of age.[4]

As mentioned above, one MEN 2B (codon 918 mutation) patient with MTC and persistent disease after surgery was only one-year old. In previous reports, MTC was reported in a patient with this mutation within the first six months of life. Furthermore, persistent disease has been reported within the first year of life.[22-24] Therefore, the consensus guidelines[11] recommend a total thyroidectomy together with a central lymph node dissection before the age of 6 months in MEN 2B patients. Lymph node metastases have been found in a 2.7-year-old child. The one-year old (codon 918) who had persistent disease in our series, did not undergo a central neck dissection. The one patient who died in our series had MEN 2B syndrome (Met918Tyr mutation) and had been operated at an age of 12.

Children with a risk level 2 (codon 634, 611, 618 or 620 mutation) have a high risk of developing MTC. Some reports have suggested that patients with 634 mutations may be regarded as a separate group with a higher risk of MTC.[9, 15] When we regarded codon 634 as a separate group, no recurrences of MTC were found in risk group 2 (codon 611, 618 and 620). However, knowing that elevated preoperative calcitonin levels and/or suspicion of lymph node involvement yield an increased risk of recurrent disease, supports the advice by the consensus meeting to perform surgery below the age of 5.[11]

It is generally believed that children with a relatively low risk (risk level 1) of developing MTC, due to a germline mutation at codon 609, 768, 790, 791, 804 or 891 should undergo a total thyroidectomy as well. The biological behavior of MTC in this group is less aggressive which is confirmed in our cohort. The only patient in this group did not have MTC. Controversy remains regarding the age at which surgery should be performed. While some believe that patients in this category should be operated on before the age of 5, others advocate surgery in this risk group before the age of 10 [11]. However, patient numbers in our series were too small to draw any conclusions.

Limitations of this study are the retrospective nature and its associated disadvantages like missing data as laboratory studies, in two cases the *RET* mutation and indications for central neck dissection. Furthermore, as can be expected, we found that patients operated in the "pre-*RET* era" were older than those in the "*RET* era". A larger proportion of these patients from the "pre-*RET* era" were not operated on at an appropriate age and had more lymph node metastases. However, when we excluded patients from the "pre-*RET* era", the same factors were still associated with persistent disease.

Prophylactic thyroidectomy at a younger age is associated with less recurrence or metastasized MTC in patients harboring a *RET* mutation. Recurrent disease was only observed in patients with the 634 and 918 codon mutations who did not undergo total thyroidectomy at an appropriate age. Therefore it seems justifiable to operate patients in the very high-risk group at an early age, as is currently recommended. All patients with codon 634 mutations should be treated equally.

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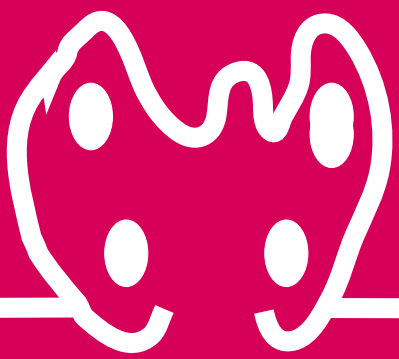

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PART 2
Parathyroid Glands

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Chapter 6

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The optimal surgical treatment for primary hyperparathyroidism in men 1 patients; a systematic review

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Abstract

Objective

The optimal surgical approach for patients with primary hyperparathyroidism (pHPT) and Multiple Endocrine Neoplasia 1 (MEN1) is controversial. We sought to determine the optimal type of surgery for pHPT in MEN1.

Methods

We collected data on clinical presentation, surgery and follow-up for MEN1 patients with pHPT at the University Medical Center Utrecht and affiliated hospitals between 1967-2008. Furthermore, we performed a systematic review of literature and meta-analysis. Surgical procedures were classified into less than subtotal (<SPTX) versus subtotal(SPTX) and total parathyroidectomy(TPTX).

Results

Fifty-two patients underwent primary surgery for pHPT, 29 had <SPTX, 17 SPTX, and 6 TPTX. Recurrent pHPT was most frequent after SPTX (65%) followed by <SPTX (59%). Persistent disease was most frequent after <SPTX (31%). Time to recurrence was 61 months longer after SPTX than after <SPTX. Although recurrent pHPT was not seen after TPTX, permanent hypoparathyroidism developed in 67% of these patients. The meta-analysis showed that after SPTX and TPTX, patients had the lowest risk of persistent and recurrent pHPT. TPTX had the highest risk of permanent hypoparathyroidism. Large non-comparative studies showed low recurrence rate after SPTX and TPTX.

Conclusion We believe that SPTX is the best surgical therapy for pHPT in MEN1. MEN1 patients with pHPT should not be treated with <SPTX because of the unacceptable high rate of recurrent and persistent pHPT.

Introduction

The Multiple Endocrine Neoplasia syndrome type 1 (MEN1) is a rare autosomal dominant inherited disorder. MEN1 is caused by a germline mutation on *MEN1* gene on chromosome 11. In the Netherlands the prevalence is estimated to be 2-3/100.000.[1] Patients with MEN1 are prone to developing endocrinopathies. These endocrinopathies are primary hyperparathyroidism (pHPT), pancreatic endocrine tumours (PETs) and pituitary adenomas. Other less frequent manifestations are adrenocortical adenomas, neuro-endocrine tumours of the stomach, thymus and bronchus. Hyperparathyroidism is the most prevalent occuring in 78-90% of MEN1 patients.[2, 3] It is often the first presentation of MEN1 syndrome.[1] These patients tend to be younger than patients with sporadic pHPT. Most commonly, pHPT presents during the second and third decade.[1, 4] PHPT can be both asymptomatic and symptomatic. Signs and symptoms may consist of bone abnormalities, mental changes, weakness, nefrolithiasis, and marked hypercalciemia.[4] MEN1 related pHPT tends to be more aggressive than sporadic pHPT and usually manifests as multiglandular disease.[5]

The treatment of pHPT is primarily surgical. The goals for surgery are restoring calcium levels to normal permanently, while preventing hypoparathyroidism and minimizing the number of reoperations.[4,6] Parathyroidectomy reduces the risk of kidney stones, fractures (improved bone mineral density) and potential cardiovascular morbidity. It may even improve quality of life. In MEN1 patients who also have a gastrinoma, parathyroidectomy may reduce the gastrin production.[7] Controversy exists on the optimal surgical strategy. Most authors have advocated subtotal (SPTX) (resection of 3-3 ½ parathyroid glands) or total parathyroidectomy (TPTX) with autotransplantation (resection of 4 glands).[4, 8, 9] There is a high risk of recurrence after surgical intervention, even after extensive surgery. The downside of extensive surgery is the increased risk of permanent hypoparathyroidism (hypocalcaemia) and recurrent laryngeal nerve injury. Acute hypoparathyroidism may cause mild to severe neuromuscular symptoms ranging from neuromuscular irritability to seizures. Yet, less extensive surgery yields a higher risk of recurrent disease requiring reintervention, which increases the risk of complications even more.

To the best of our knowledge, no reports have published a meta-analysis of the results reported in the current available literature to compare different surgical therapies on recurrent and persistent pHPT and permanent hypoparathyroidism.

The aim of this study was to determine the optimal surgical therapy for pHPT in patients with MEN1. To this end, we evaluated our experience with surgical treatment in these patients and carried out a meta-analysis.

Methods

From the MEN1 database at the University Medical Center Utrecht (UMCU), the Netherlands, patients diagnosed with pHPT between 1967 and 2008 were selected. Patients were included in the MEN1 database if they had genetically proven MEN1, or three out of five manifestations of MEN 1 or one out of five manifestations and a first-degree family member. The medical records of these patients were reviewed.

Since the UMCU is a tertiary referral center not only patients who were primarily treated at our hospital were included. Also patients who were initially treated at other institutions and later referred to our institution were included.

The diagnosis of pHPT was confirmed when serum calcium or ionized calcium levels were raised in combination with raised or inadequately suppressed PTH levels (normal reference value: 1.0-7.0 mmol/L). We have chosen to use ionized calcium levels (normal reference value: 1.15-1.32 mmol/L) in our analysis, as this became the standard parameter measured routinely since 1993 in our hospital. Before 1993 we used the serum calcium levels to diagnose hyperparathyroidism.

Operative technique

Throughout the years, our operative strategy has evolved. In the past, only subtotal or total parathyroidectomy with autotransplantation were performed. In subtotal parathyroidectomy, 3 – 3 ½ parathyroid glands (SPTX) were resected during a bilateral cervical exploration after identification of all parathyroid glands. In total parathyroidectomy 4 glands were resected (TPTX). In TPTX one (partial) gland was used as a graft for autotransplantation into the brachioradial muscle of the non-dominant fore-arm. For this technique we used the fresh tissue during the same operation. Minimally invasive parathyroidectomy (MIP) has been performed in selected cases in order to reduce the morbidity of surgery and postponing more radical surgery. A MIP can only be performed if the affected parathyroid gland can be localized preoperatively with identical location of an enlarged gland on both ultrasound and sestamibi scan. We did not routinely perform thymectomy. We classified the surgical strategy based on operation reports and medical records.
Surgical outcome was defined as the initial success after surgery, persistence and recurrence rate, and complications with permanent hypoparathyroidism in particular. Surgical cure was defined as a normalization of serum (ionized) calcium levels and PTH for a period of at least six months after the surgical procedure. If not, it was classified as persistent pHPT. Permanent hypoparathyroidism was defined as hypocalcemia persisting beyond the first 6 months after surgery and requiring substitution with calcium and an active form of vitamin D. Both duration of follow-up and time to recurrence were calculated in months. The duration of follow-up was defined as the time between the first surgical procedure and the date of final contact with the patient.

Meta-analysis & Systematic Review

We searched Medline (1966-2008), Embase (1980-2008), the Cochrane database of systematic reviews and the Cochrane Central Controlled Trials Register (2008) using predefined search terms. (‘Appendix A’) On the basis of title and abstract we selected 126 studies. Only studies (n=12) that compared different surgical treatments of pHPT were included. (Figure 1) We also included the results of our study. Pooling was done for the following groups: patients who underwent resection of less than 3 parathyroid glands (<SPTX), 3-3 ½ parathyroid glands (SPTX), or

all parathyroid glands (TPTX) including autotransplantation. We studied outcome of surgery between different groups. Outcome was defined as the risk of persistent pHPT, recurrent pHPT, and permanent hypoparathyroidism. To determine the risk of recurrent pHPT, patients who had persistent pHPT were excluded. We performed a 2-step meta-analysis. First, we studied if less than 3 parathyroid glands resected (<SPTX) or 3 or more glands resected (SPTX/TPTX) had a better outcome. Secondly, we compared SPTX to TPTX. Odds-ratio (OR), the 95% confidence interval (95% CI), and p-value were calculated. The I^2 test was used to check for quantative heterogeneity. This test measures the proportion of inconsistency between studies that cannot be explained by chance alone. Additionally, we included the results of the non-comparative studies in the results section.

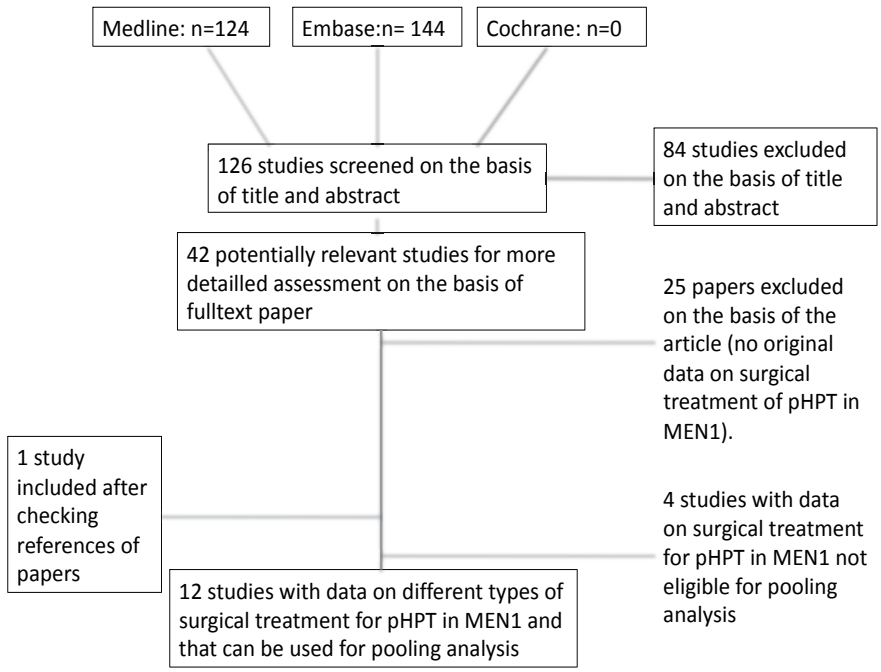


Figure 1. Flowchart of selection of articles screened and included in meta-analysis

Statistical analysis

SPSS 16.0 (SPSS Inc. Chicago, Illinois) was used for statistical analysis. Review manager 5.0 software (Cochrane Collaboration) was used for the meta-analysis. Depending on distribution, numerical data are depicted as mean +/- standard deviation (SD) or median with its interquartile range (IQR). Presented percentages are calculated on the basis of the available data. When appropriate, the X^2-test was used for statistical analysis of data. To determine if the surgical procedure was associated with recurrent disease, univariate analysis was performed. For

this purpose, patients were divided into 3 groups; 1. <SPTX (less than 3 glands resected), SPTX (3 – 3 ½ glands resected and TPTX (total parathyroidectomy, 4 glands resected).

Results

From the MEN1 database, 54 patients were identified who had pHPT. Twenty-one of them were men (39%). Ten patients were asymptomatic. Forty-one patients (76%) had one or more of the following symptoms: nefrolithiasis, mood disorders, fatigue or gastro-intestinal complaints. In three cases this was unknown. Baseline characteristics are shown in Table 1.

Table 1. Patient characteristics of pHPT in MEN 1 (n=54)

	Surgical treatment				
Patient characteristics (units)*	< 3 SPTX	SPTX 3 – 3 ½	TPTX	Overall	P-value
Age (years) †	35 (+/-12)	32 (+/-11)	29 (+/-8)	34 +/- 11	n.s.
Preoperative Ca^{++}mmol/l	1.40 (+/-0.07)	1.39 (+/- 0.08)	1.45 (+/- 0.07)	1.40 +/- 0.07	n.s
Preoperative PTH mmol/l	8.60 (+/-4.18)	7.60 (+/-3.28)	9.16 (+/-4.14)	8.40 +/- 3.90	n.s

*Data are shown in mean +/- standard deviation (SD) †Mean age at the time of Surgery
Ca^{++} mean ionized calcium Levels, PTH Parathyroid Hormone Levels
<SPTX less than 3 parathyroid glands resected , SPTX (3 – 3 ½) subtotal parathyroidectomy with 3 – 3 ½ parathyroid glands resected, TPTX total parathyroidectomy
n.s. not significant

Fifty-two patients underwent a primary surgical treatment either at our hospital (n=36) or another hospital (n=16). Two other patients did not undergo surgical treatment. Eight patients underwent a MIP, 21 underwent <SPTX, 17 SPTX and 6 TPTX. Three times, during an initial MIP, PTH levels remained elevated and the surgical procedure had to be converted to a conventional bilateral neck exploration with a TPTX either in the same session or a (few) day(s) later due to logistical reasons. An overview of the surgical procedures is depicted in Table 2. After the primary surgery, ten patients (19%) developed permanent hypoparathyroidism. In most cases, this occurred after total parathyroidectomy. One patient had a transient recurrent laryngeal nerve injury and another patient had a laryngeal nerve injury of an unknown duration.

Eleven patients (21%) developed hypercalcemia within six months, indicating persistent disease. Twenty-eight patients (54%) developed a recurrence after a median of 121 months (IQR 47-201). Time to recurrence was 61 months shorter in patients who had undergone <SPTX (93 months) than in patients who had SPTX (154 months), although this was not significant. None of the patients treated with TPTX recurred. One patient did develop persistent disease after TPTX.

Table 2. Overview of surgical treatment in patients with pHPT and MEN1 (n=52, postoperative laboratory results and complications per surgical treatment.

	Surgical Treatment				
	< SPTX	SPTX 3 – 3 ½	TPTX	Overall	P-value
Number of procedures (%)	29*	17*	6	52	
Postoperative Ca^{++}(mmol/l)	1.25 (+/- 0.12)	1.13 (+/-0.13)	1.07 (+/-0.76)	1.19 +/- 0.14†	0.011
Postoperative PTH (mmol/l)	5.70 (+/-4.26)	2.97 (+/- 1.72)	2.35 (+/-2.99)	4.42 +/- 3.70‡	0.070
Complications					
Hypoparathyroidism					0.003
Transient	0	3 (18%)††	1 (17%)	4 (8%)	
permanent**	2(7%)††	4 (24%)††	4 (67%)	10 (19%)	
unknown	1 (3%)††	0	0	1 (2%)	
duration					n.s.
Recurrent	1 (3%)	1 (6%)	-	2 (4%)	
Laryngeal nerve injury	-	1 (6%)	-	1 (2%)	
transient					
permanent					

* 8 out of the 29 <SPTX underwent MIP (minimally invasive parathyroidectomy). Two patients underwent delayed conversion to bilateral neck exploration. One of the patients in the SPTX group, underwent a MIP, but was immediately converted to a SPTX because of an insufficient drop in PTH (<50%).
Ca^{++} ionized calcium levels, PTH parathyroid hormone levels
<SPTX less than 3 parathyroid glands resected, SPTX (3 – 3 ½) subtotal parathyroidectomy with 3 – 3 ½ parathyroid glands resected, TPTX total parathyroidectomy
† Overall preoperative Ca^{++} levels are significantly higher than postoperative Ca^{++} levels, p=0.0001.
‡ Overall preoperative PTH levels are significantly higher than postoperative PTH levels, p=0.002.
** Permanent hypoparathyroidism is defined as a duration of hypoparathyroidism of 6 months or longer.
†† Of the patients with hypoparathyroidism, 3 out of 4 patients after <SPTX developed recurrent pHPT and 4 out of 6 patients after SPTX 3-3 ½ developed recurrent disease.

Outcome of surgical treatment for persistent and recurrent disease in follow-up is shown in Table 3. Thirty-one percent of the patients with <SPTX had persistent disease. Additionally, 59% of patients with <SPTX, developed recurrent disease. After SPTX, one of the patients had persistent pHPT (7%), but 65% developed recurrent disease after a median of 13 years. Although none of the six patients with TPTX developed recurrent pHPT, one patient had persistent pHPT. Patients who had TPTX had the highest risk of permanent hypoparathyroidism (67%) compared to <SPTX (7%) and SPTX (25%), p=0.003 (Table 2).

The number of recurrences in individual patients ranged from 1 to 8 times. We performed 25 operations for persistent or recurrent pHPT. The number of reoperations per patient ranged from one to seven times. During the second operation, one parathyroid gland was resected in 14 patients, 2 glands in 5 patients and 3 glands in 1 patient. No parathyroid glands could be identified in 3 patients. After secondary surgery, calcium levels initially normalized in 19 patients (76%). Three patients developed hypoparathyroidism, two of whom had permanent hypoparathyroidism.

In 13 patients with persistent or recurrent disease no reintervention was performed.

The patient who developed 8 episodes of recurrence underwent 7 reinterventions in both the cervical region and the arm where the autotransplant graft was located. She now suffers from a permanent unilateral laryngeal nerve injury. She was the only patient in our series who had supranumery glands. Median follow-up was 121 months (IQR 47-201). At the end of follow-up 9 patients had died, six because of metastasized neureoendocrine tumours, one because of metastasized melanoma and two of an unknown cause.

Table 3. Outcome of surgical treatment for pHPT in MEN1. Cure, persistent and recurrent pHPT.

	Surgical Treatment				
	< SPTX	SPTX 3 – 3 ½	TPTX	Overall	P-value
Follow-up in months median (IQR)	99 (44-162)	144 (71-207)	16 (4-236)	121 (47-201)	n.s.
Cure (%)	20 (69%)	13 (93%)	5 (83%)		n.s.
Persistent pHPT*	9 (31%)	1 (7%)	1 (17%)	11 (22%)	n.s.
Recurrent pHPT	17 (59%)†	11 (65%)	-	28 (54%)	0.010
Time to Recurrence in months (range)	93 (44-164)	154 (46-207)	-	127 (34-194)	0.088
Number of patients who underwent Reintervention (%)	16 (55%)	8 (47%)	1		
Reintervention number				25 (48%)	n.s.
1				16 (31%)	
2				3 (6%)	
3				5 (9%)	
7				1 (2%)	
Second reoperation (Number of glands resected)					
0	2	1	0		
1	7	6	1		
2	5	0	0		
3	1	0	0		
Autotransplantation	7	4	-		
Unknown	1	1			
Final TPTX status	6	6			
Outcome Second Reoperation					
Permananent Hypoparathyroidism	2	2	0		
Recurrent pHPT	5	5			

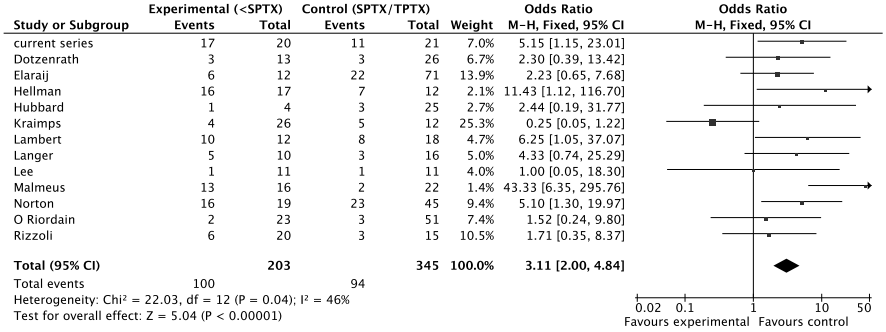
IQR interquartile range
<SPTX less than 3 parathyroid glands resected
SPTX (3 – 3 ½) subtotal parathyroidectomy with 3 – 3 ½ parathyroid glands resected, TPTX total parathyroidectomy
*Hypercalciemia within six months after surgical intervention, † All 8 MIP developed recurrent pHPT, n.s. not significant

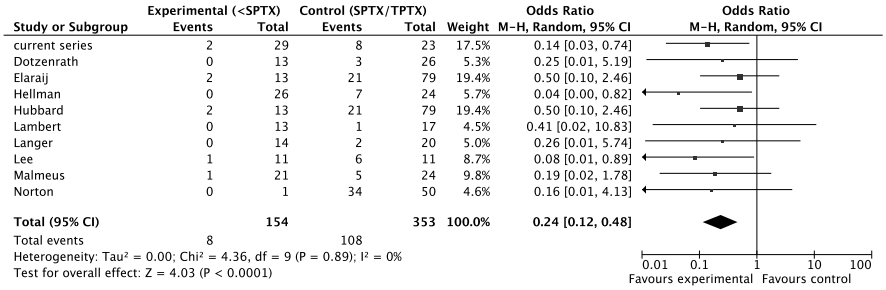
Meta-analysis & Systematic Review

First we compared patients who had <SPTX to patients with SPTX or TPTX. After <SPTX, patients had a significantly higher risk of developing recurrent and persistent pHPT than patients with SPTX or TPTX. The odds-ration for recurrent pHPT was 3.11 (95% CI 2.00-4.84) for patient who underwent <SPTX (Figure 2A). Patients with <SPTX had a significantly lower risk of developing permanent hypoparathyroidism (OR 0.24 (95% CI 0.24-0.48) (Figure 2B) and a higher risk of persistent pHPT (Appendix C1).

Figure 2A. Comparison of <SPTX versus SPTX/TPTX for 'recurrent pHPT'.

Figure 2B. Comparison of <SPTX versus SPTX/TPTX for permanent hypoparathyroidism.





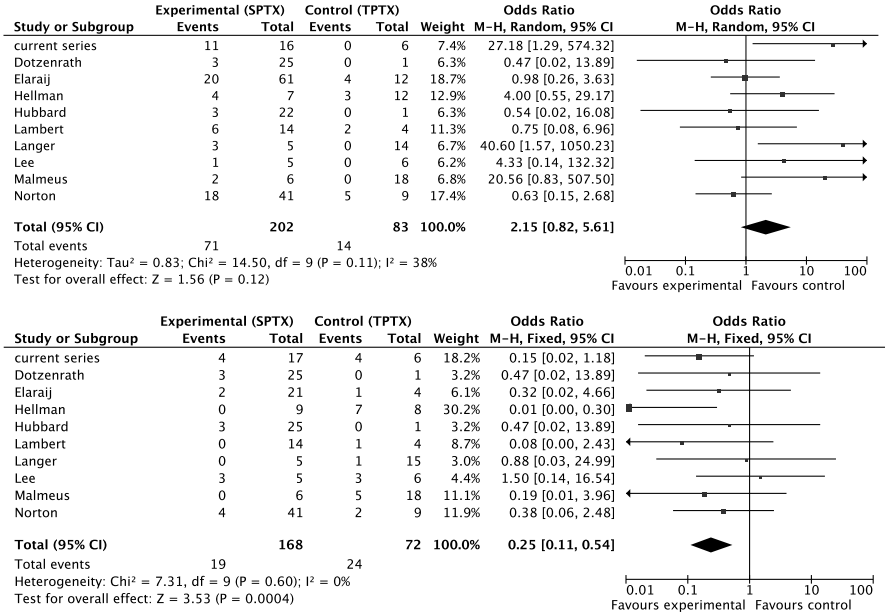
After <SPTX patients are 3.11 times more likely to develop recurrent disease than after SPTX/TPTX (95% CI 2.00-4.84, p<0.0001). Patients who had persistent pHPT have been excluded from this analysis.
After <SPTX patients have a significantly lower risk of developing permanent hypoparathyroidism than after SPTX/TPTX (OR 0.24 95% CI 0.12-0.48, p<0.0001).
<SPTX less than 3 parathyroid glands resected; SPTX 3-3 ½ parathyroid glands resected; TPTX total parathyroidectomy with autotransplantation; SPTX and TPTX are analyzed together.

Secondly, we compared patients who had SPTX to patients who had TPTX. Patients with SPTX did not have a significantly higher risk of developing recurrent pHPT than patients with TPTX (OR 2.15 95%CI 0.82-5.61, p=0.12) (Figure 3A). Neither did they have a higher risk of persistent pHPT (Appendix C2). After SPTX, patients had a significantly lower risk of permanent hypoparathyroidism than after TPTX (OR 0.25 95%CI 0.11-0.54, p=0.0004) (Figure 3B). The I^2 test revealed moderate heterogeneity between the study outcomes on recurrent pHPT (percentage of total variation across

the studies not due to chance alone was 38 and 46%). The heterogeneity for study outcomes on permanent hypoparathyroidism was small (0%). An overview of the outcomes of surgery from studies that were included in meta-analysis and those that could not be included are shown in Appendix B.

Figure 3A. Comparison of SPTX versus TPTX on 'recurrent pHPT'.

Figure 3B. Comparison of SPTX versus TPTX for 'permanent hypoparathyroidism.



After SPTX, patients do not have a significantly higher risk of developing recurrent pHPT than after TPTX (OR 2.15 95% CI 0.82-5.61, p=0.12). Patients who had persistent pHPT have been excluded from this analysis.
After SPTX, patients have a significantly lower risk of developing permanent hypoparathyroidism than after TPTX (OR 0.25 95% CI 0.11-0.54, p=0.0004).
SPTX 3-3 ½ parathyroid glands resected; TPTX total parathyroidectomy with autotransplantation

Most non-comparative studies presented large study populations. The overall recurrence rates are difficult to compare because of different techniques. One series showed an overall recurrence rate of 7.6% after SPTX (n=66) with bilateral thymectomy and <SPTX (<3). Another series of 100 patients had a failure rate of 26% after <SPTX (n=37) and combined failure rate of 11% after SPTX (n=43) and TPTX (n=11) after a follow-up period of 4.6 years. A series of 51 patients who underwent 45 TPTX with thymectomy had only five recurrences all in the autografts after 6.7 years of follow-up. The rate of permanent hypoparathyroidism in that series was 22%.
One large series of only patients who underwent reoperations for pHPT in MEN1 had a recurrence rate of 27% after 72 months. Only 2 out of 75 patients had permanent recurrent laryngeal nerve injury after their reoperation.

Discussion

This study confirms the difficulties in the management of primary hyperparathyroidism in MEN1 patients. Contradictive findings have been reported on the surgical treatment. In our study, recurrent pHPT was most frequently seen after SPTX, yet the follow-up was long compared to other series (Appendix B). Even though none of the patients developed recurrent pHPT after TPTX, the majority developed permanent hypoparathyroidism (67%). Persistent disease was most frequently seen after <SPTX. These results and those of the meta-analysis and systematic review confirm that <SPTX should not be performed because <SPTX has the highest risk of persistent and recurrent disease.

We propose subtotal parathyroidectomy (SPTX) to be the preferred treatment for HPT in MEN1.

However, some caution must be taken into account when interpreting these data. There were no randomized controlled trials and most series were retrospective. Furthermore, the duration on follow-up was different in these studies. For permanent hypoparathyroidism only few studies were available. The main limitation of our study is its retrospective nature. Some data were unavailable and there may have been confounding by indication for the type of surgery. Unfortunately, the results of the large non-comparative studies could not be included, which could have had a significant effect in the meta-analysis. An international randomized controlled trial with a long-term follow-up would be necessary to answer some of the remaining questions. Nonetheless, such a study design would be very problematic to accomplish in view of the rarity of this disease.

