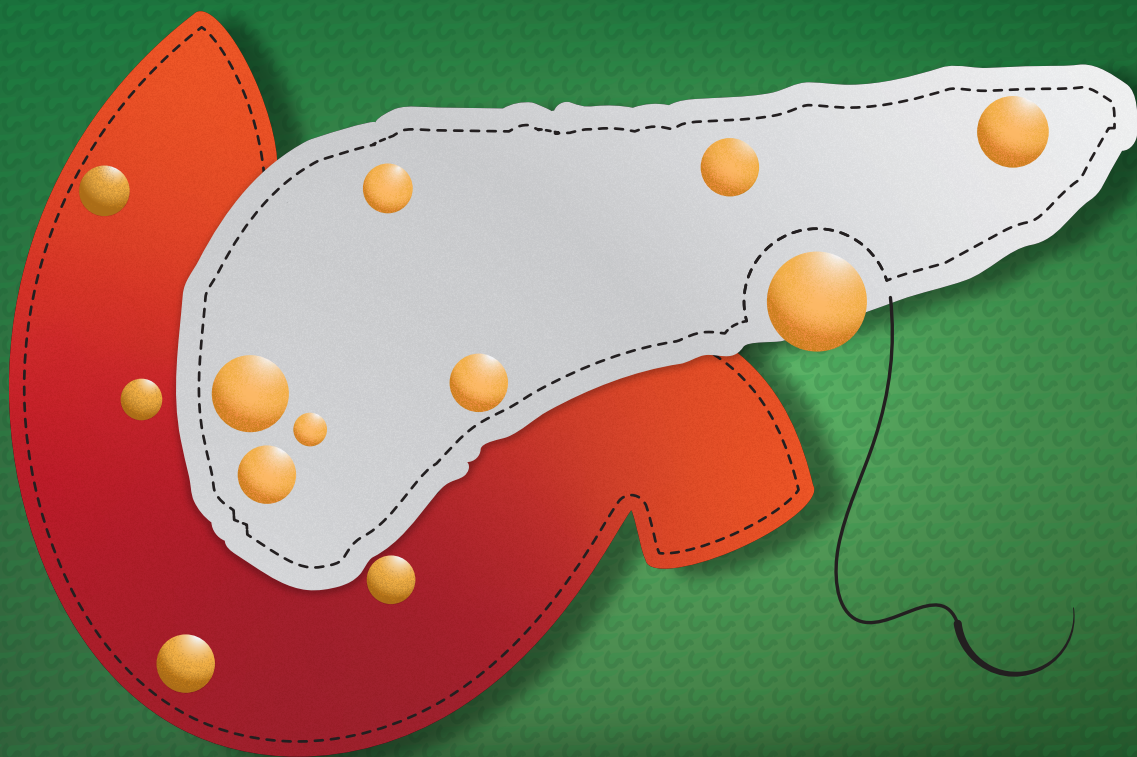


**TAILOR-MADE TREATMENT OF PATIENTS WITH
MULTIPLE ENDOCRINE NEOPLASIA
TYPE 1-RELATED
DUODENOPANCREATIC
NEUROENDOCRINE TUMORS**



DIRK-JAN VAN BEEK

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Tailor-made treatment of patients with multiple endocrine neoplasia type 1-related duodenopancreatic neuroendocrine tumors

PhD thesis, Utrecht University, The Netherlands

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Op maat gesneden behandeling van patiënten met multiële endocriene neoplasie type
1-gerelateerde neuro-endocriene duodenum- en pancreastumoren
(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. H.R.B.M. Kummeling,
ingevolge het besluit van het college voor promoties
in het openbaar te verdedigen op

donderdag 2 juni 2022 des middags te 4.15 uur

door

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geboren op 10 augustus 1992
te Rotterdam

Promotoren

Prof. dr. M.R. Vriens

Prof. dr. G.D. Valk

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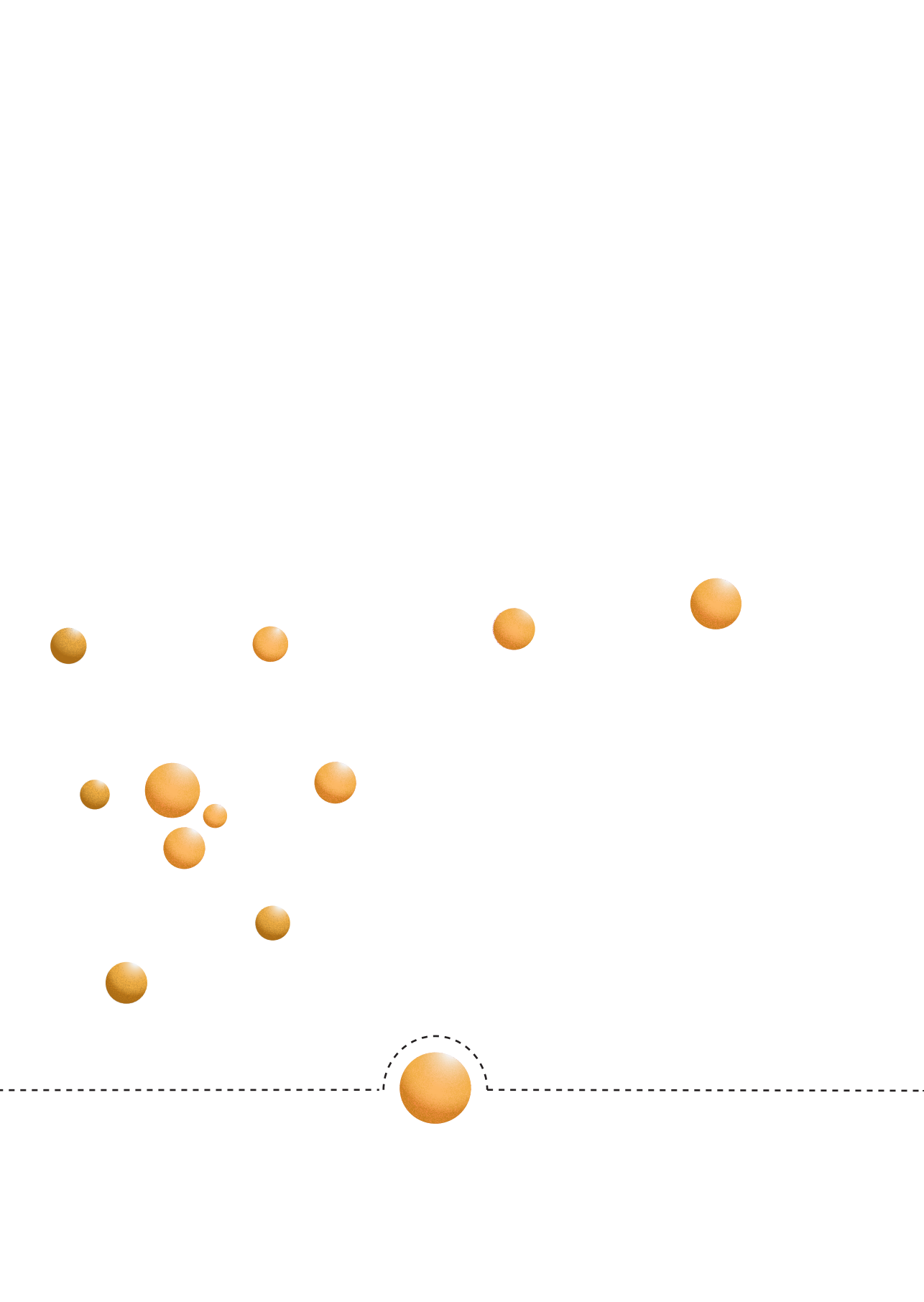
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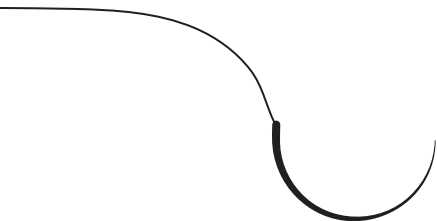
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CHAPTER I

General introduction and outline of this thesis



The neuroendocrine cells of the pancreas – the pancreatic islets – were discovered in 1869 by Paul Langerhans.¹ A simple benign tumor, or so called adenoma, arising from an islet of Langerhans was first reported by George Nicholls in 1902.² In 1907, Siegfried Oberdorfer was the first to identify a neuroendocrine tumor (NET).³ Pancreatic NETs (pNETs) are rare with an estimated incidence of less than 1 per 100,000 in the United States of America and European countries including the Netherlands.^{5–9} Less than 10% of the pNETs are associated with a hereditary syndrome.^{11,12} These hereditary syndromes include multiple endocrine neoplasia type 1 (MEN1), von Hippel-Lindau (VHL), Tuberous Sclerosis complex and neurofibromatosis complex type 1.^{11–13} The majority of the familial tumors occur in the setting of MEN1, which will form the body of this thesis.¹³

Multiple endocrine neoplasia type 1 (MEN1)

The simultaneous occurrence of primary hyperparathyroidism caused by parathyroid adenomas, duodenopancreatic neuroendocrine tumors (dpNETs), and/or pituitary adenomas was first described by Erdheim in 1903 and thereafter reported in several case reports during the first half of the 20th century.^{14–17} Nevertheless, it took until 1954 before it was Paul Wermer to describe multiple cases within two succeeding generations of a single family, thereby proving evidence for a familial occurrence.¹⁸ The syndrome was subsequently named Wermer syndrome after its founder, but is nowadays widely known and recognized as MEN1.

Multiple endocrine neoplasia type 1 is an autosomal dominant tumor syndrome, which implies that 50% of the offspring of patients with MEN1 will be affected.¹⁹ The syndrome is very rare with an estimated prevalence of 1 to 10 per 100,000 people.²⁰ The underlying gene encoding for MEN1 was mapped to chromosome 11q13 in 1988 and 1989, and the *MEN1* gene was identified in 1997.^{20–24} Since the discovery of the gene, hundreds of mutations have been identified.²⁵ The *MEN1* gene consists of 10 exons that encode the 610-amino acid protein product named menin.²⁰ Menin is thought to interact with proteins that are involved in cell transcription, genome stability, cell division and proliferation.^{25–27} The *MEN1* gene functions as a tumor suppressor gene, indicating that tumor formation follows Knudson's two-hit hypothesis – a germline mutation with an additional second somatic mutation leading to inactivation of the wild-type allele and thus loss of heterozygosity involving chromosome 11q13.^{26,28–30} The identification of the *MEN1* gene has offered the opportunity to perform early genetical testing after birth and to reassure those without MEN1. In those with the disease, early diagnosis and subsequent timely treatment of MEN1-related manifestation can be performed to reduce morbidity and mortality.^{31,32} Nevertheless, no genotype-phenotype correlation has been established up to today and therefore screening programs cannot be tailored for individual patients.

Clinically, the classical triad of MEN1 includes parathyroid adenomas, dpNETs and pituitary adenomas. The prevalence of these manifestations ranges from 77–93%, 41–57% and 30–48% and are 87%, 56% and 44%, in the Netherlands.^{19,33–37} Besides, patients have an increased risk for NETs of thymic, bronchial or gastric origin, adrenal tumors, skin and subcutaneous tumors, smooth muscle tumors and breast cancer.^{34,38} Patients with MEN1 have a reduced life expectancy of approximately 10 years as compared to the general population.³⁷ The decrease in life expectancy is primarily caused by metastases of dpNETs and therefore, increased understanding of these tumors will improve outcomes for patients with MEN1.

This thesis will elaborate on research strategies for rare diseases (Part I) and the diagnosis (Part II), prognosis (Part III) and surgical therapy (Part IV) for dpNETs in patients with MEN1.

Research strategies in rare diseases

A disease is considered to be rare if the prevalence is less than 1 in 2000 of the population in Europe, which is comparable to most definitions applied worldwide.³⁹ Worldwide, an estimated total number of more than 300 million patients is affected by one of the 6000 registered rare diseases.⁴⁰ Rare diseases frequently have a genetic cause, which leads to symptom and disease manifestation in childhood. In addition, most patients cannot be cured and therefore treatment should be aimed at increasing life expectancy and optimizing the quality of life of patients affected by such a disease. However, the medical community generally lacks facilities, resources, general knowledge and therapeutic options for rare diseases.⁴¹ MEN1 is an example of a hereditary rare disease with a lack of resources and understanding of the disease.

At the beginning of the 21st century several single and small multicenter studies aimed to address specific clinical topics in MEN1.^{19,33,42–49} However, confronted by a deficit of medical and scientific knowledge in MEN1, the DutchMEN Study Group (DMSG) was initiated in 2008. The DMSG is a collaboration between all University Medical Centers in the Netherlands and the MEN1 patient advocacy group. At the start, the aim of the DMSG was to provide evidence-based answers to clinical questions related to patients with MEN1. Conducting randomized controlled trials to answer therapeutic questions is almost impossible due to the low number of eligible patients for inclusion as well as the low yearly incidence of events in individual patients. Cohort studies, although prone to various forms of bias such as selection bias, information bias and confounding by indication, are the next best level of evidence.^{50,51} In general, population-based cohort studies overcome limitations of single center cohort studies, but have specific challenges.⁵² Nevertheless, for rare diseases, research methods for observational are far less developed.⁵³ **Chapter 2** provides an overview and stepwise approach on research strategies and observational study methods for rare

diseases, guided by examples on MEN1, to come to meaningful answers for clinical questions. This chapter describes potential pitfalls and challenges and subsequent methods to handle these in detail during different study phases.

Duodenopancreatic neuroendocrine tumors in MEN1

Metastasized dpNETs are the leading cause of death in MEN1 and significantly reduce life expectancy.^{34,37,54} These tumors represent a heterogeneous group regarding symptomatology, localization, risk of malignancy and prognosis.²⁶ The age-related penetrance reaches 80% by the age of 80.^{37,47} A subgroup of tumors excessively produces hormones leading to distinct clinical syndromes, which are referred to as functioning pNETs, whereas others do not produce hormones and are considered as non-functioning (NF-pNETs). Duodenal gastrinomas and pancreatic insulinomas are the most frequently observed functioning tumors. Overall, NF-pNETs are the most prevalent.²⁶ Due to the mutation in the *MEN1* gene, patients develop multiple tumors throughout the duodenum and pancreas complicating clinical management. Therapy should be aimed at maintaining a good quality of life by relieving symptoms associated with excessive hormone production as well as preventing liver metastases.³² Surgical resection is the cornerstone of curative therapy, but is not indicated in all patients since the majority of tumors follows an indolent course.³² At present, MEN1 clinical practice guidelines, European Neuroendocrine Tumor Society (ENETS) guidelines and North American Neuroendocrine Tumor Society (NANETS) guidelines are used in clinical practice.^{32,55,56} The present recommendations for the treatment of MEN1-related dpNETs in these guidelines are presented in Table 1. Treatment decisions may differ substantially based on the choice of guideline.

Diagnosis

Considering the high age-related penetrance and potential malignant degradation of dpNETs in MEN1, screening is aimed at early diagnosis and subsequent timely initiation of treatment.³² In this respect, MEN1 clinical practice guidelines recommend annual biochemical plasma screening including gastro-intestinal hormones and suggest annual imaging of the pancreas and duodenum by computed tomography (CT), magnetic resonance imaging (MRI), or endoscopic ultrasound (EUS).³² Non-functioning pNETs are the most frequently diagnosed dpNETs within the screening program. Due to their malignant potential, an evidence-based protocol for diagnosis and follow-up of NF-pNETs is demanded. In addition, functional imaging – somatostatin receptor scintigraphy (SRS) and [gallium 68 octreotate (68Ga)]-labeled somatostatin analogs positron emission tomography (PET-CT) – is emerging and is increasingly available. However, its added value in the screening program is unknown. In **Chapter 3**, the literature was systematically reviewed and critically appraised regarding the added value of tumor markers and imaging modalities for the diagnosis of NF-pNETs

Table 1. Guideline recommendations for the management of MEN1-related dpNETs

	MEN1 2012 ³²	ENETS 2016 ⁵⁵	NANETS 2020 ⁵⁶
NF-pNET	Consider surgery for tumors that are more than 1 cm in size and/or demonstrate significant growth over 6–12 months.	Routine surgical exploration not generally recommended for pNETs ≤2 cm or NF-pNETs on imaging studies. In patients with pNETs >2 cm, enucleation/local resection at surgery is possible in many patients, while pancreatoduodenectomy is reserved for specific selected cases.	NF-pNETs smaller than 1 cm can be observed while tumors larger than 2 cm should generally be resected.
Insulinoma	Surgery.	Surgery when metastatic disease is not present. Enucleation or limited resection remain the procedure of choice.	Surgery when possible and a dominant lesion.
Gastrinoma	Medical management (proton pump inhibitor) in the majority of patients. Surgery may be considered in experienced centers, since surgery might improve the cure rate. Whipple pancreatoduodenectomy is not suggested for the majority of patients because of an increased operative mortality and long-term morbidity.	Routine surgical exploration not generally recommended for pNETs ≤2 cm or NF-pNETs on imaging studies. In patients with pNETs >2 cm, enucleation/local resection at surgery is possible in many patients, while pancreatoduodenectomy is reserved for specific selected cases.	Medical management may be considered in many cases of gastrinomas. Surgical resection for MEN1 patients with hypergastrinemia may be most reasonable in patients with lymph node metastases, poorly controlled symptoms, or in those with pNET-dominant disease.