Most authors agree that pHPT in MEN1 is caused by multiglandular disease.[6{Tonelli, 2007 #3, 9-13] The theory that MEN1-related pHPT develops asymmetrically and in time all parathyroid glands can become hyperplastic or develop adenomas is based on the observation that there is a high risk of developing recurrent pHPT when limited resections are performed.[5, 6] Most authors therefore recommend SPTX or TPTX. [5, 8, 11, 13-15] SPTX is generally considered to be superior to TPTX because the risk of permanent hypoparathyroidism is much lower. However, there might be an increased risk of recurrence compared to TPTX since some parathyroid tissue remains in situ. The controlled-cohort studies and case series that we identified in our systematic review of literature showed the recurrence rate of pHPT was high after SPTX (3-3 ½), ranging from 12 up to 67% after eight years of follow-up (Appendix B). The non-controlled series had large study populations and more favourable outcome in recurrence rates for SPTX and TPTX, but follow-up rates were shorter than in our series. In our series the recurrence rate was 65% after a long median follow-up of 12 years. This risk of recurrence increases with time and does not reach a plateau phase.[8] After total parathyroidectomy with autotransplantation, recurrence rates of 4-55% have been reported.[8, 13, 15-17 14] The higher recurrence rate after SPTX in our series may be explained by the longer follow-up period than in other (non-comparative) series and the fact that in our series a cervical thymectomy was seldom performed.

The risk of severe and permanent hypoparathyroidism is the highest after TPTX with autotransplantation. It ranges from 13 to 47% in literature and was 67% in our

series. After subtotal parathyroidectomy this risk ranges from 0 to 22%.[13, 15] Patients with <SPTX are the least likely to develop permanent hypoparathyroidism.[11] These findings are confirmed by our meta-analysis. If patients with permanent hypocalcaemia require lifelong vitamin D and calcium supplements, this may well affect their quality of life. If left untreated, longstanding hypoparathyroidism may lead to extrapyramidal disorders (Parkinson and dementia), skin disorders and cataract. These risks have to be balanced against the benefits of curing the patient by performing extensive parathyroid resections. Especially, since the time to develop recurrent disease is long after SPTX and a reoperation is often only required after several years.
In contrast to the multiglandular disease theory, some authors have found uniglandular disease in MEN1.[18, 19] In a small number of our patients, we performed minimally invasive parathyroidectomy (MIP) to reduce complications and mainly at patients own request. This practice has been abandoned after the poor results in pHPT in MEN1. In our experience and others', the recurrence rate in these patients after MIP is unacceptably high, 100%.[6, 20]Additionally, there is a higher risk of persistent disease after <SPTX, which was 31% in our series.[11] However, some have better results with cure rates of approximately 70% after 48 months[18] and 100% after 5 years.[19]

The possibility of supranumery parathyroid glands should also be considered in the surgical treatment of pHPT in patients with MEN1. Although, some authors have not found supranumery glands, [9] these may occur in up to 0-30% of MEN1 patients (Appendix B). Not only supranumery glands can be found, but ectopic ones as well up to 33%.[21] These ectopic glands are most often located in the thymus.[9] Some authors advocate that during cervical exploration in these patients, a routine thymectomy should be performed to search for supranumery and ectopic glands. [3, 6, 9, 11, 14, 22, 23] After SPTX with thymectomy calcium levels return to normal more often (84.1%) than after SPTX without transcervical thymectomy (57.6%, p 0.0001).[24] In our series thymectomy was rarely performed. Some authors even recommend removal of the fatty tissue in the central compartment to remove all (ectopic) parathyroid tissue.[15]

Finally, one series that only studied reoperations for pHPT in MEN1 in a large case series had a recurrent laryngeal nerve injury rate of 2% and a permanent hypoparathyroidism rate of 16%.[10] In our series only one patient, who underwent 7 reoperations, developed permanent recurrent laryngeal nerve injury.

Conclusion

Given he currently available evidence, limited resections of less than 3 glands should not be part of a primary operation for pHPT in MEN1 patients, since the persistence and recurrence rates are too high. SPTX might be the best surgical treatment for patients who have MEN1 and pHPT, despite the higher risk of recurrent pHPT.

Acknowledgement

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Appendices

Appendix A. Predefined search terms
Regarding: MEN 1
"MEN 1" OR "MEN1" OR "MENI" OR "MEN I" OR "multiple endocrine neoplasia type 1" OR "multiple endocrine neoplasia syndrome type 1"

Regarding primary hyperparathyroidism
"Hyperparathyroidism, Primary"[Mesh] OR "Parathyroid Neoplasms"[Mesh] OR "primary hyperparathyroidism" OR "hyperparathyroidism" OR "HPT" OR "parathyroid adenoma*" OR "parathyroid hyperplasia"

Regarding surgical procedure
"Parathyroidectomy"[Mesh] OR "parathyroid surgery" OR "parathyroidectomy" OR "total parathyroidectomy" OR "subtotal parathyroidectomy" OR "conventional neck exploration" OR "unilateral neck exploration" OR "minimally invasive adenomectomy"

Appendix B. Summary of outcome of primary surgical treatment of pHPT in MEN1 patient in literature.

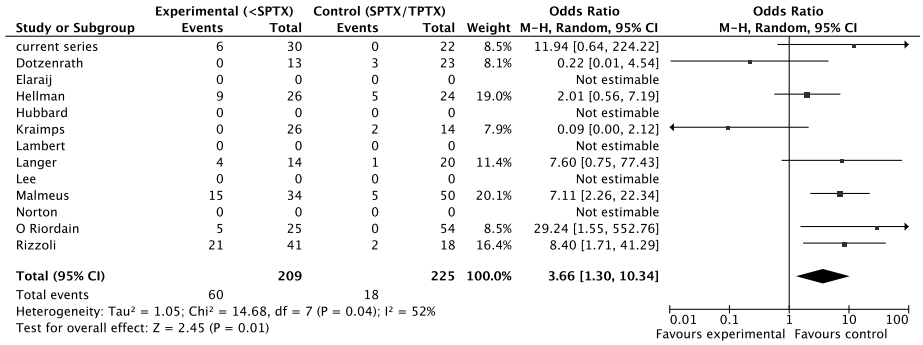
Study by author, year and study type & N patients with pHPT MEN1	Type of Surgery†	Outcome of Surgery n= and %			Duration of Follow-up (FU) *
Included in pooling analysis		*Persistent pHPT*	*Recurrent pHPT*	*Permanent hypoparathyroi-dism*	
Dotzenrath 2001 Retrospective cohort study N=38	<SPTX n=13 SPTX n=25		N=3 N=3	N=2 N=3	Mean overall FU 54 (12-180) in months
Elaraj 2003 Retrospective cohort study N=92	<SPTX n=13 SPTX n=63 TPTX n=16		N=6 (46%) N=20 (61%) N=4 (16%)	15% 26% 46%	<SPTX 5.3 SPTX/TPTX 6.1 in years
Hellman 1998 Retrospective cohort study N=50	<SPTX n=26 SPTX n=9 TPTX n=15	N=9 (35%) N=2 (22%) N=0	N=16 (61%) N=4 (44%) N=3 (20%)	N=0 N=0 N=15 (100%)‡	8.2 +/-3.9 9.1 +/-3.9 5.2 +/-2.8 in years
Hubbard 2006 Retrospective cohort study N=28	<SPTX n=4 SPTX n-21 TPTX n=4		N=1 N=1 N=2	N=0 N=2 N=1	152 (8-285) 62 (8-192) 167 (18-226) in months

Kraimps 1992 Retrospective case series N=38 MEN 1 N=4 MEN 2A	<SPTX n=26 SPTX n=14	N=0 N=2 Overall n=2	N=4 N=5 Overall n=9	Overall n=4 (10%)	8 years (1-46)
Lambert 2005 Retrospective cohort study N=37	<SPTX n=16 Thym§ 4/16 SPTX n=16 Thym 5/16 TPTX 5 Thym 3/5		N=12 (92%) N=6 (43%) N=2 (50%)	 N=1	4, FU in n=13 4.6, FU in n=14 4.6, FU in n=4 in years
Langer 2004 cohort study N=34	<SPTX n=14 SPTX n=5 TPTX n=15				132 (6-240) 151 (84-264) 36 (6-192) in months
Lee 2005 Retrospective cohort study N=22	<SPTX n=11 SPTX n=5 TPTX n=6		N=0 N=1 N=0	N=1 N=3 N=3	7.0 (0.5-19.5) 6.9 (1.5-15.5) 7.7 (2-11.5) in years
Malmeus 1986 Retrospective cohort study N=39	<SPTX n=21 SPTX n=6 TPTX n=3	N=5 N=0 N=0	N=13 N=2 N=0	N=1 N=0 N=3	Overal 6.5 (1-14) in years
Norton 2008 Prospective cohort study N= 84	<SPTX n=35 SPTX 40 TPTX 9	N=15 (44%) N=5 (12%) N=0	N=16 (47%) N=18 (44%) N=5 (55%)	N=1 (2%) N=4 (10%) N=2 (22%)	20.7 (+/-1.9) 14.5 (+/-1.5 9.9 (+/-1.5)
Riordain 1992 Retrospective cohort study N=84	<SPTX n=30 SPTX n=54	N=5 N=0	N=2 N=3	Overal N=7 (8%)	Overal 6.7 (2.5-11.1) in years
Rizzoli 1985 Retrospective cohort study N=61	<SPTX n=41 SPTX/TPTX n=20	N=21 (66%) N=2 (25%)	N=7 N=3		7.8 (1.3-12) in years
Not included in Pooling analysis					
Arnalsteen 2002 Retrospective cohort study N=83	<SPTX n=13 SPTX n=66, (+bilateral thymectomy n=55 of 66)		Reoperation n=4 Reoperations n=5 Overall recurrence 7.6%**	 13%	37 (+/-34) 50 (+/-54) no significant difference in FU
Burgess 1998 Retrospective case series N=37	SPTX n=37	N=3 (8%)	N=7 (19%) Overall recurrence rate after 8 years 67%	N=9 (24%)	8 years

Cougard 1994 Retrospective case series n=100	<SPTX n=37 SPTX n=43 TPTX n=11		Overall 'failure' 21.8% <SPTX 26% SPTX/TPTX 11%		4.6 years
Kivlen 2001 Retrospective case series N=75 with recurrent pHPT and MEN1 and reoperations	N=94 reoperations Neck exploration n=79 (84%) Median sternotomy 3 (3%) Autograft resection n=12 (13%) Procedures for persistent pHPT n=39 (42%) for recurrent pHPT n=55 (58%)		Cure or permanent hypopara-thyroidism n=58 of 64 pts (91%) Recurrent pHPT n=17 (27%) after median follow-up of 72months	N=10 (16%)	
Tonelli 2007 Retrospective case series N=51	TPTX + thymectomy n=45	N=0	N=5 (autograft recurrences)	N=10 (22%)	80 (+/-62) in months

*Duration of follow-up is reported in mean +/- standard deviation (SD) or median (range) in months or years
† Fewer than 3 glands resected (<SPTX), Subtotal parathyroidectomy (SPTX), Total parathyroidectomy (TPTX)
‡ Pemanent hypoparathyroidism, but recovery after one to seven years
§ Thym= thymectomy
**Reason for exclusion pooling analysis was the unclear recurrence rate.

Appendix C1. Comparison of <SPTX versus SPTX/TPTX on 'persistent pHPT'.
Appendix C2. Comparison of SPTX versus TPTX for 'persistent pHPT'.

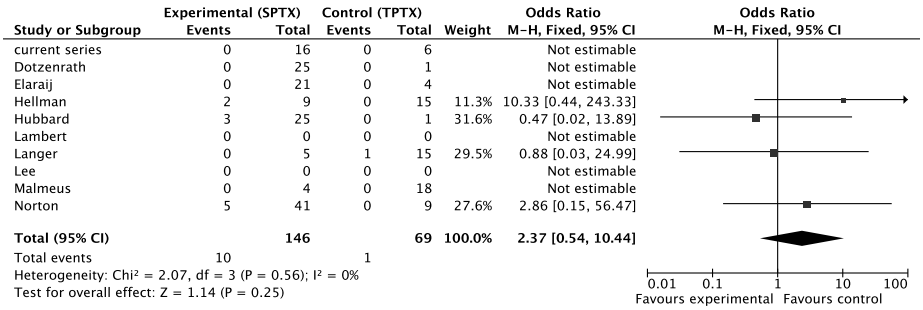


Study or Subgroup	Experimental (<SPTX) Events	Total	Control (SPTX/TPTX) Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
current series	6	30	0	22	8.5%	11.94 [0.64, 224.22]
Dotzenrath	0	13	3	23	8.1%	0.22 [0.01, 4.54]
Elaraij	0	0	0	0		Not estimable
Hellman	9	26	5	24	19.0%	2.01 [0.56, 7.19]
Hubbard	0	0	0	0		Not estimable
Kraimps	0	26	2	14	7.9%	0.09 [0.00, 2.12]
Lambert	0	0	0	0		Not estimable
Langer	4	14	1	20	11.4%	7.60 [0.75, 77.43]
Lee	0	0	0	0		Not estimable
Malmeus	15	34	5	50	20.1%	7.11 [2.26, 22.34]
Norton	0	0	0	0		Not estimable
O Riordain	5	25	0	54	8.5%	29.24 [1.55, 552.76]
Rizzoli	21	41	2	18	16.4%	8.40 [1.71, 41.29]
Total (95% CI)		**209**		**225**	**100.0%**	**3.66 [1.30, 10.34]**
Total events	60		18			

Heterogeneity: Tau² = 1.05; Chi² = 14.68, df = 7 (P = 0.04); I² = 52%
Test for overall effect: Z = 2.45 (P = 0.01)

Odds Ratio M-H, Random, 95% CI

0.01 0.1 1 10 100
Favours experimental Favours control



Study or Subgroup	Experimental (SPTX) Events	Total	Control (TPTX) Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
current series	0	16	0	6		Not estimable
Dotzenrath	0	25	0	1		Not estimable
Elaraij	0	21	0	4		Not estimable
Hellman	2	9	0	15	11.3%	10.33 [0.44, 243.33]
Hubbard	3	25	0	1	31.6%	0.47 [0.02, 13.89]
Lambert	0	0	0	0		Not estimable
Langer	0	5	1	15	29.5%	0.88 [0.03, 24.99]
Lee	0	0	0	0		Not estimable
Malmeus	0	4	0	18		Not estimable
Norton	5	41	0	9	27.6%	2.86 [0.15, 56.47]
Total (95% CI)		**146**		**69**	**100.0%**	**2.37 [0.54, 10.44]**
Total events	10		1			

Heterogeneity: Chi² = 2.07, df = 3 (P = 0.56); I² = 0%
Test for overall effect: Z = 1.14 (P = 0.25)

Odds Ratio M-H, Fixed, 95% CI

0.01 0.1 1 10 100
Favours experimental Favours control

After <SPTX, patients are more likely to develop persistent pHPT than after SPTX/TPTX (OR 3.66 95% CI 1.30-10.34, p=0.01).After SPTX, patients do not have a significantly higher risk of developing persistent pHPT than after TPTX (OR 2.37 95% CI 0.54-10.44, p=0.25).
<SPTX less than 3 parathyroid glands resected; SPTX 3-3 ½ parathyroid glands resected; TPTX total parathyroidectomy with autotransplantation;

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Chapter 7

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Long-term outcome of surgical management of primary hyperparathyroidism in multiple endocrine neoplasia type 2A patients

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Abstract

Background

The multiple endocrine neoplasia type 2A (MEN 2A) syndrome is a rare inheritable syndrome that leads to primary hyperparathyroidism in approximately 20% of patients. The most beneficial surgical approach for this condition is not yet clear. The aim of this study is to evaluate the best surgical strategy in PHPT in MEN 2A patients after a long-term follow-up.

Methods

Twenty patients with MEN 2A and PHPT were included in the study. Data of these patients were retrospectively reviewed. Surgical procedures were classified into minimally invasive parathyroidectomy (MIP), conventional neck exploration (CNE) with excision of one or two enlarged glands and selective resection of enlarged gland(s) during a total thyroidectomy.

Results

Sixteen of the twenty patients underwent primary surgery for parathyroid disease between 1979 and 2009. Six patients underwent MIP, four patients underwent CNE combined with resection of enlarged gland(s) and six patients underwent resection of enlarged gland(s) during a total thyroidectomy. Thirteen patients were initially cured after the primary operation. Three patients had persistent PHPT. Two patients developed recurrent PHPT. After MIP, none of the patients developed recurrent PHPT during 4.3 years of follow-up. Only one patient had persistent PHPT. Five patients had hypoparathyroidism. After MIP only one patient had transient hypoparathyroidism. One patient had a transient recurrent laryngeal nerve injury after MIP.

Conclusion

Of the available surgical therapies, minimally invasive parathyroidectomy is a feasible approach for PHPT in MEN 2A patients. MIP has low rates of persistent and recurrent PHPT and the complications are minimal.

Introduction

Multiple endocrine neoplasia syndrome type 2A (MEN 2A) is an autosomal dominant inherited disorder caused by a mutation in the RET oncogen on chromosome 10. This disorder can be diagnosed by genetic screening before manifestations exist.[1] Almost all patients with the syndrome develop medullary thyroid carcinoma (MTC). Therefore, these patients should undergo prophylactic total thyroidectomy according to their RET mutation as recommended by the current guidelines. Thirty to forty percent of patients develop pheochromocytomas and 20-30% of patients develop primary hyperparathyroidism (pHPT) in the course of the disease.[2] The latter is in contrast to patients with multiple endocrine neoplasia syndrome type 1 (MEN 1), who have an 78-100% risk of developing PHPT at a relatively young age.[3-12] PHPT in MEN 2A generally presents after the third decade of life.[13]

Primary hyperparathyroidism as part of MEN 2A has a diverse clinical spectrum. Firstly, parathyroid gland disease can be diagnosed in the laboratory at an early age if parathyroid glands are resected during (prophylactic) total thyroidectomy for C-cell hyperplasia or MTC. Histological examination of these parathyroid glands may show hyperplasia or adenoma. Secondly, pHPT can be diagnosed during routine biochemical screening (elevated serum calcium in combination with an inadequately non-suppressed or elevated parathyroid hormone (PTH) level) for follow-up of MEN 2A. Finally, patients can present with signs and symptoms of pHPT (e.g. renal stones or bone disease).[14]

Several factors hinder making the right choices in the treatment of pHPT in MEN 2A patients. First, pHPT in MEN 2A is a very rare disease with a different, less aggressive course than in MEN 1. Second, the incidence of multiple parathyroid gland disease is variable in pHPT in MEN 2A. Furthermore, supranumerary glands may be present and affected. Hence, recommendations on surgical therapy in MEN 2A-related pHPT are contradictive. Some authors[15] advocate total parathyroidectomy combined with autotransplantation of parathyroid tissue. Others[14, 16-19] are in favor of only selective resection of the enlarged parathyroid gland(s). Previous reports have included relatively few patients with MEN 2A and hyperparathyroidism.[14, 16, 17, 19-21] In most reports outcome of surgical treatment was analyzed in conjunction with patients with MEN 1.[16-20]

The purpose of this study was to evaluate the outcome of surgical treatment for pHPT in a large cohort of patients with MEN 2A treated at the University Medical Center of Utrecht (UMCU) during long-term follow-up. Our outcome of surgical treatment of PHPT in MEN 2A is compared to the available literature.

Methods

This retrospective cohort study includes patients with pHPT identified from the MEN 2A database from the UMCU between 1979 and 2009.

We defined MEN 2A as the presence of two or more presentations of the syndrome (MTC, pheochromocytoma, pHPT) or a MEN 2A germline mutation. Patients with MEN 2A were included if they had either biochemical evidence of pHPT (elevated serum calcium in combination with an inadequately non-suppressed or elevated

parathyroid hormone (PTH) level) or enlarged parathyroid glands during the operation for total thyroidectomy. In the regular management and surveillance of the MEN 2A population, patients are screened on the presence of biochemical evidence of pHPT on an annual or six monthly bases. Data on patient characteristics at baseline, RET proto-oncogene mutation, surgical treatment, and outcome were collected. We based outcome after surgery mainly on laboratory findings (serum ionized calcium and parathyroid hormone levels). Serum ionized calcium levels are checked (two-) monthly during the first six months after surgery to trace the presence of persistent hypercalciemia or hypoparathyroidism. After six months patients are included in the regular surveillance program for MEN 2A patients, as mentioned above, to detect recurrent hyperparathyroidism. Surgical cure was defined as a return to normal levels of serum calcium for a period of at least six months after operation. Recurrence was defined as a return of the hypercalcemia after surgical cure. Persistence was defined as hypercalciemia that remained after surgery or returned within six months after surgery. Hypoparathyroidism and nerve damage were considered complications of surgery. Hypoparathyroidism was defined as a decreased serum ionized calcium of less than 1.15 mmol/L (<4.60 mg/dL) or total calcium of less than 2.2 mmol/L (<8.8 mg/dL) or the use of calcium and/or vitamin D supplements. Hypoparathyroidism was considered permanent if it persisted for more than six months after surgery. Damage to the recurrent laryngeal nerve (RLN) was considered to be permanent if it was still present at final follow-up.

Patients were treated by minimally invasive parathyroidectomy (MIP), conventional neck exploration (CNE) combined with excision of enlarged glands, and resection of only the enlarged gland(s) during a total thyroidectomy. The MIP technique used at our institution is performed in selected cases if a parathyroid adenoma has been localized in the same location on sestamibi scan and ultrasound (US) preoperatively. A MIP is typically carried out by first marking the incision site directly above the adenoma using US. A small (≤ 3 cm) horizontal incision is made, followed by dissecting the route towards the adenoma: anteromedial border of the sternocleidomastoid muscle (retracted laterally), lateral margin of the strap muscles (retracted medially), carotid sheeth (gently retracted laterally), and the thyroid gland, firmly held anteromedially to expose the adenoma. The nerve is nearly always easily identifiable during this procedure. Intra-operative PTH measurements (ioPTH) are routinely carried whenever a MIP is performed. A significant drop of more than 50% of the intra-operative PTH level compared to the most recent pre-operative level is considered as successful.[22]

Conventional neck exploration is defined as the systematic bilateral exploration of all four parathyroid glands whenever possible. The RLN is always identified and used as landmark during the exploration, but a nerve-monitoring device is not used routinely. All pathologically enlarged, hyperfunctioning parathyroid tissue is removed. Ipsilateral thymectomy is only performed whenever an inferior parathyroid gland cannot be identified during CNE. Due to logistic reasons, ioPTH is not used routinely in CNE. If deemed necessary, histological confirmation of parathyroid tissue is sought. We defined the histopathological diagnosis of the resected glands as adenoma or hyperplasia of the parathyroid glands. The findings of a rim of normal

tissue surrounding enlarged parathyroid tissue makes the diagnosis adenoma more likely. The absence of this rim of normal tissue or the presence of two or more enlarged parathyroid glands makes hyperplasia more likely.
The duration of follow-up was defined as the interval between the date of primary surgery and the date of last contact or the last measured serum calcium level.

We used SPSS, version 16.0, SPSS Inc. Chicago, Illinois, to describe the population. Data are shown as mean or median based on their distribution. For the description and analysis of the patients who underwent surgery, we excluded those who did not undergo surgery. An ANOVA analysis was applied to calculate the statistical difference in mean follow-up between intervention groups.

Results

From the MEN 2A database, 130 MEN 2A patients were identified, 23 of whom (18%) had PHPT. Of three patients insufficient information was available and therefore they were excluded from the present study. The study population consisted of 20 patients. The median age at diagnosis of PHPT was 39 years (range: 20 to 66). There were eleven men and nine women. The Cys634Arg mutation was detected in half of the patients. Eight patients were symptomatic. Primary hyperparathyroidism was detected by routinely measuring serum calcium levels in ten patients. In four patients PHPT was diagnosed during thyroidectomy for MTC. No serum calcium or PTH levels were measured preoperatively in the four latter patients. Patient characteristics are shown in Tables 1A & 1B.

Table 1A. Patient characteristics at baseline

Variable	Number of patients (percentage of total (n = 20))
Men/Women	11(55%)/9 (45%)
Mutation	
Cys634Arg	10 (50%)
Cys634Tyr	3 (15%)
Cys634Trp	1 (5%)
Codon 618	1 (5%)
Unknown	5 (25%)
Symptoms of PHPT	8 (40%)
Fatigue	4 (20%)
Nefrolithiasis	3 (15%)
Bone pain or osteopenia	4 (20%)
Muscle weakness	1 (5%)
Pre-operative ionized calcium level*	1.36 mmol/L (range: 1.33 to 1.42)
Pre-operative PTH level#	9.3 pmol/L (range: 5.5 to 26.0)
Median age at diagnosis of PHPT	39 years (range: 20 to 66)

PTH: parathyroid hormone
PHPT: primary hyperparathyroidism
*Reference value Ca^{2+} 1.15-1.32 mmol/L

Table 1B. Other MEN2A associated features (n=20)

Feature	Data, Number (%)
MTC (treatment: total thyroidectomy)	19 (95)
Pheochromocytoma (treatment: adrenalectomy)	17 (85)
Pruritus	1 (5)
Cutaneous lichen amyloid	2 (10)
Lipoma	1 (5)
Angiolipoma	1 (5)

MTC Medullary Thyroid Carcinoma

Out of 20 patients, 16 underwent primary surgery for parathyroid disease in the period between 1979 and 2009 (Table 2). Of the surgical patients eleven underwent primary surgery at the UMCU and five in referral hospitals. Six patients underwent minimally invasive parathyroidectomy (MIP), four patients underwent a conventional neck exploration combined with excision of one or two enlarged glands and six patients underwent selective resection of the enlarged gland(s) during a total thyroidectomy. An overview of the number of resected glands and the histological examination at primary surgery is listed in Table 2. As stated earlier, intra-operative PTH measurements are currently routinely carried out whenever a MIP is performed, however for logistic reasons ioPTH was only performed in two of our six MIP patients.

Table 2. Overview primary surgical procedure for hyperparathyroidism in MEN 2A

Operation/No. of patients (total n=16)	No. of patients (N) with number of glands resected	Pathology			Localisation of glands
		Adenoma	Hyperplasia	None	
MIP*/6	N=6	5	1	0	Adenoma: right cranial (N=2), left caudal (N=3). Hyperplasia: left caudal
Conventional neck exploration with resection of enlarged glands/4	N=3; 1 gland resected N=1; 2 glands resected	1	3	0	Adenoma: left caudal Hyperplasia: left cranial, right cranial, right cranial + caudal, right paratracheal
Resection of glands during total thyroidectomy/6	N=1; 0 glands resected* N=4; 1 gland resceted N=1; 2 glands resected	1	4	1	Adenoma: left caudal Hyperplasia: left caudal, right cranial, left + right cranial

MIP Minimally Invasive Parathyroidectomy
*No glands identified

The mean overall follow-up after the primary surgery for the total group was 9.6 years (range: 2 months to 26.7 years). The mean follow-up per group was 5.0 years

for MIP, 7.8 years for the conventional neck exploration combined with selective excision of enlarged glands and 16.1 years for selective resection of the enlarged gland(s) during a total thyroidectomy. These differences in mean follow-up are statistically significant (p=0.04).
Overall, 13 patients (81%) were cured (Table 3), with the highest cure rate obtained after MIP. Three patients had persistent PHPT, one after MIP, one after conventional neck exploration (one gland resected) and one after total thyroidectomy (no enlarged glands resected, because none were found). Two patients developed recurrent disease. One of these developed recurrent disease 10.3 years after removal of one gland during total thyroidectomy. The other patient developed recurrence 11.2 years after removal of one gland by conventional neck exploration.

Table 3. Surgical outcome after primary surgery for hyperparathyroidism in MEN 2A

Operation	Number of						Mean follow-up in years *
	Patients	Successfully treated patients	Recurrent pHPT	Persistent pHPT	Hypocalciemia (transient/ permanent)	Nerve injury (transient/ permanent)	
MIP	6	5	0	1	1 (transient)	1 (transient)	5.0 (±5.8)
Conventional neck exploration with resection of enlarged glands	4	2	1	1	2 (1 unknown, 1 transient)	1 (transient), 1 phrenic (permanent)	7.8 (±6.2)
Resection of glands during total thyroidectomy	6	4	1	1	2 (permanent)	0	16.1 (±6.2)

pHPT, Primary hyperparathyroidism; MIP, minimal invasive parathyroidectomy
*The mean follow-up between the different surgical groups was significantly different, p= 0.04

We carried out additional analysis to study the potential relationship between RET mutation and outcome after primary surgery. Eight of the sixteen operated patients were carriers of the Cys634Arg mutation. We found that four out of five patients with recurrence or persistence of hyperparathyroidism after primary surgery had the Cys634Arg mutation.

Complications occurred in nine patients following primary surgery, consisting of transient recurrent laryngeal nerve injury in two patients, phrenic nerve injury in one patient and persisting hypoparathyroidism in five patients (Table 3). Transient hypoparathyroidism occurred in one patient after a minimally invasive procedure. Two patients had hypoparathyroidism after conventional neck exploration with removal of one and two glands respectively (of unknown duration and transient, respectively). Two patients had permanent hypoparathyroidism after total thyroidectomy, with removal of one and two glands, respectively. One patient had

immediate postoperative failure (calcium and PTH levels remained elevated after removal of a right cranial adenoma) and underwent successful re-exploration one week later, with removal of a hyperplastic gland located on the left side caudally in thymic tissue. Four patients died during follow-up. Two of them died of non-MEN 2A-related malignancy and two of unknown causes.

Results for re-operations

Three patients underwent re-operations for persistent or recurrent PHPT. One patient was operated a total of three times for primary hyperparathyroidism. Primary surgery included removal of a hyperplastic gland (600 mg), located in the left cranial position during total thyroidectomy in 1982. She developed asymptomatic recurrence of hyperparathyroidism in 1993. Secondary surgery was performed in 1996 because the patient was pregnant and consisted in resection of an adenoma (520 mg) located left caudally. A MIP was performed in 2008 (recurrence 1998) with removal of another hyperplastic gland (940 mg) located right caudally. Eight months after tertiary surgery serum ionized calcium and PTH levels are within the normal range.

Another patient had persistence after removal of a hyperplastic gland located left caudally during CNE in 1979. The right and left cranial glands were also located by intra-operative biopsy, but were not resected. She underwent a second conventional neck exploration in 1982. An adenoma (left cranial) and a hyperplastic gland (right caudal) were resected and partially autotransplanted in the right brachioradial muscle. The right caudal gland was not found during the latter procedure. Patient was cured after the second CNE after a follow-up of 11.3 years.

A third patient underwent a conventional neck exploration in 1994 with removal of a hyperplastic gland (right cranial) and an adenoma (location not described). No other glands were found. In 2005 the patient underwent a re-exploration of the right side of the neck during which recurrence medullary thyroid cancer was removed. She underwent a MIP in 2007 (left cranial hyperplastic gland) for recurrence hyperparathyroidism 10 years after primary surgery. A right-sided re-exploration was performed in 2009 for recurrence hyperparathyroidism (November 2008). Intra-operative ultrasound showed a definite large gland on the right side of the neck, located between the right carotid artery and trachea. The gland was resected and partially auto-transplanted in the right lower-arm. No definite histopathological diagnosis concerning hyperplasia or adenoma could be made. The latest postoperative serum ionized calcium level was 1.33 mmol/l and serum PTH level was 13 pmol/l.

Discussion

Our series is one of the largest series to report results of surgical therapy for primary hyperparathyroidism in patients with MEN 2A with a long duration of follow-up. From the data we conclude that MIP is the procedure of choice for MEN 2A patients at our institution. None of the patients who underwent a MIP developed recurrent PHPT and only one patient had persistent PHPT. Complications were few and transient. Yet, we should keep in mind that follow-up in this group was significantly shorter.

MIP has a minimal risk of damage to surrounding tissues. Furthermore, MIP provides

better cosmetic results, patient comfort and a shorter duration of hospital stay.[22] The MIP technique has become the standard of care for sporadic PHPT in institutions with significant experience with the procedure. Its role in the setting of familial PHPT is evolving. MIP can be performed if preoperative imaging studies are concordant. In the current time of genetic screening and prophylactic total thyroidectomy at young age, almost all MEN 2A patients have undergone some form of neck surgery for MTC before the presentation and surgery of PHPT. This was the case in all patients who underwent a MIP in our series. Taking the postoperative fibrosis and scarring after neck surgery into account, a minimally invasive procedure is preferred over more invasive techniques. For example, the risk of recurrent laryngeal nerve injury is minimized. It also reduces the chance of permanent hypoparathyroidism, which can have a dramatic impact on the quality of life.

Our results suggest that PHPT in MEN 2A is generally not an aggressive disease. Hypercalcemia was commonly mild. PHPT was asymptomatic in 60% in our series and 42% to 84% of cases in retrospective studies.[13-15, 19-21] PHPT is rarely the first diagnosed endocrinopathy in MEN 2A (15% in our series). In most patients, MEN 2A-related PHPT was present in the third and fourth decade which was also seen in other series.[14, 17, 20, 21]

Three patients had persistent PHPT and two patients developed recurrent hypercalcemia. The somewhat unfavorable results of persistent PHPT after surgery in our series may be related to the fact that some patients were operated on at the beginning of the 1980s (in a time when MEN diseases were less well appreciated) and at other hospitals with less experience in operating on patients with multi-gland disease. Unfortunately, the latter is not always preventable because of the rarity of the syndrome. This emphasizes however the importance to recognize MEN 2A correctly and refer patients with MEN 2A to tertiary referral centers.
We found relatively low rates of persistent and recurrent PHPT after selective gland resection during a long follow-up period. This supports our belief that patients with MEN 2A-related PHPT often have single gland disease instead of multiglandular disease, as is the case in patients with MEN1. We believe that a more conservative approach with selective resection of enlarged glands is justified rather than a subtotal or total parathyroidectomy. This is in line with most reports on PHPT in MEN 2A (Table 4). An overview of the results of primary surgery in MEN 2A-related PHPT obtained in worldwide studies is given in Table 4. Surgical cure rates of 83% and recurrence of 3% have been reported after selective resection of only enlarged glands.[23] In several series selective parathyroidectomy resulted in an immediate postoperative cure rate of 100%. Recurrence rates in those series varied from 0-14% after a follow-up of 5.8-8.0 years.[14, 18, 21] In another series of 54 patients with MEN 2A and PHPT, 29 underwent resection of only macroscopically enlarged glands. In that series 6.9% had persistent disease and after 6.4 years of follow-up 13.8% developed recurrent disease.[13] Another small series of four patients who underwent selective parathyroidectomy showed neither persistent nor recurrent disease after 8.0 years of follow-up. However, 25% of the patients had hypoparathyroidism.[5, 20] We

found a recurrence rate comparable to the ones found in these studies. However, in our series the duration of follow-up was longer.

Contrary to the aforementioned studies, Herfarth et al.[15] advocate total parathyroidectomy and autotransplantation of one of the glands in the forearm as the procedure of choice. In this study none of the five patients treated with total parathyroidectomy developed recurrence after a mean follow-up of 16.3 years. Recurrence was seen in 14% after selective gland resection and in 25% after subtotal parathyroidectomy. The rate of hypoparathyroidism was almost identical after selective resection of enlarged glands (one or two) and after total parathyroidectomy with autotransplantation (19% and 20%).
We also found a rather high rate of postoperative hypoparathyroidism in our MEN 2A patients (31%). In two patients hypoparathyroidism was transient (one patient after MIP, and one after conventional neck exploration). Permanent hypoparathyroidism after neck exploration most likely reflects inadvertent parathyroid injury during operation. Since permanent hypoparathyroidism developed in two patients who had their parathyroid glands resected during total thyroidectomy, the hypoparathyroidism could also be the result of damage to the glands during thyroidectomy. The transient cases of hypoparathyroidism are probably the result of suppressed normal parathyroid tissue. This is caused by constant exposure to hypercalcemia as a result of autonomously functioning hyperplastic or adenomatous parathyroid glands with an elevated calcium set-point. The complication rate after MIP was low, with one case of transient recurrent laryngeal nerve injury.
We carried out an additional analysis to study the potential relationship between RET mutation and outcome after primary surgery. However, the relatively large number of patients with a Cys634Arg mutation in the total group and the relatively small number of patients in the subgroups did not allow us to make solid conclusions regarding this relationship.
The histopathological diagnosis of the resected parathyroid glands may be under influence of selection bias of the operating procedure. During MIP, exploration is minimal and not all parathyroid glands are explored. Only if ioPTH does not drop (performed in two cases) a more extensive exploration is undertaken. If more than one or two glands are found and affected, it is often regarded as hyperplasia, which is often the case in bilateral neck explorations. Whereas in MIP, only one gland is removed, which is often considered to be an adenoma.

Although this is one of the largest studies on surgery in MEN 2A-related PHPT, the number of patients per group was small and patients were not randomized. We had a relatively low number of patients with PHPT among a total group of 130 MEN 2A patients. MIP at our institution is considered only if imaging studies are concordant. In our series the results may therefore show confounding by indication. In addition, follow-up after MIP was statistically significant shorter than after other procedures.
The results of our study reflect the MEN2 population in the region of the UMCU. This may lead to underscoring of comments on surgery in patients with MEN2A and PHPT from centers in other regions but with similar RET mutations. Nonetheless, we

advocate MIP as an adequate surgical procedure for MEN 2A patients with PHPT.

Conclusion

Minimally invasive parathyroidectomy is a feasible approach for primary hyperparathyroidism in MEN 2A patients. MIP has low persistence and recurrence rates and complications are minimal.

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PART 3
Pancreas

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Chapter 8

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Surgical treatment of pancreatic endocrine tumours in patients with multiple endocrine neoplasia type 1 syndrome. A systematic review

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Abstract

Aim

Gathering available evidence to answer the following questions: (I) what are the indications for surgery in pancreatic endocrine tumours (PETs) in MEN1 patients? (II) if any, what type of surgery is warranted?

Introduction; PETs are the main cause of death in MEN1. Type and timing of their surgical treatment is controversial.

Methods

Based on title and abstract, studies were selected. Two reviewers performed inclusion, data-extraction and quality-assessment. Heterogeneity prevented pooled analysis.

Results

Thirty-six articles, mostly case-series, were included. Those with controlled designs were mainly of low methodological quality. *Insulinomas:* when operating most patients with hyperinsulinism cure rates were 69-100%. Although cure rates appeared higher after distal pancreatic resection than after conservative surgery in one study, other case-series show cure rates of 100% after varying strategies including enucleation. *Gastrinomas:* Outcome in tumours >30mm was worse. Biochemical cure rate was 0% when operating patients with tumours >=25mm compared to 64-77% after operating earlier combined with extensive resections. Survival did not differ. Duodenal gastrinomas were frequently enountered (11-76%) warranting duodenotomy. *Non-functioning tumours (NF-PETs):* Risk for metastases seemed to increase with tumour size. Survival was worse in tumours >30mm. Recurrence after surgery for PETs =<20m was 33-60%. Extensive resections resulted in 50% cure rate.

Conclusions

Since methodological quality of the evidence is low, results must be interpreted cautiously. *Indications:* it seems reasonable operating all MEN1-related insulinomas, gastrinomas>25mm and if aiming for biochemical cure, gastrinomas >10mm. Surgery for NF-PETs =<20mm may be considered. *Type of resection:* Extensive resections for insulinomas and gastrinomas led to biochemical cure more often. However, survival did not differ. Type of resection for NF-PETs remains debatable.

Introduction

Multiple endocrine neoplasia type 1 (MEN1) is a rare autosomal dominant inherited endocrine tumour syndrome, first described by Wermer in 1954.[1] It is characterised by the development of parathyroid hyperplasia or adenomas, pituitary adenomas, pancreatic endocrine tumours (PETs), adrenal gland adenomas and neuroendocrine tumours (NET) of the bronchus, thymus, and gastro-intestinal tract.

The responsible tumour suppressor gene was identified in 1997. It is located on the long arm of chromosome 11q13 and encodes for the Menin protein, which is a transcription regulator.[2] Reports regarding genotype-phenotype correlation in MEN1 vary. In contrast to a specific genotype-phenotype correlation, recent studies do show a relationship between type and location of mutations and clinical expression of the MEN1 syndrome[3-5]. The clinical significance of these results has yet to be determined. Clinically manifest PETs occur in 35-75% of the patients with MEN1[6-16]. Histological examination reveals that 90-100% of the operated patients with MEN1 have PETs[17, 18]. The different types of PETs can be classified by the autonomous hormones they secrete. The most common PETs are gastrinomas producing gastrin (Zollinger-Ellison syndrome), insulinomas (insulin) and non-functioning PETs (no specific hormone). Other peptides frequently detected in the presence of PETs are chromogranins, pancreatic polypeptide, vasoactive intestinal peptide, glucagon, and calcitonin[19]. Also, numerous microadenomas may be present in the entire pancreas[20, 21]. PETs are the main cause of death in MEN1 patients[22, 23]. Much controversy exists about their management[24, 25]. Many surgical strategies have been proposed, varying from conservative approaches (i.e. only resecting tumours >30mm) to more aggressive approaches[12, 26, 27]. A recent consensus statement of the European Neuroendocrine Tumor Society (ENETS) about the management of PETs is based on consensus between experts[28]. Especially with regard to MEN1 gastrinomas and non-functioning tumours, experts concluded that the available evidence is limited and controversy still exists[29, 30]. The focus of these guidelines lies on digestive neuroendocrine tumours in general and the specific recommendations for MEN1 patients are limited[28-32].

Yet, PETs in patients with MEN1 have a different natural course than sporadic PETs: compared with sporadic PETs, those in MEN1 occur at a younger age and tend to be multiple. Hence, there is a need for more specific and evidence-based recommendations about the management of PETs in patients with MEN1.

The purpose of this systematic review was to gather the best available scientific evidence to answer the following questions. 1. What are the indications for pancreatic surgery in pancreatic endocrine tumours in patients with the MEN1 syndrome? 2. What type of surgery is warranted?

Methods

Studies that evaluated surgical management of pancreatic endocrine tumours (gastrinomas, insulinomas, VIPomas, somatostatinomas, glucagonomas and non-functioning tumours) in people aged 18 years or older with MEN1 were considered for inclusion. They were included if they studied the effect of pancreatic surgical

interventions on cure, complications and/or survival. Studies that reported about surgical treatment of sporadic and MEN1 related PETs, were included if MEN1 PETs data were reported separately and could be used for analysis. A search was conducted in Medline (1966-2009), Embase (1980-2009), the Cochrane database of systematic reviews and the Cochrane Central Controlled Trials Register (2009) using predefined search terms (see Table 1). There were no language restrictions. Randomized controlled trials (RCT), case-control studies, prospective and retrospective cohort studies, controlled before-and-after designs, interrupted time series designs, prospective and retrospective case series (>5 patients) were all included. Bibliography tracking of identified articles was performed.

Table 1. Search terms

Medline
(Multiple endocrine neoplasia syndrome type 1 OR MEN 1 syndrome OR Multiple endocrine neoplasia syndrome type 1 OR multiple endocrine neoplasia) AND (Endocrine pancreatic tumor* OR Endocrine pancreatic tumour* OR Pancreatic endocrine neoplasia* OR Hereditary endocrine pancreatic tumor* OR Hereditary endocrine pancreatic tumour* OR Pancreatic Functioning tumor* OR Pancreatic Functioning tumour* OR Pancreatic non functioning tumor* OR Pancreatic non functioning tumour* OR Functioning tumor* pancreas OR Functioning tumour* pancreas OR non functioning tumor* pancreas OR non functioning tumour* pancreas OR neuroendocrine tumor* pancreas OR neuroendocrine tumour* pancreas OR neuroendocrine pancreatic tumor* OR neuroendocrine pancreatic tumour* OR Adenoma, Islet Cell OR Carcinoma, Islet Cell OR Gastrinoma) AND (Pancreatectomy OR pancreaticojejunostomy OR Surgery OR Surgical Procedures, Operative OR Pancreas surgery OR Pancreatic surgery OR Pancreatectomy OR Surgery OR Surgical OR Surgical treatment OR surgical intervention OR surgical management)
Embase
(‘Multiple endocrine neoplasia syndrome type 1’ OR ‘MEN 1 syndrome’ OR ‘Multiple endocrine neoplasia syndrome type 1’ OR ‘multiple endocrine neoplasia’) AND (‘Endocrine pancreatic tumor*’ OR ‘Endocrine pancreatic tumour*’ OR ‘Pancreatic endocrine neoplasia*’ OR ‘Hereditary endocrine pancreatic tumor*’ OR ‘Hereditary endocrine pancreatic tumour*’ OR ‘Pancreatic Functioning tumor*’ OR ‘Pancreatic Functioning tumour*’ OR ‘Pancreatic non functioning tumor*’ OR ‘Pancreatic non functioning tumour*’ OR ‘Functioning tumor* pancreas’ OR ‘Functioning tumour* pancreas’ OR ‘non functioning tumor* pancreas’ OR ‘non functioning tumour* pancreas’ OR ‘neuroendocrine tumor* pancreas’ OR ‘neuroendocrine tumour* pancreas’ OR ‘neuroendocrine pancreatic tumor*’ OR neuroendocrine pancreatic tumour* OR ‘Islet Cell Adenoma’ OR ‘Islet Cell Carcinoma’ OR ‘Gastrinoma’) AND (Pancreatectomy OR pancreaticojejunostomy OR Surgery OR ‘Surgical Procedures’ OR ‘Pancreas surgery’ OR ‘Pancreatic surgery’ OR Pancreatectomy OR Surgery OR Surgical OR ‘Surgical treatment’ OR ‘surgical intervention’ OR ‘surgical management’)

One reviewer (JS) determined whether retrieved references with the search were relevant by screening their titles and abstracts. Subsequently, two reviewers (JS & CP) independently assessed the eligibility of the full texts of the selected articles by filling out predefined in- and exclusion scoring forms and comparing them. If they disagreed about in- or excluding an article, consensus was reached by discussion. From the included studies we recorded study designs, patient characteristics, intervention, outcome, duration of and loss to follow-up. We used standard data

extraction sheets to score each study independently for methodological quality using study design-specific scoring lists based on checklists developed by the Dutch Cochrane Center (*www.cochrane.nl/index.html*), with equal weighting being applied to each criterion. In case the reviewers would disagree the co-reviewers (MV & GV) had the deciding vote. Items about quality were scored as 'done', 'not done', or 'not reported'. Only the answer 'done' would increase the quality score. This score was transformed into one of the levels of evidence as defined by the Oxford centre for evidence-based medicine (Phillips et al. 2001): (1a) systematic review of RCTs; (1b) individual RCT; (2a) systematic review of cohort studies; (2b) individual cohort study; (2c) "outcomes" research, ecological studies; (3a) systematic review of case-control studies; (3b) individual case-control study; (4) case series.
Kappa statistics for in- and exclusion were used to calculate the level of interobserver agreement. For data extraction and methodological quality percent agreement was calculated.

The data that were extracted from the included articles were summarized in evidence tables on a study-by-study narrative basis. Each tumour type had its own table. (see Appendix A-D) When different PETs were present in one cohort, but could not be analyzed separately per tumour type, they were discussed in the section 'combined tumours'. If information about survival and metastatic disease was unavailable or could not be related to an indication for surgery or type of surgical resection, biochemical cure or recurrence were used as intermediate endpoints. The heterogeneity of study designs, interventions, follow-up periods and outcome made it impossible to pool these results. All authors partook in drawing conclusions and making recommendations.

Results

The search yielded 1074 hits, including doubles. 178 studies were selected after application of in- and exclusion criteria to titles and abstracts, 36 after applying the criteria to the full texts (Figure 1).

The kappa coefficient of inter-observer agreement on in- and exclusion criteria was 0.88 (95% CI 0.80-0.95). Agreement on information extraction was 97%, on methodological quality 94%. Evidence tables for each specific tumour type can be found in the appendices (Appendix A-D).
Most studies were case-series and the few available cohort studies were of low methodological quality mainly because the study groups were not comparable leading to a low level of evidence.

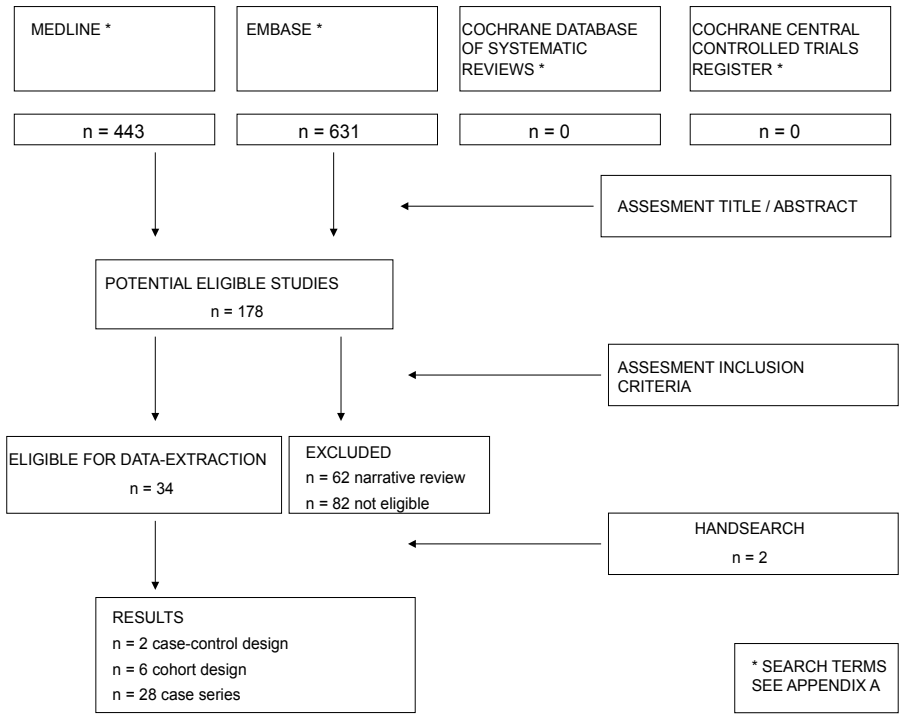

Figure 1. Search results

Insulinoma

Data about insulinomas were retrieved from four retrospective case series (level of evidence 4), which were published in five different papers[33-37]. Additionally we extracted data from six case series (level of evidence 4), published in seven different papers that reported on different kinds of PETs[24, 38-43] (Appendix A).

In only three studies the indications for surgery are explicitly reported, being 'presence of insulinoma' in two[40, 43] and 'insulinoma and absence of diffuse liver metastases' in the third[24]. 93-100% of the patients with insulinomas in the included studies were operated.

In almost all included studies, 50-100% of the patients had multiple PETs[24, 34-42]. Whether this always represents multiple insulinomas is unclear, because only four authors report that the multiple tumours were indeed multiple insulinomas, confirmed by positive immunohistochemistry staining[33, 36, 39, 41, 42].

One study (published in two papers) showed no long-term (follow-up ten years) tumour recurrence after distal pancreatic resection (DPR) (n=12) (removal >75% of the pancreas with enucleation of visible tumours in the pancreatic head) in contrast to the recurrence rate of 40% after more conservative surgery (n=5)[35, 37] The other case series reporting solely on insulinomas showed surgical cure rates of 69, 92 and 92% after 9 years [33, 34, 36]. The 92% cure rates were obtained after large DPRs (>80%) with or without enucleation, but also pancreaticoduodenectomies (PD) or

total pancreatectomies (TP)[33, 36]. In the series with the cure rate of 69%, recurrence was most often observed after enucleation. After DPR the cure rate was 75%[34].
In six more recent small case series from which we extracted data on insulinomas, cure rates were 100% in a total of 22 patients. Sixteen of these patients had 50% or more of their pancreas resected, while four underwent an enucleation, one a corpus resection and in two patients the type of resection was not reported [24, 38-43]. Differences in cure rates between the different studies might be attributed to temporal differences.

Gastrinoma
Four controlled studies (level of evidence 3b, 3b, 4 and 4) and eight case series that were published in nine different papers, (level of evidence 4), were included that solely presented data on gastrinomas [44-56]. Four case series that reported about different kinds of PETs were included and specific data on gastrinomas were extracted [24, 41-43]. (Appendix B)
Most studies show that multifocality is a common feature of gastrinomas (40-97%) [45, 49, 51, 53, 54, 56]. In one case series only isolated duodenal gastrinomas were encountered[43], but in most case series pancreatic and duodenal tumours were both present (9-72%). Because of this most authors routinely performed duodenotomy [24, 41, 42, 44, 46, 49, 51-56].
In one case-control study surgery itself was studied as a prognostic factor and no association between surgery and prolonged survival was seen [44]. The surgical patients more often had tumours ≥ 30 mm and follow-up in the surgical group was significantly longer than in the non-surgical group.
In two case-control studies tumour size >30mm was shown to be an adverse prognostic factor. Large tumours were associated with liver metastases in the first study [44] and aggressive growth in the second study. This aggressive growth was associated with decreased survival [46]. In contrast, Bartsch et al. did not find an association between tumour size and behaviour in their case series [24].
In two prospective case series patients were operated if tumours surpassed 25-30 mm without having diffuse liver metastases. These patients underwent operations varying from enucleation to DPR and Whipple resection. This surgical strategy led to a 100% 10-year survival in one study and 86% in the other study. In patients who were not operated on (tumours < 25mm) the 10-year survival was 100%. [51, 52] In contrast to the good survival rates, no biochemical cure was observed in both studies and 6% of the operated patient developed liver metastases [51].
Two other case series reported a more aggressive surgical strategy in which all patients without diffuse liver metastases were operated on (irrespective of tumour size)[24] or all patients with hypergastrinemia[43]. In one study large resections (DPR+/- enucleation) were performed in 55% of the patients. This resulted in 64% biochemical cure (normal fasting gastrin and secretin tests) and >90% survival after a mean follow-up period of ten years [24]. In the other study, only large resections were performed (large DPR+/- enucleation or pylorus-preserving pancreaticoduodenectomy (PPPD) leading to 77% biochemical cure three to five months after surgery and 81% overall survival[43]. Thompson et al. operated on patients with localised gastrin secretion,

determined by transhepatic selective venous gastric sampling[55, 56]. He performed only DPR's leading to biochemical cure rates (normal fasting gastrin) of 91%-100%[55, 56]. In two small case-series, it was observed that local tumour recurrence occurred only after local excision[24, 55].

Non-functioning tumours

Two retrospective cohort studies (level of evidence 2b and 4) and one case series (level of evidence 4) that only presented data on NF-PETs were included[57, 58]. Additionally, three case series (level of evidence 4) that reported on different kind of PETs were included, from which we extracted data on NF-PETs[24, 41, 43]. (Appendix C)
One cohort study showed a significant association between the size of NF-PETs and the occurrence of (lymph node and/or distant) metastases and survival[57]. The risk of synchronous metastases increased with increasing tumour size, from four percent in tumours ≤10mm to 43% in tumours >30mm. Survival was worse for patients with distant metastases (34 versus 98% 8-year survival) and for patients with tumours larger than 30mm [57]. The other cohort study showed that for tumours ≤20 mm, metastases were not associated with tumour size[59]. One study showed that tumours smaller than 20mm showed no growth during follow-up, whereas patients with tumours larger than >35mm developed new tumours and metastases [58].
One cohort study studied whether early surgery when tumours are smaller than 20mm is indicated. Surgery was compared with watchful waiting in patients with tumours ≤20mm.[57] No benefit of surgery (enucleation 30%, PD 7%, DPR + enucleation 53% and subtotal pancreatectomy 7%), in terms of recurrence and occurrence of metachronous metastases, was found in this cohort. However, baseline characteristics and follow-up differed significantly between both groups [57]. Follow-up in the conservative group was half as long as follow-up in the surgical group. This probably led to an underestimate of the benefit of surgery. This study also showed that recurrence rates were not different between enucleation and major resection.
In three cases series a total of 14 patients underwent a major resection (DPR, with enucleation when indicated, subtotal pancreatectomy or PD) for their NF-PETs. These operations resulted in a 50% cure rate and no disease-specific mortality after a maximum of 6.5 years follow-up [24, 41, 43]. The fourth case series did not adequately describe the relation between surgery and outcome.

Other pancreatic endocrine tumours (glucagnomas, somatostatinomas, VIPomas)

From the relevant case series, eleven patients had glucagonomas, seven VIPomas and two somatostatinomas[24, 34, 39, 40, 60-64]. From these studies no specific information for further analysis could be retrieved.

Combined tumours

Two cohort studies (level of evidence 2b) [12, 60] and six case series (level of evidence 4) were included [61-66]. Because evidence from these studies is meager, only the most relevant results are reported here. More detailed results are presented in appendix D.

The first cohort study analysed the effect of several prognostic factors on overall survival (OS) and progression free survival (PFS). They found younger age, functional tumours and no metachronous metastases to be associated with a better OS. In patients without metastases withholding surgery was associated with decreased OS[60]. In this cohort 89% underwent a DPR, PD or TP. This resulted in recurrence of disease in five out of 31 patients with a pancreatic remnant. Two of the four patients treated with enucleation developed recurrent disease. Within the surgical group, pancreatic resection (PPPD, PD or TP) was associated with an improved PFS when compared with enucleation[60]. Overall ten-year survival in this cohort was 75%, median follow-up 4.3 years[60]. In the other cohort study, surgery was performed on all patients with biochemical evidence of a PET without radiological evidence of metastases. Biochemical evidence was defined as two independent endocrine tumour markers raised on two separate occasions. All but one patient underwent a DPR. [12]. They compared biochemical recurrence in patients who were symptomatic with patients who were asymptomatic. This strategy led to 100% survival in the asymptomatic group versus 83% in the symptomatic group, after a follow-up of six and seven years respectively [12]. Biochemical recurrence was 60% in asymptomatic patients and 83% in symptomatic patients [12].

From the uncontrolled studies, two of the five case series reported that they could not find an association between tumour size and the presence of metastases [61, 63]. One case series studies patients who were re-operated for PETs. The operations that were performed were DPRs +/- enucleation. Three out of five patients with gastrinomas were biochemically cured, but all developed new NF-PETs. One of the three patients with NF-PETs was cured. Only one patient (with a VIPoma) developed liver metastases. The overall survival was 100%.

Reported complications of pancreatic surgery

Pancreatic surgery may cause minor and major complications. Common complications that were reported were diabetes mellitus, pancreatic fistulas, anastomosis leakage, abscess, wound infection, pancreatitis, gastro-intestinal motility disorders and malabsorption. The complication rates varied from 0% to 52%. Patients who undergo major pancreatic resection have a higher chance of developing diabetes mellitus. In one case-series 6 out of 7 patients developed insulin dependent diabetes mellitus after DPRs and subtotal pancreatectomy.[58] Complications were poorly reported in studies on surgery for gastrinomas. Those who did, reported complication rates between 37 and 52%[24, 43, 51]. Most studies did not discriminate between major and minor resections and complications rates. Some did report complication rates per specific resection. After DPR major complications (mainly fistulas, followed by wound infection, abscess and anastomosis leakage) varied from 19-50%[12, 24, 43, 61, 65]. One study reported one case of peri-operative death. Complication rates after reoperation were higher than after primary surgery 57% versus 37%[24, 43, 51].

Discussion

This systematic review focuses exclusively on the surgical treatment of PETs in patients with MEN1.

The most important conclusion of our 'lege artis' performed systematic review is that the evidence for the surgical strategy in PETs in MEN1 is meagre. Most evidence comes from uncontrolled studies. The few studies with a controlled design are of low methodological quality. Both are prone to (selection and publication) bias. Furthermore, most study groups were small, indications for the surgical intervention were inadequately reported and the exact type of intervention was often unspecified. In most studies, primary outcomes such as survival and development of metastases could not be correlated to the intervention. Therefore, biochemical cure or recurrence rates were used as intermediate outcomes.