Abbreviations: *dpNET* duodenopancreatic neuroendocrine tumor, *ENETS* European Neuroendocrine Tumor Society, *MEN1* multiple endocrine neoplasia type 1, *NANETS* North American Neuroendocrine Tumor Society, *NF-pNET* non-functioning pancreatic neuroendocrine tumor, *pNET* pancreatic neuroendocrine tumor

in MEN1. In addition, a systematic review was performed with the aim to assess the growth rate of MEN1-related NF-pNETs and to establish their age-related penetrance.

Imaging

In light of the imaging-based screening program, the 2001 and 2012 clinical practice guidelines suggested imaging once every three years and annually respectively.^{31,32} However, both of these guidelines lack a recommendation regarding the preferred imaging modality. Ideally, the choice for an imaging modality is guided by the diagnostic accuracy of pNETs as well as local lymph node and liver metastases, the precision of tumor size estimation, invasiveness, side-effects and costs. Furthermore, once a lesion suspicious for a MEN1-related pNET is identified, the clinical question remains as to whether histopathological tumor confirmation is demanded before patients are referred for operative resection or a watchful waiting strategy is initiated.

The ENETS guidelines suggest EUS-guided biopsies in patients with sporadic NF-pNETs, but no specific recommendations are presented for MEN1.¹³ A recent consensus report lacked a conclusion on if and when to perform EUS-guided biopsies for MEN1-related

pNETs.¹⁴ **Chapter 4** assesses the use and overall diagnostic accuracy of pancreatic imaging studies and the added value of pancreatic fine needle aspirations (FNA) for the diagnosis of MEN1-related PanNETs in a population-based cohort. In addition, **Chapter 4** estimates the diagnostic accuracies of MRI versus CT in a contemporary cohort.

Imaging-based tumor size

Apart from the diagnosis of NF-pNETs, surgical indications for NF-pNETs are primarily based on tumor size (Table 1). Most NF-pNETs smaller than 2 cm have an indolent natural course and can be safely managed without surgery.^{57–60} Therefore, the 2 cm cut-off is frequently used for surgical decision making in clinical practice nowadays.^{55,56} Pancreatic surgery, however, is associated with a substantial risk of complications.⁶¹ This underscores the importance of an accurate size estimation; underestimation of tumor size may lead to a prolonged period of watchful waiting, whereas overestimation may hypothetically lead to unnecessary surgery with the associated risk of complications. A single center study from an expert center reported that preoperative tumor size of MEN1-related pNETs was frequently overestimated.⁶² In the debate on the preferred imaging modality (CT, MRI or EUS), the ability to accurately determine tumor size should be taken into account. **Chapter 5** describes the reliability and agreement of radiological and pathological tumor size of pNETs in patients with MEN1 in a population-based cohort.

Gastrinomas

Peptic ulcerative disease leading to gastrointestinal bleeding was another major clinical manifestation within the mosaic of endocrinopathies occurring within MEN1. In 1955, the surgeons Zollinger and Ellison linked the concurrent existence of gastric hypersecretion, peptic ulcerative disease in atypical locations, and islet cell tumors of the pancreas.⁶³ This syndrome was named the Zollinger-Ellison Syndrome (ZES) and these gastrin-producing tumors were referred to as gastrinomas. Gastrinomas were thought to arise from the gastrinoma triangle – an anatomic triangle defined by the junction of the cystic and common bile duct superiorly, the junction of the second and third portions of the duodenum inferiorly, and the junction of the neck and body of the pancreas medially.⁶⁴ The original description of the ZES – a pNET, peptic ulcerative disease and hypergastrinemia – was challenged in 1990 since histopathological studies observed that gastrinomas in patients with MEN1 generally arise from the submucosa of the duodenum and more rarely from the pancreas.^{65–68} Moreover, gastrinoma precursor lesions were found in the duodenum.^{69–71} Besides the duodenal gastrinomas, patients are affected by diffuse background adenomatosis of the pancreas.^{49,72–74}

Proton pump inhibitors (PPIs) and Histamine Type-2 Receptor Antagonists in the 1970's changed the perspectives of patients with MEN1-related gastrinomas. Their widespread

use decreased the number of deaths caused by ZES-related complications and caused a shift towards deaths attributed to the malignant degradation of dpNETs.^{34,42,43,54,75} Altogether, the predominant duodenal origin altered the view on the surgical management of patients with MEN1-related gastrinomas, such that patients would at least need a duodenal exploration – i.e., duodenotomy with local excision of submucosal gastrinomas.^{45,49,76,77} Nevertheless, the latter approach leads to a high rate of persistent or recurrent hypergastrinemia.^{78,79} Therefore, (total) duodenectomy is needed to achieve biochemical cure.^{80,81}

Considering the high prevalence of gastrinomas in patients with MEN1, the shift from gastric acid hypersecretion-related complications to the malignant nature of dpNETs as cause of death in MEN1 and the need for major surgery, more in-depth knowledge on the natural history and prognostic factors for survival of gastrinomas in patients with MEN1 is demanded. Several studies reported 10-year survival rates of 88% to 100% regardless of therapy.^{45,80,82–84} However, the majority of these studies are derived from a single expert center, the National Institute of Health and a multicenter cohort study from France which included patients from 1959 until 1995, long before the widespread use of PPIs.^{78,82,83,85,86} Although the disease-specific survival has been reported to be excellent, a subgroup of patients with an aggressive disease course has been described.⁸² Higher fasting serum gastrin levels have been reported in MEN1 in patients with more extensive burden disease.^{45,54} Moreover, in sporadically occurring gastrinomas, baseline fasting serum gastrin levels provided valuable prognostic information.⁸⁵ **Chapter 6** presents survival and prognostic factors, including fasting serum gastrin, for survival within the Dutch population-based cohort of patients with MEN1-related gastrinomas.

Metastatic Patterns

The high diagnostic accuracy of ⁶⁸Ga-Dota PET/CT has increased the detection rate of peripancreatic lymph node metastasis.⁸⁷ As patients with MEN1 usually have multiple dpNETs, the origin metastasis is often unknown. Anticipated metastatic patterns could aid surgical decision making regarding the procedure of choice and the extent of surgery. In addition to multifocal radiological disease, histopathological duodenopancreatic resection specimens often contain multiple pNETs, many microadenomas (<0.5cm) and minute and small duodenal gastrinomas.^{72,73} In these patients, appropriate staging according to Tumor, Node and Metastasis (TNM) staging systems is impossible if primary tumors and metastasis cannot be reliably related. The latter limits adequate prognostication of patients with MEN1. To gain explore metastatic patterns of multifocal MEN1-related dpNETs, we investigated the relatedness of 137 primary dpNETs and microadenomas and 36 matched locoregional and distant metastases of 10 patients with MEN1 in **Chapter 7**.

Tumor biology

Metastasized dpNETs are the leading cause of death.^{34,37,54} Tumor size is still regarded as the single and most important prognostic factor.^{47,58–60} Pancreatic neuroendocrine tumors in MEN1 are thought to arise from the pancreatic islets, although the ductal epithelium has also been reported as origin.^{88,89} Loss of heterozygosity of the wild-type MEN1 allele is observed in pNETs, microadenomas and mono-hormonal endocrine cell clusters.⁸⁸ Although it is generally assumed that pNETs share a common origin, different survival rates are reported between patients with a NF-pNET and those with an insulinoma.^{34,60,90} The assumption that insulinomas have a more favorable prognosis as compared to patients with NF-pNETs is assigned to a relatively small tumor size, early symptomatology with subsequent treatment, or because of better differentiated tumors.⁹¹ However, at present no studies have assessed a potential difference in survival between patients with MEN1-related NF-pNETs and those with insulinomas, also taking important prognostic factors such as tumor size and tumor differentiation into account. In **Chapter 8** an international, multicenter study was performed including patients with a resected MEN1-related NF-pNET or insulinoma – thereby providing histopathological data regarding size and differentiation – to assess whether the prognosis differs between these groups. In addition, survival and factors associated with liver metastases-free survival were assessed to come to meaningful advice regarding post-operative counseling and follow-up specifically for NF-pNETs and insulinomas.

Insulinomas

Insulinomas are the most frequently occurring functioning pNETs in MEN1 and approximately 10–15% of patients will be affected.^{26,37,92} These insulin-producing tumors lead to life-threatening hypoglycemia. The combined occurrence of signs or symptoms of hypoglycemia with concomitant biochemical endogenous hyperinsulinemic hypoglycemia and relief of symptoms after glucose administration is known as Whipple's triad, which is pathognomonic of insulinomas.⁹³ Whipple's triad is ideally triggered and confirmed during a 72-hour supervised fast test.^{94,95}

Surgical resection is regarded as the only curative therapy.^{32,55,56} Localization of the insulinoma(s) is challenging considering the multifocality of pNET in MEN1. Therefore, more extensive resections were initially proposed, such as a 80% resection of the pancreas to the left of the superior mesenteric/portal vein with subsequent enucleations of pNETs in the pancreatic head.^{92,96} Although persistent and recurrent hypoglycemia seem uncommon after this aggressive approach, the procedure is associated with pancreatic insufficiency.^{92,96,97} An insulinoma often is the first manifestation of MEN1 and a common surgical indication in children and adolescents with MEN1, which hampers the preservation of long-term pancreatic function.^{98–101}

Guidelines lack evidence-based recommendations regarding the extent of surgery. Persistence and recurrence of hypoglycemia have been reported in 25 – 50% after an

enucleation and in 2.6 – 20% after extensive resections.^{49,97,102} However, most studies were limited by a single center design, relatively low sample size and patients were often included more than 30 years ago, thereby hampering comparisons between surgical strategies.^{49,96,97,102–104} Although insulinomas occur in approximately 1 out of 10 patients with MEN1, the annual number of patients undergoing surgical resection is very low. Single center studies or even population-based cohort studies will lack statistical power. To overcome limitations others have faced, the International MEN1 Insulinoma Study Group was founded to allow a large multicenter cohort study including 46 hospitals from the Netherlands (DMSG), France, Germany, Italy and North America. **Chapter 9** presents the procedure-specific long-term risk of insulinoma recurrence after surgery for MEN1-related insulinomas in a comprehensive international cohort.

Duodenopancreatic surgical procedures

Portraying the increasing incidence of pNETs, the number of pancreatic surgeries for pNETs performed annually has increased over the past decades in high-volume centers from Europe and the United States of America.^{105,106} Nevertheless, less than 1 out of 10 pancreatic operations is performed for a pNET in the Netherlands.¹⁰⁷ Atypical resections, such as enucleations, are a feasible and safe technique for pNETs. The most frequently performed procedures for solitary pNETs are listed below:

- Enucleations: the local resection of a pNET without concomitant resection of (healthy) surrounding pancreatic tissue.
- Distal pancreatectomy: a resection of the left side/the tail (and sometimes body) of the pancreas. The procedure can be performed with simultaneous resection of the spleen or as a spleen-preserving distal pancreatectomy.
- Whipple or pylorus-preserving pancreatoduodenectomy (PPPD): for tumors located in the pancreatic head that are technically unfeasible for an enucleation or within the duodenum, a pancreatic head resection is demanded. The pancreatic head, duodenum, gallbladder, part of the bile duct and, in case of a classical Whipple, also the pylorus of the stomach are resected. Anastomoses are formed between the small bowel and (1) main pancreatic duct – the pancreaticojejunostomy; (2) bile duct – the hepaticojejunostomy; (3) stomach – the gastrojejunostomy.
- Total pancreatectomy: a complete resection of the entire pancreas, with or without total duodenectomy by definition leading to endocrine and exocrine pancreatic insufficiency.

Due to the multifocality of dpNET in MEN1, extensive procedures such as the Thompson procedure – i.e., distal pancreatectomy to the level of the superior mesenteric vein, enucleation of tumors in the pancreatic head, duodenotomy with local excision of tumors in the duodenum and peripancreatic lymph node dissection – were proposed to achieve cure and

reduce the risk of recurrence.⁶⁷ Nowadays, combined resections, such as multiple enucleations, a distal pancreatectomy plus enucleation, a Whipple/PPPD plus enucleation or a Whipple/PPPD plus distal pancreatectomy, are frequently performed.

The choice of surgical procedure for sporadic, but particularly MEN1-related dpNETs, should involve:

- Radical tumor resection;
- Prevention of clinically relevant tumor recurrence;
- Maintenance of pancreatic function;
- Correction of any tumor-related symptoms;
- Minimize procedure dependent morbidity and mortality.⁴⁹

Surgical morbidity and mortality

Pancreatic surgery is associated with a mortality rate of 3.6% in the Netherlands.¹⁰⁷ Mortality is related to annual center volume.^{108,109} After the nationwide centralization and required annual case load mortality decreased in the Netherlands.¹⁰⁸ For example, in 21 international expert centers mortality was 1.6% after ‘low-risk’ pancreatoduodenectomies.¹¹⁰ Although mortality rates have decreased due to centralization, these high risk procedures are associated with high rates of morbidity.¹¹⁰

Historically, complications after surgical procedures were impossible to compare due to differences in surgical populations, but most importantly due to a lack of systematic assessment and grading of complications which could be compared between studies. In 2004, Clavien and Dindo developed the first classification for complications, which ranks complications by severity based on the treatment applied, and captures complications within five grades (Table 2).¹¹¹ The classification was tested in 6336 patients and proved to be simple, reproducible, logical, useful and comprehensive and is nowadays widely accepted and increasingly being used.^{111,112} The system was originally developed to grade the severity of the single most severe complication. However, patients might develop multiple complications of lesser severity, which might contribute to an overall higher cumulative burden of the ‘lesser’ complications than one single ‘more severe’ complication. From this respect the cumulative complication index (CCI) was developed and validated to calculate the cumulative burden of complications by adding up every single complication, weighted for their respective severity.^{113,114}

Despite the developments in the classification of general surgical complications, complications of pancreatic surgery specific complications could not be sufficiently classified. Therefore, the International Study Group of Pancreatic Surgery (ISGPS) and the International Study Group of Liver Surgery (ISGLS) developed definitions and classifications for pancreatic surgery-associated complications. The most common pancreatic surgery-specific complications include:

Table 2. Clavien-Dindo classification of surgical complications and respective weights of the comprehensive complications index^{111,113}

Grade	Definition	CCI Weight*
Grade I	Any deviation from the normal postoperative course without the need for pharmacological treatment or surgical, endoscopic, and radiological interventions. Allowed therapeutic regimens are: drugs as antiemetics, antipyretics, analgesics, diuretics, electrolytes, and physiotherapy. This grade also includes wound infections opened at the bedside	300
Grade II	Requiring pharmacological treatment with drugs other than such allowed for grade I complications Blood transfusions and total parenteral nutrition are also included	1750
Grade III	Requiring surgical, endoscopic or radiological intervention	
Grade IIIa	Intervention not under general anesthesia	2750
Grade IIIb	Intervention under general anesthesia	4550
Grade IV	Life-threatening complication (including CNS complications)** requiring IC/ICU management	
Grade IVa	Single organ dysfunction (including dialysis)	7200
Grade IVb	Multiorgan dysfunction	8550
Grade V	Death of a patient	-

*The CCI = (sum of the weights)/2. Patients with a grade V complication automatically receive the maximum score of 100.

**Brain hemorrhage, ischemic stroke, subarachnoidal bleeding, but excluding transient ischemic attacks.

Abbreviations: CCI comprehensive complications index, CNS central nervous system, IC intermediate care, ICU intensive care unit.