Insulinoma

In surgery for insulinomas it is important to treat the hormonal excess syndrome as well as the tumour itself. Insulinomas larger than 5 mm can cause hypoglycemia with adrenergic and neuroglycopenic symptoms. These patients can be cured by performing surgical resection [21]. From the available evidence it can be concluded that every patient with an insulinoma should undergo surgical resection [24, 33-43]. Current guidelines of the ENETS recommend tumour enucleation and peroperative exclusion of the presence of additional tumours. A central or distal pancreatic resection is only recommended when enucleation is unfeasible due to the tumour location [31].

The available evidence, however, suggests that performing a mere enucleation might leave possibly present undetectable microadenomas in situ and may therefore lead to recurrent disease and endanger cure. In a direct comparison one study showed that DPR +/- enucleation of tumours in the pancreatic head leads to a better cure rate than enucleation alone. In the other studies mostly large resections (DPR, PD or TP depending on tumour location) are responsible for the cure rates. However, survival rates were not reported for the different surgical strategies. Major pancreatic resections yield a higher risk of developing DM. It is unclear whether other complications are more frequent as well. This should be kept in mind when considering a type of resection.

Gastrinoma

Since duodenal (submucosal) gastrinomas are almost always present in patients with gastrinomas, the available literature suggests that duodenotomy with tumour excision should routinely be performed.

The current ENETS guidelines state that the role of surgery as well as the type and timing of surgery is still controversial in MEN1 related ZES. Although the authors underline that the efficacy of surgery in patients with tumours >20 mm is unproven, they do recommend it. At the same time, they advise against a Whipple resection in general [30].

Our results suggest that survival rates are indeed satisfactory when the indication for surgery is set at a tumour size of ≥ 25-30 mm. But with this indication no biochemical cure is observed, which indicates these patients still harbour tumour [51, 52]. With

a more aggressive strategy – operating as soon as tumours surpass 10 mm (or perhaps even all gastrinomas) – biochemical cure can be accomplished [24, 43, 55, 56]. The literature also shows that biochemical cure was only accomplished after performing large DPR's or Whipple resections [24, 41-43, 55, 56]. Thus according to the yet available evidence operating early and more extensively results in a higher biochemical cure rate. However, no increased survival rates are seen with this aggressive strategy, which makes recommendation of aggressive surgery difficult.

Non-functioning pancreatic endocrine tumours
Patients with tumours >30 mm seem to have a worse survival than patients with smaller tumours, but results are contradictory [24, 57]. The hypothesis that early surgery may be beneficial for these patients is incorporated in the ENETS consensus statement which states that in patients with multiple tumours prophylactic surgery aims to remove the lesions before malignancy develops [29]. However, in the yet available literature no evidence is found for the effectiveness of surgery in patients with tumours ≤20 mm [24, 43, 57].
Current guidelines do not provide clear-cut recommendations for surgical strategy in NF-PETs and due to the scant amount of evidence neither can we. We concur that early surgery (≤20 mm) is an option because larger tumours seem to have an increased risk of metastases and lower survival. Given the available evidence, no additional recommendations can be made about the type of resection.
Other pancreatic endocrine tumours Due to the lack of evidence no conclusions regarding these tumours could be drawn.

Combined tumours Outcome from studies that consider different PETs as one tumour group may be treated as supporting evidence. These studies confirm that withholding surgery decreases survival. It was also observed that large resections increase the chance to cure, but effects on overall survival were not reported.

Complications
The risk of complications seems acceptable, although not every study reports those. If complications are reported, it is often done inadequately. Complication rates in series vary from 0-52% of which pancreatic fistulas and DM are the most frequent in the studies reports. From the available studies, only few discriminated complications between minor and major pancreatic resections. Major resections had a higher postoperative rate of DM, which is obvious. Only one case of peri-operative death was found in these studies. Literature on pancreatic surgery shows that mortality is often <4% in high volume centers[67]. Main complications after pancreatic surgery are delayed gastric emptying, DM, pancreatic fistulas, abscess, wound and infection. In one study for pancreatic resections for NET in a high volume centre, pancreatic fistulas were observed more frequently after enucleation of a tumour in the head than after PD or DPR[68].

Conclusion

The available literature is of low methodological quality and does not provide clear-cut indications for surgery. However, it seems reasonable to operate on all patients with insulinomas and patients with gastrinomas >25 mm. Operating on patients with gastrinomas between 10 and 25 mm seems defendable given the available evidence. There is no evidence that early surgery (tumours ≤20, mm) of non-functional tumours improves cure and survival. However, the possible association between a heightened risk of metastases and larger tumour size may favour surgery. In insulinomas and gastrinomas more extensive operating strategies may be adopted. A duodenotomy ought to be performed when operating on patients with gastrinomas, because submucosal duodenal tumours are often found. In non-functional tumours, it remains debatable, which type of surgery is preferable, because in the available studies mostly large resections were performed.

Although survival rates of MEN1 patients with PETs are relatively good with conservative surgery, more aggressive surgical resection may improve the chance of (biochemical) cure. However, it remains important that as to day no survival benefit of early and aggressive surgery is demonstrated.

There is a need for high quality controlled studies that will enable researchers to determine which strategies lead to improved quality of life, higher survival and lower complication rates.

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PART 4
Adrenal Glands

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Chapter 9

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Retroperitoneal endoscopic versus conventional open adrenalectomy; a cost-effectiveness analysis

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Abstract

A retrospective cost-effectiveness analysis was carried out, comparing retroperitoneal endoscopic and conventional adrenalectomy, which is based on a large group of patients, restricted in tumor size for the purpose of ensuring comparability.

Methods

Between 1990 and 2004, 61 patients underwent either endoscopic or open adrenalectomy for tumors smaller than 6cm. A short-term-base-cost-effectiveness analysis was performed to evaluate costs per day prevented and a sensitivity analysis was calculated.

Results

Average recovery time, post-operative stay and resumption to oral intake were significantly shorter in the endoscopic group, while operative time was longer. No major complications occurred in either group. In 3 endoscopic procedures conversion was indicated. The base-cost-effectiveness ratio was €15.41 per day post-operative stay prevented by the endoscopic approach. The number needed to treat with endoscopic adrenalectomy to prevent one week of post-operative stay is 2.

Conclusion

Retroperitoneal endoscopic adrenalectomy may be considered a cost-effective procedure compared to conventional open adrenalectomy.

Introduction

Laparoscopic adrenalectomy has become the therapy of choice for adrenal tumors smaller than 6cm. Gagner was the first to describe the lateral transabdominal approach for laparoscopic adrenalectomy.[1] This minimally invasive approach is suitable for resection of the adrenal gland because of the accessibility of the adrenal gland's blood supply and since most tumors are small and benign. Many authors since then have affirmed and emphasized the safety and effectiveness of laparoscopic adrenalectomy compared to conventional open approach.[2-6] In laparoscopic adrenalectomy hospital stay is shorter, fewer post-operative complications occur, less blood transfusions are needed and post-operative recovery is shortened.[7-12] Since 1998 the posterior endoscopic approach has become the standard technique for resection of adrenal masses smaller than 6cm in the University Medical Center in Utrecht, the Netherlands

Once safety and efficacy of laparoscopic adrenalectomy have been established, cost aspects become a matter of interest.[13, 14] Especially since laparoscopic adrenalectomy is often seen as an expensive procedure due to its extensive operating time and costly surgical equipment. Only a few authors have performed a cost-analysis on laparoscopic adrenalectomy versus conventional open adrenalectomy.[3, 15, 16] However, these studies lack sufficiently large patient groups and did not match for tumor size, resulting in an indication bias favoring the laparoscopic group. Furthermore, no sensitivity analyses were performed to determine the (relative) importance of the cost variables. Here we describe a cost-effectiveness analysis of both endoscopic and conventional open adrenalectomy by economic evaluation standards in a larger, tumor size matched cohort including a sensitivity analysis in a single tertiary center.

Methods

Study design

In our study we included 61 patients who underwent adrenalectomy - either conventionally open or endoscopically. From January 1998 until the dec of 2003, 32 patients underwent endoscopic adrenalectomy. We use the retroperitoneal approach based on the technique described by Walz et al.[12]

Data Collection

Data of 61 patients were obtained retrospectively through hospital records (e.g. patient characteristics, operative data and postoperative data). These operations were compared to a historical control group of conventional open adrenalectomies performed between 1990 and 1997. All patients from this group with tumors larger than 6cm were excluded since these patients would not have been eligible for the endoscopic procedure. Thus both cohorts were restricted in tumor size for the purpose of ensuring comparatability. Laparoscopic resection was performed via the posterior retroperitoneal approach in 27 patients and through a lateral retroperitoneal approach in 5 patients. In the conventional group, an anterior abdominal approach was used in 21 and a posterior retroperitoneal approach in 10.

Hospital admission time until discharge, stay on recovery unit, time to oral intake, and occurrence of complications were assessed. The cost-effectiveness evaluation was made from a healthcare payer's perspective. Prospective randomization was considered impossible due to the low incidence of this group of disorders and hardly ethical in an era where endoscopic adrenalectomy is preferred by the majority of patients.

Cost assessment

This study is based on a micro-costing principle. A micro-costing principle is a detailed cost-overview in contrast to a global cost-overview (gross-costing). We have chosen for this principle to evaluate the interventions and cost-units separately. Therefore all resources are regarded separately as units, measured and valued to obtain a detailed overview of all costs. Pre-operative consultation, laboratory testing, sterilization and sterile material for operation equipment and follow-up consultation were excluded, for these categories were considered to be equal in both groups. However, costs for disposable materials in endoscopic procedures were regarded separately.

For the greater part, unit costs were provided by the department of financial administration of the University Medical Center of Utrecht (The Netherlands).Table 1. Costs per patient were calculated by multiplying volume by unit costs, including 41% overhead costs. Surgical procedure costs include salary costs of operation staff (bottom-up principle), costs of pre-operative imaging, surgical supplies, stay at recovery unit, and complication costs. Cost of post-operative stay includes costs of nursing and medical staff. Costs for intensive care were calculated according to the guidelines of the Dutch Ministry of Health, Welfare and Sports issued in 2004.[13] All costs were calculated in Euros (1 EURO is the equivalent of 1.34USD).

Table 1. Unit Costs*

Unit	Unit Cost
Medical personnel	
Surgical staff (/min)	€ 3.15
Surgical nurses (/min)	€ 1.18
Anesthesiologist (/min)	€ 2.01
Anesthesiology nurse (/min)	€ 0.59
Disposables	€ 602.08
Imaging	
Ultrasonography	€ 44
CT scan	€ 163
MRI	€ 182
Recovery nurse (/min)	€ 0.50
Nursing unit	
Nursing staff (/day)	€ 124
Medical Staff (/day)	€ 45
Blood transfusion (/packed cell)	€ 179
Intensive care (/min)	€ 0.69

*Overhead costs are 41% of unit costs (including central departments, cleaning service, material, building, gas, water, electricity, et cetera)

Statistical Analysis

Baseline characteristics were compared between the endoscopic and open adrenalectomy group (Table 2). Continuous variables were expressed as a mean (±standard deviation (SD)) or median (interquartile range (IQR), defines the middle 50%(25-75%)). The outcome between the two groups were compared using the Student's T-test or Mann-Whitney U test when data were abnormally distributed. $P<0.05$ was considered statistically significant. When data could not be retrieved from hospital records, they were imputed. Imputation is a legitimate method for estimating the expected value when some data cannot be retrieved. This is based on the mean or median in the entire population enabling a more reliable analysis. This was performed in the following categories: n=5 time to oral intake, n=8 time to anesthesia, n=10 preoperative imaging cost.

In all analyses, the dataset with imputed values was used, except for the Monte Carlo sensitivity analysis mentioned below.[17]

The average effect per patient was defined as days of post-operative stay. The mean difference is presented with the corresponding 95% confidence interval. The number of patients needed to treat with endoscopic adrenalectomy in order to prevent one week of post-operative stay was calculated by using the reciprocal of the average difference in post-operative stay expressed in weeks.

A short-term base-case cost-effectiveness analysis was performed to evaluate the costs per day of post-operative stay prevented by the endoscopic approach as compared to the conventional open adrenalectomy. A cost-effectiveness ratio was calculated and one-way sensitivity analyses were used to explore the relative impact of the effect parameter as well as the individual cost parameters on that ratio. Cost parameters were varied over a range of 50 to 150% of the actual costs and the effect parameter was varied over its 95% confidence interval.

Furthermore, a 10,000 iteration Monte Carlo simulation varying cost and effect parameters simultaneously over pre-defined ranges was performed. From this analysis, the median costs per day of post-operative stay prevented by endoscopic adrenalectomy can be deducted. Furthermore, the result of each iteration is plotted graphically.

Results

General characteristics

Age, sex, and tumor side were similar between the laparoscopic and open group. The mean tumor size in the endoscopic group was slightly smaller than in the open conventional group (2.7cm vs. 3.2cm; $P<0.2$) As shown in Table 2, all other variables including tumor type and in both groups were similar.

Table 2. Patient and tumor characteristics according to surgical procedure

	Laparoscopic adrenalectomy (n=32)	Open adrenalectomy (n=29)
Age at surgery in years[1]	43.2 (15.0)	45.8 (13.0)
Gender		
male	37.5%	44.8%
female	62.5%	55.2%
Tumor localization		
left	68.8%	37.9%
right	28.1%	51.7%
bilateral	3.1%	10.3%
Tumor size in centimeters[1]	2.7 (1.1)	3.2 (1.3)
Tumor type		
pheochromocytoma	53.1%	51.7%
adrenal cortical adenoma	37.5%	48.3%
other	9.4%	-

[1]Mean (standard deviation)

Volume comparison

The median operative time for endoscopic adrenalectomy was 70 minutes longer than that of a conventional open adrenalectomy (130(I*QR* 103-150) versus 60(IQR 50-70) minutes, p<0.001). As a consequence, the median total time in the operation room for preoperative preparations, operation and immediate postoperative care was also longer in the endoscopic group (213 (IQR 195-250|) versus 115 (IQR 102-139) minutes, p<0.001). On average, right-sided endoscopic adrenalectomy took 20 minutes longer than left-sided adrenalectomy. Operative times in bilateral open resections took 75, 95 (both open resections) and 200 minutes (endoscopic), respectively.

The time spent on the induction of anesthesia did not differ between groups with a mean of 25 minutes in the endoscopic versus 22 in the open conventional group (p>0.5). In contrast, post-operatively, patients who underwent endoscopic adrenalectomy spent 77 minutes less on the surgical recovery unit than patients who underwent conventional open adrenalectomy (225(IQR 150-415) vs. 302 (IQR 182-1348) minutes, p=0.08). The patients in the endoscopic adrenalectomy group resumed oral intake sooner after the procedure (median 1(IQR1-2) days versus 2(IQR1-3), p<0.001).

Complications

Three patients treated endoscopically, required conversion to an open operation. In one patient the adrenal gland could not be visualized because of excessive fat, in another case the adrenal gland was firmly adherent to the renal hilum due to peritumoral inflammation, while the third patient developed peritoneal rupture during inadvertent coughing, which made subsequent visibility inadequate.

No peri-operative mortality was encountered in either group. In the laparoscopic group none of the patients developed major complications or required blood transfusions post-operatively. In the conventional open group seven patients required blood

transfusions (total: 15 units of blood). Complications in the open group included two patients with a pneumothorax, one with a pneumonia and two wound infections. The two patients with a wound infection had Cushing's syndrome as the presenting diagnosis.

Base-case cost-effectiveness analysis

On average, patients who underwent endoscopic adrenalectomy generated € 3,542 direct medical costs, including € 2,604 for the surgical procedure (table 3). Average direct medical costs were € 3,485 in the open adrenalectomy group, including € 1,678 for the surgical procedure. The € 926 higher costs for the surgical procedure in the laparoscopic group was partially compensated for by € 869 lower post-operative stay costs. Endoscopic adrenalectomy was more effective in terms of post-operative hospital stay: post-operative stay was on average 3.7 (95%CI: 2.3-5.0; *P*<0.001) days shorter in the laparoscopic group compared to the open adrenalectomy group (3.9 versus 7.6).

The base-case cost-effectiveness ratio of endoscopic versus open adrenalectomy was € 15.41 per day of post-operative stay prevented. By performing endoscopic adrenalectomy, postoperative stay is reduced and days of post-operative stay are prevented. The base-cost-effectiveness analysis shows that in general it would cost €15.41 per day which could be prevented by performing endoscopic adrenalectomy. The number needed to treat to prevent one week of post-operative hospital stay was 2.

Table 3. Average costs and effect on post-operative stay of laparoscopic vs open adrenalectomy; university medical center utrecht 1990-2002[1]

	Laparoscopic adrenalectomy	Open adrenalectomy	Difference
Cost per patient			
Surgical procedure[2]	2,604	1,678	926
Post-operative stay[3]	938	1,808	-869[4]
Total direct medical costs	3,542	3,485	57
Effect per patient			
Days of post-operative stay	3.9	7.6	-3.7

[1]Costs are expressed in euros and are based on 2002 Cost Prices
[2]Includes salary cost of surgeon, anesthesiologist, operating room and anesthesiology assistants, costs of pre-operative imaging, surgical supplies, stay at recovery unit, complication costs, and overhead costs
[3]Includes nursing staff and medical staff on nursing unit, and overhead costs
[4]Individual figures of post-operative stay do not add up to reported difference due to rounding

Sensitivity analysis

The cost-effectiveness ratio is influenced (sensitive to) by changes in several variables (figure 1, Tornado diagram). The influence of these variables on the cost-effectiveness ratio has been analyzed per variable. The most important parameter is cost per day of post-operative stay. If the cost per day of post-operative hospital stay increases by 6.6% (amounting to € 180 per day), endoscopic would equal open adrenalectomy costs since the post-operative stay is longer in the open group. The

same holds true for a 10.2% drop in average operation staff costs, a 36.1% increase in recovery costs (amounting to € 0.68 per minute) as well as a 139.0% increase in average pre-operative imaging costs.

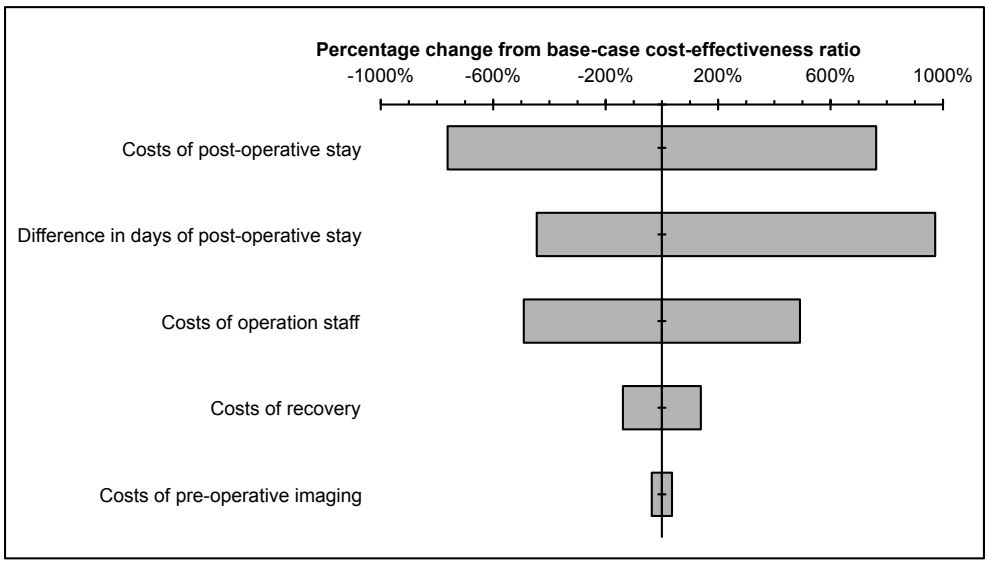

Figure 1. Tornado diagram for cost per day of post-operative stay prevented by laparoscopic versus open adrenalectomy.

Tornado diagrams graphically display the results of sensitivity analyses regarding changes in the cost-effectiveness ratio (CE-ratio) when the value of individual factors are varied keeping the remaining factors constant. Results are presented as percentual change from the base-case cost-effectiveness ratio (€15.41 per day postoperative stay prevented), where a positive change favors open adrenalectomy. Individual cost items are varied over 50 to 150% of base-case costs of the specific item, whereas the difference in postoperative stay is varied according to the boundaries of the 95% confidence interval. The factor, displayed as a bar, at the top of the diagram (cost of post-operative stay) has the largest effect if changed on CE-ratio and the lowest bar (cost on preoperative imaging) the smallest effect. If cost factors are varied, this only affects the numerator of the CE-ratio, leading to a symmetrical bar. If the effect estimate (difference in days of postoperative stay) is varied according to the boundaries of its 95% confidence interval (-5.0 to -2.3), this affects both the numerator and denominator of the CE-ratio (costs / days of postoperative stay prevented), leading to an asymmetrical bar. This asymmetry means that a certain decrease in the difference of postoperative days prevented leads to a larger increase of CE-ratio favoring the open group, than a similar increase in the difference of postoperative days prevented would have on the decrease of cost-effectiveness ratio favoring the endoscopic group.

The cost-effectiveness ratio is calculated between the two limits of the 95% confidence interval (95% CI). At the lower limit of the 95% CI of the difference in days of post-operative stay (5 days prevented by endoscopic adrenalectomy), endoscopic adrenalectomy costs € 165 per day of postoperative stay prevented, whereas at the upper limit of the 95% CI (2.3 days saved by endoscopic adrenalectomy) performing endoscopic adrenalectomy would save € 53 per day of postoperative stay prevented

compared to the open adrenalectomy strategy. If the difference in days of post-operative stay increases from 3.7 to 3.9 days, both intervention strategies would cost the same (cost-equal).

A Monte Carlo simulation varying cost and effect parameters simultaneously with 10.000 iterations showed that in the mid 50% of simulations this cost-effectiveness ratio lay between saving €47 and costing €90 per day extra of post-operative stay prevented. The costs for endoscopic surgery were slightly higher. However, the days for postoperative stay were shorter than in the open conventional group. The analysis showed, that when the postoperative stay after endoscopic adrenalectomy would even be shorter, the number of prevented days would go up, and the cost would go down. Figure 2.

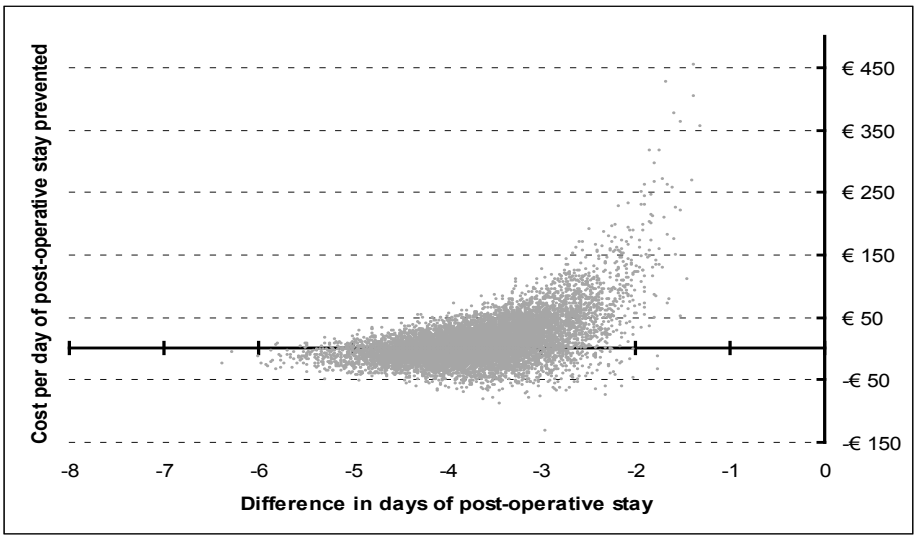

Figure 2. Results of a Monte Carlo simulation.

Depicted are the individual results of 10,000 iterations with regard to differences in days of post-operative stay and cost per day of post-operative stay prevented by laparoscopic versus open adrenalectomy. A positive cost value favors open adrenalectomy. A negative difference in days of post-operative stay favors laparoscopic adrenalectomy.
Exactly at the mean difference in days of postoperative stay prevented (i.e. 3.7 days), the base-case cost-effectiveness ratio is €15.41, lying above the horizontal axis (depicting a cost-effectiveness ratio of 0). Therefore, most simulated cost-effectiveness (CE) ratios at and around this value of postoperative days prevented are above the horizontal axis (at -3.7, 60% of simulated CE ratios are above the horizontal axis). Within the range of -5 to -2.3 days difference in postoperative days (the 95% confidence interval of the effect estimate), on average 57.3% of simulated CE ratios are above the horizontal axis (which is 100% at exactly -2.3 days and 12.5% at exactly -5 days).

Discussion

This cost-effectiveness analysis is the first to address cost comparison in tumor size matched large groups. Furthermore, we have included a sensitivity analysis, which has not been carried out in any of the studies published hitherto. This study shows that retroperitoneal endoscopic adrenalectomy is a cost-effective procedure compared to conventional open adrenalectomy.

Since laparoscopic and conventional open adrenalectomy are not expected to have the same outcome in effectiveness, cost effectiveness analysis is an appropriate method to use for comparative purposes.[13]

Hospital admission, duration of stay on the recovery unit and time to oral intake were significantly shorter in the endoscopic group. Major complications did not occur in the laparoscopic group in contrast to the conventional open group. The cost-effectiveness analysis and sensitivity analysis show that in order to prevent one week of post-operative stay 2 patients need to be treated laparoscopically. A short-term base-case cost-effectiveness analysis was performed to evaluate the costs per day of post-operative stay prevented by the laparoscopic approach as compared to the conventional open adrenalectomy. The estimated costs to prevent one post-operative day prevented by endoscopic approach as compared to conventional open adrenalectomy in this study were €15. When allowed for uncertainty may even gain up to about €50 or cost about €100. Endoscopic operative times were significantly and more than twofold longer than those of the conventional open method. However, with increasing experience we noticed a decline in operative time. When taking this learning curve into account, one can assume that cost in the endoscopic group will decrease with time when the average operative time decreases favoring endoscopic adrenalectomy. The 32 endoscopic procedures were all performed by one surgeon experienced in endoscopic surgery. This situation does reflect reality in surgical practice and is an honest reflection of our experience. Although reported operative times vary, most authors report more or less similar operative times as in our series.[2, 3, 5, 8, 18] The significant difference between laparoscopic and open operative times can be explained in several ways. Firstly, we have included only smaller tumors in the open group in contrast to most other series. This facilitates resection in open conventional adrenalectomy. Secondly, the significant extra time in the operating room in the endoscopic group is used for adequate preoperative positioning and draping. Other important differences between endoscopic adrenalectomy and open conventional adrenalectomy are recovery unit stay and postoperative hospital stay. The longer operative times in the open group for bilateral adrenalectomy did not affect the overall difference between groups significantly (only 3 min).

Although prior studies demonstrated cost-effectiveness for laparoscopic adrenalectomy, these were not matched for tumor size thereby possibly introducing an indication bias.[3, 16, 18] One might expect that when larger tumors are included in the analysis incision sizes could be larger, operative times prolonged, resection more complex due to malignant growth. This could lead to prolonged post-operative recovery time, more intra-operative blood loss and complications in the analysis with a disadvantage for the open group. In addition the present study has included a relatively large number of patients in both groups, thereby increasing the strength of

the results.
Despite these advantages, the retrospective nature of this study is not ideal. It is obvious a historical control group has methodological disadvantages. In contrast to what might be expected, no decrease in admission time was observed throughout the years involving our analysis. It is therefore not certain that reported differences are not confounded or are actual causal, but other changes in surgical care over the years can not be excluded. Unfortunately, the small number of patients undergoing adrenalectomy and the already described efficacy of laparoscopic adrenalectomy does not allow prospective randomization, which would have been necessary to perform an optimal analysis. We have chosen one approach for all patients. A more individualized approach might even further reduce costs associated with endoscpic adrenalectomy, provided experience in the various techniques is sufficient.

This cost-effectiveness analysis is regarded from a hospital perspective. Although societal costs are likely to differ, researching these costs is problematic. Nonetheless, it is safe to assume that societal costs will be less in the laparoscopic than in the conventional open group. This assumption is based on data from other minimally invasive procedures (eg. laparoscopic appendectomy and cholecystectomy) showing earlier resumption of work and all day activities.[19]
In conclusion, retroperitoneal endoscopic adrenalectomy is a cost-effective procedure compared to open conventional adrenalectomy.

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Chapter 10

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Posterior retroperitoneoscopic adrenalectomy, safe and effective

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Abstract

Aim

The aim of this study was to review our experience with the posterior retroperitoneoscopic adrenalectomy (PRA). This is the procedure of choice for adrenal tumours in our institution.

Method

Between 1997 and 2008, 112 PRAs were performed in the UMCU. Data were retrieved from our prospectively collected database including data on patient demographics, the surgical procedure, complications and hospital stay.

Results

One hundred and twelve PRAs were carried out successfully in 105 patients, including 7 bilateral adrenalectomies. Most patients with unilateral adrenal disease had pheochromocytoma (n=39), 16 of these were part of Multiple Endocrine Neoplasia Syndrome type 2 (MEN2); 21 patients had Cushing's syndrome and 20 had Conn's syndrome. Mean Body Mass Index (BMI) of the operated patients was 27 (range 17.5-55). On average the operation took 110 min (45-240) with a mean blood loss of 30 ml. Median tumour size was 3.1 cm (0.4 - 7.5 cm). In two patients the PRA had to be converted to open surgery. Seven patients experienced postoperative complications (major n=2, minor n=5). One patient needed a reoperation. The average hospital stay was 3 days. We observed a learning curve with a significant decrease in operative time throughout the years.

Conclusion In experienced hands PRA is a safe and effective surgical technique for adrenal gland tumours up to 6-7cm with a minimal complication rate.

Introduction

Laparoscopic adrenalectomy was first described in 1992 by Gagner et al.[1, 2] After its introduction, the advantages of laparoscopic adrenalectomy compared to open adrenalectomy soon became apparent: less blood loss, fewer complications, less postoperative pain and a shorter hospital stay.[3-6] Hence, laparoscopic adrenalectomy has become the gold standard for the treatment of adrenal gland tumours smaller than 6cm.[3, 7-9] Relative contra-indications for the laparoscopic technique include larger tumours, signs of local invasion and primary adrenal malignancies.