- Postoperative pancreatic fistula (POPF): an abnormal communication between the pancreatic duct epithelium and another epithelial surface containing pancreas-derived enzyme-rich fluid, either related to a leak of a pancreatic-enteric anastomosis or originating from the raw pancreatic surface.^{115,116}
- Post-pancreatectomy hemorrhage (PPH): hemorrhage after pancreatic surgery which can occur early (≤ 24 hours after the operation) or late (≥ 24 hours), intraluminal or extraluminal, and mild or severe bleeding.¹¹⁷
- Delayed gastric emptying (DGE): the inability to return to a standard diet by the end of the first postoperative week and includes prolonged nasogastric intubation (> 3 days or the need to reinsert the nasogastric tube for persistent vomiting after postoperative day 3).¹¹⁸
- Bile leakage: bile leakage from the bilioenteric anastomosis or from injury of the bile ducts defined as fluid with an increased bilirubin concentration in the abdominal drain or in the intra-abdominal fluid on or after postoperative day 3, or as the need for radiologic intervention (i.e., interventional drainage) because of biliary collections or relaparotomy resulting from bile peritonitis.¹¹⁹
- Chyle leakage: lymphatic leakage, defined as output of milky-colored fluid from a drain, drain site, or wound, on or after postoperative day 3, with a triglyceride content ≥ 110 mg/dL or ≥ 1.2 mmol/L.¹²⁰

Roughly, the severity of complications is graded as A, B or C, where grade A complications generally do not have clinical consequences, grade B complications demand a change in the postoperative management and grade C complications require a relaparotomy or invasive intervention(s) and might lead to multiple organ failure and/or life-threatening situations. Grade B/C complications are generally considered as clinically relevant.

Studies investigating complications after pancreatic surgery for MEN1-related dpNETs observed rates of complications ranging from 26 – 58%.^{80,97,121–125} However, complications were not systematically addressed according to accepted classification systems in the majority of studies. The only study which overcame this major limitation is a previous study from the DMSG – including 61 patients undergoing a (duodeno)pancreatic resection for a NF-pNET – which documented a severe complication (Clavien-Dindo ≥ 3) in 19 of 58 patients (33%).⁶¹ No factors were associated with the occurrence of a severe complication. Grade B/C POPF, DGE, PPH, bile leakage occurred in 25%, 16%, 5% and 3%, respectively. The majority of procedures in this series included distal pancreatectomies and enucleations (80%), whereas the most severe complications seemed to occur after major surgery (i.e., Whipple/PPPD or total pancreatectomy). In addition, specific data on complications after major surgery are particularly important considering the multifocality of tumors involving both the pancreas as well as the duodenum. Particularly in these patients, the choice of timing and extent of surgery is a risk-benefit balance analysis guided by the oncological benefits against the risks of potential complications and long-term side effects. However, data on complications after major surgery are scarce and lacked systematic classification according to accepted criteria.^{80,121,122,125} Therefore, **Chapter 10** describes the incidence, severity and cumulative burden of complications after major surgery for dpNETs in patients with MEN1. In **Chapter 11** these findings are placed into perspective and future improvements are discussed.

A postoperative pancreatic fistula is the most frequently occurring pancreatic surgery-specific complication. Several studies observed a higher incidence of POPF after surgery for pNETs as compared to other pancreatic diseases and pNETs were reported as an independent risk factor for POPF after pancreatoduodenectomy and after distal pancreatectomy.^{126–130} Potential explanations include the high rate of atypical pancreatic resections, i.e., enucleations, central pancreatectomies or combined procedures, a soft pancreas which induces a higher exocrine activity with more enzyme-rich pancreatic fluid, a main pancreatic duct of less than 3 mm, more side branches of the main pancreatic duct and a reduced suture holding capacity.^{131,132} Therefore, complication data specifically after surgery for pNETs are demanded to improve patient counseling. Procedure-specific outcomes and risk factors for complications after surgery for pNETs in two ENETS Centers of Excellence were assessed in **Chapter 12**. In addition, the occurrence and severity of complication in patients with (multifocal) MEN1/VHL were compared with patients with (solitary) sporadic pNETs

and patients with functioning pNETs as compared to NF-pNETs.

The overall aim of this thesis is to improve care for patients with MEN1-related dpNETs. Data to guide important decisions regarding patient selection for surgery are limited. Moreover, if surgery is considered, we aim to provide surgeons with evidence to justify the choice for the optimal surgical procedure guided by data on early complications and long-term disease-related outcomes.

AIMS PER CHAPTER

PART I RESEARCH STRATEGIES

Chapter 2

- To describe observational study methods for rare diseases, describing the stepwise process from clinical research questions to scientific answers, guided by the DutchMEN Study Group research strategies.

PART II DIAGNOSIS

Chapter 3

- To systematically review the diagnostic accuracy of biomarkers for the diagnosis of NF-pNETs in patients with MEN1.
- To systematically review the diagnostic accuracy of imaging modalities (conventional [CT, MRI or EUS] and functional [¹¹¹Indium SRS or ⁶⁸Gallium labeled PET/CT]) for the diagnosis of NF-pNETs in patients with MEN1.
- To systematically review the growth rate of NF-pNETs in MEN1.
- To systematically review the age-related penetrance of NF-pNETs in MEN1.

Chapter 4

- To assess the use and overall diagnostic accuracy of pancreatic imaging studies for the diagnosis of pNETs in patients with MEN1.
- To assess the diagnostic accuracy of MRI and CT for the diagnosis of pNETs in MEN1 in a contemporary cohort.
- To estimate the added value of pancreatic fine needle aspirations for the diagnosis of pNETs in MEN1.

Chapter 5

- To assess the reproducibility, in terms of reliability and agreement, of radiological and pathological tumor size of resected pNETs in patients with MEN1 from a population-based cohort.

PART III PROGNOSIS

Chapter 6

- To assess survival of patients with MEN1-related gastrinomas.
- To assess prognostic factors for survival of patients with MEN1-related gastrinomas.

Chapter 7

- To identify metastatic patterns of multifocal duodenopancreatic neuroendocrine tumors in patients with MEN1.

Chapter 8

- To assess if patients with a resected MEN1-related NF-pNET have a different prognosis than those with a resected MEN1-related insulinoma.
- To assess factors associated with liver metastases-free survival in patients with resected NF-pNETs and those with resected insulinomas.

PART IV SURGICAL THERAPY

Chapter 9

- To investigate the risk of hypoglycemia or insulinoma recurrence after surgery for MEN1-related insulinoma in a comprehensive international cohort.

Chapter 10

- To assess the incidence, severity and cumulative burden of postoperative complications and pancreatic function after major duodenopancreatic surgery in a population-based cohort of patients with MEN1.
- To identify pre-operative and intra-operative factors associated with a severe complication.

Chapter 11

- To assess the future perspectives of major surgery in MEN1.

Chapter 12

- To assess procedure-specific outcomes and risk factors for complications after surgery for pNETs in two ENETS Centers of Excellence.
- To compare complications of patients with (multifocal) MEN/VHL-related versus (solitary) sporadic pNETs.
- To compare complications of patients with a functioning pNETs versus those with NF-pNETs.

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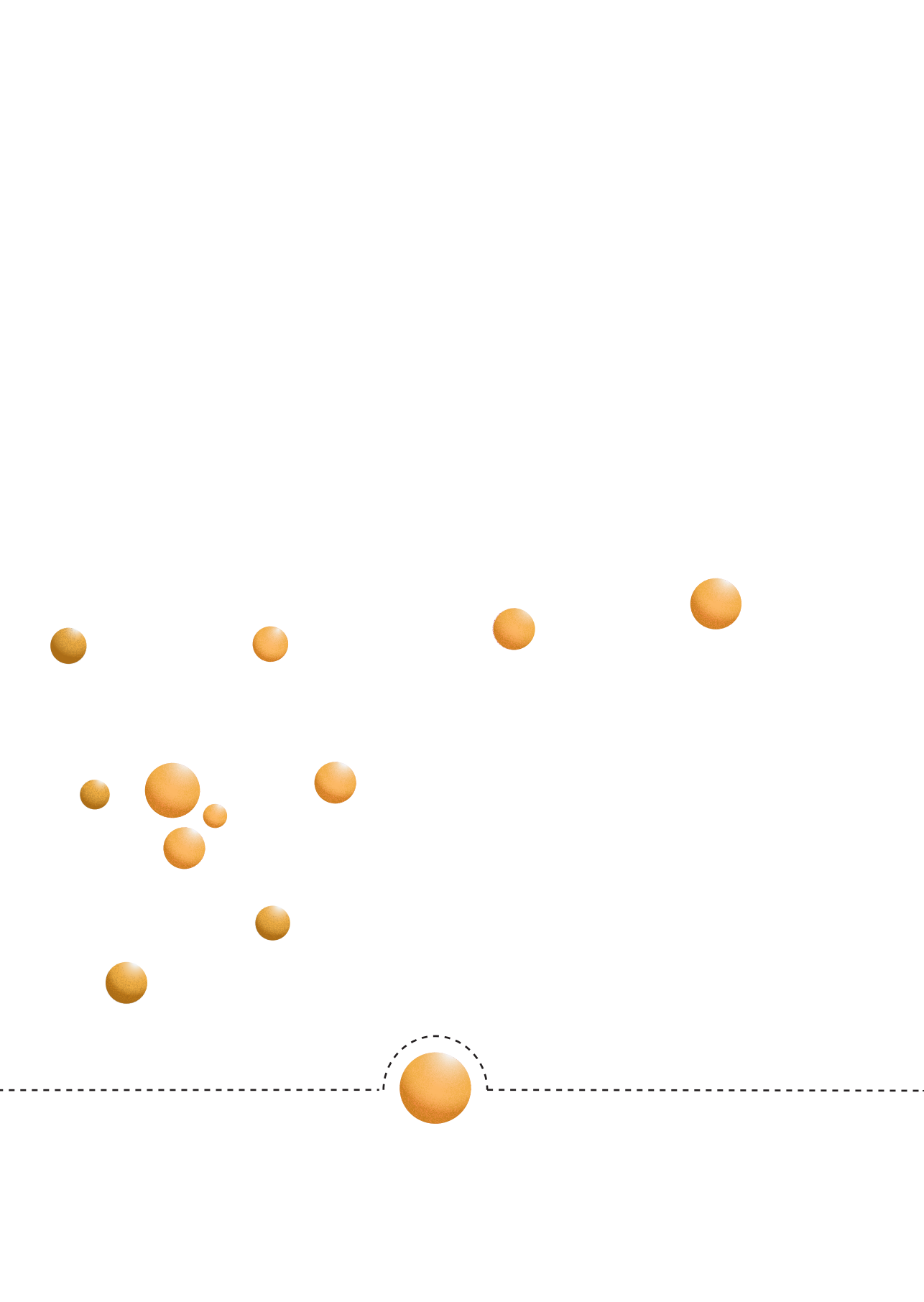
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PART I

RESEARCH STRATEGIES



CHAPTER II

'Quality in, quality out', a stepwise approach to evidence-based medicine for rare diseases promoted by MEN1

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D.J. van Beek, R.S. van Leeuwen, C.R.C. Pieterman, M.R. Vriens, G.D. Valk



ABSTRACT

Rare diseases pose specific challenges in the field of medical research to provide physicians with evidence-based guidelines derived from studies with sufficient quality. An example of these rare diseases is multiple endocrine neoplasia type 1 (MEN1), which is an autosomal dominant endocrine tumor syndrome with an estimated occurrence rate of 2-3 per 100,000. For this complex disease, characterized by multiple endocrine tumors, it proves difficult to perform both adequate and feasible studies. The opinion of patients themselves is of utmost importance to identify the gaps in the evidence-based medicine regarding clinical care. In the search for scientific answers to clinical research questions, the aim for best available evidence is obvious. Observational studies within patient cohorts, although prone to bias, seem the most feasible study design regarding the disease prevalence. Knowledge and adaptation to all types of bias is demanded in the strive for answers. Guided by our research on MEN1 patients, we elaborate on strategies to identify sufficient patients, to maximize and maintain patient enrollment and to standardize the data collection process. Preferably, data collection is performed prospectively, however, under certain conditions data storage in a longitudinal retrospective database with a disease-specific framework is suitable. Considering the global challenges on observational research on rare diseases, we propose a stepwise approach from clinical research questions to scientific answers.

INTRODUCTION

Rare diseases that affect less than one in 2000 people, pose challenges in supporting patients and physicians with evidence based guidelines of sufficient quality(1). Medical decision making becomes challenging when guidelines are scarce or the underlying scientific evidence is meager.

Multiple endocrine neoplasia type 1 (MEN1) (OMIM 131100) is an autosomal dominant disease with an estimated occurrence rate of 2-3 per 100,000(2). Due to the complexity of the disease, which is characterized by the development of multiple endocrine tumors already at an early age, developing evidence-based guidelines is a challenge(3). Most patients suffer from the classical triad of primary hyperparathyroidism (pHPT), duodenopancreatic neuroendocrine tumors (dpNETs) and/or pituitary adenomas. The prevalence for pHPT, dpNETs and pituitary tumors are 87, 56 and 44%, respectively in the Dutch population(4). Other encountered neoplasms include adrenal tumors, neuroendocrine tumors (NETs) of thymic, bronchial or gastric origin, skin and subcutaneous tumors, smooth muscle tumors and breast cancer(5, 6). Life expectancy of MEN1 patients is reduced compared to the general population(4). The prognosis of patients depends on early tumor detection and subsequent targeted interventions to prevent disease progression, making lifelong screening and intensive monitoring necessary(7).

Randomized controlled trials studying interventions and the optimal follow-up are almost impossible because of the low number of eligible patients for inclusion as well as the low yearly incidence of events in individual patients. Cohort studies are prone to various forms of bias such as selection bias, information bias and confounding by indication(8, 9).

Confronted by the principles of evidence-based medicine and need for high-quality scientific evidence regarding follow-up and interventions in MEN1, in the Netherlands in 2007, a retrospective MEN1 database was carefully designed. The aim was to answer multiple research questions which were based on the clinical dilemmas MEN1 patients and their treating physicians encountered in daily practice. The aim of this project was to provide patients and physicians with valid data. Considering the complexity of MEN1, a longitudinal database with a disease-specific framework was a necessity. Whereas strategies for conducting randomized controlled trials on rare diseases have been described, research methods for observational studies are far less developed(10). Guided by the fruitfulness of our longitudinal database and experience on this topic, this article will elaborate on our research strategy and observational study methods for rare diseases, describing the stepwise process from clinical research questions to scientific answers.

Clinical dilemma and theoretical study design

Formulating research questions and determining study design

Biomedical research consists of three main phases: formulating a research question, the collection of data based on these questions and the analysis of data. In recent years, the importance of the opinion of patients affected by the disease in this process is increasingly acknowledged. The subsequent study design is guided by a well-structured research question, addressing the study domain (patients with symptoms or a certain disease), determinant (diagnostic test, factor or therapy) and outcome of interest.

MEN1 formulated research questions

Clinical guidelines are only as good as the evidence and judgments they are based on(11). Even though there was a MEN1 consensus statement at the time, the scientific evidence underlying recommendations regarding screening and treatment of the different MEN1 manifestations was not always sufficient(12).

Our research group was confronted by a paucity of data on the natural course of the different MEN1 manifestations, prognostic factors, a genotype-phenotype relation, the timing and effect of therapy and recommendations based on strong evidence for periodical screening for these manifestations. These topics were the basis of the first set of research questions (Table 1). In addition, we consulted the patient advocacy group in this stage of the process, as to which questions they deemed important to study. The patient advocacy group considered quality of life as an important topic to study. More specifically, they considered questions regarding frequency and content of follow-up visits, effects and complications of surgery (e.g. hypoparathyroidism after (sub)total parathyroidectomy) and survival of dpNETs important topics since these affect quality of life. In a process of informed shared decision-making, research questions and study aims were formulated thereafter.

Study population

A single center patient population

In the University Medical Center (UMC) Utrecht, approximately 80 MEN1 patients were identified in 2007, which can be regarded as a ‘large’ rare disease population. However large, this study population lacked power to detect meaningful differences. In addition, we considered the population as a possible source of selection bias, since our center is a national center of expertise in MEN possibly leading to a case-mix with more advanced stages of the disease. Improper selection of patients, not being representative for the target population, leads to selection bias. In general, minimizing selection bias is attempted by including as many patients as possible in terms of percentage from similar hospitals, regarding level of patient care, during the recruitment phase.

Table 1. DMSG MEN1 study questions

Manifestation	MEN1 Study goal	Research questions	Study objectives
pHPT			
pHPT	Timing and effect of treatment	Patient advocacy group	What is the optimal surgical strategy for multiple endocrine neoplasia type 1 (MEN1)-related primary hyperparathyroidism (pHPT)? What is the course of postoperative hypoparathyroidism? Is genotype is associated with persistent/recurrent pHPT?
NETs			
Thymus and lung NET	Natural course	DMSG Patient advocacy group	What is the prevalence, tumor growth, and survival of Thymus and lung NETs in an unselected MEN1 population with long-term follow-up?
NET	Prognostic factors	Subsequent questions	Is there an association between blood type O and the occurrence of neuroendocrine tumors in the national Dutch MEN1 cohort?
pNET, tumor markers	Evidence-based screening	DMSG Patient advocacy group	What is the diagnostic accuracy of chromogranin A (CgA), pancreatic polypeptide (PP), and glucagon for pNET in MEN1?
dpNET	Prognostic factors	DMSG	What is overall survival and what are prognostic factors for patients with liver metastases from DP-NETs?
pNET	Natural course	DMSG Patient advocacy group	What is the natural history of small (<2cm) non-functioning pNETs in MEN1? What are effect modifiers for tumor growth of small (<2cm) non-functioning pNETs in MEN1?
pNET	Timing and effect of treatment	DMSG	Is surgery for Multiple Endocrine Neoplasia type 1 (MEN1) related nonfunctioning pancreatic neuroendocrine tumors effective for improving overall survival and preventing liver metastasis?
pNET	Timing and effect of treatment	DMSG	What are short and long-term morbidity after pancreatic surgery for multiple endocrine neoplasia type 1 (MEN1)-related nonfunctioning pancreatic neuroendocrine tumors?
pNET	Timing and effect of treatment	Subsequent questions	What are outcomes of robot-assisted and laparoscopic spleen-preserving pancreatic surgery in MEN1 patients?
pNET	Prognostic factors	Subsequent questions	Is there an association between WHO grade and the development of liver metastases in pNETs in MEN1 patients?
pNET	Prognostic factors	Subsequent questions	What is the role of p27Kip1 and p18Ink4c in pancreatic neuroendocrine tumor development in MEN1 patients?
pNET	Prognostic factors	Subsequent questions	What are promoter methylation profiles in pNETs in MEN1?
Thyroid incidentalomas	Prevalence and natural course	Subsequent questions	What is the prevalence of thyroid incidentalomas in MEN1 patients compared with nonMEN1 patients? Is thyroid tumorigenesis MEN1-related?
Pituitary tumors			
Pituitary tumors	Natural course	DMSG Patient advocacy group	What are the results of systematic pre-symptomatic PIT screening in MEN1? What are the outcomes after long-term follow-up of PITs with emphasis on nonfunctioning microadenomas diagnosed by screening in MEN1?
Effect of screening			
Screening	Evidence-based screening	DMSG	What is the effect of genetic screening on outcome in multiple endocrine neoplasia type 1 (MEN1)?

Table 1 continues on page 36

Table 1 continued from page 35

Manifestation	MEN1 Study goal	Research questions	Study objectives
Screening	Evidence-based screening	DMSG	Is there a lag time from MEN1 diagnosis of the index case to MEN1 diagnosis of family members? Is a time lag in MEN1 diagnosis associated with an increased morbidity and mortality risk?
Screening	Genotype-phenotype	DMSG	What is the clinical course of MEN1 mutation-negative patients with two out of the three main MEN1 manifestations and mutation-positive patients during long-term follow-up?
Quality of life			
Quality of life	Quality of life	Patient advocacy group	Do MEN1 patients have fear of disease for themselves or for family members? Is there an association between MEN1-related fear and health-related quality of life? What are risk factors for fear of disease occurrence in MEN1?
Other			
Breast cancer	-	Subsequent questions	What is the incidence of breast cancer in the Dutch longitudinal MEN1 database? What is the role of <i>MEN1</i> in human breast cancer?
Breast cancer	-	Subsequent questions	What are risk factors involved in early-onset elevated breast cancer in MEN1?

Abbreviations: *DMSG* Dutch MEN Study Group, *MEN1* multiple endocrine neoplasia type 1, *pHPT* primary hyperparathyroidism, *CgA* Chromogranin A, *PP* Pancreatic Polypeptide, *pNET* pancreatic neuroendocrine tumor, *NET* neuroendocrine tumor, *PIT* Pituitary, *dpNET* duodenopancreatic neuroendocrine tumor

Collaboration at a national level

In the Netherlands, MEN1 patients are commonly treated in a UMC (tertiary referral center) because of the necessity of a trained and dedicated multidisciplinary team(13). To include a representative sample of MEN1 patients and to increase sample size, a nationwide collaboration, known as the *DutchMEN Study Group (DMSG)*, was initiated in 2008(14). The DMSG consists of endocrinologists from every Dutch UMC, a consulting endocrine surgeon and representation from the patient advocacy group. Moreover, other specialist members of the multidisciplinary team are closely involved, which is in line with MEN1 guidelines to optimize patient care(13). The DMSG program, including patients from all Dutch UMCs, has led to the inclusion of over 90% of the total Dutch MEN1 population (> 400 participants) making it a true representation of Dutch MEN1 patients(15).

Patient identification

Before enrolling patients, a consistent diagnosis in accordance with guidelines is important. In this manner, a restricted population is created and selection bias is minimized. Using a standard identification method, MEN1 patients were identified by hospital diagnosis databases review. MEN1 diagnosis was based on clinical, familial or genetic criteria, based on clinical practice guidelines(12, 13). Less than 10% of the genetically diagnosed MEN1

patients is not included in the registry and only one person refused to participate. The high participation rate (over 90%) has also been found in a survey of patients with leukodystrophies, another rare disease(16). However, for MEN1 populations, this registry participation rate is globally unique and thereby leading in the field.

Strategies to maximize patient enrolment

The urge for international data registries is expressed by The European Union Committee of Experts on Rare Diseases(17). Patients with rare diseases are generally easily accessible for participation in international data registries(16, 18). Since MEN1 is an autosomal dominant trait, patients' children have a 50% chance of inheriting the disease(2). Patients are well aware of the high disease morbidity and decreased life expectancy since the disease 'runs in the family'. Patients maximize their contribution to medical research and subsequent clinical care for their affected relatives and other MEN1 patients.

Nevertheless, before patients are willing to engage in medical research, the physician-patient interaction is important(16, 19). In addition, to enroll a maximum number of patients, the study goals should be of direct importance to patients and their families. Active involvement of the national patient advocacy group in the DMSG from the stage of designing the research questions contributed to the high participation rate of patients.

Obtaining informed consent

The study protocol for the DMSG database and subsequent studies was approved by the Medical Ethical Committees of all UMCs in the Netherlands. The requirement to obtain individual informed consent was waived because of the retrospective and observational design. However, all patients received a letter including information regarding the collection and storage of clinical data and the possibility to refuse. From 2016 onward, the clinical database was continued prospectively including the collection of biobank materials (clinical biobank). Before inclusion in the clinical biobank, patients are informed by telephone or during an outpatient clinic visit including written patient information and asked to provide written informed consent. In addition, patients are informed about the possibility to withdraw their informed consent at any given time in the future.

Strategies to maintain patient participation

Studies with a necessary long-term follow-up are vulnerable to losing patients during follow-up. This potentially leads to follow-up bias, especially when the loss to follow-up differs between groups. Since MEN1 is a chronic disease in which patients have an ongoing risk for tumor development, patients consult endocrinologists annually, reducing follow-up bias to a minimum. However, patients that move to another place might change academic treatment center, decide to transfer to a local hospital or quit the follow-up regimen

altogether. With respect to the nationwide collaboration of all Dutch UMCs in the DMSG, loss to follow-up of patients is mostly prevented. Changing academic center will not end nor interrupt data collection, because registry entry will continue from the new treatment hospital. Nevertheless, effort must be made to ensure regular physician consultations. DMSG strategies to minimize loss of follow-up include the physician-patient relation, and moreover, regular physician consultation is also promoted by the patient advocacy group.

Strategies to establish the study population of the Dutch MEN1 registry are summarized in Table 2.

Table 2. DMSG study population strategies

Study step	Recommendation
Sample size and minimize selection bias	<ul style="list-style-type: none"> - Nationwide collaboration - Including comparable hospitals, e.g. tertiary referral centers - Multicenter research - Start (supra)national study group
Patient identification	<ul style="list-style-type: none"> - Consistent diagnosis according to guidelines - Standardized identification method in hospital diagnosis databases
Patient enrolment	<ul style="list-style-type: none"> - Formulate study goals that are of direct importance to patients - Patient advocacy group involvement from the start <ul style="list-style-type: none"> • Recruitment of patients among members • Prioritize research agenda/ study questions • Provide information among members/patients • Familiarize medical research among members • Yearly national and regional patient and specialist meetings - Disease biology <ul style="list-style-type: none"> • Autosomal dominant disorder • Families willing to help affected relatives <ul style="list-style-type: none"> ▪ Knowledge on morbidity and mortality - Informed consent
Patient participation	<ul style="list-style-type: none"> - Routine clinical care <ul style="list-style-type: none"> • Trained and dedicated multidisciplinary treatment team • One contact person for each institute • Physician-patient relation • Annual clinical consultation due to risk of tumor onset - In case of hospital change - Nationwide collaboration - Continue registry in new treatment center - International workshop on Multiple Endocrine Neoplasia (WorldMEN 2016, Utrecht, The Netherlands) including patient sessions - Patient advocacy group <ul style="list-style-type: none"> • Promote annual clinical consultation • Yearly national and regional patient and specialist meetings • Distribute research findings among patients • Formulate new patient oriented questions

From patients to data

The importance of data collection

The goal of the data collection process is to gather data which give true and objective reflections of patients' conditions. Incorrect data or inconsistent data collection leads to information bias; therefore, the process of data collection must be carefully designed, conducted and preferably secured. Before the actual collection of data, it is important to understand the nature of the data, to classify the data and to decide to which extent data are collected. The quality of data is guided by validity and precision. Systematic errors in data collection are dangerous, since these lead to irreversible damage to the study's internal validity(20).

Database design

The construction of a structured database and the valid and precise collection of data were critical research steps (Table 3). Even though some single-center MEN1 studies were conducted in the UMC Utrecht, a well-structured process of data collection was absent(21, 22). In addition to the nationwide expansion of the study population, multicenter, nationwide collaboration on data collection was essential. Considering the urge for quick answers to clinical questions and the low disease prevalence, studies with prospective data collection seemed utopia. Therefore, a retrospective database was the first step to answer multiple research questions.

After setting the basis for national multi-institutional data gathering, the actual process of data collection and storage, based on national consensus, was the next step. Since multiple research questions were formulated for different MEN1 manifestations, a database to store individual patient data was carefully designed according to the disease characteristics and the formulated research questions. Many research questions addressed the frequency and timing of screening, thus knowledge of the natural history was demanded. Longitudinal data provide insight in the natural disease course. Therefore, data were collected retrospectively every quarter from 1990 to 2016.

This longitudinal, retrospective design was further developed to a MEN1 disease-specific framework. MEN1 patients have a lifetime risk to develop multiple endocrine tumors in multiple organs, each with a different penetrance. Since, there is no clear age-related penetrance for every manifestation and manifestations can occur at any given age, lifelong screening for all MEN1 manifestations is required(7, 23). Consequently, the process of diagnosis, therapy and follow-up is an ongoing, repeated and simultaneous process for MEN1-related neoplasms.

Variable selection

In line with the disease's complexity, a wide range of variables needed to be included in the database. Annual consultations include clinical and biochemical screening by specialized

Table 3. DMSG recommendations for data storage and data collection

Study step	Recommendation
Data storage	<ul style="list-style-type: none"> - Web-based database <ul style="list-style-type: none"> • Easily accessible from every hospital during data collection - Confidentiality <ul style="list-style-type: none"> • Pseudo-anonymize patients • E.g. convert dates to quarters (e.g. 1 Q 2010 for all dates from January 1st up to and including March 31st 2010)
Database design	<ul style="list-style-type: none"> - Urge for relatively quick answers <ul style="list-style-type: none"> • Retrospective database design - Study group consensus on database design - Research questions on natural course of disease <ul style="list-style-type: none"> • Longitudinal database design • Quarterly collection of data - Develop a disease-specific framework - Epidemiological background: diagnostic, etiologic, prognostic and/or therapeutic aims - Longitudinal design <ul style="list-style-type: none"> • Repetitive collection of the same variables
Variable selection	<ul style="list-style-type: none"> - General data and considerations <ul style="list-style-type: none"> • General patient data or demographics • Raw or primary data • Complex diseases and multiple research questions: more variables demanded - Select disease-related variables <ul style="list-style-type: none"> • Screening programs • Different disease manifestations • Biochemical, radiological, surgical and pathology data - Study group consensus on variables - Plan on how variables, such as laboratory values, should be collected in different hospitals - Plan on how the 'raw' data can potentially be analyzed
Data collection	<ul style="list-style-type: none"> - General steps <ul style="list-style-type: none"> • Collect raw data • Compulsory variables • Implement constraints in the database • Data capture control among data collectors • One principal investigator <ul style="list-style-type: none"> ▪ Discuss uncertainties ▪ Document and store these decisions - Develop a central protocol to facilitate standardized data collection <ul style="list-style-type: none"> • Data are consistently captured appropriately - Minimize number of data collectors at a time <ul style="list-style-type: none"> • Data collectors will gain familiarity with the database • Select data collectors with affinity for the project, e.g. PhD students using the data for their thesis • Provide data collectors with enough time to collect data

endocrinologists. Hormonally active tumors secrete different hormones, depending on the tumor's origin. Therefore, biochemical screening ranges from 72-h fast tests for pancreatic insulinomas to serum calcium levels for primary hyperparathyroidism. Radiological screening differs both in frequency and modality for different manifestations(13). Extensive additional diagnostic procedures may be performed when patients are suspected of a manifestation. Therapeutic interventions range from medical treatment to surgical resections, guided by manifestation, patient characteristics and disease stage.

Based on these factors and the formulated research questions a dataset was developed for each specific MEN1 manifestation (pHPT, dpNETs, pituitary tumors, NETs (of stomach, bronchus and thymus), and adrenal lesions) including biochemical, radiological, surgical and pathology data (Table 4). Besides, datasets for general data on patient characteristics, medication use and previous medical history were designed. Multiple versions of these datasets were discussed among the study group participants and thereafter the final, total dataset was agreed upon.

Ahead of the collection of biochemical data from multiple hospitals, the planning on 'how to' collect these variables is essential, since clinical laboratories often harbor different measurement methods, leading to different units of measurement and reference values. Therefore, involvement of clinical chemistry laboratories of all participating centers is important during the design of the database. In addition, to compare these values during data analysis, a useful strategy is to express the values as upper level of the normal of the reference value, which is widely used in medical research.