The most frequently used endoscopic approach for adrenalectomy is the lateral transabdominal route.[8, 10-14] The advantages of this technique are a good exposure and the availability of anatomical landmarks. Disadvantages are the higher chance of injury to intraperitoneal organs, abdominal adhesions and a longer operating time. Alternative to the lateral transabdominal approach, several other approaches have been reported, including the anterior transabdominal, lateral retroperitoneal and posterior retroperitoneal approaches. Because of the limited incidence of adrenal gland neoplasms, the number of comparative studies between the different approaches is restricted. The few reports show minimal differences.[4, 15-17]

As the adrenal glands are located posteriorly in the retroperitoneum, it would appear logical to approach them directly from posterior through the retroperitoneal “space”. This approach was first reported by Walz et al.[18-20] The potential advantages of this method are the direct route to the adrenals and a low chance of injury to abdominal organs. An additional advantage is the possibility of performing a bilateral extirpation without repositioning the patient.[18, 21, 22] Because of the limited working area in this approach, the technique seems more difficult and building experience takes time.[20]

The aim of this study was to evaluate and report our experience with this posterior retroperitoneoscopic adrenalectomy (PRA) in the second largest (monoinstitutional) series worldwide.

Methods

Between October 1997 and December 2008, 105 patients underwent a posterior retroperitoneoscopic adrenalectomy (PRA) at the University Medical Center Utrecht. Indications for PRA were functional tumours, non-functional tumours generally smaller than 6cm without signs of local invasion on preoperative imaging, and solitary metastases to the adrenals. Functional tumours included pheochromocytomas and adrenal adenomas causing Cushing’s disease, Conn’s disease or testosterone overproduction. All non-functional tumours larger than 4cm were considered eligible for surgery.

Data on patient characteristics, surgical procedure and outcome were retrieved from our prospectively assembled database. All procedures were carried out by, or under the supervision of, one surgeon (IBR). Operative time was measured from skin incision to applying wound dressings.

Statistical analysis

Patient characteristics are shown as frequencies and percentages. Data are reported with means or median depending on the distribution of data. Means are reported

with standard deviation (+/-SD) and range, and median with interquartile range (IQR). Furthermore, operative times were studied for a possible learning curve. For statistical analysis, SPSS 16.0, SPSS Inc. Chicago, Illinois was used.

Diagnostics

Preoperative diagnosis was based on a complete medical history, physical examination, laboratory tests and imaging studies. Hypercortisolism was established with a 1 mg dexamethasone suppression test and measuring the 24-hours urine's cortisone. ACTH level in plasma was measured to differentiate between ATCH-dependent and independent Cushing. Primary hyperaldosteronism was characterized by hypertension, hypokaliemia, increased plasma aldosterone and decreased plasma renin levels. Increased catecholamine levels in plasma and catecholamine metabolites (metanephrines) in 24-hours urine confirmed the diagnosis of a pheochromocytoma. A complete hormone analysis was performed in case of an incidentaloma. To localize adrenal tumours and measure the size, a CT-scan and/or MRI were performed. None of the patients that had Conn's disease underwent selective venous sampling.

Preoperative preparation

All patients with a pheochromocytoma were treated with preoperative α-& ß-blockage. All these patients were operated under stringent monitoring (central venous line, arterial line, etc.) In case of Conn's disease, electrolyte disturbances were corrected and blood pressure was regulated with spironolactone combined with extra medication if necessary. Patients with Cushing's syndrome were treated with a perioperative hydrocortisone stress schema. No prophylactic antibiotics were administered.

Operative technique

A slightly modified technique from the one previously described by Walz was used.[19] PRA is performed under general anesthesia. The patient is positioned in a prone position with the hips maximally flexed in order to create sufficient distance between the 12th rib and the iliac crest. The 12th rib along with the back muscles and iliac crest are marked with surgical ink followed by the trocar incisions. For bilateral procedures, the sites are symmetrical (Figure 1). Below the tip of the 12th rib a 2cm transverse incision is made.

Next, subcutaneous tissue and muscles are separated. This is followed by digitally creating a space posterior from the kidney guided by its lower pole. The retroperitoneal space is increased by a dilatation balloon. Two additional trocars are placed centimeters cranially of the iliac crest with a maximal lateral space between them. Finally a blunt tip trocar is placed in the first incision and the retroperitoneal space is insufflated with CO_2. The retroperitoneal space is insufflated up to a pressure of 15 mmHg. In (morbidly) obese patients the maximum pressure that is used is 20 mmHg.

A 0°scope is used routinely. Guided by the diaphragm, the peritoneal sac is moved

aside. Next, the adrenal gland can easily be identified by the dark yellow color and its consistency (Figure 2).

Occasionally, a fourth trocar can be placed to obtain more exposure but this is rarely necessary. The adrenal gland is dissected from its surroundings, both bluntly and with the LigaSure TM from the upper/lateral border to the plane with the kidney and finally the hilus. After the adrenal vein is identified, it is clipped and divided. The entire adrenal gland is removed using Endocatch® retrieval bag. The surgical site is checked for hemostasis and the trocars are removed under view. The muscle fascia is closed and the skin is closed intracutaneously. No drains are used.

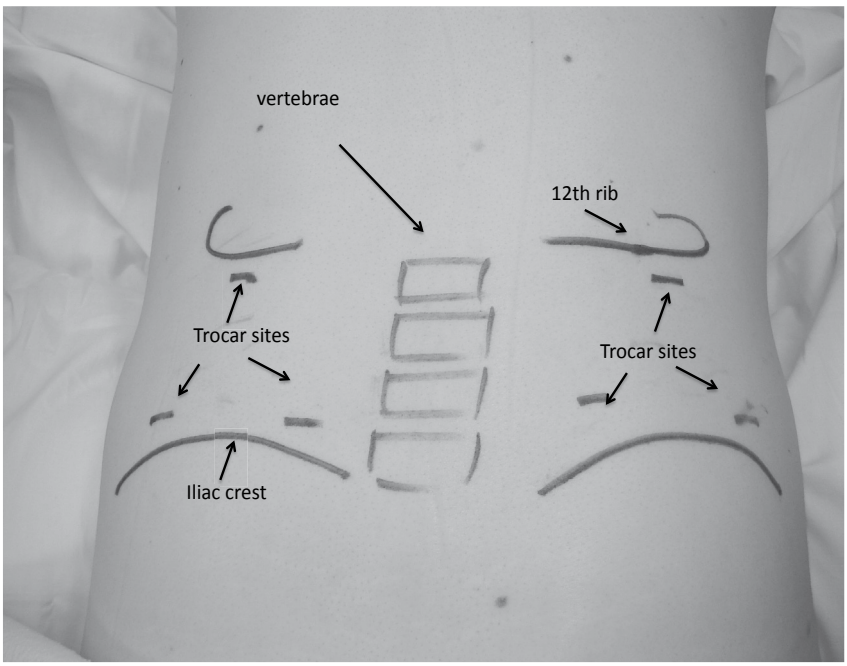

Figure 1. Trocar sites and anatomical landmarks for bilateral posterior retroperitoneoscopic adrenalectomy

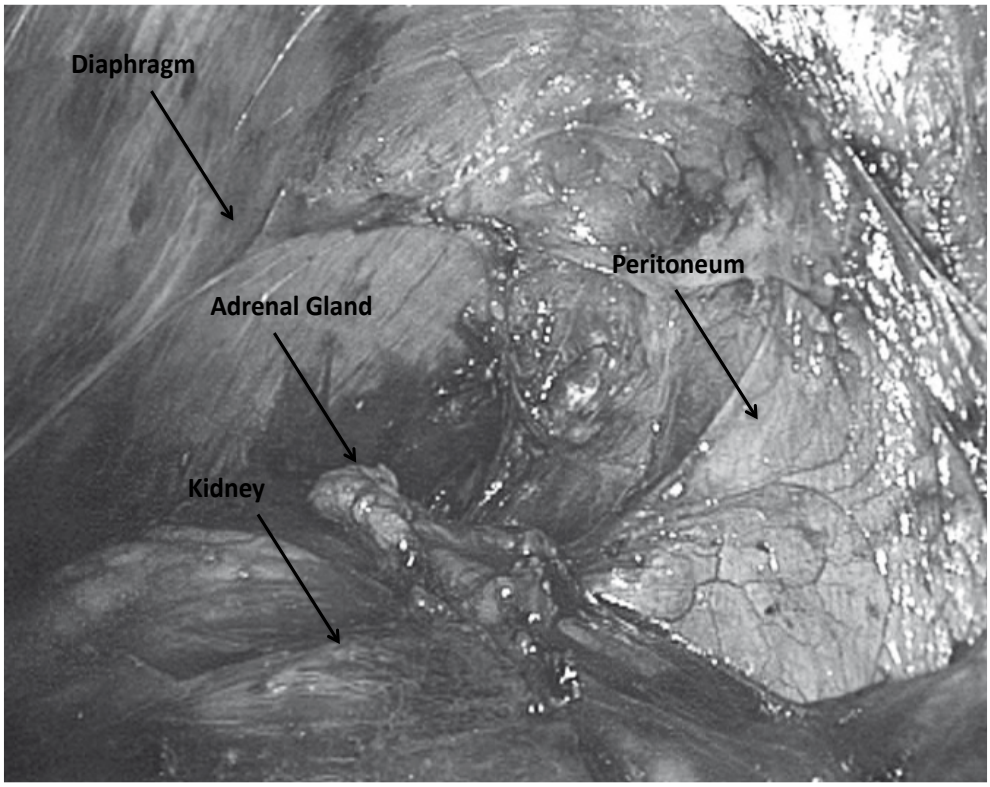

Figure 2. Operative Field PRA on the left side; Scope-view

Results

Between 1997 and December 2008, 112 posterior retroperitoneoscopic adrenalectomies were performed in 105 patients. Seven patients underwent bilateral adrenalectomy. In 5 patients, a subtotal adrenalectomy was performed to preserve adrenal gland function. These patients were MEN2 patients with either unilateral or bilateral pheochromocytoma. Patient characteristics are shown in Table 1. The mean age was 46 years (range 9-74). The mean Body Mass Index (BMI) was 27kg/m^2 (17.5-55). One patient with a BMI of 55 was successfully operated without complications. Four patients underwent posterior retroperitoneoscopic adrenalectomy for solid tumours larger than 6cm. In none of these patients it was clear on preoperative imaging that the tumour was larger than 6cm. Two of them had an ACTH producing neuroendocrine tumour causing bilateral adrenal hyperplasia, which led to uncontrollable hypercortisolism. During the same study period, 44 conventional open adrenalectomies were performed. Indications for these open procedures were tumours larger than 6 cm or a suspected primary adrenal malignancy (adrenocortical carcinoma). One patient underwent a robotic assisted adrenalectomy. Details on these procedures have not been studies further.

Table 1. Patient Characteristics in Posterior Retroperitoneoscopic Adrenalectomy.

Characteristics	n=
Patients (n=)	105
Left: Right (n,%)	65 (62%): 40 (38%)
Unilateral	98
Bilateral	7
Subtotal adrenalectomy	5
Tumour Size (cm) mean (+/SD)	3.1 (+/- 1.6) (IQR 2-4cm)
BMI^ kg/m^2 (range)	27 (IQR 23-29kg/m^2)
Indications unilateral	
Pheochromocytoma	39
MEN# 2A	15
MEN 2B	1
Von Hippel-Lindau	1
SDHD*	2
Cushing (ACTH independent)	21
Conn	20
Testosteron producing tumour	1
Incidentaloma	12
Suspected Metastasis†	2
Other/non functioning tumour	3
Indication bilateral	
Cushing (ACTH dependent)	5
Pheochromocytoma (MEN 2A)	2

*SDHD mutation in SDHD gene succinate dehydrogenase complex subunit D
#MEN Multiple Endocrine Neoplasia syndrome
^BMI Body Mass Index
† Of the two suspected metastases, only one proved to contain malignancy upon final histology of a clear cell renal carcinoma

The mean operative time of the unilateral cases was 110 minutes (SD ± 36, range 45-240). After 30 procedures the mean operative time had decreased significantly from 130 minutes (SD+/- 30) to 100 minutes (SD +/- 35) ($P<0.05$). After 40 procedures it decreased to 95 minutes and the final cases took 90 minutes on average (Figure 3). A BMI of 30 kg/m^2 and higher did not appear to affect operative time significantly ($p=0.706$). Operative data are summarized in Table 2.
Two conversions (2%) to open conventional surgery were necessary due to an insufficient view of the operative field. No perioperative mortality occurred. During the operation, 6 patients with pheochromocytomas developed systolic blood pressures of $\geq$ 200 mmHg despite α-& ß- blockage. Postoperatively, one patient required re-exploration for persistent bleeding from the muscular part of a trocar insertion site. Another patient developed congestive heart failure, which was successfully treated conservatively. Minor complications occurred in 5 patients, including flank hematoma, and superficial wound infection. Median hospital stay was 3 days (IQR 2-5).

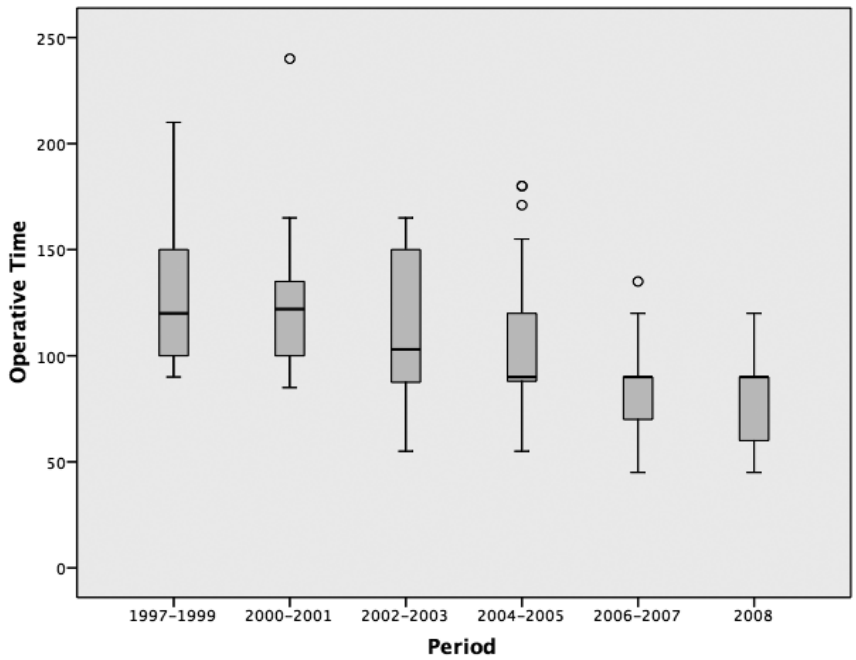

Figure 3. Learning Curve; mean operative time per time period in years. Bilateral procedures and conversions were excluded from the analysis

Table 2. Operation Details

Operation Details		Interquartile Range (IQR)
Operative time unilateral (min)	110	90-130
Left adrenalectomy	107	90-132
Right adrenalectomy	114	90-130
BMI<25	109	90-121
BMI 25-30 (Overweight)	118	90-150
BMI>30 (Obesity)	110	90-133
Operative Time Bilateral	175	150-300
Blood loss (cc)	30	0-50
Conversion (n=)	2	
Postoperative hospital admission (days)	3	2-5
Complications postoperatively (n=)		
Severe	2	
Mild	5	
Late complications (n=)		
Persistent Hypertension	7	
Chronic pain symptoms	3	
Mortality	0	

Final histology of the patients who had an incidentaloma showed seven adenomas,

one case of hyperplasia, one pheochromocytoma, one myelolipoma, one haematoma and one normal adrenal gland. None of the tumours except for the one renal cell carcinoma metastasis were concluded to be malignant at final histology.

During follow-up 3 patients complained of wound pain, which eventually subsided. Two other patients developed bulging of the flank wall ("hernation"). Seven patients had persistent hypertension after PRA, six of whom had Conn's disease preoperatively. All 6 patients who had Conn's disease preoperatively and persistent hypertension after the procedure did have a decrease of their blood pressure. Although the exact values have not been documented, the hypertension in all patients was noted as improved. None of the patients had persistent hypokalemia.
Except for the patient with metastatic adrenal lesion, none of the patients have developed any signs of malignant disease during follow-up including the patient who had a testosteron-producing tumour. After one year of follow-up there were no signs of metastatic disease in this patient. The patient with the renal cell carcinoma metastasis had a follow-up period of one year in which he did not develop new metastatic disease. One of the two patients, who had a neuroendocrine ACTH producing tumour, died of metastatic disease from the neuroendocrine tumour three years after the bilateral adrenalectomy (adrenal hyperplasia > 6cm).

Discussion

In this second largest mono-institutional series in literature, 112 posterior retroperitoneoscopic adrenalectomies (PRA) are reviewed. PRA appears to present a safe and effective approach for endoscopic adrenalectomy in the majority of adrenal tumours. Similar to the Walz group experience, no "collateral" damage to other/adjacent organs was observed.[19] PRA was associated with minimal blood loss, very low conversion & morbidity rates and no mortality. A steady learning curve was observed with a significant decrease in operative time from 130 minutes in the first cases to 90 minutes in the last cases.

Most centers use the transabdominal approach for adrenalectomies.[4, 8, 10-14, 16, 23-27] It provides good exposure and anatomical landmarks. On the other hand, apart from the advantage of a smaller risk of collateral organ damage, PRA is not impeded by adhesions caused by previous abdominal surgery. Additionally, in case of bilateral adrenal tumours the patient is already in Jack knife position and does not have to be repositioned, thus reducing total operating time.[22]

The number of complications in our series was small (2% major, 6% total)[3]. In comparison, overall complication rates in transabdominal series have been reported to vary between 3 and 20%.[3] Bleeding is the most common complication following transabdominal adrenalectomy.- Organ injury (liver, spleen, kidney, large bowel and diaphragm) is found in around 4%.[3] Port site hernia's are more common following the transabdominal approach (10%) than after PRA (rarely).[17]
Conversion to open surgery was required in 2/112 procedures, which is similar to

conversion rates in literature (~ 2-3%).[7, 11, 13, 22] Persistent hypertension after the operation was observed in 7 patients, 6 of whom had Conn's disease. However, in all six patients the hypertension did improve after surgery. In literature, persistent hypertension in Conn's disease is recognized with rates varying from 0 to 25% after adrenalectomy. This phenomenon is most likely caused by preexistent essential hypertension.[8, 11]

Although we performed PRA successfully in morbidly obese patients, sometimes difficulties were encountered in obtaining a good exposure and insufflating the retroperitoneal space. Others also found that to obtain a good exposure, high intra-cavital pressures of up to 20-24 mmHg can be necessary.[21, 22] Although this may lead to an increased central venous pressure, arterial pressure and cardiac output, we and others have not encountered such problems.[21, 22, 28] We did not observe major bleeding complications. However, should bleeding occur from the inferior vena cava, the limited space may make it difficult to control it. Therefore, careful dissection with a smooth instrument (e.g. suction device) is recommended in the vicinity of the adrenal vein.

Endoscopic adrenalectomy has been particularly advocated for tumours up to 6cm. For larger tumours, carrying a higher risk of malignancy, open resection is more often favored. Nonetheless, larger tumours have been resected endoscopically, predominantly using the transabdominal approach.[3, 23, 26, 29] In this series, sporadically tumours of up to 7.5 cm have been removed without problems. It is obvious that locally invasive growth has to be excluded on preoperative imaging in such cases.[21, 22]

Although it is standard to remove the entire adrenal gland, a subtotal adrenalectomy can be carried out.[7, 11, 22] Subtotal adrenalectomy can be performed in order to preserve adrenal function in patients with bilateral adrenal tumours or increased risk of developing a contralateral pheochromocytoma (familial syndromes like MEN2, von Hippel-Lindau disease, etc.). In our series, five subtotal adrenalectomies were performed successfully. Tumours smaller than 2cm and located peripherally are most suitable for this technique. Technically, it is not difficult. To preserve adrenal function, approximately one third of the adrenal gland must be left in situ.[7, 30]

The mean operative time in our series was shorter than that reported for the transabdominal approach.[3, 10, 31] The learning curve we observed in our series will probably continue. Walz. et al. showed a learning curve with operative time decreasing to 40 minutes per case after 560 procedures and claim that it is still ongoing.[22] In light of these findings in combination with the relative rarity of the disorder, centralization of this type of surgery is a must. In addition, a team consisting of the same surgeons, anesthesiologists and operating nurses is advocated.

The main limitation of this study is the retrospective and uncontrolled setting making a sound comparison between different techniques difficult. However, due to the relatively low prevalence of the disease, it is unlikely that sufficiently large

randomized trials will be accomplished.

In conclusion, the posterior retroperitoneoscopic adrenalectomy is a safe and effective procedure in the treatment of adrenal gland tumours of up to 6-7 cm with a minimal complication rate. Because of the evident learning curve, and the rarity of the causitive disorders this procedure requires experience and centralization.

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Chapter 11

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Clinical spectrum of pheochromocytoma

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Abstract

Background

Pheochromocytomas vary in presentation, tumor size, and in catecholamine production. Whether pheochromocytoma size correlates with hormone levels, clinical presentation, and perioperative complications is not known. The goal of this study was to determine if tumor size and hormone level correlate according to the clinical presentation at diagnosis.

Study Design

We retrospectively analyzed all patients who underwent an adrenalectomy with a diagnosis of a pheochromocytoma from February 1996 to October 2008. We grouped patients according to their clinical presentation at diagnosis (routine biochemical screening, incidentaloma, classic symptoms, pheochromocytoma crisis) and obtained preoperative radiographic tumor size and catecholamine hormone levels. ANOVA was used for the group effects and the Kruskal–Wallis rank test was used for pairwise comparison between groups with the Sidak/Bonferroni method for multiplicity adjustment according to age, tumor size, and hormone level. The Pearson correlation coefficient was then calculated to determine if hormone level correlated with tumor size.

Results

Eighty-one of 107 patients had data available for complete analysis. The average age at diagnosis for all patients was 47.1 years, and the average tumor size was 4.9 cm. The average highest hormone ratio among all patients was 27.4. Tumor size and hormone ratio levels differed among all groups ($p \leq 0.03$). A direct correlation ($p = 0.014$) was apparent between tumor size and hormone level. Complication rates also differed among the four groups of patients ($p \leq 0.02$).

Conclusions

Our study showed that tumor size directly correlates with hormone level. Smaller tumors tend to secrete lower levels of catecholamines, but larger tumors have a wider variation in secretory potential. Larger tumors, however, produced the highest hormone ratios.

Introduction

Pheochromocytomas are catecholamine-secreting tumors that may result in life-threatening complications if left untreated. The overall estimated incidence is 2 to 8 per million. The incidence of sporadic pheochromocytomas peaks in the fourth and fifth decades of life.[1, 2] Because they are derived from chromaffin cells derived from the neural crest, pheochromocytomas may arise at any region of neural crest origin.[3, 4] Pheochromocytomas can present as symptomatic tumors or incidentalomas, or be diagnosed during screening for familial syndromes (multiple endocrine neoplasia type 2, Von Hippel Lindau syndrome, neurofibromatosis type 1, paraganglioma syndrome, and mutation in succinate dehydrogenase B, C and D).[1, 4] Up to about 25% of pheochromocytomas occur as part of a familial syndrome.[2, 4-6] Forty percent of pheochromocytomas have a classic presentation that consists of episodic hypertension, headaches, diaphoresis, and flushing. Hypertensive crisis may develop in some patients resulting in cardiovascular shock with stroke, myocardial infarction, or multiorgan failure.[1] Another 40% of pheochromocytomas present with no clinical symptoms and are found incidentally during radiologic imaging studies for another indication.[1, 7]

Individual pheochromocytomas vary widely in size and in catecholamine production and urinary metabolite excretion.[8] A recent study reported that sporadic pheochromocytomas tend to be larger and produce a higher level of catecholamines compared with familial tumors.[8, 9] Yet, whether pheochromocytoma size correlates with hormone levels, clinical presentation, and perioperative complications is not known. The goal of this study was to determine if a correlation exists between tumor size and hormone level according to the clinical presentation at diagnosis.

Methods

We retrospectively analyzed the records of all patients who underwent an adrenalectomy with a diagnosis of a pheochromocytoma at the University of California, San Francisco from February 1996 to October 2008. We recorded patient demographics, preoperative catecholamine values, radiographic tumor size, and outcomes data. The Institutional Review Board at the University of California, San Francisco, approved the study.

A pheochromocytoma was defined as any adrenal tumor with urine or plasma catecholamines (epinephrine or norepinephrine) or metanephrines (metanephrine or normetanphrine) greater than 1 times the upper limits of normal and pathologic findings consistent with a pheochromocytoma. The hormone level was normalized by generating a ratio of the highest level for that hormone divided by the corresponding upper limit of normal. The level that produced the largest hormone ratio was used for analysis. Adrenal tumor size was obtained by reviewing all preoperative radiologic reports of CT or MRI scans. Measurement of the adrenal tumor consisted of length, width, and height. The largest measurement from all preoperative studies was used for analysis.

Because patients with pheochromocytomas have varying presentations, we categorized them into four groups according to their clinical presentation at the time of diagnosis. The “screening” group consisted of patients who underwent hormone

screening routinely because of a known family history of pheochromocytoma, known familial syndrome associated with an increased risk of pheochromocytoma developing, or an earlier personal history of a pheochromocytoma or paraganglioma. The "incidentaloma" group consisted of patients diagnosed with an adrenal incidentaloma during imaging for an unrelated problem with no previous symptoms related to pheochromocytoma.
The "classic" group consisted of patients presenting with "classic" symptoms of pheochromocytoma, defined as hypertension, headache, hyperhidrosis, shortness of breath, chest pain, vision changes, dizziness, or syncope. Any patient requiring hospitalization or displaying symptoms after a procedure without any end organ failure was categorized into this group.
The "crisis" group consisted of patients presenting de novo with "pheochromocytoma crisis," defined as end organ damage (myocardial infarction, cardiogenic shock, cerebral vascular accident, renal failure, pulmonary edema, or acute respiratory distress syndrome). All patients in this category required hospitalization, as expected.

Statistical analysis
Mean ± standard deviation (SD) for each specified variable was generated for all patients combined and then separately for each group. One-way analysis of variance was used to test the overall group effects on means according to age, tumor size, and hormone level. The Kruskal Wallis rank test was used for pairwise comparison between groups and the Sidak/Bonferroni correction was adopted for multiplicity adjustment for significance. A p value <0.05 was considered statistically significant. The Pearson correlation coefficient was then calculated to determine if hormone level correlated with tumor size and to determine if recurrent or metastatic disease had any confounding effect on the results.
We also determined whether preoperative or postoperative complication rates differed within each group, again adjusting for the confounding effects of clinical presentation (metastatic disease and recurrent disease). A preoperative complication was defined as any hospital admission related to the pheochromocytoma, a myocardial infarction, or a cerebral vascular accident. A postoperative complication was defined as a myocardial infarction (MI), a cerebral vascular accident (CVA), or ICU admission. These variables were defined as complications because of the direct causality from catecholamine excess, so other complications such as pneumonia, urinary tract infection, and bleeding were excluded. The Fisher's exact test was used to evaluate for these differences. Statistical significance was established at $p < 0.05$.

Results
During the study period, 107 patients underwent an open or laparoscopic adrenalectomy, 81 (76%) of whom had data available for complete analysis (Table 1). One-third of patients were categorized into the incidentaloma group, 14% into the screening group, 46% into the classic group, and 7% into the crisis group. Women accounted for 64% of all patients and more than 59% of patients within each group. The average age at diagnosis for all patients was 47.1 ± 16.8 years, and the average tumor size was 4.9 ± 2.6 cm. The average highest hormone ratio

among all patients was 27.4 ± 90.3.

Table 1. Demographic and Clinical Characteristics of 81 Patients with Pheochromocytomas

Characteristic	All patients	Incidentaloma	Screening	Classic	Crisis
n	81	27	11	37	6
Women, n (%)	52 (64.2)	16 (59.3)	8 (72.7)	22 (59.5)	6 (100)
Men, n (%)	29 (35.8)	11 (40.7)	3 (27.3)	15 (40.5)	0
Age, y, mean ± SD	47.1 ± 16.8	56.9 ±13.1*	37.8 ± 14.9†	41.9 ± 16.5	47.5 ± 10.7
Tumor size, cm, mean ± SD	4.9 ± 2.6	4.8 ± 2.2	2.6 ± 1.7†	5.3 ± 2.8	6.9 ± 1.7*
Highest hormone ratio, mean ± SD	27.4 ± 90.3	9.7 ± 10.5	6.8 ± 13.4†	30.5 ± 83.1	127.4 ± 255*

Adjusted p values for each variable: age, $p < 0.007$; size, $p < 0.004$; highest hormone ratio, $p \leq 0.03$ (except when comparing the crisis and classic groups, $p = 0.37$).
* Statistically significant highest value.
† Statistically significant lowest value.

There were some notable differences between the four groups (Table 1). The incidentaloma group was significantly older and the screening group was significantly younger (adjusted [adj] $p < 0.007$). Tumor size differed among all groups ($p < 0.004$). Specifically, the crisis group had significantly larger tumors, and the screening group had significantly smaller tumors (adj $p < 0.009$). There was also a distinguishable difference in the highest hormone ratio between the groups ($p \leq 0.03$), except when comparing the crisis and classic groups (adj $p = 0.37$). The crisis group had the highest hormone ratio and the screening group had the lowest (adj $p = 0.0325$). A direct correlation ($p = 0.014$) was apparent when comparing tumor size and hormone level of all patients (Figure 1). This correlation remained consistent when each group was analyzed separately (Figure 2).

Similar analysis performed to account for confounding variables revealed similar results. The only difference was a stronger correlation between tumor size and hormone ratio ($r = 0.3$, $p = 0.01$ with confounding variables and $r = 0.5$, $p = 0.0001$ without).

Our data also showed that bilateral pheochromocytomas, recurrent disease, and metastatic disease produce a variety of hormone levels. We found that three of four patients who presented with bilateral pheochromocytomas had a hormone ratio <10, but one had a hormone ratio of 511. Likewise, seven of eight patients with recurrent disease presented with a hormone ratio <10, but one had a ratio of 46.5. In contrast, of the three patients who presented initially with metastatic disease, two had a hormone ratio >20. Complication rates differed among the four groups of patients (Table 2).

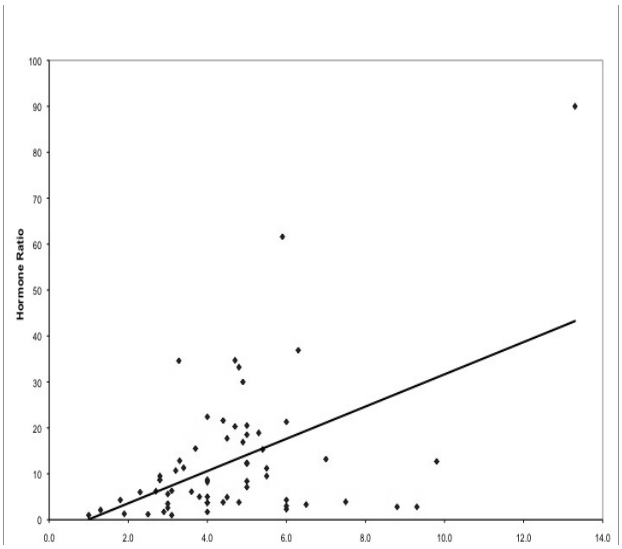

Figure 1. Scatter plot illustrating linear correlation between tumor size and the highest hormone ratio of all patients (p = 0.014).

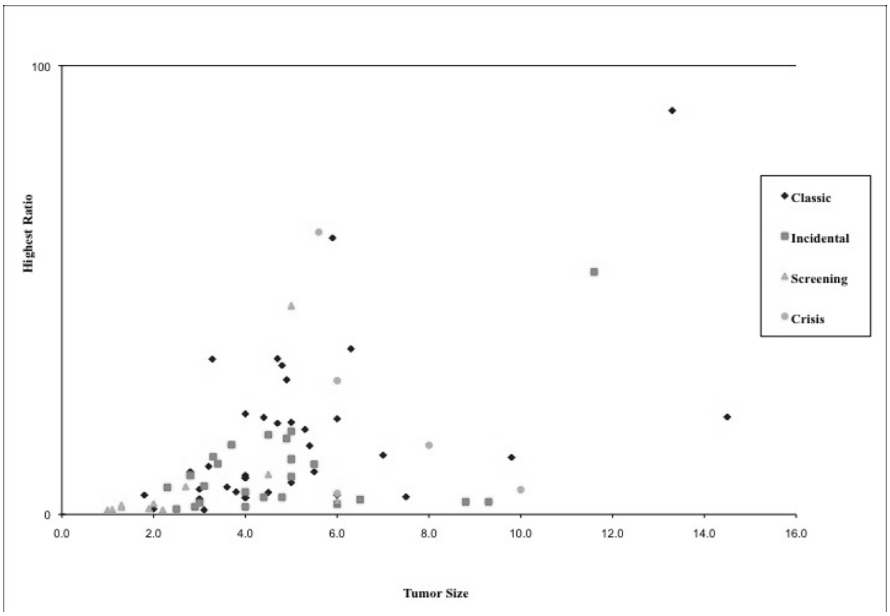

Figure 2. Scatter plot illustrating linear correlation between tumor size and the highest hormone ratio within each group.

Table 2. Comparison of Complication Rates Between Groups of Patients with Pheochromocytoma

Variable	All patients (n = 81) n (%)	Incidentaloma (n = 27) n (%)	Screening (n = 11) n (%)	Classic (n = 37) n (%)	Crisis (n = 6) n (%)
Preoperative admission	24 (29.6%)	2 (7.4%)	0 (0%)	15 (40.5%)	6 (100%)*
Preoperative Myocardial Infarction	3 (3.7%)	1 (3.7%)	0 (0%)	0 (0%)	2 (33.3%)
Preoperative CVA	3 (3.7%)	1 (3.7%)	0 (0%)	2 (5.4%)	0 (0%)
Preoperative ICU admission	11 (13.6%)	2 (7.4%)	0 (0%)	5 (13.5%)	3 (50%)*

*Denotes statistical significance of $p < 0.05$.

Patients from the crisis group required preoperative hospital admission for pheochromocytoma-related symptoms more often, and those from the screening group never required preadmission (unadjusted [unadj] $p \leq 0.02$). Preoperative MIs were more common in the crisis group, but there was a significant difference only when compared with the classic group (unadj $p = 0.02$). Although three patients suffered a preoperative CVA, this finding was not statistically significant. No patient suffered either a postoperative MI or CVA. Finally, patients in the crisis group required more ICU admissions and those in the screening group required none.

Discussion

In this study we evaluated whether the size of a pheochromocytoma correlated with the quantity of hormone secreted. Although intuitively it can be presumed that this correlation exists, no study, to our knowledge, has evaluated the specific relationship between tumor size, hormone level, and clinical presentation of the patient. We demonstrated a direct correlation between tumor size and hormone level that was present independent of the clinical presentation (Figure 1 and Figure 2). The linear correlation between tumor size and hormone level was stronger when confounding factors were excluded from analysis. Overall, hormone levels varied widely across all tumor sizes (Figure 3).

Although larger tumors tended to produce low or high amounts of catecholamines, smaller tumors had fewer tendencies to secrete high levels of catecholamines. This is evidenced by the fact that contrary to tumors >3 cm, no tumors ≤3 cm produced a hormone ratio ≥10 (unadj $p < 0.001$). Statistical significance remained when comparing tumors ≤4 cm and >4 cm at a cutoff hormone ratio of 20 (unadj $p = 0.0005$). This difference in hormone secretion may result from differences in tumor characteristics, independent of size, including cystic degeneration or necrosis of the tumor, in addition to dissimilarity in hormone metabolism.

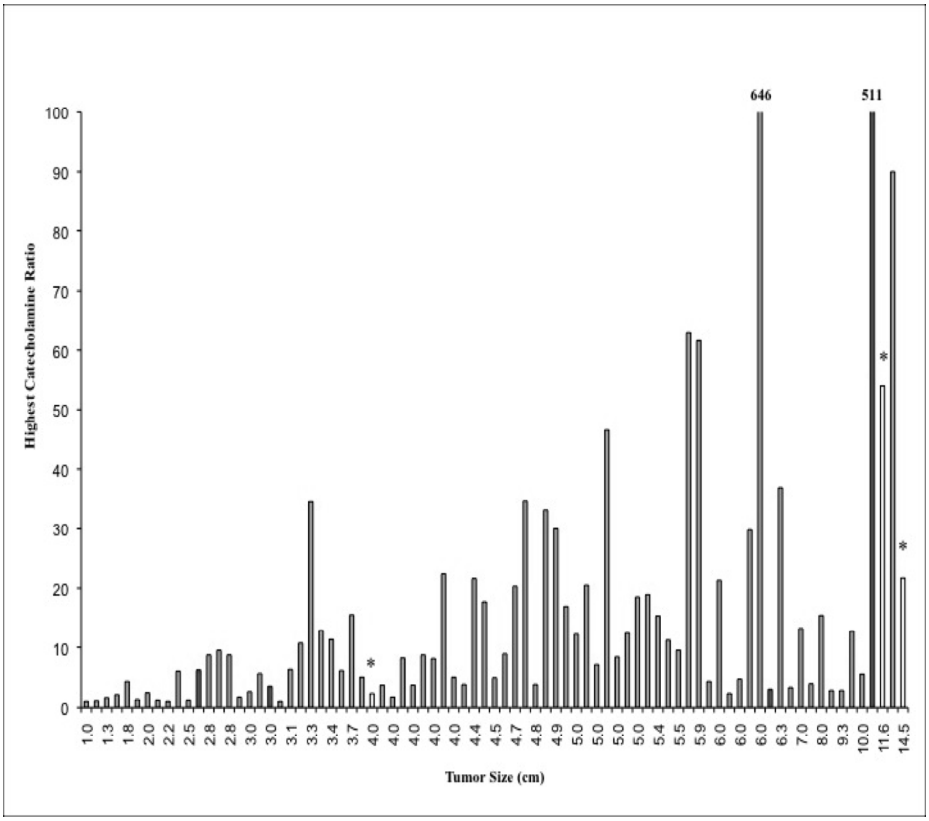

Figure 3. Graph depicting the distribution of tumor size and hormone ratio of all patients with pheochromocytoma. *Denotes patients who presented with metastatic disease. Red bars denote patients with bilateral pheochromocytomas.

Previous studies showed that tumor size weakly correlates with urinary dopamine excretion ($r = 0.28$, $p = 0.04$), but not with the level of plasma catecholamines, and the levels varied widely according to the subtype of catecholamine secreted.[10] Hormone metabolism within the tumor itself has been suggested to account for the variation in hormone production.[8] More than 90% of the elevated fractionated plasma metanephrines in pheochromocytoma result from hormone metabolism within the tumor cell and not peripheral metabolism.[11] There is also evidence that 30% of pheochromocytomas may present in a subclinical manner in which urine or serum metanephrine is elevated only one to two times above the upper limits of normal.[7] To account for these variations, we created a hormone ratio of the highest hormone (catecholamine or metanephrine) level secreted in urine or blood to allow for a more accurate analysis of the size-hormone level relation. Standardizing our data with a ratio revealed that size and hormone level directly correlate.

Interestingly, although intuitively it may seem that patients with bilateral pheochromocytomas and recurrent disease should have higher hormone ratios, our

data do not show this. The low hormone level (<10) in most patients with recurrent pheochromocytoma in our study can be accounted for by the routine screening done postoperatively, which helps diagnose recurrence before hypersecretion becomes markedly elevated. The presence of metastatic disease resulted in two of three patients producing a hormone ratio >20. Though this finding might be expected in metastatic disease, the sample size was too small to assess the correlation statistically.

We also showed that complications associated with pheochromocytoma are related to the clinical presentation. Although it may be obvious that the type of clinical presentation influences perioperative complications, no studies, to our knowledge, have examined this in a systemic fashion. We systematically analyzed complications according to clinical presentation. Patients who present in crisis, as would be expected, are at increased risk of preoperative admission, preoperative MI, and postoperative ICU admissions. Patients who present with classic symptoms of pheochromocytoma have an increased risk of requiring a preoperative admission. This difference may be accounted for by the fact that the classic patients have larger tumors and secrete a higher level of catecholamines than patients who have incidentalomas or are diagnosed by routine screening.

Our study has several limitations. First, this is a retrospective study conducted in a single institution, which, by design, has inherent limitations. Second, preoperative imaging was conducted with both CT and MRI, which can result in size measurement variations. Third, multiple radiologists interpreted the preoperative imaging scans, producing operator variation in final size determination. Fourth, we did not have a standard laboratory examination for all patients. Some patients had only plasma values, and others had only urine values and fewer had both plasma and urine values available. It is known that hormone levels differ between plasma and urine samples. We used the hormone ratio to account for and normalize this difference, but accurate correlation between tumor size and hormone level may still be affected.

In summary, our study shows that pheochromocytoma tumor size directly correlates with hormone level. This correlation is strongest with tumor sizes up to 4 cm. Our data show that smaller tumors tend to secrete lower levels of catecholamines, but larger tumors have a wider variety in secretory potential. Larger tumors, however, produce the highest hormone ratios.

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Chapter 12

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General discussion

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In this thesis, we have addressed the surgical management of several sporadic en familial endocrine tumors in the thyroid gland, parathyroid glands, pancreas and adrenal glands.

Thyroid Cancer

In *Chapter 2,* we give an overview of current clinical staging systems and biomolecular markers in thyroid cancer. In addition to fundamental research on biomarkers, the therapeutic implications will become more pronounced. Factors that predict a more aggressive PTC may warrant more aggressive surgery than total thyroidectomy alone.[1] Hence, it can be anticipated that a central lymph node dissection may be performed in patients with PTC who have a BRAF V600 mutation.[1-3] These patients have a more aggressive disease and a higher chance of recurrent disease. By performing a central lymph node dissection, recurrence in the central neck region may be prevented. In *Chapter 3*, we conclude that several specific MMPs and TIMPs are expressed in PTC. We found some differences in expression of MMPs and TIMPs between the various stages of PTC. Immunohistochemistry studies of MMPs and TIMPs should further elucidate more distinct expression patterns in different types of papillary thyroid cancer. If certain MMPs and TIMPs prove to be a predictor of more aggressive disease, like BRAF V600, a more aggressive surgical treatment (eg. a total thyroidectomy combined with a central neck dissection) could be advocated in such cases.

Another prognostic factor is the presence of metastatic lesions that are fluorodeoxyglucose-positron emission tomography (FDG-PET) positive. Whether the more aggressive nature of these FDG-PET-positive PTCs in the neck warrants more aggressive surgical therapy is unknown. It has been documented that patients with FDG-PET positive lymph node metastases have a worse survival.[4, 5] In *Chapter 4,* we describe that only patients who had a FDG-PET positive lesion died as a consequence of their disease. Nonetheless, some of these patients can still be cured by re-operation and resection of metastatic tumors in the neck or mediastinum. Therefore, we believe that localized neck lesions that are FDG-PET scan positive should be resected, regardless of size.

From literature it is known that age is an important prognostic factor in patients with MEN2 and MTC and that surgical treatment is based on age and codon mutation. In *Chapter 5*, prognostic factors in patients with MEN2 who underwent total thyroidectomy were studied. All patients who developed recurrent or persistent disease had been operated at an older age than recommended. Besides a higher age at surgery, we also showed that the presence of lymph node metastases, an elevated preoperative calcitonin level and the codon mutation/risk level are associated with a worse prognosis. Interestingly, only patients with a codon 634 and 918 mutation developed persistent and recurrent MTC. Given these findings, it is of the utmost importance to perform a total thyroidectomy in these patients at the age recommended by the guidelines[6] according to the codon mutation.

Some issues remain to be addressed in the future. Although, no differences in genotype-phenotype differences have been shown between various codon 634

mutations in the past, the presentation between patients who have a codon 634 mutation can differ.[7] There are patients who develop MTC at an older age than the age recommended for total thyroidectomy in the guidelines, while, others develop MTC at an earlier age. These latter patients may require surgery at an even younger age than recommended by the guidelines.[8] Other recent studies also advocate that patients with a mutation in codon 634 should be set apart from mutation risk level 2 and be treated earlier.[9] Larger studies with a long-term follow-up may reveal specific genotype-phenotype relationships between the different codon 634 mutations leading to a different surgical management.

Primary Hyperparathyroidism in MEN1 and MEN2A

One of the main controversies in endocrine surgery is the surgical management of primary hyperparathyroidism in patients with MEN1 and MEN2A. There is no place for medical treatment of the disease and only surgery can offer cure.[10, 11] The meta-analysis on the surgical treatment of primary hyperparathyroidism in *Chapter 6* increases the evidence for the optimal surgical treatment of hyperparathyroidism in patients with MEN1. From this study, it appears that subtotal parathyroidectomy is the 'optimal' surgical treatment despite the high recurrence rate after this procedure. Furthermore, the risk of permanent hypoparathyroidism (hypocalcaemia) is significantly lower than after total parathyroidectomy with autotransplantation.[11-13] Autotransplants do not always succeed and it may take days to months before they start producing PTH. The resulting hypocalcaemia can lead to significant morbidity with a prolonged hospital stay for intravenous calcium infusion as we have experienced in our practice. Resection of less than 3 parathyroid glands should not be performed for pHPT in MEN1, because the risk of persistent and recurrent pHPT is unacceptably high, which necessitates reoperative surgery with its associated risks. Redo surgery is often challenging to the surgeon because of scarring in the neck, which makes identification of the recurrent laryngeal nerve difficult. The rate of recurrent pHPT remains high even after the 'optimal' type of surgery, subtotal parathyroidectomy.[11-13] Yet, the time to develop recurrent disease may take years. The average time to recurrence after subtotal parathyroidectomy was 10 years in our series, during which time the patient often experience good quality of life.

Two types of studies may yield stronger evidence than is currently available.

First, a large randomized controlled, multicenter trial could offer a more reliable answer to which is the optimal type of surgical treatment of MEN1 related pHPT, but it is questionable if this is feasible. Second, the large Dutch nationwide database of the DuctchMEN workgroup containing all patients with MEN1 in the Netherlands will provide genotype-phenotype information on whether some families experience a more aggressive course of the disease than others.

Primary hyperparathyroidism in patients with MEN2A occurs much less frequently than in MEN1. Therefore, the studies that have addressed the surgical treatment contained small study populations.[14-16] In *Chapter 7*, we show that selective parathyroidectomy, also named minimally invasive parathyroidectomy (MIP), is the most favorable surgical approach in these patients as it is associated with lower

morbidity than more extensive procedures when these patients are free of recurrent disease for years a minimal invasive procedure be preferred over extensive surgery as the initial surgical procedure.
In MEN2A related pHPT, it is even more complicated to perform large trials since the incidence of the disease is so much lower than in MEN1. A longer postoperative follow-up of these patients after minimally invasive/ selective parathyroidectomy may help to elucidate whether or not this is the best surgical therapy for this disease in terms of recurrent disease. Another important aspect is that, as in MEN1, some MEN2A families demonstrate a more aggressive presentation of pHPT. It is unknown if a genotype-phenotype relationship exists. Large multicenter studies with a large number of patients are necessary to study such issues.

Pancreatic endocrine tumors in MEN1
Another controversial topic in endocrine surgery is the surgical management of MEN1 patients with PETs. To answer if ,and what type of, surgical resection (minimal or extensive resections) should be employed, we performed a systematic literature review in *Chapter 8*. The controversy on the surgical management reflects the many different studies with different and contrasting outcomes. The overall quality of these studies is low and therefore no firm conclusions can be drawn from the available literature. Some cautious recommendations however can be made.
It seems reasonable to operate on all patients with insulinomas and patients with gastrinomas >25 mm. Operating on patients with gastrinomas between 10 and 25 mm seems defendable. There is no evidence that early surgery (tumors ≤20, mm) of non-functioning-PETs (NF-PETs) improves cure and survival.
In insulinomas and gastrinomas more extensive operating strategies may be adopted. A duodenotomy should always be performed surgery for gastrinoma, since submucosal duodenal tumors are often present. In NF-PETs, it remains debatable, which type of surgery is preferable, because in the available studies extensive resections were generally performed. Although survival rates of MEN1 patients with PETs are relatively good with conservative surgery, more aggressive surgical resection may improve the chance of (biochemical) cure. Nonetheless, early and aggressive surgery has not been demonstrated to lead to improved survival.
There is a need for high quality controlled studies that will enable researchers to determine which strategies lead to improved quality of life, higher survival and lower complication rates. But will such studies be feasible? It is clear that the low level of evidence in literature combined into the systematic review of literature regarding the surgical management of PETs in MEN1 in this thesis has not yielded the solution to the existing controversy. Again, the low incidence of MEN1 and PETs preclude prospective randomized trials to compare different operative strategies. Physicians treating these patients are in need of answers to questions including: When should we operate PETs in MEN1? What PETs should we operate and at what size? What strategy should be adopted when multiple PETs are present? The DuctchMEN database will be a valuable tool to address these questions.

Posterior Retroperitoneoscopic Adrenalectomy (PRA)

Endoscopic adrenalectomy has become the golden standard for resection of adrenal gland tumors.[17, 18] In *Chapter 9*, PRA has been shown to be cost-effective compared to open adrenalectomy. In these days the laparoscopic lateral transabdominal adrenalectomy is the most popular technique for resection of adrenal gland tumors.[19, 20] In *Chapter 10,* we evaluated our experience with this PRA and showed that this approach is safe and effective for tumors up to 6-7 cm. Because of the evident learning curve observed, and the rarity of the causative disorders this procedure requires experience and centralization.[21] PRA offers an alternative to the laparoscopic lateral transabdominal adrenalectomy.[21] For now, it remains the surgeon's preference, which technique is used. A randomized trial, which would offer the best evidence for the optimal procedure seems immoral, because the endoscopic techniques have proven their benefits in other descriptive and comparative studies.[21, 22]

Clinical presentation and size of pheochromocytoma

Little is known about a possible relationship between the size of pheochromocytomas and the clinical presentation and complication.[23] The aim of *Chapter 11* the goal of the study was to evaluate whether a correlation exists between tumor size and catecholamine levels according to the clinical presentation at diagnosis.

We found that tumor size and hormone levels significantly correlate up to 4 cm. This correlation is independent of clinical presentation. Small tumors generally secrete low hormone levels and do not have the propensity to secrete high levels of catecholamines. On the other hand, larger tumors have a wider variety in secretory potential.

We also showed that complications associated with pheochromocytoma are related to the clinical presentation. Patients who present with classic symptoms of pheochromocytoma have an increased risk of requiring a preoperative admission, which may be caused by larger tumors and a higher level of catecholamines than patients who have asymptomatic pheochromocytomas. We recommend that extra care should be given to patients with larger tumors and higher catecholamine levels who have classic symptoms because they can cause major complications if left untreated.

General comments

From this thesis, it can be concluded that the management of patients who have an endocrine tumor is complex and individual. The treatment of these patients has become tailor-made more and more throughout the years. Patients with familial endocrine syndromes should be regarded as distinctly different from patients with sporadic endocrine disease and often require a different and more aggressive approach. These patients and their families are entitled to centralized care with a multidisciplinary team of endocrinologists and endocrine surgeons. The number of centers, which take care of these patients, should be kept small, in order to provide adequate experience and knowledge of the complex problems. An additional advantage of clustering patients is the possibility of conducting studies with larger populations to address some of the remaining controversies. It also provides the

possibility to incorporate research developments into the management of these patients more readily. Endocrine surgery is a dynamic field in which a surgeon is coerced to keep up with innovative clinical developments in order to offer patients the "best surgical" treatment. Translational research will shed new light on the custom-made surgical treatment.

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Chapter 13

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Summary in Dutch
Nederlandse Samenvatting

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In dit proefschrift wordt de chirurgische behandeling van verschillende endocriene tumoren besproken. Endocriene tumoren zijn tumoren die zich ontwikkelen in hormonale klieren, zoals de schildklier, bijschildklieren, bijnieren en alvleesklier. De chirurgische behandelingen van deze tumoren is in de loop van de jaren maatwerk geworden. Endocriene tumoren kunnen spontaan voorkomen bij patiënten, maar komen ook voor in het kader van erfelijke endocriene tumorsyndromen. Drie bekende erfelijke tumorsyndromen zijn het multipele endocriene neoplasie syndroom (MEN) type 1, 2A en 2B. Patiënten die lijden aan deze erfelijke tumorsyndromen hebben een verhoogd risico op het ontwikkelen van meerdere tumoren in verschillende endocriene organen. Patiënten met MEN 1 ontwikkelen vrijwel allemaal tumoren in de bijschildklieren, terwijl patiënten met MEN2A en MEN2B vrijwel allemaal een agressieve vorm van schildklierkanker ontwikkelen. Ons onderzoek is gericht op de chirurgische behandeling van zowel niet erfelijke als erfelijke endocriene tumoren. De behandeling wordt in toenemende mate aangepast op het biologische gedrag van de tumoren en de klinische kenmerken van de patiënt.

Papillair schildklierkanker is de meest voorkomende vorm van schildklierkanker. In *hoofdstuk 2* hebben we een overzicht gegeven van scoringssystemen die de prognose van papillair schildklierkanker bepalen alsmede de daarbij bekende biomarkers die van invloed zijn op het biologische gedrag. Er zijn verschillende biomarkers die in het tumorweefsel bepaald kunnen worden die een indicatie geven over de mate van agressiviteit van schildklierkanker. Met name de BRAF *V600* mutatie is een mutatie die voorkomt bij agressievere vormen van het papillair schildklierkanker.
Specifiek hebben we een groep van enzymen, de matrix metalloproteïnasen, bestudeerd om te kijken of die tot uitdrukking komen bij verschillende stadia van papillair schildklierkanker. Deze matrix metalloproteïnases (MMPs) zijn een groep enzymen die verantwoordelijk zijn van de afbraak van celverbindingen. Dat maakt het gemakkelijker voor tumorcellen om zich te verplaatsen in het gezonde weefsel, ook wel plaatselijke uitzaaiingen genoemd. In *hoofdstuk 3* hebben we gevonden dat deze MMPs aanwezig zijn in tumorweefsel van verschillende stadia van het papillair schildklierkanker. Verder zijn er aanwijzingen dat er verschillen zijn in de expressie van MMPs in verschillende stadia van de ziekte.

Verder hebben we bij papillair schildklierkanker gekeken naar de waarde van de FDG-PET-scan bij lymfeklier uitzaaiingen. Het blijkt dat patiënten die uitzaaiingen naar de lymfeklieren hebben die gedetecteerd worden met een PET-scan, zogenaamde PET positieve lymfeklieren, een slechtere prognose hebben dan patiënten met lymfeklier uitzaaiingen die niet gezien worden met een PET-scan. In *hoofdstuk 4* vergeleken we deze 2 patiënten groep met lymfklieruitzaaiingen. We zagen dat alleen patiënten overleden in de groep met PET positieve lymfeklieren. Door chirurgische verwijdering van de aangedane lymfeklieren, kunnen in enkele gevallen patiënten genezen.

Een andere vorm van schildklierkanker, medullair schildklierkanker wordt gezien bij vrijwel alle patiënten met MEN 2A en MEN 2B. In het verleden overleden veel patiënten aan de gevolgen van hun schildklierkanker omdat de ziekte in een te laat

stadium ontdekt werd. Tegenwoordig kan er met genetische screening bepaald worden of een patiënt, vaak een kind, drager is van het zieke gen. Op basis van de genafwijking kan bepaald worden op welke leeftijd deze kinderen het beste geopereerd kunnen worden. Wij hebben in *hoofdstuk 5* laten zien dat bij bepaalde genafwijkingen deze kinderen vroeg geopereerd dienen te worden. De schildklier wordt dan in zijn geheel verwijderd teneinde de ontwikkeling van de schildklierkanker voor te zijn of in een vroeg stadium te kunnen behandelen. Factoren die een rol spelen bij een slechtere prognose van deze patiënten zijn een hogere leeftijd ten tijde van de operatie, verhoogde tumormarkers voor de operatie en bepaalde genafwijkingen.

Patiënten met het erfelijke endocriene tumorsyndroom MEN1 ontwikkelen allemaal tumoren die parathormoon produceren in één, maar veelal meerdere bijschildklieren. Dit leidt tot een verstoorde calciumhuishouding waarbij het calcium in het bloed stijgt, hyperparathyreoïdie genaamd. Dit kan leiden tot o.a. osteoporose, hoge bloeddruk, stemmingsstoornissen met depressie en buikklachten. De enige behandeling is chirurgisch, maar er bestaat veel controverse over het type chirurgische behandeling. De vraag is of alleen de aangedane bijschildklier, meerdere bijschildklieren of alle bijschildklieren verwijderd dienen te worden. De kans op recidief ziekte is hoog, zelfs als 3 van de 4 klieren verwijderd worden. Echter, bij verwijderen van 4 klieren bestaat weer een hoog risico op een te laag calcium wat onbehandeld tot ernstige gevolgen kan leiden. In *hoofdstuk 6*, hebben we naar onze eigen patiënten populatie gekeken waarbij we zagen dat er een hoog recidiefpercentage na uitgebreide chirurgie (verwijderen van 3-3 ½ bijschildklier) is, maar gemiddeld pas laat, na 10 jaar. Na verwijderen van 4 bijschildklieren is de kans op een te laag calcium weer veel te groot. Uit onze begeleidende literatuurstudie en meta-analyse bleek dat het verwijderen van 3 – 3 ½ bijschildklier de beste chirurgische behandeling is van deze patiënten.
Bij MEN 2A is de hyperparathyreoïdie milder en komt slechts bij 30-40% van de patiënten voor. Ook over deze chirurgische behandeling is onduidelijkheid. In *hoofdstuk 7* hebben we aan de hand van onze relatief grote patiënten populatie laten zien dat het verwijderen van slechts 1 of 2 bijschildklieren veilig is en een laag recidief percentage geeft. Echter, we zullen deze patiënten in de tijd moeten blijven volgen.

Naast hyperparathyreoïdie, ontwikkelen 35-70% van de patiënten met MEN1 ook pancreas eilandjes cel tumoren (PETs). Deze tumoren zich in de cellen die insuline, gastrine en andere hormonen produceren in de alvleesklier. De meest voorkomende tumoren zijn gastrinomen, die gastrine produceren, insulinomen, die insuline produceren en niet-functionerende tumoren, die geen actieve hormonen produceren. De chirurgische behandeling van de tumoren is omstreden. Er bestaat twijfel over de indicatie en het tijdstip waarop deze tumoren geopereerd moeten worden en zo met welke techniek ze geopereerd moeten worden. Er bestaan grofweg twee scholen. Er is een school die afwachtend is. Patiënten met deze tumoren worden pas laat geopereerd, vaak als tumoren groter zijn dan 3 cm. Er is ook een school die zeer agressief is met opereren, die al bij kleine tumoren, grote

operaties doet. In *hoofdstuk 8* hebben we een systematisch overzicht gegeven over de chirurgische behandeling van deze pancreas eilandjes cel tumoren (PETs). Uit de literatuur hebben wij gemeend te concluderen dat het redelijk lijkt alle patiënten met een insulinoom te opereren vanwege de overproductie van insuline die kan leiden tot ernstige symptomen. Tevens valt te overwegen om alle patiënten met een gastrinoom van groter dan 2,5cm te opereren. Er is enig bewijs dat ook patiënten met gastrinomen tussen de 1 en 2,5cm geopereerd kunnen worden. Er is geen bewijs dat een operatie in een vroeg stadium van niet-functionerende tumoren (tumoren <2cm) genezing en overleving verbetert. Echter, er is een mogelijk vergroot risico op uitzaaiingen bij grotere niet-functionerende tumoren. Bij insulinomen en gastrinomen kunnen grotere operaties overwogen worden waarbij grote delen van de alvleesklier verwijderd worden. Bij alle gastrinomen dient ook de twaalfvingerige darm geopend te worden omdat er vaak ook meerdere kleine tumortjes in de twaalfvingerige darm aanwezig zijn die anders gemist kunnen worden. Bij niet-functionerende tumoren, blijft het de vraag welke type operatie de voorkeur geniet. In de beschikbare literatuur worden voornamelijk grote operaties verricht.
Alhoewel de overlevingskansen voor MEN1 patiënten met pancreas eilandjescel tumoren goed zijn, kan een agressieve aanpak met vroege en grote chirurgie de kans op genezing vergroten. Echter, tot op de dag van vandaag, heeft deze grotere chirurgie nog geen overlevingsvoordeel bewezen te hebben.