Data collection

Variations during the data collection process, also known as observer bias, are remarkably reduced by securing the data collection process, minimizing the number of data collectors and collecting only uninterpreted or 'raw' data to prevent interpretation before statistical analysis. For example, there was no variable 'primary hyperparathyroidism' but, to define this variable, the outcomes of serum calcium and parathyroid hormone levels were used, so data were collected without knowledge of the outcomes. In addition, outcomes had to be subsequently found in individual patients during data analysis, hereby including the repetitive identification of a tumor over time as the reference standard for the diagnosis of a tumor(24). A central protocol was developed, which described per variable how it should be collected, so data collectors would gain familiarity with the appropriate gathering of data and interobserver variations were reduced. In the database, constraints were implemented for the range of outcomes that could be collected, preventing mistakes in data collection because of typing errors. Although the data collection in multicenter studies is commonly performed by researchers from every institution, only one or two centrally appointed data collectors were operating at a time were. This minimized differences in data collection and enabled the collectors to gain vast experience in this large dataset, reducing intraobserver

Table 4. Database variables for each MEN1 manifestation

General data	Patient identification number, date of birth, gender, family identification number, date of death, cause of death				
MEN1 data	Date MEN1 diagnosis, basis MEN1 diagnosis, MEN1 genetic analysis, MEN1 mutation				
	Biochemical*	Imaging*	Surgery*	Pathology*	Medication*
pHPT	Calcium (ionized/total) PTH Albumin 25-OH vitamin D	<u>Conventional imaging:</u> Neck ultrasound/ CT/ MRI (number of abnormalities) <u>Functional imaging:</u> Scintigraphy	Surgery Type of surgery Parathyroid autotransplant Thymectomy Complications	Number of parathyroids Histopathology	1,25 vitamin D Calcium supplementation Cinacalcet
NETs (pancreas, duodenum, stomach, lung, thymus)	Gastrin Glucose Insulin Pro-insulin C-peptide Glucagon Pancreas polypeptide GHRH VIP Serotonin in thrombocytes 24-hrs urinary 5-HIAA Chromogranin A NSE 72-hour fasting test	<u>Conventional:</u> <i>Abdominal:</i> MRI/ CT/ gastroduodenoscopy/ EUS (number, size and location of NETs, metastases) <i>Thoracic:</i> MRI/ CT (number, size and location of NETs, metastases) <u>Functional:</u> Somatostatin receptor scintigraphy PET/CT (number, size and location of NETs, metastases)	Surgery Organ Type of surgery Lymph node dissection Metastectomy Complications	Number of NETs per organ/part of organ Size of the largest NET Immunohistochemistry: • Gastrin • Insuline • Glucagon • Pancreas polypeptide • Chromogranin A • Synaptophysin • Other Mitosis per 10 HPF Ki-67 Lymph node metastases Other metastases R-status	PPI Insulin SU-derivatives Other oral antidiabetics Somatostatin analogues Chemotherapy
Pituitary	Prolactin IGF-1 TSH FT4 Cortisol (basal/midnight) ACTH LH FSH Testosterone SHBG Estradiol Dexamethason suppression test	<u>Conventional imaging:</u> MRI/ CT (number, size and consistency of abnormalities)	Surgery Type of surgery Complications	Number of adenomas Size adenomas Type of adenoma (solitary/ cystic) Immunohistochemistry: • Prolactin • GH • FSH • LH • TSH • ACTH • Other Proliferation R-status	Somatostatin analogues Pegvisomant Dopamin agonist Vasopressin analogue Glucocorticoid Hormone replacement therapy Somatropin Testosterone Thyreomimetic agents Ketoconazol Metyrapon

Adrenal	Dexamethason suppression test	Conventional imaging.	Surgery	Number of adenomas/ carcinomas	Glucocorticoid
	24-hrs urinary (nor) metanephrines	MRI/ CT (hyperplasia, number and size of adenomas, metastases)	Type of surgery	Size adenomas	Mineralcorticoid
	24-hrs urinary cortisol		Endoscopic resection	R-status	DHEA
	Cortisol (basal, midnight)		Complications		Ketoconazol
	Plasma (nor)metanephrines				Metirapon
	Plasma renin activity				
	Aldosteron				
	ACTH				
	DHEA				
	Androstenedione				

* These data are captured every quarter from 1990 to 2014.
Abbreviations 5-HIAA 5-Hydroxyindoleacetic acid, 24-hrs 24- hours, 25-OH 25-Hydroxyvitamin, ACTH Adrenocorticotrophic hormone, CT computed tomography, DHEA Dehydroepiandrosterone, EUS endoscopic ultrasonography, FSH follicle-stimulating hormone, FTV4 free thyroxine, GH growth hormone, GHRH growth hormone releasing hormone, HPF high power fields, IGF-1 insulin-like growth factor 1, LH Luteinizing hormone, MEN1 Multiple endocrine neoplasia type 1, MRI magnetic resonance imaging, NET neuroendocrine tumor, NSE neuron-specific enolase, PET/CT positron emission tomography/computed tomography, pHPT primary hyperparathyroidism, PIT pituitary, PPI proton-pump inhibitor, PTH parathyroid hormone, R-status resection margin status, SHBG sex hormone-binding globulin, SU sulphylurea, TSH thyroid stimulating hormone, VIP vasoactive intestinal peptide

variability. The ideal geography of the Netherlands and centralizing the DMSG from Utrecht, optimized this way of data collection. All uncertainties during data collection and data capture were discussed with one principal investigator, which minimized defaults in these areas. These uncertainties were stored and are assessable at the present and in the future for the data collectors and researchers. The importance of protocolized data collection is the gathering of complete data, structurally the same in every center and for every patient, irrespective of disease severity.

Data storage

Nowadays, the use of an easily accessible electronic, web-based application facilitates the collection of data in multicenter studies. However, security issues regarding patient data are of utmost importance. Concerning confidentiality, patients were pseudo-anonymized upon inclusion in the database, where every patient received a unique identification code, which is available for future data collection at the participating hospital. Regarding the low disease prevalence, all dates were converted into a quarter of a year (e.g. 1 Q 2010 for all dates from January 1 up to and including March 31, 2010). In addition to the simplification of data collection, electronic registries offer the opportunity of direct data output for all involved researchers.

Missing data

Retrospective studies are vulnerable for missing data, which lead to bias if the missing data are related to the outcome or exposure of interest. By securing the data collection, the quarterly collection of data and the available data from annual consultations, missing data were minimized. Nevertheless, prospective data collection will further reduce missing data.

Time aspects

Longitudinal studies covering a long time span, might encounter changes in care due to increased technology and medical knowledge. In case variations in care or measurement of determinants or outcomes lead to systematic differences between patients over time, detection bias exists. Considering the numerous manifestations and the longitudinal study design, detection bias is lurking for all MEN1 manifestations, since biochemical and radiological diagnostics have gained increased sensitivity and specificity over the years. With an increase in frequency and quality of screening, manifestations are detected in earlier disease stages. Stratifying patients into birth cohorts, to minimize the effects of detection bias, has been previously performed in a longitudinal study(23). Nevertheless, by including patients from 1990 onward, differences in diagnostic opportunities and therapies are reduced, compared to studies including patients over a longer time span.

Table 5. DMSG contributions to clinical care

Title	Study objectives	Outcomes/ New insights
Multiple endocrine neoplasia type 1 (MEN1): its manifestations and effect of genetic screening on clinical outcome (7)	To determine the effect of genetic screening on outcome in multiple endocrine neoplasia type 1 (MEN1).	Genetic diagnosis is associated with less morbidity at diagnosis and at follow-up. Early genetic diagnosis might therefore lead to improvement of long-term outcome.
Primary hyperparathyroidism in MEN1 patients: a cohort study with longterm follow-up on preferred surgical procedure and the relation with genotype (25)	To identify the optimal surgical strategy for multiple endocrine neoplasia type 1 (MEN1)-related primary hyperparathyroidism (pHPT). To describe the course of postoperative hypoparathyroidism and to assess whether genotype is associated with persistent/recurrent pHPT	SPTX with bilateral Bilateral transcervical thymectomy is the procedure of choice for MEN1-related pHPT. Genotype seems to affect the chance of recurrence. Postoperative hypoparathyroidism lasting 6 months or more should not be considered permanent in MEN1.
Low accuracy of tumor markers for diagnosing pancreatic neuroendocrine tumors in multiple endocrine neoplasia type 1 patients (14)	To assess the diagnostic accuracy of chromogranin A (CgA), pancreatic polypeptide (PP), and glucagon for pNET in MEN1.	The diagnostic accuracy of the tumor markers CgA, PP, and glucagon for pNET in MEN1 is low.
Natural course and survival of neuroendocrine tumors of thymus and lung in MEN1 patients (26)	To assess prevalence, tumor growth, and survival of Thymus and lung NETs in an unselected MEN1 population with long-term follow-up.	In MEN1 patients, Thymus NETs almost exclusively occurred in males and had a very low prevalence and a high mortality. Lung NETs occurred more often than previously thought, had an indolent course, and occurred equally in both sexes. Tumor growth in males was double compared with female patients.
Breast-cancer predisposition in multiple endocrine neoplasia type 1 (6)	To clarify the role of <i>MEN1</i> in human breast cancer. To assess the incidence of breast cancer in the Dutch longitudinal MEN1 database.	Female patients with MEN1 are at increased risk for breast cancer. Our observations indicate that <i>MEN1</i> mutations are involved in human breast carcinogenesis. Intensified breast-cancer screening at a relatively young age should be considered in female patients with MEN1.
Thyroid incidentalomas in patients with multiple endocrine neoplasia type 1 (28)	To assess the prevalence of thyroid incidentalomas in MEN1 patients compared with nonMEN1 patients. To verify whether thyroid tumorigenesis is MEN1-related.	MEN1 patients do not have a higher prevalence of thyroid incidentalomas compared with primary hyperparathyroidism patients without the diagnosis of MEN1. Menin was expressed in the thyroid tumors of MEN1 patients.
No Association of Blood Type O With Neuroendocrine Tumors in Multiple Endocrine Neoplasia Type 1 (29)	To assess the association between blood type O and the occurrence of neuroendocrine tumors in the national Dutch MEN1 cohort.	An association between blood type O and the occurrence of neuroendocrine tumors in MEN1 patients was not confirmed. For this reason, the addition of the blood type to screening and surveillance practice seems not to be of additional value for identifying MEN1 patients at risk for the development of neuroendocrine tumors, metastatic disease, or a shortened survival.

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Title	Study objectives	Outcomes/ New insights
Long-Term Natural Course of Pituitary Tumors in Patients With MEN1: Results From the DutchMEN1 Study Group (DMSG) (30)	To assess the results of systematic pre-symptomatic PIT screening and subsequent long-term followup of PITs with emphasis on nonfunctioning microadenomas diagnosed by screening.	Systematic presymptomatic screening for PIT in patients with MEN1 predominantly results in detection of nonfunctioning microadenomas. Prolactinoma in patients with MEN1 responded well to medical treatment. Microadenomas grew only occasionally and after many years without clinical consequences. Frequent magnetic resonance imaging followup of nonfunctioning microadenomas in the context of MEN1 and sporadically occurring PITs therefore seems debatable.
Impact of Delay in Diagnosis in Outcomes in MEN1: Results From the Dutch MEN1 Study Group (31)	To assess whether there is a lag time from MEN1 diagnosis of the index case to MEN1 diagnosis of family members. To determine whether this lag time was associated with an increased morbidity and mortality risk.	There is a clinically relevant delay in MEN1 diagnosis in families because of a lag time between the diagnosis of an index case and the rest of the family. More emphasis should be placed on the conduct of proper counseling and genetic testing in all eligible family members.
Robot-assisted spleen preserving pancreatic surgery in MEN1 patients (32)	To describe robot-assisted and laparoscopic spleen-preserving pancreatic surgery in MEN1 patients, and to compare both techniques.	Minimally invasive spleen-preserving surgery in MEN1 patients is safe and feasible. Patients who underwent robot-assisted surgery did not require conversion to open surgery.
Early and Late Complications After Surgery For MEN1-related Nonfunctioning Pancreatic Neuroendocrine Tumors (33)	To estimate short and long-term morbidity after pancreatic surgery for multiple endocrine neoplasia type 1 (MEN1)-related nonfunctioning pancreatic neuroendocrine tumors (NF-pNETs).	MEN1 NF-pNET surgery is associated with high rates of major short and long-term complications. Current findings should be taken into account in the shared decision-making process when MEN1 NF-pNET surgery is considered.
MEN1 redefined, a clinical comparison of mutation-positive and mutation-negative patients (4)	To describe and compare the clinical course of MEN1 mutation-negative patients with two out of the three main MEN1 manifestations and mutation-positive patients during long-term follow-up.	Mutation-positive and mutation-negative MEN1 patients have a different phenotype and clinical course. Mutation-negative patients develop MEN1 manifestations at higher age and have a life expectancy comparable with the general population. The apparent differences in clinical course suggest that MEN1 mutation-negative patients do not have true MEN1, but another MEN1-like syndrome or sporadic co-incidence of two neuro-endocrine tumors.
Prognostic factors for survival of MEN1 patients with duodenopancreatic tumors metastatic to the liver: results from the DMSG (34)	To determine overall survival (OS) and prognostic factors for patients with liver metastases from DP-NETs.	Despite the fairly indolent course of DP-NET liver metastases in MEN1 patients, half of the population was deceased after 10 years. Sex and tumor load at diagnosis of liver metastases are possible prognostic factors for worse survival.

Title	Study objectives	Outcomes/ New insights
Management of MEN1 Related Nonfunctioning Pancreatic NETs: A Shifting Paradigm: Results From the DutchMEN1 Study Group (35)	To assess if surgery for Multiple Endocrine Neoplasia type 1 (MEN1) related nonfunctioning pancreatic neuroendocrine tumors (NF-pNETs) is effective for improving overall survival and preventing liver metastasis.	MEN1 patients with NF-pNETs <2cm can be managed by watchful waiting, hereby avoiding major surgery without loss of oncological safety. The beneficial effect of a surgery in NF-pNETs 2 to 3cm requires further research. In patients with NF-pNETs >3cm, watchful waiting seems not advisable.
MEN1-Dependent Breast Cancer: Indication for Early Screening? Results From the Dutch MEN1 Study Group (36)	To assess whether other risk factors are involved to identify MEN1 at greatest risk for early-onset elevated breast cancer.	The increased breast cancer risk in MEN1 carriers was not related to other known breast cancer risk factors or familial cancer history, and therefore breast cancer surveillance from the age of 40 years for all women with MEN1 is justifiable.
Prognostic value of WHO grade in pancreatic neuro-endocrine tumors in Multiple Endocrine Neoplasia type 1: Results from the DutchMEN1 Study Group (38)	To assess the prognostic value of WHO grade in MEN1-related pancreatic neuroendocrine tumors.	High mitotic count is correlated with poor prognosis in MEN1 patients with large non-functioning pNETs.
Long-Term Natural Course of Small Nonfunctional Pancreatic Neuroendocrine Tumors in MEN1- Results From the Dutch MEN1 Study Group (24)	To assess long-term natural history of small NF-pNETs and its modifiers in the Dutch MEN1 population.	The majority of small NF-pNETs are stable at long-term follow-up, irrespective of the underlying MEN1 genotype. A subgroup of tumors is slowly growing but cannot be identified on clinical grounds. In this subgroup, tumors with missense mutations exhibited faster growth. Additional events appear necessary for pNETs to progress. Future studies should be aimed at identifying these molecular driving events, which could be used as potential biomarkers.
Expression of p27Kip1 and p18Ink4c in human multiple endocrine neoplasia type 1-related pancreatic neuroendocrine tumors (39)	To assess the role of role p27Kip1 and p18Ink4c in MEN1-related pancreatic neuroendocrine tumor development.	These findings indicate that loss of p18Ink4c, but not p27Kip1, is a common event in the development of MEN1-related pNETs. Restoration of p18Ink4c function through CDK4/6 inhibitors could be a therapeutic option for MEN1-related pNETs.
High Fear of Disease Occurrence Is Associated With Low Quality of Life in Patients With Multiple Endocrine Neoplasia Type 1: Results From the Dutch MEN1 Study Group (41)	To assess whether MEN1 leads to psychological distress because of fear of disease occurrence (FDO), and affects quality of life.	The majority of patients with MEN1 have FDO for themselves and even more for their relatives. This psychological distress is associated with a lower health-related quality of life. Therefore, in the medical care for MEN1, emphasis should also be placed on FDO and quality of life.
DNA methylation profiling in MEN1-related pancreatic neuroendocrine tumors reveals a potential epigenetic target for treatment (42)	To determine promoter methylation profiles in MEN1-related pNETs.	Promoter hypermethylation is a frequent event in MEN1-related and sporadic pNETs. Targeting DNA methylation could be of therapeutic value in MEN1 patients with advanced pNETs.

Abbreviations: *DMSG* Dutch MEN Study Group, *MEN1* multiple endocrine neoplasia type 1, *pHPT* primary hyperparathyroidism, *SPTX* Subtotal parathyroidectomy, *CgA* Chromogranin A, *PP* Pancreatic Polypeptide, *pNET* pancreatic neuroendocrine tumor, *NET* neuroendocrine tumor, *NF-pNETs* Non-functioning pancreatic neuroendocrine tumor, *PIT* Pituitary, *DP-NET* duodenopancreatic neuroendocrine tumor, *FDO* Fear of disease occurrence

The DMSG contributions to patient care

Up till now, publications from the retrospective database have already answered many of our research questions and clinical management has changed Table 5 (4, 6, 7, 14, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39). Moreover, the retrospective study design proved to be suitable, since a long latency between exposure and outcome exists. Most important findings from the DMSG research include the decreased life expectancy, the natural course of NETs (lung, thymus and pancreas) and pituitary adenomas, the effect of periodical screening and the surgical outcomes including complications of non-functioning pancreatic NETs(4, 14, 24, 26, 30, 33, 35). Another example from our database is the increased breast cancer risk for women with MEN1 compared to the general population (Risk Ratio of 2.8)(6).

Current state and future perspectives

Designing a prospective database

Prospective data collection will further reduce the number of missing data, increase the quality of data, enable the collection of baseline characteristics and unknown confounders. Therefore, the database is continued prospectively to enhance future MEN1 research based on the outcomes of the retrospective database. The design of the prospective database focuses on user-friendliness by using a new Web-based platform, which offers the opportunity to physicians to enter data during routine care and to automatically capture laboratory values from patient hospital records. Consequently, according to research findings, new research questions and advances in diagnostic and therapeutic regimen, several variables are added such as the breast cancer screening, bone density measurements, nuclear imaging and therapy and surgical techniques. In addition, some indicator variables are added for the manifestations, so cross-sectional prevalence estimates can easily be obtained, which are useful for planning supranational research projects and imaginably a global RCT in the future. In addition to clinical data collection, patient material is obtained and stored in the biobanks of all UMCs to enable future basal and translational research. Assembled MEN1 patient materials include blood (serum, EDTA and citrate plasma and DNA) and tissue (formalin-fixed paraffin-embedded and fresh frozen) of biopsies and resected specimen(40). Materials are preferably obtained during routine patient care.

The Parelsnoer Institute

This clinical database and biobank initiative is now part of the Parelsnoer Institute (PSI) (<http://www.parelsnoer.org/page/en/Home>) which is part of the Netherlands Federation of UMCs(40). Within the framework of PSI, individual UMC clinical biobanks store patient materials according to a standardized national biobanking protocol, covering all phases of biobanking: collection, pre-analysis, registration, processing and storage of the samples(40).

These data are centrally stored and linked to the clinical patient data. Clinical data are either manually captured, however, automatic data capture options are available for certain variables, such as laboratory tests(40). The connection between clinical and biobank data offers the unique opportunity to study genetic and epigenetic factors driving hereditary NETs. Identification of these factors in familial tumors, could in the future be extrapolated to sporadic NETs to identify NETs with an unfavorable prognosis and offer specific new targets for therapeutic opportunities.

CONCLUSION

Guided by our MEN1 experiences, we propose a stepwise approach from clinical research questions to scientific answers (Table 6). This experience can guide others planning to start a database for rare diseases. Involvement of the patients themselves from the beginning leads to meaningful research questions guiding clinical care and, in addition, increases the participation rate, thereby minimizing selection bias. Thereafter, the protocolized and standardized process of data collection and data storage into a disease-specific database enables the collection of homogeneous data and reducing information bias. Ongoing prospective clinical data collection and the collection of biobank materials has commenced in 2016, which will further increase the quality of the data and enables clinical epidemiological and translational research in the near future. This will directly impact patient care and provide new insights into MEN1 in the future.

Table 6. DMSG overview of study phases and recommendations.

Study phase	Recommendation
1. Formulating research questions	
Formulate research questions	Involvement of patient advocacy group
2. Patient inclusion	
a. Increase sample size	National multicenter collaboration and study group
b. Identify patients	<ul style="list-style-type: none"> - Diagnosis according to clinical practice guidelines - Standardized identification method in participating centers
c. Maximize patient enrollment	<ul style="list-style-type: none"> - Formulate patient relevant study aims - Involvement of patient advocacy group <ul style="list-style-type: none"> • Recruitment of patients among members • Familiarize medical research among members
d. Maintain patient participation	<ul style="list-style-type: none"> - Continue registry entry in new treatment center - Optimize physician-patient relation in routine clinical care - Patient advocacy group <ul style="list-style-type: none"> • Distribute research findings among patients • Yearly patient and specialist meetings
3. Data storage and data collection	
a. Data storage	<ul style="list-style-type: none"> - Web-based database - Maintain confidentiality in accordance with local legislation
b. Database design	<ul style="list-style-type: none"> - Consider study design - Consider epidemiologic type of research questions - Develop a disease-specific framework
c. Variable selection	<ul style="list-style-type: none"> - Raw data - Select disease-related variables - Reach consensus on data in study group - Plan how variables, such as laboratory values, should be collected in different hospitals
d. Data collection	<ul style="list-style-type: none"> - Develop a central protocol to facilitate standardized data collection - Minimize number of data collectors at a time
4. Designing a prospective database based on outcomes of the retrospective database	
a. User-friendliness	- Automatic data capture from hospital records
b. Supranational collaboration	- Add indicator variables of manifestations for cross-sectional identification of patients to plan supranational studies
c. Continuously update database	- Include new variables based on new research questions or advances in care
5. Biobanking	
a. National collaboration	<ul style="list-style-type: none"> - Establish central organization/ collaboration between UMCs - Create centralized biobanking protocol including all phases: collection, pre-analysis, registration, processing and storage of the samples - Sites of tissue collection and storage <ul style="list-style-type: none"> • E.g. in individual UMC biobanks
b. Decide on materials	<ul style="list-style-type: none"> - Choices on which patient materials to collect <ul style="list-style-type: none"> • Blood, tissue, feces, urine and others - Plan on how to collect materials <ul style="list-style-type: none"> • Routine patient care or in research setting
c. Link to clinical data	- Preferably implement link to clinical data

Collaborators

All individuals listed as collaborators participated in the DutchMEN Study Group from its establishment, thereby putting significant effort in the formation of the DMSG, patient recruitment on a national level, formulation of research questions and variable selection. Members of the DutchMEN Study Group (DMSG) at its establishment in alphabetical order:

P.H. Bisschop, MD, Ph.D., Amsterdam UMC, Amsterdam, The Netherlands.

I.H.M. Borel Rinkes, MD, Ph.D., University Medical Center Utrecht, Utrecht, The Netherlands.

O.M. Dekkers, MD, Ph.D., Leiden University Medical Center, Leiden, The Netherlands.

M.L. Drent, MD, Ph.D., Amsterdam UMC, Amsterdam, The Netherlands.

B. Havekes, MD, Ph.D., Maastricht University Medical Center, Maastricht, The Netherlands.

W.W. de Herder, MD, Ph.D., Erasmus Medical Center, Rotterdam, The Netherlands.

A.R.M.M. Hermus, MD, Ph.D., Radboud University Medical Center, Nijmegen, The Netherlands.

A.N.A. van der Horst-Schrivers, MD, Ph.D., University Medical Center Groningen, Groningen, The Netherlands.

J. de Jong, President of the Dutch MEN patient advocacy group (Belangengroep MEN), the Netherlands.

H.F.A. Vasen, MD, Ph.D., Netherlands Foundation for the Detection of Hereditary Tumours, the Netherlands.

B.A. Zonnenberg, MD, Ph.D., University Medical Center Utrecht, the Netherlands.

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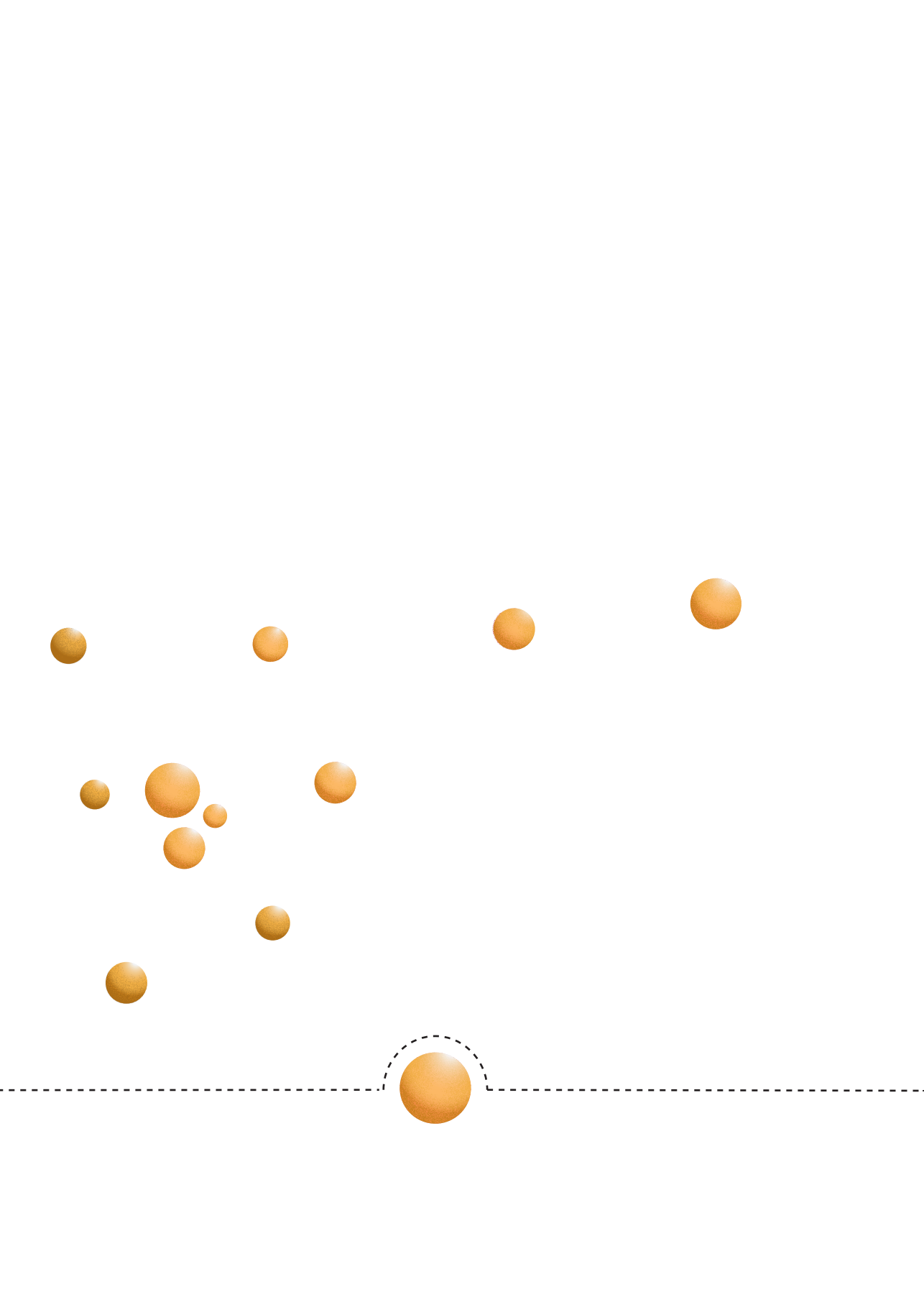
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PART II

DIAGNOSIS



CHAPTER III

Diagnosing nonfunctional pancreatic NETs in MEN1: the evidence base

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M.J.C. van Treijen, D.J. van Beek, R.S. van Leeuwen, M.R. Vriens, G.D. Valk



ABSTRACT

In multiple endocrine neoplasia type 1 (MEN1), nonfunctional pancreatic neuroendocrine tumors (NF-pNETs) are the most frequently diagnosed NETs and a leading cause of MEN1-related death. The high prevalence and malignant potential of NF-pNETs outline the need for an evidence-based screening program, as early diagnosis and timely intervention could reduce morbidity and mortality. Controversies exist regarding the value of several diagnostic tests. This systematic review aims to evaluate current literature and amplify an up-to-date evidence-based approach to NF-pNET diagnosis in MEN1. Three databases were systematically searched on the diagnostic value of biomarkers and imaging modalities. Twenty-seven studies were included and critically appraised (modified Quality Assessment of Diagnostic Accuracy Studies). Another 12 studies, providing data on age-related penetrance and tumor growth, were included to assess the optimal frequency and timing of screening. Based on current literature, biomarkers should no longer play a role in the diagnostic process for NF-pNETs, as accuracies are too low. Studies evaluating the diagnostic value of imaging modalities are heterogeneous with varying risks of bias. For the detection of NF-pNETs, endoscopic ultrasound (EUS) has the highest sensitivity. A combined strategy of EUS and MRI seems to be the most useful. Gallium 68 octreotate- DOTA positron emission tomography-CT could be added if NF-pNETs are diagnosed to identify metastasis. Reported growth rates were generally low, and two distinct phenotypes were observed. Surveillance programs should focus on and be adapted to the presence of substantial growth in NF-pNETs. The optimal age to start screening must yet be determined, as insufficient evidence for an evidence-based recommendation was available.

INTRODUCTION

Multiple endocrine neoplasia type 1 (MEN1) is a rare familial tumor syndrome, primarily caused by germline mutations in the MEN1 gene, encoding the tumor-suppressor protein menin [1]. Glandular hyperplasia and neoplastic endocrine tumors of the pituitary, parathyroid glands, duodenum, and pancreas form the major manifestations of the syndrome. Other manifestations of MEN1 are neuroendocrine tumors (NETs) of gastric, bronchial, or thymic origin; breast cancer; adrenal adenomas; and cutaneous manifestations, such as lipomas, collagenomas, and facial angiofibromas [2,3].

NETs are manifest in MEN1, and particularly, thymic carcinoid and duodenopancreatic NETs (dpNETs) cause a decreased life expectancy in MEN1 [4–6]. dpNETs are the most prevalent NETs and can be divided in functional, e.g., hormone producing, and nonfunctional. Nowadays, nonfunctional pancreatic NETs (NF-pNETs) are the most frequently diagnosed NETs in MEN1 and a leading cause of MEN1-related death [7,8]. NF-pNETs cause symptomatic disease in only up to 13% of patients, despite their multicentric appearance [9]. The high prevalence and malignant potential outline the need for an evidence-based screening program to diagnose NF-pNETs at an early stage to enable meticulous follow-up and timely intervention to prevent metastasized disease. Studies focusing on sporadically occurring NF-pNETs are difficult to extrapolate to MEN1-related NF-pNETs, which are characterized by their multifocal occurrence and a more indolent course of disease in contrast to their sporadic counterparts. Moreover, the onset in MEN1 is at a younger age, and NF-pNETs are diagnosed in an earlier stage because of the screening programs [9,10]. This illustrates the importance to substantiate guidelines providing recommendations for MEN1 patients, based on evidence derived from MEN1 populations. Current guidelines advise MEN1 mutation analysis already at the age of five and subsequent presymptomatic screening for MEN1 manifestations [3]. Because of their “silent” behavior and the correlation between metastases and tumor size [7], identification of NF-pNETs depends on sensitive biochemical biomarkers and imaging modalities [5,7].

The use of biochemical markers for the diagnosis of NF-pNETs is currently under debate, as the most recent studies on biomarkers reported low diagnostic accuracies for pNETs in MEN1 [11,12]. In addition to biochemical testing, clinical practice guidelines recommend diagnosis and surveillance of NF-pNETs by anatomical imaging modalities, such as CT scan, MRI, or endoscopic ultrasound (EUS) [3]. Functional imaging, such as somatostatin receptor scintigraphy (SRS) and [gallium 68 octreotate (^{68}Ga)]-labeled somatostatin analogs positron emission tomography (PET; ^{68}Ga -dodecanetetraacetic acid (DOTA) PET-CT), is emerging, and therefore, the best approach to NF-pNETs needs to be re-evaluated. In addition, recent studies showed insights in the very low growth rate of small NF-pNETs, fueling the discussion on timing and frequency of surveillance [13,14].

The diagnosis of small NF-pNETs with a possible indolent course of disease creates a high risk for unnecessary and expensive screening and consequently, a high burden for the patients. Whereas consensus on the indications for surgery could not be established in 2012 [3], current cohort studies give substantial evidence that a conservative approach for tumors up to 2 cm fits within treatment goals to reduce morbidity and mortality associated with metastatic disease [14,15]. This frames the screening dilemma in MEN1: imaging modalities should reliably detect tumors below the cutoff of 2 cm, but the indolent behavior of a large proportion of NF-pNETs could lead to overdiagnosis. The current guideline dates from 2012 and gives rise to the need of an evidence-based approach for diagnosis and follow-up in MEN1 [3]. Recently, controversies in the diagnostic approach in MEN1 were outlined, but a systematic overview of up-to-date literature on NF-pNETs is lacking [16–18]. We systematically reviewed and critically appraised the present literature on the diagnostic value of biochemical biomarkers and various imaging modalities to diagnose NF-pNETs in patients with MEN1. In addition, we evaluated the optimal timing of follow-up by reviewing current literature on the age-related penetrance and tumor growth of NF-pNETs in MEN1.

METHODS

Search Strategies

The electronic bibliographic databases Medline/Pubmed, Embase, and Web of Science were searched December 2017 to review systematically current literature on the diagnostic value of biomarkers and imaging modalities for NF-pNET in MEN1 patients. Keywords are reported in Table 1, and the complete search string is documented in Supplemental Material 1. To gain insight into the penetrance and behavior of NF-pNETs in MEN1 and subsequently answer the question on the optimal timing and frequency of follow-up, a third search was operated, also including our study domain (MEN1 patients). The literature searches were reviewed by an experienced librarian. Database subject terms, such as Mesh terms (Medline) and Emtree terms (Embase), were used as appropriate. Selection of articles was restricted to English, Dutch, German, and French, and for original research, there was no restriction for the year of publication of the studies.