De chirurgische behandeling van bijniertumoren is in de jaren 90 verschoven van een klassieke open operatie naar een minimaal invasieve operatietechniek, de laparoscopie oftewel kijkoperatie. Al snel werd aangetoond dat de laparoscopie aanzienlijke voordelen had ten opzichte van de klassieke open operatie. De opnameduur in het ziekenhuis werd korter, patiënten hebben minder pijn en er is minder bloedverlies.
In *hoofdstuk 9* hebben we een kosteneffectiviteit analyse verricht waarbij we de klassieke open operatie hebben vergeleken met de minimaal invasieve posterieure retroperitoneale bijnierverwijdering, oftewel bijnierextirpatie. Wij vonden dat de opnameduur in het ziekenhuis korter was en het bloedverlies minder was in de laparoscopie groep. De operatieduur was wel langer, maar de extra kosten van een laparoscopische operatie wegen op tegen de voordelen van de operatie voor de patiënt. Wij concluderen dan ook dat de endoscopische operatie, de posterieure retroperitoneale bijnierextirpatie kosteneffectief is.
Er zijn verschillende toegangsroutes naar de bijnieren, van voren via de buik, van de zijkant en van achteren via de rugzijde. De meest gebruikelijke wijze is via de buikzijde. In *hoofdstuk 10* laten we de ervaring zien met de retroperitoneale bijnierextirpatie, posterieure retroperitoneale adrenalectomie. Dat is een techniek waarbij de bijnieren via de rugzijde worden benaderd, wat de meest directe route naar de bijnieren is. In deze grote serie, laten we zien dat deze techniek een veilige techniek is met weinig complicaties. Wel is het een techniek die een leercurve heeft. Het is dan ook van belang dat deze operatie alleen door of onder begeleiding van ervaren chirurgen wordt verricht.

Een specifieke bijniertumor is het feochromocytoom, een tumor uitgaande van het bijniermerg. Deze produceren hormonen als adrenaline, noradrenaline en dopamine. Deze tumoren kunnen asymptomatisch zijn, maar ook leiden tot levensgevaarlijke hoge bloeddruk en zelfs tot shock. In *hoofdstuk 11* hebben we voor het eerst aangetoond dat de grote van de feochromocytomen gerelateerd is aan de hoogte van de hormoonproductie en complicaties.

Samenvattend kunnen we stellen dat endocriene tumoren een wijd klinisch spectrum hebben. De chirurgische behandeling is aangepast aan de biologische factoren van de tumoren en de klinische kenmerken van deze patiënten. De chirurgische behandelingen van deze tumoren is in de loop van de jaren maatwerk geworden.

Chapter 14

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Dankwoord

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Curriculum Vitae Auctoris

Jennifer Marijke Janneke Schreinemakers was born on February 4th 1981 in Eindhoven. In 1999, she graduated cum laude from the Gymnasium at the Sint Augustinium College in Eindhoven. In 1999, she was admitted to Utrecht University's medical school. During her rotations, she started research with Prof.Dr. Inne Borel Rinkes on adrenal gland tumors. Eventually, this research led into her PhD research project. After graduating from medical school in 2005, she worked as an intern in the Sint Antonius Hospital's general surgery department. In January 2007, she started the general surgery residency program at the University Medical Center Utrecht (Prof.Dr. I.H.M. Borel Rinkes). From January 2009 until June 2009, she worked as a research fellow in the Endocrine Surgery service at the University of California San Francisco with Prof.Dr. Orlo H. Clark. Upon returning in the Netherlands, she continued her residency program at the Amphia Hospital in Breda. (Dr. L. van der Laan en Dr. J.H. Wijsman).

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14. Meer de SG, Schreinemakers JM, Zelissen PM, Stapper G, Sie-Go DM, Borel Rinkes IH, Vriens MR. A ten-year evaluation of cytologic adequacy of 1611 FNAC specimens. Submitted

List of Abbreviations

ACTH	Adrenocorticotropic Hormone
BMI	Body Mass Index
BRAF	BRAF *V600* mutation
CEA	Carcinoembryonic antigen
CNE	Conventional Neck Exploration
DPR	Distal Pancreatic Resection
Enucl	Enucleation
FDG-PET	Fluorodeoxyglucose- Positron Emission Tomography
MEN	Multiple Endocrine Neoplasia Syndrome
MEN1	Multiple Endocrine Neoplasia Syndrome Type 1
MEN2, MEN2A &2B	Multiple Endocrine Neoplasia Syndrome Type 2, 2A and 2B
MIP	Minimally Invasive Parathyroidectomy
MMP	Matrix Metalloproteinasis
MTC	Medullary Thyroid Carcinoma; Medullary Thyroid Cancer
NF-PET	Non Functioning Pancreatic Endocrine Tumor
ioPTH	Intraoperative PTH
OS	Overall Survival
PD	Pancreaticoduodenectomy
PET	Pancreatic Endocrine Tumor
PET	Positron Emission Tomography
PFS	Progression Free Survival
pHPT	Primary Hyperparathyroidism
PP	Pancreatic Polypeptide
PPPD	Pylorus Preserving pancreaticoduodenectomy
PRA	Posterior Retroperitoneoscopic Adrenalectomy
PTC	Papillary Thyroid Carcinoma; Papillary Thyroid Cancer
PTH	Parathormone
RET	REarranged during Transfection, proto-oncogene
RLN	Recurrent Laryngeal Nerve
SDHD	SDHD gene succinate dehydrogenase complex subunit D
<SPTX	Less than Subtotal Parathyroidectomy
SPTX	Subtotal Parathyroidectomy
TIMP	Tissue Inhibitor Metalloproteinasis
TP	Total Pancreatectomy
TPTX	Total Parathyroidectomy
US	Ultrasound
VIP	Vasoactive Intestinal Peptide
ZES	Zollinger-Ellison Syndrome

APPENDICES (CHAPTER 8)

Appendix A. Insulinomas

Reference Study design and quality	Study population and characteristics and indications for surgery	Tumour characteristics at baseline	Interventions	Outcome category	Result primary outcome + statistics at follow-up	LoE	Comments
Part 1: evidence from uncontrolled studies dealing solely with insulinomas							
Box Som van 1995 Retrospective case series Clearly defined cases, intervention and outcome	n=26 MEN1 insulinoma + surgical treatment France 1967-1994, GTE registry mean age: 32 yrs (10-57) M:F 31:69% FU: 9 yrs Indications for surgery: NA	Size: NA Multiplicity: NA Location: Head 47% Body 60% Tail 73% Metastases: Lnn n=2	Surgery: n=26 Operations: n=31 Reoperations n=4 DPR n=16 + enucl. head n=3 + enucl. multiple n=2 Subtotal (90%) pancreatectomy n=1 Enucl. n=7 Multiple enucl. n=2 PD n=1 TP n=3 (with synchronous ZES) Hemihepatectomy right sided n=1	Complications Recurrence Metastases (metachronous) Cure Mortality Loss to FU	Complications: DM n=3 (after TP) Abscess n=1 Anastomotic leakage n=1 Pancreatic cyst n=1 Persistent disease n=2 after: enucl. n=1 DPR n=1 Recurrence n=1 after 10 yrs Other recurrence ZES n=6 after enucl. Metastases Lnn n=4 LM n=1 Cure after operation 92% Mortality 0% Loss to FU n=1	4	same study group + partially the same data as Cougard 2000, less pts
Cougard 2000 Retrospective case series Clearly defined cases, intervention and outcome	n=44 MEN1 insulinoma France 1960-1996, Hôpital Edouard-Herriot mean age: 36.4 yrs M:F 36:74% FU (mean): 9 yrs Indications for surgery: NA	Size: NA Multiplicity: 79% Location Diffuse 86% Head 7% Unknown 7% Other tumours Gastrinoma n=8 Glucagonoma n=6 VIPoma n=1 Metastases:(baseline/FU) n=6 Lnn n=2 (insulinoma)	Surgery: n=41 TP n=3 Enucl. head n=8 + gastrectomy n=1 PD + enucl. tail n=1 DPR >80% n=26 Unkown n=3 Combined with enucl. duodenum: n=1	Complications Cure	Complications DM n=3 2 pts had persistent ZES Insulinoma cure n=25/36 (69%) Persistent disease n=5 n=2 → reoperation n=1 DPR n=1 TP Recurrence n=3 Metastases n=2 after enucleation most recurrence after DRP 75% cure Cure gastrinoma n=3 (37%)	4	Same data, same study group as Box Som 1995, more pts

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		LM n=4 gastrinoma n=3 VIPoma n=1		Mortality Loss to FU	Mortality n=3 (not insulinoma related) Loss to FU n=5 (12%)		
O'Riordain 1994 & Rasbach 1985 Retrospective case series Clearly defined cases, intervention and outcome	n=19 MEN1 insulinoma + surgical therapy USA 1970-1991, Mayo Clinics median age: 24 yrs (9-55) M:F 58:42% FU (median): 10.3 yrs (1.7-18.8) Indications for surgery: NA	Size: 2-40 mm (available for first 12 pts) Multiplicity: 89% No.(median): 6 (2-16) Location: Head 24% Uncinate process 17% Body 24% Tail 35% Proximal tumours 6% Distal tumours 39% Both 56% Metastases: lnn n=2 n=1 unresectable	Surgery: n=18 DPR > 75% n=12 + enucl. n=6 + limited lnd n=1 enucl. (after previous subtotal resection) n=1 TP n=1 Conservative surgery n=5 minimal DPR n=3 + enucl. n=2 enucl. n=2	Complications Long-term recurrence Postoperative cure* Survival Mortality	Complications: TP DM 100% Exocrine deficiency 100% DPR Permanent DM 8% Transient DM 8% Gastrointestinal motility disorder 8% Pancreatitis 8% Long-term recurrence after: DPR >75%: 0% conservative surgery: 40% Postoperative mortality: 0% Postoperative cure 94.4% 4,8, 12 yrs survival 94.4, 94,4, 85.0%. death n=4 insulinoma related 25% MEN1-related 75%	4	Publications from same study group and medical center.
Proye 2000 Retrospective case series Clearly defined cases, intervention and outcome	n=13 MEN1 insulinoma France 1971-2002, Lille mean age: 36.5 yrs M:F 62:38% FU: NA Indications for surgery: no signs of local invasion.	Size: NA Multiplicity: 100% Location (radiological): Head n=4 Tail n=18 Operative and histological: Head n=3 Tail n=110 Metastases: NA	Surgery: n=13 DPR ± enucl. n=12 Enucl. n=1	Cure Reoperations Metastases (metachronous)	Cure n=12 (92%) Persistant disease n=1 Reoperations n=3 Insulinoma head n=2 Metachronous gastrinoma duodenum n=1 Metastases LM n=1 after insulinoma n=1 after gastrinoma	4	-
Part 2: extracted evidence from uncontrolled studies dealing with different types of PETs							
Bartsch 2005	n=26 MEN1 + PET + surgery n=5 insulinoma	No. (all PET): 3 (1-5)	Surgery: n=5	Complications (all pts n=26)	Overall Complications 52%, after: PPPD n=4	4	Good quality case series.

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Prospective case series Clearly defined cases, intervention and outcome	Germany 1981-2004, Philipps-University Marburg mean age (at diagnosis): 34 yrs (13-52) M:F 60:40% FU (after operation): 88 mth (10-241) Metastases: 0% Indications for surgery: No diffuse LM		Enucl. n=2 DPR + enucl. n=2 Corpus resection n=1	 Relation tumoursize and metastases Recurrence and metastases (metachronous) Survival	Fistula n=3 DPR +/- enucl. +/- duodx n=17 Fistula n=8 Abscess n=2 Bleeding n=2 Enucl. +/-duodx n=7 Fistula n=1 Abscess n=2 DM n=1 No relationship tumour size and metastasis recurrence 0% metastases 0% metachronous NF-PET n=1 25% Overall survival n=24 (96%) disease related mortality 0%		
Kaczirek 2002 Retrospective case series Clearly defined cases, intervention and outcome	n=6 MEN1/PET n=4 insulinoma Austria 1981-2001 mean age (at first presentation) 44.3 yrs M:F NA FU (mean, after PET surgery): 81 mth ± 50.2 Indications for surgery: NA Type of Surgery: Depending on site: DPR or enucl. Multiple tumours: DPR + enucl. Large tumour head: PD	Size: 15-25 mm Multiplicity: n=2 (50%) Location: head n=2 body n=2 tail n=2 Metastases lnn n =1 LM n=0	Surgery: n=4 DPR n=2 + enucl. n=1 Enucl. n=2	Complications Cure rate Survival	Postoperative complications 0% Biochemical cure 100% Disease related mortality 0%	4	
Samaan 1989	n=14 MEN1 + PET n=2 insulinoma	Size: n=1: 4-24 mm	Surgery: n=2 50% DPR n=2	Recurrence	Recurrence 0%	4	No indications for surgery, not all

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Retrospective case series Poorly defined cases, intervention and outcome.	USA 1971-1988, MD-Anderson age: 28 and 29 yrs M:F 1:1 FU: 1,5 and 7 yrs Indications for surgery: NA	n=1: insulinoma 1-25 mm + NF-PET 50 mm Multiplicity: 100% No. of insulinomas: n=1: 3 n=1: 7 location: n=1 tail n=1 head + tail Metastases: 0%		Mortality	Mortality n=0		procedures specified
Sheppard 1989 Prospective case series Clearly defined cases, intervention and outcome	n=9 MEN1 + PET n=2 insulinoma USA 1983-1988, NIH Bethesda age: 26 and 56 yrs M:F 1:1 FU: 3 and 27 mth Indications for surgery: insulinoma	Insulinoma n=2 Size largest tumour: n=1 30 mm n=1 40 mm Multiplicity: n=1 Location: Head n=1 Body n=1 Metastases insulinoma: 0	Surgery: n=2 Type of surgery: NA	Cure	Cure n=2 (100%)	4	No surgical intervention specified.
Thompson 1995 & Thompson 1998 Retrospective case-series Clearly defined cases and intervention. Outcome poorly defined.	n=40 MEN1 + functional PETs + surgical resection n=6 Insulinoma 1978-1997 Michigan Medical Center, USA age NA M:F NA FU: 5-18 yrs Indications for surgery: NA	Insulinoma n=6 Size: NA Multiplicity: 83% Location: NA Metastases (lnn): n=1 (17%)	Surgery: n=6 DPR + enucl. n=6	Cure Survival	Cure 100% Survival 100% Overall survival: 5;10;15 yrs: 97;94;94% Deaths: MEN1 unrelated	4	a lot of missing data publications from the same study group and center.
Tonelli 2006 Case series	n=16 MEN1 + PET n=3 insulinoma Italy,	Insulinoma n=3 Size (mm): 20; 22; NA No.: 1 Location: NA	Surgery: n=3 Insulinoma only: DPR + enucl. n=1	Complications (overall)	major complications 37% pancreatic fistula and/or pancreatico-jejunal anastomosis leak n=4 abdominal abscess n=1	4	-

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Clearly defined cases, intervention and outcome	1992-2003, Florence mean age: 36 yrs (29-40) M:F 67:33% FU: 6-108 mth (all pts) Indications for surgery: insulinoma	Metastases 0%	Insulinoma + gastrinoma PPPD + enucl. n=1 DPR + enucl. + duodx n=1	Cure Survival	dehiscence n=1 late complications 31% IDDM + cholangitis n=3 malabsorption n=2 Cure insulinoma 100% Overall survival: 81% MEN1 related mortality: 0%		

* postoperative cure was defined as no symptoms and normal fasting glucose for at least 6 mth

Abbreviations used in alphabetical order: DM diabetes mellitus; duodx duodenotomy; DPR distal pancreatic resection; enucl. enucleation of tumour; FU follow-up; GTE Le Groupe d'étude des Tumeurs Endocrines; IDDM insulin dependent diabetes mellitus; LM liver metastases; lnd lymph node dissection; lnn lymph nodes; LoE level of evidence; MEN1 multiple endocrine neoplasia type 1; M:F male: female; mth months; NA not available; NIH National Institutes of Health; No. number; PD pancreaticoduodenectomy; PET pancreatic endocrine tumour; PPPD pylorus preserving pancreaticoduodenectomy; pts patients; TP total pancreatectomy; USA United States of America; VIP vasoactive intestinal peptide; yrs years; ZES Zollinger Ellison Syndrome.

Appendix B. Gastrinomas

Reference Study design and quality	Study population and characteristics	Tumour characteristics at baseline	Indications for surgery	Interventions and control conditions	Outcome category	Result primary outcome + statistics at FU	LoE	Comments
Part 1: evidence from controlled studies dealing solely with gastrinomas								
Cadiot 1999 Retrospective case-control Study quality: 6/7	n=77 MEN1/ZES Exclusion criteria: synchronous LM, FU < 12 mth, postoperative death Studygroups: LM + / LM – stratification tumour size: <30mm / 20-30mm / >30mm France 1959-1995 median age: 41 yrs (21-72) M:F 61:39%	Size pancreas: ≥ 30mm: n=10 20-30mm: n=5 <20 mm: n=42 No.: NA Location (n=63): Pancreas only 52% Duodenum only: 3% Both: 16% Both + lnn: 10% Pancreas + lnn: 13% Lnn only: 6 LM n=0 (exclusion criterium)	NA	Surgery n=48 DPR ± lnd ± enucl.n=32 PD n=5 TP n=1 Enucl. n=3 Laparotomy without tumour n=6 Antrum gastrinoma + lnd n=1 Irradical resection n=8	Metachronous LM Mortality	Metachronous LM n=8 (10.4%) n=7 after macroscopic radical resection Multivariate analysis: PET ≥ 30 mm associated with LM (RR 29.0; 95% CI, 3.2-260.7; p=0.0003). NS: ZES, diagnosis < 1980, gender, centre and surgery. Overall mortality: 17% Mortality LM + 62.5% LM - 11.6% Associated with poor survival: ZES diagnosis <1980 (RR 8.24; 95% CI 1.67-40.60, P=0.01) Age at ZES diagnosis (RR 1.08; 95% CI 1.03-1.14, p =0.001)	3b	No indications for surgery No relation specified between outcome and type of surgery

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	Median FU (mth): LM+: 88 (18-191) LM–: 100 (12-366) NS							
Gibril 2001 Prospective case-control Study quality: 7/7	n=57 MEN1/ZES USA 1972-2000, NIH Maryland group 1 n=49: non-aggressive growth (<25% in volume per mth, ± LM) group 2 n=8: aggressive growth (>25% or new lesions) mean age (at ZES diagnosis): 1. 38 yrs ± 2 (17-60) 2. 31 yrs ± 3 (23-49) M:F 1. 53:47% 2. 37:63% mean FU: 8 yrs (0.3-27)	PET size (mm) (operated pts) 1. 18 ± 2 2. 53 ± 9 Location 1 vs 2 Pancreas only 9(26%)/2 (29%) Duodenal only 16 (47%)/ 2 (29%) both 3 (9%) / 3 (43%) unknown 6 (18%)/0 (0%) Metastases 1 vs 2 LM 1 (2%)/ 2 (25%) Unclear if syn- or metachronous: BM 0/ 3 (38%) Lnn 18 (53%)/ 6 (86%)	Resection tumour size > 25 mm Partial hepatectomy in minimal LM No resection in diffuse LM >1987 transillumination duodenum	Surgery n=41 (72%) 1. n=34 (69%) 2. n=7 (88%) NA surgical procedure	Factors associated with aggressive disease Metastases Survival (5 yr)	Factors High fasting gastrin p=0.0001 BM p= 0.019 LM p<0.0001 Gastric carcinoid p=0.024 Tumour size > 30 mm p<0.0001 LM group1/group2: n=4 (8%)/ n=6 (75%) Disease specific 5yr- survival 1 vs 2 1. 100% (92-100) 2. 88% (53-98) p=0.0012	3b	No information on resection type Surgery is not the subject of this analysis Cytoreductive surgery is not analysed
Jensen 1998 Retrospective cohort Study quality: 4/8	n=45 MEN1/ZES USA; NIH mean age (at diagnosis): 37.8 yrs ± 1.6 M:F 49:51% mean FU: 8.2 yrs ± 0.9	Size: NA No.: NA Location (operated pts) pancreas 64% duodenum 53% lnn 7% Metastases: Lnn 53% LM: 4%	NA	Explorative laparotomy 63%	Metastases Survival	LM 14% Survival 5,10, 15 yrs 93%, 93%, 93% pts without LM 96%, 96%, 96%	4	No tumour features, no indication and no intervention specified.
Melvin 1993 Retrospective cohort Study quality: 4/8	n=76 gastrinoma n=19 MEN1/ZES USA Ohio State University	Size: NA No.: NA Location: pancreas only n=4	NA	Surgical intervention n=28 in 17 pts Pancreatic surgery: resection and/or enucl.	Cure rate after pancreas surgery Survival	Cure (fasting gastrin): n=1 (9%) Survival rate 5,10,15, 20 yrs 94, 75, 61,58%	4	Little information on tumour characteristics and surgical

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	mean age: 45 yrs M:F 74:26% FU: ≥10 yrs	Metastases: Lnn: n=3 LM/distant: n=6		n=11 gastrectomy n=12		10 yrs survival: no metastases 100% Lnn 67% Distant 40%		intervention.
Part 2: evidence from uncontrolled studies dealing solely with gastrinomas								
Deveney 1983 Case series Clearly defined cases, intervention and outcome.	n=52 ZES n=15 MEN1/ZES Surgical exploration for gastrinoma USA >1960, University of California Characteristics (all pts n=52): Age: 15-67 yrs M:F 52:48% FU: 10 mth-10 yrs	Size NA No. Single n=1 Multiple n=6 No tumour n=3 Unknown n=5 Location: NA Metastases: NA	Contraindications for surgery: Metastases Multiplicity Before cimetidin Infiltrating tumour head	All pts had laparotomy n=4 resection TP n=1 (insulinoma + gastrinoma) DPR n=2 Enucl. n=1 n=3 no tumour found n=8 no resection	Cure rate	1/4 cured (after TP)	4	Resection in only 4 pts missing data
Nikou 2005 Retrospective case series Clearly defined cases and outcome. Intervention poorly defined.	n=11 MEN1/ZES Greece Mean age: 51.8 yrs (31-66) M:F 45/55% mean FU: 6.1 yrs (2.1-8.5)	Size largest pancreas tumour: 27mm (15-40) Mean no.: Pancreas 1.3 (1-2) Duodenum 1.9 (1-3) Location (n) pancreas:9 head 6 body 2 tail 1 Duodenum: 12 Metastases: Lnn 45% Liver 45%	NA	Surgery n=6 (54.4%) DPR n=1 (solitary lesion tail) DPR + enucl. + duodx n=1 (multiplicity duodenum/ pancreas) PD n=1 (large gastrinoma head) duodx n=3	Metastases Disease free survival Mortality	LM n=2 4/11 disease free n=3 after resection >50% 6/11 alive with disease n=3 after surgery death 1/11 (9%)	4	No relationship between type of surgery and outcome Indications for surgery are unclear
Norton 2001 Prospective case series	n=81 MEN1/ZES Group 1 n=25: tumours < 25 mm Group 2 n=48: tumours ≥	Available for group 2 Pancreatic tumour size mean ±SD (range) (mm): overall 26 ± 3 (5-60)	(1) Tumour ≥ 25 mm (2) no diffuse LM (3) functional syndrome other	Type of surgery group 2A/2B: Enucl. 12/39% DPR 24/58%	Complications	Early (<30 days) complications: 29% (2A=2B) Late (>30 days) complications: 10% (2A=2B)	4	Outcome was not related to surgical strategy

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Clearly defined cases, intervention and outcome.	25 mm no diffuse LM 2A: single tumour < 60 mm n=17 2B: multiple tumours or single tumour ≥ 60 mm n=31 Group 3 n=8: diffuse LM USA ≥ 1972, University of California mean age (at onset ZES): 35 yrs ± 1 M:F 43:57% FU: from onset ZES: 15.2 yrs (± 1.1) from surgery: 6.9 yrs (± 0.8)	2A: 22 ± 3 (10-40) 2B: 26 ± 3 (5-60) No. PETs: 3.7 ± 0.7 Multiplicity: single 25% multiple 68% Location: Pancreas 29% Duodenum 19% Both 44% Solitary lnn 6% Location pancreas: Head 21% Body 31% Tail 63% Metastases: Lnn 69% LM 8%	than ZES Head: enucl. Body + tail: enucl. if possible Multiplicity:DPR head + multiplicity: PD Isolated LM: resection (wedge/ segment or lobectomy) Routinely lnd >1987 transillu-mination duodenum	PD 0/6% Duodx. 53/68% Lnd 53/68% Liver resection: 0/13%	Metastases % disease-free Survival	metastases: LM group 1 vs. group 2 0% vs. 6% Lnn: NA % disase free 2A/2B: immediate 35/10 (p=0.039) > 5 yr: 0/0 survival 10, 15 yr: 1: 100, 100% 2A: 100,100% 2B: 100,89% 3: 88, 52% significantly better in 1 and 2 vs 3 (p < 0.03)		Same research group, same centre as Norton 1999 Different design
Norton 1999 Prospective case series Clearly defined cases, intervention and outcome.	n=151 ZES n=28 MEN1/ZES + surgical resection USA 1981-1998, University of Califomia mean age: 43 yrs ± 14 M:F 50:50% Mean FU (after first operation) 7 yrs ± 5	Size ≤ 10 mm 52% >10 mm 48% No.: NA. Location: Pancreas 68% Duodenum 57% Lnn only 4% Unknown 7% Metastases: Lnn 61% LM 14%	tumour on imaging > 30 mm after 1987 routinely transillumination duodenum + duodx	Type of surgery: Biopsy only 3% DPR 38% Enucl. 69% PD 3% Hepatic resection 6% duodx in 50%	DFS immediately and after 5 yrs Overall survival	Immediately: 16% After 5 yrs: 6% Relapse: 100% Overall survival: 5,10 yrs 100,86%	4	Multiple operations per patient were performed Same research group, same centre as Norton 2001 Different design
Ruszniewski 1993 & Mignon 1993 Case series Clearly defined	n=45 MEN1/ZES France 1959 -1989 mean age (at diagnosis):	Size: NA No.: (available for n=26) solitary 27% multiple 73% 2-8 tumours n=7 >10 n=12	no surgery n=9: LM and lung metastases n=5 Unknown n=4 Further	total gastrectomy n=19 tumour surgery: n=36 blind DPR n=15 (negative laparotomy) PD: n=3	Complications	Complications: mortality: 9% (<1970) hemorrhage 11% sepsis 8% group1 n=24: no tumour at discharge	4	No indications for surgery provided Interventions not specified

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cases, intervention and outcome.	43.8 yrs ± 11.6 M:F 64:36% median FU: 64 mth (range 0.1-252)	 Location (operated pts n=36) pancreas only 38% duodenum only 8% both 14% no tumour 19% within pancreas head: 35% body: 8 % tail: 38% Diffuse: 19% Location (non-operated pts n=9): pancreas only 22% duodenum only 0% both 11% no tumour 22% within pancreas head: 22% body: 11% metastases: LM 16% Isolated lnn 18%	laparotomy in all pts without diffuse LM Operative technique: IOUS-, transillumination-, systematic duodenum palpation +. Cauda/ corpus: DPR Caput: enucl. PD: unenucleable head tumour Duodenal wall tumour: duodx		Metachronous metastases Cure rate Overall survival	group 2 n=14: residual tumour LM 16% isolated lnn 2% isolated lung 2% Cure: group 1 n=1 (14%) Overall survival: Total group 5, 10 yrs (± SD): 67 ± 7%; 63 ± 10%. No difference between group 1 and 2		Publications from same study group and medical centre; presenting the same data.
Thompson NW 1992 Retrospective case series Clearly defined cases, intervention and outcome	n=11 MEN1/ZES + surgery USA 1976-1991, Michigan Medical Centre mean age (at treatment): 39 yrs M:F 36:64% FU: 0.25-14 yrs	Size: NA Other PETs: 100% Location gastrinoma: Pancreas only 36% Duodenum only 36% Both 27% Metastases: Lnn: n=6 (55%) LM: n=0	No LM Regional gastrin secretin by THVS Operative technique: ≥ 1986 routinely duodx	DPR ± enucl. head/uncinate process + duodx + lnd n=7 Enucl. n=4 gastrectomy (early years) n=2	Recurrence Cure rate Survival	Recurrence: After DPR: 0% After enucl. 75% reoperation n=2 → disease free n=1 Biochemical cure: normal basal gastrin n=10 normal secretin test n=4 Survival: n=10 Death unrelated cause	4	No tumour characteristics
Thompson NW 1989 Retrospective case series	n=6 MEN1/gastrinoma + surgery without gastrectomy or TP	Size mm: PET 10-110 Multiplicity 67%	Indication for surgery: regional gastrin secretion as	DPR n=1 DPR + splen. n=2 DPR + enucl. n=2 + duodx n=1	Cure rate	normal serum gastrin at end FU: n=6 (100%) transient gastrin rising during	4	Selection bias: 2 pts were included in this case series