Study Selection

Original studies assessing the diagnostic value of biomarkers or imaging modalities for the diagnosis (NF-)pNETs in patients with MEN1 were eligible for inclusion. In addition, articles were selected if tumor growth and/or penetrance were studied. Studies that included both sporadic NF-pNETs and MEN1-related NF-pNETs were eligible if it was possible to

Table 1. Keywords

Tumor markers for diagnosis NF-pNETs in MEN1				
Biomarker OR Chromogranin A OR pancreatic polypeptide OR glucagon	AND	Neuroendocrine tumor OR endocrine tumor OR NET OR non-functioning tumor	AND	Pancreas OR duodenopancreatic OR gastroenteropancreatic OR pNET
Imaging for diagnosis NF-pNETs in MEN1				
Imaging OR CT OR MRI OR EUS OR Ultrasonography OR Scintigraphy OR PET	AND	Neuroendocrine tumor OR endocrine tumor OR NET OR non-functioning tumor	AND	Pancreas OR duodenopancreatic OR gastroenteropancreatic OR pNET
Growth Rate and Penetrance of NF-pNETs in MEN1				
Multiple endocrine neoplasia type 1 OR MEN1 OR Werner syndrome OR hereditary	AND	Neuroendocrine tumor OR endocrine tumor OR NET OR non-functioning tumor	AND	Pancreas OR duodenopancreatic OR gastroenteropancreatic OR pNET

Searches were conducted in December 2017. Abbreviations: CgA, chromogranin A; PP, pancreatic polypeptide.

extract data for MEN1-related (NF-)pNETs separately. We excluded reviews, case reports, and studies including only functional dpNETs. Functional dpNETs were defined as tumors with biologically active hormone secretion and consequently, distinct clinical syndromes or symptoms, e.g., gastrinomas, insulinomas, and glucagonomas. NF-pNETs were NETs without a distinct clinical syndrome as a result of excessive hormone production. pNETs immunoreactive to other gastrointestinal hormones without a clinical syndrome are regarded as NF-pNETs. To minimize selection bias, studies with five or less MEN1 NF-pNET patients were excluded.

Data Extraction

All identified articles were entered in Covidence® and after the removal of duplicates, independently screened on title and abstract by two authors (M.J.C.v.T. and D.-J.v.B.). Thereafter, independent full text review of potentially relevant studies was performed, and studies were selected if eligibility criteria were fulfilled (M.J.C.v.T. and D.-J.v.B.). Authors resolved any disagreements by consensus and when unsuccessful, with the help of a third reviewer (G.D.V.). Reasons for exclusion at full text screening were recorded. All included articles were cross referenced for additional relevant articles. Diagnostic accuracy measures, sensitivity, specificity, positive predictive value (PPV), negative predictive value, and area under the curve (AUC) were obtained from the included studies. In case these measures were not provided, data were obtained, and 2x2 contingency tables were calculated. Thereafter, sensitivity and specificity were calculated using the standard formulas: sensitivity (%) = true positives/(true positive + false negative); specificity (%) = true negative/(true negative + false positive). In addition, NF-pNET growth rates and the age-related penetrance of NF-pNETs were obtained. With the consideration of the rarity of the MEN1 syndrome and

the expected heterogeneity between studies, as a result of long inclusion periods, differences in patient care, and patient characteristics, narrative data analysis was preferred over meta-analysis.

Risk of Bias Assessment

Study and patient characteristics were retrieved from the included articles. Included articles on biomarkers and imaging modalities were critically appraised using a modified Quality Assessment of Diagnostic Accuracy Studies tool by two reviewers independently (M.J.C.v.T. and D.-J.v.B.) [19]. Quality Assessment of Diagnostic Accuracy Studies addresses four important domains: patient selection, index test, reference standard, and flow and timing (Supplemental Material 2). We developed a risk of bias tool to appraise critically studies assessing the growth rate in NF-pNETs (Supplemental Material 3). Based on the Quality In Prognosis Studies tool [20] for prognostic studies, four important domains and subsequent criteria were formulated: study participation and attrition, identification of NF-pNETs, outcome measurement, analysis, and reporting. To grade the strength of recommendations and quality of evidence, we used the Grading of Recommendations, Assessment, Development, and Evaluation system [21,22].

RESULTS

Biochemical Tumor Markers

A total of 4281 studies were identified in the databases, of which 519 were duplicates (Fig. 1a) [23]. After removal of duplicates, 3762 studies were screened on title/abstract, and subsequently, full texts were retrieved for 46 potentially relevant studies. Eventually, 11 studies were included for risk of bias assessment.

Characteristics of the included studies are reported in Table 2. Overall, considerable heterogeneity was observed in study designs and in study populations. The majority of studies was derived from single center patient populations, whereas only one study assessed the biomarker accuracy in a multicenter population-based cohort [12]. Several studies used a case-control design to observe possible differences in tumor markers among MEN1 (NF-) pNET patients, MEN1 patients, sporadic pNET patients, and/or healthy controls. Most studies focused on a single tumor marker.

The methodological quality of the studies and their risk of bias varied among the studies (Table 3). In all studies, except for de Laat *et al.* [12], patient selection could have introduced bias. Exclusion criteria [e.g., proton-pump inhibitor (PPI) use or chronic kidney failure], selection of certain subgroups (e.g., preoperative estimation of tumor markers), or a case-control design could have overestimated biomarker accuracy. In almost all included studies,

Table 2. Study characteristics of included biomarker studies.

Authors, Year, Ref.	Country	Single/Multicenter	Population	No. MEN1 Patients	MEN1 Tumor Markers	MEN1 (NF-) pNET	Index Test(s)	Reference Test
De Laat <i>et al.</i> , 2013 (12)	The Netherlands	Multicenter	Population-based cohort	274	159	159	CgA n=81 PP n=73 Glucagon n=94	Pathology. If not available CT/MRI/EUS
Granberg <i>et al.</i> , 1999 (24)	Sweden	Single center	Case control	36	36	27	CgA	CT/ US
Langer <i>et al.</i> , 2001 (30)	Germany	Single center	Case control	23	12	12, 6 NF-pNET	PP (stimulated)	Pathology, CT/SRS/ EUS, biochemistry
Lewis <i>et al.</i> , 2012 (28)	USA	Single center	Cohort	52	52	52	CgA n=4 PP n=30 Glucagon n=29	Pathology
Mutch <i>et al.</i> , 1997 (29)	USA	Single center	Cohort	459	202	20	PP	CT/MRI/SRS/ Selective angiography
Nehar <i>et al.</i> , 2004 (25)	France	Single center	Case control	34	34	22, 11 NF-pNET	CgA	CT/EUS
Perrachi <i>et al.</i> , 2003 (26)	Italy	Single center	Case control	25	25	16, 6 NF-pNET	CgA	?
Stridsberg <i>et al.</i> , 1995 (27)	Sweden	Single center	Case control	11	11	?	CgA	Pathology
Qui <i>et al.</i> , 2016 (11)	USA	Single Center	Cohort	293	113	55 pNET, 58 Non pNET	CgA n=79 PP n=63 Glucagon n=24	Pathology. If not available CT/MRI/EUS/SRS

N= total number, US ultrasonography

the reference standard or patient flow could have introduced bias, or insufficient data were available to score these risks. Most studies scored a high risk of bias for the reference test, because of variation in reference standards within the study (including reference standards with low accuracy), lack of blinding, or the latency between index and reference test. This is the result of retrospective research on a rare syndrome where data usually were collected in the course of patient care without standardization.

Chromogranin A

Six studies evaluated chromogranin A (CgA) as a diagnostic tumor marker in MEN1 patients [11,12,24–27]. Two studies had maximum applicability for this review [11,12]. de Laat *et al.* [12] had the lowest overall risk of bias and estimated the accuracy of CgA in 81 consecutive Dutch MEN1 patients. AUC for CgA was 0.48 with a reported sensitivity of 33% [12]. Subgroup analysis showed only a slight improvement of accuracy in patients without PPI

use compared with those with PPI: AUC 0.56 vs AUC 0.47, respectively. The accuracy of CgA (AUC 0.66) for metastatic disease was evaluated, as well with a sensitivity of 53%. Qiu *et al.* [11] evaluated CgA in 79 patients. Reported AUC was 0.60, but patients on PPI were excluded from the analysis. No correlation was observed between CgA and tumor size, tumor load, or tumor stage nor an association with overall survival.

The three remaining studies at higher risk of bias, especially because of the selection of patients and size of the study (Table 3), concluded insufficient accuracy for CgA as a screening biomarker to identify (early) pancreatic involvement in MEN1, and none of the included studies advised CgA as a screening tool for diagnosis and staging. Sensitivity in these studies ranged from 27% up to 70%, and a low specificity was reported. No data were presented on the accuracy of CgA as a marker for progressive disease.

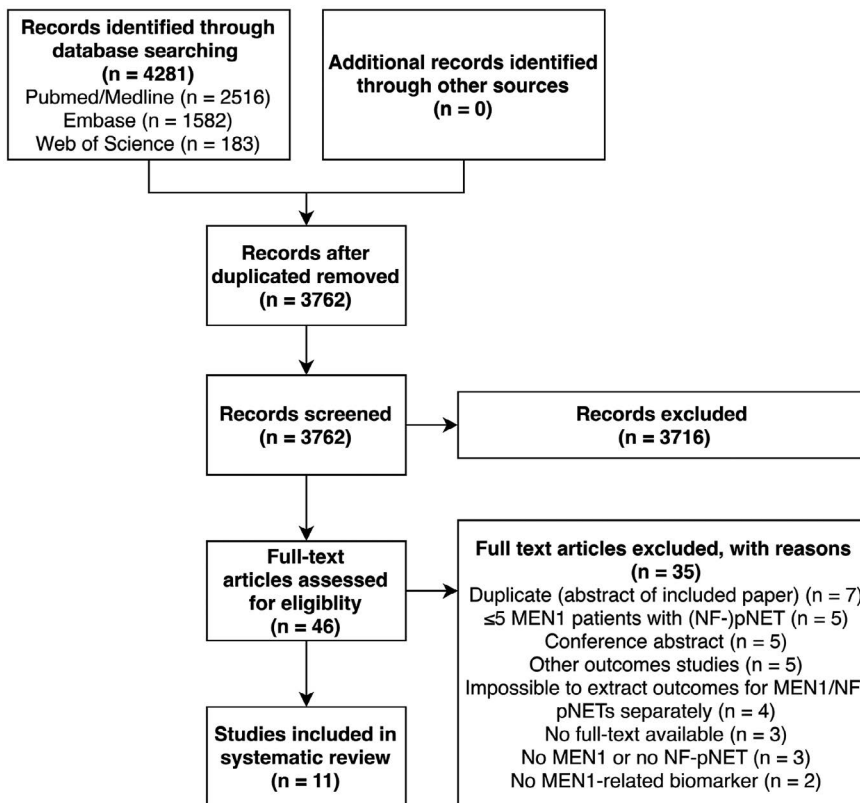


Figure 1A. Preferred Reporting Items for Systematic Reviews and Meta-Analyses flow diagram for identified biomarker studies

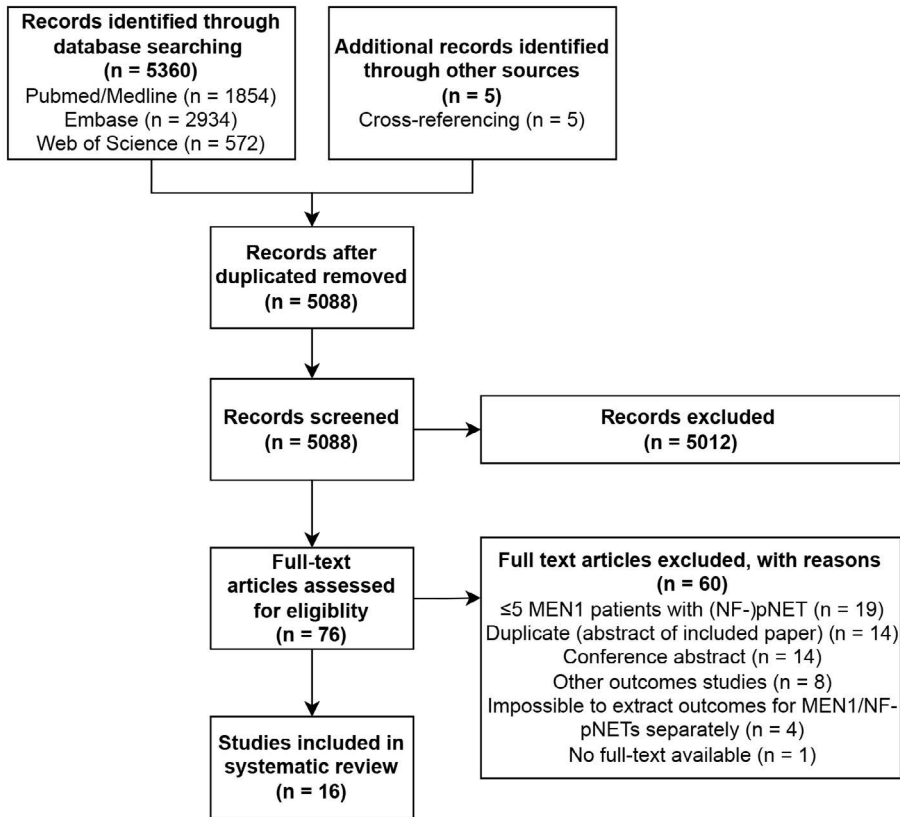


Figure 1B. Preferred Reporting Items for Systematic Reviews and Meta-Analyses flow diagram for identified imaging studies

Based on two studies with maximum applicability, low risks of bias, and equivalent reported outcomes, we conclude an inadequate diagnostic value for CgA. Therefore, CgA should not be routinely used in MEN1 NF-pNET screening programs.

Pancreatic polypeptide

Four studies evaluated pancreatic polypeptide (PP) [11,12,28,29], of which two studies met applicability criteria for this review (Table 3). de Laat *et al.* [12] reported a sensitivity of 36%, specificity 74%, and AUC 0.64 for the diagnosis of a pNET. Qiu *et al.* [11] reported the same AUC (0.64) for PP. For metastatic disease, a sensitivity of 50%, specificity of 74%, and AUC of 0.73 were reported [12]. No correlation was found between PP and tumor size, number of tumors, or tumor stage, but PP levels correlated with age and functional status of pNET. No association was found with survival [11]. Both studies concluded an

unsatisfactory diagnostic value. Lewis *et al.* [28] reported a decline in PP after surgery in 81% of the population. This assumes a correlation with tumor load, but no quantitative data were shown. Therefore, it remains unclear whether the postoperative decrease in PP had any clinical relevance. Mutch *et al.* [29] published PP outcomes in 202 patients with MEN1 and reported specificity of 88% and sensitivity of 95%. However, metrics were likely to be overestimated as a result of verification bias, as only patients with elevated PP levels and eight patients with normal fasting plasma PP, but with clinical suggestive symptoms, received radiographical evaluation (reference standard; $n = 28$). One study evaluated the serum PP after a standard meal stimulation test in patients with MEN1 [30] and concluded that a meal stimulation test was not reliable and added no extra information on the presence of pNETs.

In conclusion, current literature does not substantiate the use of PP as a diagnostic for NF-pNETs in MEN1.

Glucagon

Three studies reported outcomes on glucagon in MEN1-related pNETs (Table 2). Reported AUCs were 0.77 and 0.58 [11,12]. One study reported a sensitivity and specificity of 43% and 73% [12] in 94 patients. No significant correlation was found among tumor size, number of tumors, tumor stage, or location in 24 patients [11], but another study reported a moderate but significant correlation between ^{68}Ga -dodecanetetraacetic acid tyrosine-3-octreotate (DOTATATE)-avid tumor volume of pNETs to plasma glucagon levels in 25 patients with

Table 3. Risk of bias for included studies assessing the diagnostic value of biomarkers for pNETs in MEN1.