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Clearly defined intervention and outcome.Cases poorly defined	USA 1978-1988, Michigan Medical Centre mean age: 36 yrs (27-46) M:F 2:4 (33:67%) mean FU: 7 yrs (5 mth-12 yrs)	Location: pancreas only n=4 pancreas + duodenum: n=2 Location within pancreas: head n=3 body n= 4 tail n= 1 Metastases: lnn n=2	determined by THVS in 4 pts NA in 2 pts	Enucl.+ duodx n=1	Survival	hypercalcemia: n=2 normal secretin test: n=1 survival: 100%		based on postoperative results
Thompson JC 1983 Retrospective case series Clearly defined cases. Intervention and outcome poorly defined.	n=27 ZES n=11 MEN1/ZES USA ≥ 1970, University of Texas Medical Branch mean age (at diagnosis): 38 yrs M:F 6:5 (55:45%) mean FU (all pts) 45 mth	Size: NA No.: multiple n=7 single n=2 no tumour found n=2 Location: Pancreas only: n=7 Duodenum only: n=1 both: n=1	NA Standard operative procedure: enucleation Duodenal tumour :duodx Isolated LM: metastasectomy	Exact procedures: NA	Metastases Cure rate Survival DFS	LM: n=1 Lnn: NA Biochemical cure: n=0 Survival: 5;10 yrs: 87%; 87%. 1 disease related death (LM), 1 unrelated death.	4	Gastrectomy was the main intervention.
Part 3 extracted evidence from uncontrolled studies dealing with different types of PETs								
Bartsch 2005 Prospective case series Clearly defined cases, intervention and outcome	n=26 MEN1 + PET n= 11 gastrinoma + surgery Germany 1981-2004, Philipps-University Marburg mean age: 42 yrs (29-52) M:F 55:45% FU (after surgery): ZES 123 mth (38-213)	Size: 12 mm (3-25) No.: PETs 2 (1-5) Location: duodenum only 9% pancreas only 19% both 72% Metastases: lnn n=5 (45%) LM n=1 (9%)	No evidence of diffuse LM	Gastrinoma DPR + enucl. n=4 + duodx n=3 PPPD n=2 Enucl. + duodx n=4 + wedge resection liver n=1 Duodx n=1	Complications (all pts n=26) Recurrence and metastases	Complications: 52% after PPPD n=4 Fistula n=3 DPR ± enucl. ± duodx n=17 Fistula n=8 Abscess n=2 Bleeding n=2 Enucl. ± duodx n=7 Fistula n=1 Abscess n=2 DM n=1 Recurrent ZES: n=4 (36%) after duodx lnn metastases n=2 (18%)	4	Good quality case series

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					Biochemical cure Survival Relation tumoursize and metastases	metachronous: NF-PET n=9 (82%) Insulinoma n=1 (9%) →DPR + enucl. biochemical cure (normal gastrin + secretin test): n=7 (64%) PPPD n=4 (100%) Overall survival: n=24 (96%) Disease related mortality: 0% No relationship tumour-size and metastasis		
Thompson NW 1998 Retrospective case series Clearly defined cases, intervention and outcome	n=40 MEN1 + functional PETs + surgical resection n=34 gastrinoma USA 1978-1997, Michigan Medical Centre mean age: 39.4 yrs M:F 44:56% maximum FU: 18 yrs	Size: NA Multiplicity: 97% Location: duodenal n=30 pancreatic n=12 both n=8 Metastases: lnn: 43% All but 2 from duodenal primary n=2 concomitant somatostatinoma and insulinoma	NA	Surgery: all pts (n=34) DPR + enucl. + duodx + lnd	Recurrence/ metastases Biochemical cure Survival	Recurrence: n=4 Metastases: LM n=1 (wedge resection, after 1 yr disease-free) Lnn: NA Biochemical cure: gastrin 68% secretin 33% Survival: 5;10;15 yrs: 97;94;94% Deaths: MEN1 unrelated	4	Same centre, same author as Thompson 1995 A lot of missing data
Thompson NW 1995 Retrospective case series Clearly defined cases and intervention. Outcome poorly	n=26 MEN1 + PET n=17 gastrinoma Inclusion gastrinoma: no LM; with surgery 1978-1993 USA, Michigan Medical Centre	Size: NA Multiplicity: NA Location: Pancreas n=10 Duodenum n=13 Both n=6	Pts without diffuse LM	Surgery: n=17 Gastrinoma DPR n=13 Enucl. n=4 afterwards DPR n=2 for insulinoma	Metastases Cure Survival	LM: 0% biochemical cure: fasting gastrin: 65% negative secretin test: 29% Overall survival: 100%	4	a lot of missing data Same centre, same author as Thompson 1998.

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defined.	mean age: 36.7 yrs M:F 35:65% FU: 1-16 yrs	Lnn metastases: 46% (all duodenal primary)						
Tonelli 2006 Case series Clearly defined cases, intervention and outcome	n=16 MEN1 + PETs n=13 gastrinoma Italy 1992-2003, Florence mean age: 41 yrs (26-63) M:F 46:54% FU (all pts n=16): 6-108 mth	Size mm (range): 1-18 (5 NA) No. (range):1-7 Additional NF-PET n=12 range 2-40 Location: Pancreatic 0% Duodenal 93% Lnn: 7% Gallbladder: 7% Biliary confluence: 7% Metastases: Lnn n=6 LM n=1 Omental n=1	All hyper-gastrinemia	Surgery: all pts (n=13) Insulinoma + gastrinoma PPPD + enucl. n=1 DPR + enucl. + duodx n=1 Gastrinoma only: PPPD n=1 PD n=7 + tail resection n=1 + liver segment resection n=1 + liver metastectomy n=1 Extended PD n=2 + liver segment resection n=1 TP n=1	Complications (overall) Metastases Biochemical cure Survival	major postoperative complications 37% pancreatic fistula ± pancreatico-jejunal anastomosis leak n=4 abdominal abscess n=1 dehiscence n=1 late complications 31% IDDM + cholangitis n=3 malabsorption n=2 Lnn n=2 LM n=0 Biochemical cure: (fasting gastrin + secretin test) 77% (3-5 mth after surgery) Survival: 81% Disease related mortality: 0%	4	-

Abbreviations used in alphabetical order: BM bone metastases; CI confidence interval; DFS disease free survival; DM diabetes mellitus; DPR distal pancreatic resection; duodx duodenotomy; enucl. enucleation of tumour; FU follow-up; IDDM insulin dependent diabetes mellitus; IOUS intra-operative ultrasound; LM liver metastases; lnd lymph node dissection; lnn lymph nodes; LoE level of evidence; MEN1 multiple endocrine neoplasia type 1; M:F male: female; mth months; NA not available; NF non functioning; NIH National Institutes of Health; No. number; NS not significant; PD pancreaticoduodenectomy; PET pancreatic endocrine tumour; PPPD pylorus preserving pancreaticoduodenectomy; pts patients; RR relative risk; SD standard deviation; splen. splenectomy; THVS transhepatic selective venous gastric sampling; TP total pancreatectomy; USA United States of America; yr year; yrs years; ZES Zollinger Ellison Syndrome.

Appendix C. Non functioning tumours

Reference Study design and quality	Study population and characteristics	Tumour characteristics at baseline	Indications for surgery	Interventions and control conditions	Outcome category	Result primary outcome + statistics at follow-up	LoE	Comments
Part 1: evidence from controlled studies dealing soleley with non-functioning tumours								
Triponez 2006a	n=108 MEN1 + isolated NF-PETs	(mean ± SD, range) Size: 21 ± 18 mm, 3-90	NA	Pancreatic surgery n= 43 (40%)	Factors associated with	Size (mm) > 30 vs ≤ 10 (p< 0.01);	4	No clear indications for

Retrospective cohort Study quality: 1/8	France 1956-2003, GTE registry age (at MEN1 diagnosis): 36.2 ± 14 yrs M:F 35:65% FU (after diagnosis): 4.3 ± 3.3 yrs (0.1-16)	No.: 2.9 ± 2.3, 1-10 Location: NA Metastases: n=21 (19%): Lnn 14% LM 67% Lung 10% Other 10% Synchronous 15%		Curative n=32 (75%) Non-curative n=11 (25%) Type of surgery: PD n=5 (12%) DPR + enucl. n=7 (17%) DPR n=11 (26%) Subtotal pancreatectomy n=3 (7%) Enucl. n=9 (21%) biopsie ± intestinal and/or biliary derivation n=7 (17%) No surgery: n=65 (60%)	shorter survival Metastases (metachronous) Survival Average life expectancy	> 30 vs. 10-30 (p < 0.01). No difference ≤ 10 vs 10-30 (p=0.39) non-curative vs. curative surgery (p < 0.01). No difference none vs. curative surgery (p=0.15) Metastases 5% 8 yrs survival distant metastases 34% (95% CI 6-62%). No distant metastases 98% (95% CI 75-100%) NF-PET vs no PETs: 68.5 (95% CI 64-72.9) yrs vs 77.1 (95% CI 74.4-79.9) yrs (p < 0.01) NF-PET vs gastrinoma 68.5 vs 71.4 (p=0.99)		surgery no surgery group not comparable with curative surgery group
Triponez 2006b Retrospective cohort Study quality: 5/8	n=65 MEN1 + NF-PETs ≤ 20 mm France 1956-2003, GTE registry. surgery group age (at MEN1 diagnosis): 36.0 ± 15.4 yrs FU: 6.7 ± 4 yrs conservative group age (at MEN1 diagnosis): 35.6 ± 13.0 yrs FU: 3.3 ± 2.6 yrs FU differed significantly (p=0.001)	Surgery group n=15 Size: 14.1 mm ± 4 No.: 4.8 ± 2.2 Location: Head 27%, Body 7%, Tail 27%, combination 40% Metastases n=3 (20%) Lnn n=3 (20%) LM n=1 (7%) Conservative n=50 Size: 11.7 mm ± 4.2 No.: 2.7 ± 2.1 Location: Head 16%, Body 14%, Tail 14%, combination 34% NA 22% Metastases n=2 (4%) Lnn n=0 LM n=2 (4%) Overall	NA	Surgery n=15 Biopsie n=1 Enucl. n=5 PD + Ind n=1 DPR ± enucl. n=7 + Ind n=1 Subtotal pancreatectomy n=1 No surgery n=50 watchful waiting	Complications Recurrence/ disease progression Metastases (metachronous)	Complications: Mortality perioperatively 0% No statistical difference in disease progression or mortality between groups. Surgery group Recurrence n=5 (33%) Conservative group Disease progression: n=6 (16%) Surgical group: Pts with major resection more recurrence than pts with enucleation. No statistical measure. Metastases Surgery group n=15 LM n=1 (7%) Conservative n=50 LM n=1 (2%)	2b	groups at baseline not comparable

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		Multiplicity 80% Metastases surgery vs. conservative p=0.044)			Death	Surgery group n=2 disease related Conservative group n=1 other MEN1 related (NET thymus)		
Part 2: evidence from uncontrolled studies dealing solely with non-functioning tumours								
Sakurai 2007 Retropective case series No clear relationship between surgery and outcome	n=14 MEN1 + NF-PETS (n=26 NF-PETs) Japan 1995-2004, Shinshu University School of Medicine mean age: 48 yrs (29-59) M:F 50:50% FU: 78 mth (27-115)	NF-PET size (mean): Overall: 20 mm (range 5-78) Surgery group: 37 mm (15-78) Multiplicity: Surgery group: 3/7 Conservative group: 4/7 Location Head n=7 Body n=9 Tail n=10 Metastases: n=1 LM	NA	Surgery: n=8 for NF-PET n=6 2pts refused surgery; 1 was eventually operated after 9 yrs FU Subtotal pancreatectomy n=1 DPR n=3 DPR + enucl. n=1 Enucl. n=1 Enucl. n=1 for insulinoma PD n=1 for gastrinoma	Complications Tumour growth in pts with conservative treatment Recurrence and metastases (metachronous) Mortality	Impaired Glucose Tolerance 6/7 after pancreatectomy 1/7 after enucl. or no surgery Tumours <20 mm, no growth during FU 5/6 pts with tumours ≥ 35mm developed new NF-PETS or metastases 1/8 pts with tumours ≤ 25 mm developed new NF-PETS LM n=1 Mortality n=1 due to LM	4	No clear complication rates, indications for surgery and outcome not related to surgery
Part 3: extracted evidence from uncontrolled studies dealing with different types of PETs								
Bartsch 2005 Prospective case series Clearly defined cases, intervention and outcome	n=26 MEN1 + PET + surgery n=9 NF-PET + surgery Germany 1981-2004, Philipps-University Marburg mean age (NF-PET): 39 yrs (20-68) M:F (NF-PET) 56:44% FU (NF-PET)after operation: 43 mth (5-139)	Size(median) 31 mm (8-110) No.(median) 5 (1-11) Location: NA Metastases:n=1 lnn (11%)	No diffuse LM and NF-PET >10 mm	DPR n=9 + enucl. n=6	Complications (overall) Recurrence and metastases (metachronous)	Complications 52% after: PPPD n=4 Fistula n=3 DPR ± enucl. ± duodx n=17 Fistula n=8 Abscess n=2 Bleeding n=2 Enucl. ± duodx n=7 Fistula n=1 Abscess n=2 DM n=1 NF PET: Recurrence n=5 (56%) Reoperation n=3 local recurrence n=2 lung metastasis n=1	4	Good quality case series.

					Survival	No relationship tumour size and metastasis Overall survival n=24 (96%) disease related mortality 0%		
Thompson 1995 Retrospective case series Clearly defined cases, intervention. Outcome not clearly defined.	n=26 MEN1 + PET n=3 NF-PET Inclusion NF-PET: random selection USA University of Michigan. Mean age (NF-PET): 39 yrs (22-54) M:F (NF-PET) 33:67% FU (NF-PET): 2-3 yrs	Size: 30-130 mm Multiplicity: n=2 (67%) Location: neck + body n =1 duodenum + distal pancreas n=1 (concomitant gastrinoma) Head, body + tail n=1 Metastases: n=0	NA	Surgery: all pts NF-PET DPR ± enucl.: n=3 + Duodx/Ind n=1 (concomitant gastrinoma)	Recurrence and metastases (metachronous) Survival	NF-PET Recurrence + LM: n=1 (33%) Overall survival 100%	4	Selection bias NF-PETs a lot of missing data Same centre, same author as Thompson 1998.
Tonelli 2006 Case series Clearly defined cases, intervention and outcome	n=16 MEN1 + PET + surgery n=2 NF-PET + surgery Italy 1992-2003, University of Florence. age (NF-PET): 63 and 58 yrs M:F (NF-PET) 100:0% FU (all pts): 6-108 mth	Size (largest): 10 and 32 mm No.: 1-9 Location: NA Metastases (syn- or metachronous) Lnn: n=2 (100%) LM: n=1 (50%)	gastrin ↑ Insulinoma PET>10 mm	NF-PET PD, tail resection and liver resection: n=1 PPPD n=1	Complications (all pts) Cure Survival	major complications 37% pancreatic fistula and/or pancreatico-jejunal anastomosis leak n=4 abdominal abscess n=1 dehiscence n=1 late complications 31% IDDM + cholangitis n=3 malabsorption n=2 Cure NF-PET 50% Survival: 81% Disease related mortality: 0%	4	-

Abbreviations used in alphabetical order: CI confidence interval; DM diabetes mellitus; DPR distal pancreatic resection; duodx duodenotomy; enucl. enucleation; FU follow-up; GTE Le Groupe d'étude des Tumeurs Endocrines; IDDM insulin dependent diabetes mellitus; LM liver metastases; lnn lymph nodes; lnd lymph node dissection; LoE level of evidence; MEN1 multiple endocrine neoplasia type 1; M:F male: female; mth months; NA not available; NF non functioning; No. number; PD pancreaticoduodenectomy; PET pancreatic endocrine tumour; PPPD pylorus preserving pancreaticoduodenectomy; pts patients; SD standard deviation; USA United States of America; yrs years

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Appendix D. Combined tumours

Reference Study design and quality	Study population and characteristics	Tumour characteristics at baseline	Indications for surgery	Interventions and control conditions	Outcome category	Result primary outcome + statistics at follow-up	LoE	Comments
Part 1: evidence from controlled studies dealing with different types of PETs								
Kouvaraki 2006 Retrospective cohort study Study quality: 7/8	n=55 MEN1 and confirmed PETs USA MD Anderson Cancer Centre median age (at PET diagnosis) 37 yrs (8-69) M:F 51:49% median FU: 4.3 yrs (4mth-38yrs)	Tumour type: NF-PET n=20 (36%) Gastrinoma n=21 (38%) Insulinoma n=12 (22%) Glucagonoma n=2 (4%) median tumour size: 28mm (6-130) No.: multiplicity 71% single 24% unknown 5% Location: NA Metastases 13% LM n=5 (9%) lung n=1 (2%) bone n=2 (4%)	Indication for surgery: NA No surgery due to: Pt refusal n=4 Locally advanced PET n=1 Distant metastases n=10 Concomitant carcinoid n=2	Prognostic study; the effect of factors on OS and PFS is studied Surgery n=38 (69%) TP n=3 PD + enucl. n=4 DPR + enucl. n=27 Enucl. n=4 Wedge resection liver n=2 Duodx. (gastrinoma) n=9 (56%)	Complications Effect of factors on OS and PFS Recurrence Metastases Survival	IDDM n= 12 (34%; 35 pts at risk, 3 had IDDM prior to surgery) PD n=2/4, DPR n=8/27, TP n=2/3 Better OS: younger age (p 0.0003), functional tumours (p=0.037) no synchronous metastases (p=0.002) Decreased OS (pts without metastases, n=48) NF-PETs (p=0.021) no surgical resection (p=0.026) female sex (p=0.034) age at diagnosis (p=0.015) Type of surgery (resection vs. enucl.) independently associated with better PFS Recurrence (n=35 at risk) n=7 (20%) after DPR n=5/27 after enucl. n=2/4 Metastases (n=36 at risk) distant metastases n=5 (14%) OS: 10 yrs 75% (59-88%) PFS: 10 yrs 62% (43-80%) Death: n=16 Metastatic PET n=9 Metastastic NET (unclear origin) n=3 Metastatic NET thymus n=1	2b	-

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						NA n=2 Alive n=39 AWD n=12 ANED n=26 Lost to FU n=1		
Skogseid 1996 Prospective cohort study Study quality: 6/8	n=20 MEN1 + PET without evidence of metastases Sweden University Hospital Uppsala 2 cohorts: asympt. (n=8) mean age: 33 yrs mean FU: 6.0 yrs (0.5-11.0) M:F 63:37% sympt. (n=12) mean age: 51 yrs mean FU: 7.3 yrs (0.5-17.0) M:F 50:50%	Asymptomatic group: preoperatively: 87% raised stimulated PP 62% raised chromogranin A 12% raised basal gastrin Size: 9 mm (5-50) No.(mean): 5.6 duodenal tumours n=0 metastases: n=0 Symptomatic group: n=7 ZES n=3 hypoglycaemia n=1 WDHA-syndrome n=1 abdominal pain + weight loss No. (mean): 4.3 size: 11 mm (5-30) duodenal tumours: n=3 metastases: lnn n=3 LM n=2	All pts with increased levels of pancreatic endocrine tumour markers (2 independent markers on 2 separate occasions) without evidence of metastases	Asympt. group: DPR + splen. n=6 DPR + splen. + enucl. n=2 Duodx: n=4 Sympt. group: DPR n=11 + enucl. n=4 Enucl. n=1 Duodx n=6 All pts: IOUS	Complications Biochemical cure and recurrence Metastases Survival	complications: mortality 0% wound infection n=2 (10%) major n=5 (25%) (IDDM, intestinal perforation, mild pancreatitis, pseudocyst) Asymptomatic group Postoperative normalization tumour markers 5/8 (63%) asympt. recurrence 60% Cure 40% no metastases during FU Symptomatic group: Postoperative normalization n=6 (50%) recurrence 83% sympt. recurrence 50% no additional metastases during FU Asymptomatic group 100% Symptomatic group: 83% (17% death due to disease progression)	2b	Lead time bias. Same research group, same centre as Grama 1992
Part 2: evidence from uncontrolled studies dealing with different types of PETs								
Fendrich 2006 Retrospective case series Clearly defined cases, intervention and outcome	n=33 reoperation for PET n=9 MEN1 + recurrent PET and/or metastases Germany 1987-2004, Philipps-University, Marburg age (MEN1): 36 yrs	Tumour type: Insulinoma n=0 Gastrinoma n=5 (56%) NF-PET n=3 (33%) VIPoma n=1 (11%) Size (sporadic + MEN1) 39 mm (2-250) Metastases (MEN1): lnn metastases: n=1	Indication for reoperation: no tumour found; newly developed PETS or local/ distant metastases Indication for initial surgery:	Reoperative Surgery (if initial surgery failed) n=13 Reoperations: Metastasis resection Distant n=4 Lymph node n=1 pancreatic and/or duodenal resections: n=8	Complications	Sporadic and MEN1 combined; complication rate was similar Overall: 36% after 1st operation 57% after 2nd operation transient fistula 32% peri-operative mortality 4.8% (overall) 3% after 1st 29% after 3rd operation peri-operative mortality MEN 1, n=0	4	Outcome not related to type of surgery Same research group, same centre as Bartsch 2005.

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	mean FU (sporadic + MEN1): 124 mth (16-384) ZES: 142 mth (36-384) NF-PETS: 142 mth (16-236)	lung metastases: n=1	Gastrinomas All NF-PET >10 mm VIPoma All PET metastases Synchronous/ metachronous metastases without diffuse LM when >90% of metastatic disease can be resected	Initial operations (all including Ind): ZES: Duodx ± enucl., DPR ± enucl. VIPoma: Splenopancreatectomy NF-PET: DPR ± enucl.	Cure rate Survival	ZES 3/5 pts biochemically cured 5/5 new NF-PETs (<10 mm) development, no LM NF-PET 1/3 pts cured, no LM VIPoma: developed LM; 3x resection of LM Distant metastasis resection lung, n=1 free of detectable disease overall survival MEN1: 100%		
Gauger 2003 Retrospective case series Clearly defined cases, intervention and outcome	n=15 MEN1 no symptoms of PET USA 1994-2002, University of Michigan median age: 29 yrs (15-63) M:F 47:53% FU: 50 mth (2-95)	Tumour type: Gastrinoma and NF-PET raised gastrin n=9 EUS: PETs n=14 mean size largest: 21 mm (6-65) Multiplicity n=12 No. (median): 3 (1-5) Location: Distal 60% Head and uncinate process 23% Body 17% Lnn metastases n=1 (7%)	NA; Raised serum gastrin → + duodx	Studied intervention is EUS operations n=13 (87%) DPR + splen. n=12 (92%) + enucl. n=9 PPPD + limited DPR n=1	Complications Biochemical cure Metastases Survival	Complications wound infection n=1 (8%) fistula n=5 (38%) biliary fistula n=1 (8%) recurrent raised gastrin 100% metastases n=0 overall survival 100% loss to FU n=1	4	Other intervention than surgery studied
Grama 1992 Retrospective case series Clearly defined cases, intervention and outcome	n=33 MEN1 + PET Sweden ≥ 1973, multicentre mean age: 44 yrs (13-65) M:F 48-52%	Insulinoma n=7 (21%) Size largest: 25 mm (10-40) Multiplicity: 43% Metastases: 0% Gastrinoma, n=19 (58%) Size largest: 20 mm (7-80) Multiplicity: 71% Metasteses: 37%	NA Insulinomas all Gastrinomas Contraindica-tions: Diffuse LM, no tumour found	Surgery n=27 tail resection n=8 + enucl. n=2 DPR n=10 Whipple n=3 Enucl. n=6 Combined with:	Complications Cure rate	Complications Morbidity n=5 (19%) Pancreatic fistula/abscess n=3 Duodenojejunal perforation n=1 Leaking anastomosis n=1 Insulinoma Insulinoma cure n=7 (100%) Relapse pancreatic tumour markers	4	Indications for surgery not clearly described Outcome not related to type of surgery

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	mean FU: 8 yrs (1-16)	lnn n=3 LM n=3 both n=1 NF-PET n=7 (21%) Size largest: 67 mm (10-200) Multiplicity: 57% Metastases: 43% LM + Lnn n=2 LM 1 Location overall: Distal 80% Head 20%	NF-PET Large tumour 200 mm not operated on	Wedge resection liver n=2 Lnd n=3 Duodx n=5	Metachronous metastases Survival	n=2 LM + ZES n=1 Survival: 100% Gastrinoma Cure postoperative n=8 (57%), 100% relapse Hyperinsulinism n=2 (14%) LM n=2 (14%) Survival n=14 (74%) Death due to progressive disease with LM n=5 NF-PET LM n=1 (14%) Survival n=3 (42%) Death due to progressive disease with LM n=4		Same research group, same centre as Skogseid 1996
Lairmore 2000 Retrospective case series Clearly defined cases, intervention and outcome	n=21 MEN1, PET + surgical treatment USA 1993-1999, Washington University School of Medicine, Missouri mean age (at surgery): 44.5 yrs (19-73) M:F 48:52% FU (mth) after: Enucl.: 25 PD: 34 Non Whipple: 18	Tumour type: NF-PET + PPoma: n=15 Insulinoma: n=3 Gastrinoma: n=1 VIPoma: n=1 Mean size: 18 mm (1-80mm) No.: NA Location: head: 21% neck: 6.3% uncinate process 10% body: 29% tail: 25% duodenal wall 4% Metastases lnn 7 (33%) LM/distant 2 (9.5%)	Tumours ≥ 10 mm or functioning tumours	DPR ± enucl. n=11 PD ± enucl. n=5 Enucl. n=5 + duodx in ZES + lnd in ZES / malignancy LM: wedge resection liver n=2 cure n=1 diagnosis n=1	Complications Tumoursize vs metastases Recurrence Survival	DPR n=11 Pancreatic/bile leak n=1 Abscess n=1 Wound infection n=1 PD n=5 Pancreatic/bile leak n=2 Abscess n=1 Wound infection n=1 Respiratory failure n=1 Death n=1 Enucl. n=5 Abscess n=1 Transfusion RBC n=1 Other major complications n=2 NS: association between size and metastases at surgery Recurrence n=2 After PD 1/5 After enucl. 1/5 Overall survival 19/21 (90%) Perioperative death n=1 Unrelated death n=1	4	-

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Ligneau 2001 Retrospective case series Clearly defined cases, intervention and outcome	n=10 pts with cystic PETs n=6 MEN1 + cystic PETs + surgery France 1982-1999, Hôpital Edouard-Herriot mean age: 39 yrs (20-56) M:F 83:17% mean FU + range: 38 mth (15-85)	Tumour type: gastrinoma n=3 glucagonoma n=1 insulinoma n=1 NF-PET n=1 somatostatinoma n=1 mean size largest (cystic PETs): 45 mm (10-100) mean no. (including solid): 4 (2-12) location (cystic PET): head n=1 body n=2 tail n=5 metastases: lnn n=2 LM n=0	NA	DPR n=4 PD n=1 TP n=1	Recurrence Metastases Survival	Recurrence n=1 after PD Metachronous metastases LM/distant n=1 Survival 5/6 (83%) 1 death after LM	4	Although solid tumours were present, they were not included in the analysis.
Lowney 1998 Retrospective case series Clearly defined cases, intervention and outcome	n=48 MEN1 + PET USA 1969-1998, Washington University School of Medicine, Missouri median age (at diagnosis): 41.7 yrs (6-72) M:F 48-52% median FU (since operation/diagnosis): 3.1 yrs (0.3- 28)	Tumour type: NF-PET 50% Gastrinoma 25% Insulinoma 21% VIPoma 4% Size: <10 mm: n=9 10-20 mm: n=18 >20 mm: n=23 No.: NA Location: NA Metastases: Lnn n=11 (23%) LM n=2 (4%)	NA	Surgery n=43 (90%) DPR n=27 PD n=7 Enucl. n=8 Lap. DPR n=1 33% of gastrinoma had duodx	Relationship tumour size, tumour type and metastatic potential Recurrence/ Metastases	Metastatic rate at baseline per tumour size (imaging): <10 mm 25% 10-20 mm 33% >20 mm 28% pathological size <10 mm 33% 10-20 mm 11% >20 mm 35% relationship tumour size and metastases NS. Metastatic rate at baseline per tumour type Gastrinoma 42% Insulinoma 17% NF-PET 21% VIPoma 100% Recurrence n=4 reoperation n=2	4	Publication from same study group and medical centre; Lairmore 2000

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					Survival	LM metachronous n=1 (insulinoma) Survival n=46 (96%) death: unrelated causes		

Abbreviations used in alphabetical order: ANED alive no evidence of disease; asympt. asymptomatic; AWD alive with disease; DPR distal pancreatic resection; duodx duodenotomy; enucl. enucleation; EUS endoscopic ultrasound; FU follow-up; IDDM insulin dependent diabetes mellitus; IOUS intraoperative ultrasound; Lap. laparoscopic; LM liver metastases; Ind lymph node dissection; Inn lymph nodes; LoE level of evidence; MEN1 multiple endocrine neoplasia type 1; M:F male: female; mth months; NA not available; NET neuro-endocrine tumour; NF non functioning; No. number; NS not significant; OS overall survival; PD pancreaticoduodenectomy; PET pancreatic endocrine tumour; PFS progression free survival; PP pancreatic polypeptide; pt patients; pts patients; splen. splenectomy; sympt. symptomatic; TP total pancreatectomy USA United States of America; VIP vasoactive intestinal peptide; WHDA watery diarrhea hypokalemia achlorhydria; yrs years; ZES Zollinger Ellison Syndrome

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