Authors, Year	Biomarker	Risk of Bias				Applicability		
		Patient Selection	Index Test	Reference Standard	Flow and Timing	Patient Selection	Index Test	Reference Standard
De Laat <i>et al.</i> , 2013 (12)	CgA, PP, glucagon	+	+	?	-	+	+	+
Qui <i>et al.</i> , 2016 (11)	CgA, PP, glucagon, gastrin	-	+	?	-	+	+	+
Granberg <i>et al.</i> , 1999 (24)	CgA	-	+	-	-	+	+	-
Mutch <i>et al.</i> , 1997 (29)	PP	-	+	-	-	-	+	+
Nehar <i>et al.</i> , 2004 (25)	CgA	-	-	+	-	-	+	+
Perrachi <i>et al.</i> , 2003 (26)	CgA	-	-	?	?	-	+	?
Langer <i>et al.</i> , 2001 (30)	Meal stimulation test	-	+	-	-	-	+	-
Lewis <i>et al.</i> , 2012 (28)	PP, gastrin, glucagon	-	+	+	?	-	-	+
Stridsberg <i>et al.</i> , 1995 (27)	CgA	-	-	+	?	?	+	+

Abbreviations: +, low risk/low applicability concerns; 2, high risk/high applicability concerns; ?, unclear.

MEN1 ($r = 0.5$) [31]. This study was not included in the risk of bias evaluation, as the diagnostic value for diagnosis of a pNET was not assessed, but the correlation between tumor markers and maximum standardized uptake value was assessed. Nevertheless, a large proportion of glucagon levels was within reference range, whereas only patients with proven NETs were included, confirming the low diagnostic sensitivity [31]. In 29 of the 56 cases studied by Lewis *et al.* [28], elevated glucagon levels were observed in 24 (83%).

Based on low accuracies and lack of correlation with disease status in the first two studies, we conclude that glucagon cannot play a vital role in MEN1 screening programs for NF-pNETs.

Other biomarkers

Gastrin was evaluated by some included studies, but gastrinomas are beyond the scope of this review.

Two studies estimated the diagnostic value of a combination of biomarkers. Qiu *et al.* [11] found an AUC of 0.60 for the combination of CgA, PP, and gastrin. This result is consistent with de Laat *et al.* [12], who reported an AUC of 0.59 for CgA, PP, and glucagon. Therefore, we conclude that the combined use of biomarkers is not of added value to the use of the individual biomarkers for the diagnosis of NF-pNETs in MEN1 patients.

No studies on the diagnostic value of circulating tumor cells or molecular markers, such as micro RNA and mRNA of cell-free DNA in MEN1-related pNETs, were encountered in this study using our search strategy.

Imaging

The search strategy yielded 5360 results (Fig. 1b). After the removal of duplicates, 5083 were screened on title and abstract, of which 71 potentially relevant articles were selected for full-text screening. Sixteen studies were included for risk of bias assessment.

Except for one study [32], all included articles were single-center studies. Seven studies collected the data prospectively [32–38].

Most studies reported results on EUS, followed by CT, SRS, ^{68}Ga -DOTAPET-CT, and MRI (Table 4). Seven studies had no concerns regarding applicability for this review (Table 5). There was a high concern on applicability for patient selection in most studies because of the high proportion of included functioning dpNETs or because a surgical cohort was analyzed. Most studies scored a high risk of bias on flow and timing because of different reference standards within the study population and the lack of standardization of index and reference test in the majority of studies.

Table 4. Study characteristics of included imaging studies.

Author, year	Country	Single/ Multicenter	Study Design and Data Collection	No. MEN1 Patients	MEN1/ pNET	MEN1 NF-pNET	Index Test	Reference Test
Albers <i>et al.</i> , 2017 (33)	Germany	Single center	Cross-sectional, prospective data collection	33	33	31	⁶⁸ Ga- DOTATOC PET-CT	MRI/EUS
Barbe <i>et al.</i> , 2012 (32)	France	Multicenter	Cross-sectional, prospective inclusion/ data collection	90	90	90	MRI/EUS	MRI/EUS
Camera <i>et al.</i> , 2011 (40)	Italy	Single center	Cross-sectional	14	9	?	CT	Pathology (n=4), EUS
Gauger <i>et al.</i> , 2003 (43)	USA	Single center	Cross-sectional, retrospective data collection	66	15	13	EUS	Pathology
Goroshi <i>et al.</i> , 2016 (39)	India	Single center	Retrospective data collection	18	13	6	⁶⁸ Ga- DOTANOC PET/CT	CT/ Pathology
Hellman <i>et al.</i> , 2005 (44)	Sweden	Single center	Cross-sectional, retrospective data collection	25	25	23	EUS 5-HTP PET (selectively)	CT, US (n=3) Pathology (n=8)) Rest biochemical
Kornaczewski Jackson <i>et al.</i> , 2017 (46)	Australia (Tasmania)	Single center	Retrospective data collection	49	25	12	¹⁸ F-FDG PET/ CT	Pathology, CT, ultrasound, EUS, MRI
Langer <i>et al.</i> , 2004 (34)	Germany	Single center	Prospective data collection	36	22	13	EUS, CT, ¹¹¹ In SRS	Pathology or clinical FU
Lastoria <i>et al.</i> , 2016 (45)	Italy	Single center	Cross-sectional	18	11	?	⁶⁸ Ga- DOTATATE PET/CT	Pathology or clinical FU
Lewis <i>et al.</i> , 2012 (28)	USA	Single center	Cross-sectional, retrospective data collection	52	52	?	¹¹¹ In SRS, CT, MRI, EUS	Pathology
Morgat <i>et al.</i> , 2016 (35)	France	Single center	Cross-sectional, prospective data collection	19	19	?	⁶⁸ Ga-DOTA- TOC PET/CT, ¹¹¹ In SRS, CT	Pathology, CT/MRI/EUS/ ¹⁸ FDG PET/ CT
Skogseid <i>et al.</i> , 1998 (41)	Sweden	Single center	Cross-sectional, retrospective data collection	25	25	13	CT, US, MRI, angiography, SRS	Pathology
Van Asselt <i>et al.</i> , 2015 (36)	The Netherlands	Single center	Cross-sectional study, prospective data collection	41	35	?	EUS, ¹¹ C-5-HTP PET	Pathology, CT/MRI
Waldmann <i>et al.</i> , 2009 (37)	Germany	Single center	Prospective data collection	35	24	18	CT, SRS, EUS	Pathology
Wamsteker <i>et al.</i> , 2003 (42)	USA	Single center	Cross-sectional study, retrospective data collection	65	13	11	EUS	Pathology
Yim <i>et al.</i> , 1998 (38)	USA	Single center	Prospective data collection	29	?	?	¹¹¹ In SRS	Pathology, CT/MRI/ arteriogram

Abbreviations: ¹¹C-5-HTP, ¹¹C-5-hydroxytryptophan; ¹⁸FDG ¹⁸F-Fluorodeoxyglucose

Table 5. Risk of bias for included studies assessing the diagnostic value of imaging modalities for pNETs in MEN1.

Authors, Year	Imaging	Risk of Bias				Applicability		
		Patient Selection	Index Test	Reference Standard	Flow and Timing	Patient Selection	Index Test	Reference Standard
Albers <i>et al.</i> , 2017 (33)	EUS/ MRI/ ⁶⁸ GaPET/CT	+	-	-	+	+	+	+
Barbe <i>et al.</i> , 2012 (32)	EUS / MRI	+	+	-	+	+	+	+
Lastoria <i>et al.</i> , 2016 (45)	⁶⁸ Ga PET/CT	+	?	-	-	+	+	+
Van Asselt <i>et al.</i> , 2015 (36)	MRI, CT, EUS, SRS, 11C-5-HTP PET	-	+	-	-	+	+	+
Morgat <i>et al.</i> , 2016 (35)	⁶⁸ Ga PET/CT, CE-CT, SRS	+	+	-	-	+	+	+
Gauger <i>et al.</i> , 2003 (43)	EUS	+	+	-	-	+	+	+
Hellman <i>et al.</i> , 2005 (44)	EUS	+	?	-	-	+	+	-
Goroshi <i>et al.</i> , 2016 (39)	⁶⁸ Ga PET/CT, CT	+	-	+	-	-	+	+
Wamsteker <i>et al.</i> , 2003 (42)	EUS	-	+	+	-	+	+	+
Kornaczewski Jackson <i>et al.</i> , 2017 (46)	¹⁸ FDG PET/CT	+	?	-	-	-	+	+
Langer <i>et al.</i> , 2004 (34)	EUS, CT, SRS	+	+	-	-	-	+	+
Lewis <i>et al.</i> , 2012 (28)	EUS, CT, MRI, SRS	-	+	+	?	-	+	+
Camera <i>et al.</i> , 2011 (40)	CT	+	-	-	-	-	+	+
Waldmann <i>et al.</i> , 2009 (37)	EUS, SRS, CT	+	-	-	-	-	+	+
Skogseid <i>et al.</i> , 1998 (41)	CT, SRS	-	+	+	?	-	+	+
Yim <i>et al.</i> , 1998 (38)	SRS	-	?	+	-	-	-	+

Abbreviations: CE, contrast-enhanced.

Conventional imaging

CT

Sensitivity varied between 54% and 81%, with a specificity of 50% (Table 6) [28,34,35,37,39–41]. Langer *et al.* [34] reported 54% sensitivity in a group of patients with dpNET with surgery as a reference standard. All patients with a false-negative CT had small duodenal or pancreatic gastrinomas (largest 14 mm). Lewis *et al.* [28] reported the highest sensitivity (81%) with a PPV of 96% on preoperative CT. Eight of the 43 CTs were negative, with the largest missed pNET measuring 4 cm. In a prospective series of 19 consecutive patients with MEN1 suspected for dpNETs undergoing ⁶⁸Ga-dodecanetetraacetic acid–tyrosine-3-octreotide (DOTA-TOC) and contrast-enhanced CT, the reported sensitivity and specificity of CT were 60% and 50%, respectively. However, on a per-lesion basis, solely of the pancreas, CT revealed 37 of the 46 lesions (80%) identified by ⁶⁸Ga-DOTA-TOC PET scanning [35]. All included studies had high risks of bias, but most results were uniform. Studies reported inferior diagnostic values of CT compared with ⁶⁸Ga-DOTA PET-CT [35,39] and EUS

Table 6. Accuracy of Imaging Modalities

Author, Year	EUS	MRI	CT	SRS	⁶⁸ Ga-PET/CT
Albers <i>et al.</i> , 2017 (33)					
n	27	27			27
Sensitivity, %	100%	74%			78%
Barbe <i>et al.</i> , 2012 (32) [‡]					
n	75	67			
Sensitivity, %	83%	74%			
Lastoria <i>et al.</i> , 2016 (45)					
n					11
Sensitivity, %					100%
Van Asselt <i>et al.</i> , 2015 (36) ^{*‡}					
n	35			35	
Sensitivity, %	97%			51%	
Morgat <i>et al.</i> , 2016 (35) [†]					
n			76	76	76
Sensitivity, %			60%	20%	76%
Specificity, %			50%	50%	100%
Gauger <i>et al.</i> , 2003 (43)					
n	13				
Sensitivity, %	92%				
Hellman <i>et al.</i> , 2005 (44)					
n	22/8				
Sensitivity, %	64%/50%				
Goroshi <i>et al.</i> , 2016 (39) [†]					
n			13		13
Sensitivity, %			63%		100%
Wamsteker <i>et al.</i> , 2003 (42) [†]					
n	10				
Sensitivity, %	82%				
Langer <i>et al.</i> , 2004 (34)					
n	16		13	17	
Sensitivity, %	75%		54%	71%	
Lewis <i>et al.</i> , 2012 (28)					
n	35	8	43	32	
Sensitivity, %	100%	88%	81%	84%	
Camera <i>et al.</i> , 2011 (40) [†]					
n			11		
Sensitivity, %			78%		
Skogseid <i>et al.</i> , 1998 (41) [∞]					
n			15/10	15/10	
Sensitivity, %			57%/20%	75%/0%	
Waldmann <i>et al.</i> , 2009 (37)					
n	20		24	24	
Sensitivity, %	100%		62%	54%	
Yim <i>et al.</i> , 1998 (38) [†]					
n				16	
Sensitivity, %				58%	

Abbreviation: n, number of included patients in the study.

‡ Results from analysis for pNETs > 1cm.

* Not every patient received an MRI or CT (either MRI or CT), so sensitivity could not be extracted.

‡ sensitivity based on per-lesion analysis in n patients.

no reference standard was described for the index test. Results in table are distracted from the article with biochemical signs (n=22) / histopathology (n= 8) as reference standard.

∞ =population and sensitivity for major disease / limited disease

[28,34,37]. The reported differences in accuracy between CT and SRS were varying (Table 6), and no direct comparison was made between MRI and CT. Studies describing the characteristics of missed lesions on CT reported small sizes, mostly below 17 mm, but a few exceptions were recorded [28,34,35].

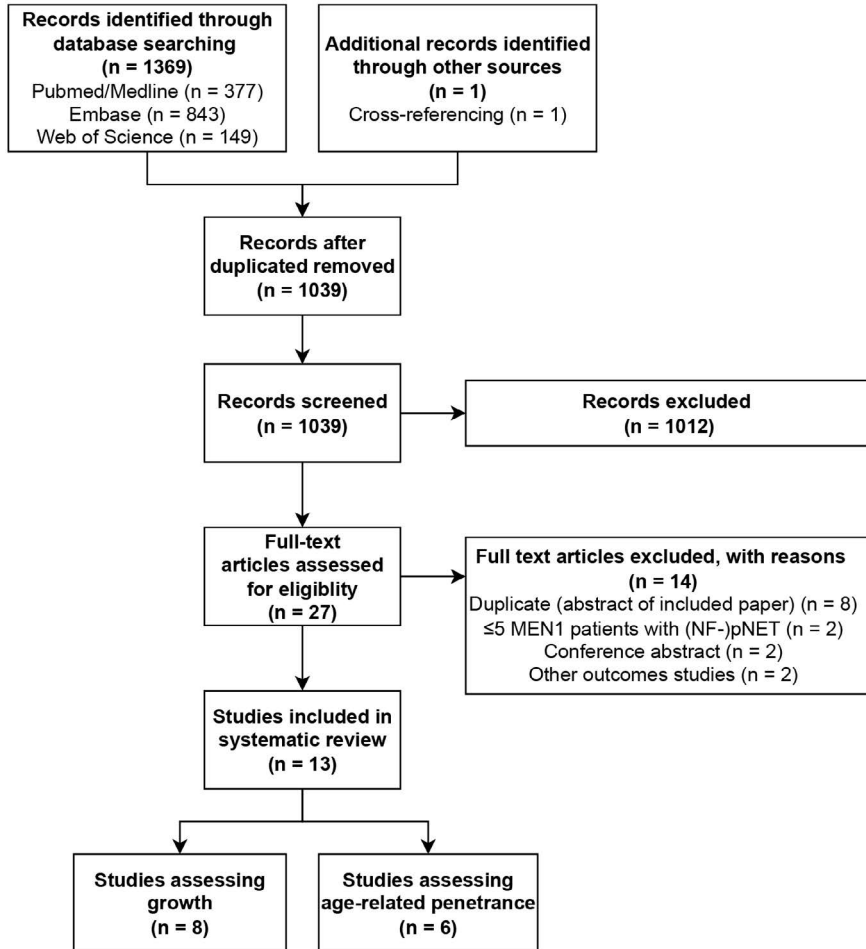


Figure 1C. Preferred Reporting Items for Systematic Reviews and Meta-Analyses flow diagram for identified studies on growth and penetrance

MRI

Three studies evaluated the diagnostic value of MRI with reported sensitivities varying between 74% and 88% [28,32,33] and a PPV of 100% [28]. Barbe *et al.* [32] compared the diagnostic value of MRI (1.5 T) with EUS in 90 consecutive patients with MEN1 to study the concordance of both modalities for the detection of pNETs ≥ 10 mm. MRI and EUS (reference standard was the combination of both) identified 57 (63%) patients with tumors between 10 and 20 mm. Overall, sensitivities for EUS and MRI were 84% and 81%, but for tumors >20 mm, sensitivities were 65% for EUS and 85% for MRI, respectively. Barbe *et al.* [32] concluded that for the detection of ≥ 10 mm pNETs, EUS is superior to MRI, but the latter performed homogeneous throughout the entire the pancreas, whereas 94% of all tumors missed by EUS were in the body and tail. Therefore, they advised that both should be performed at the initial evaluation [32]. It is important to note that the diagnostic value may be underestimated, as imaging outcomes were classified as negative, whereas a tumor <10 mm was identified on one of the modalities ($\sim 25\%$ of patients). Albers *et al.* [33] compared EUS, ^{68}Ga -DOTA PET-CT, and MRI (1.5 T) and reported MRI sensitivity of 74% on a patient-based level. The authors also performed a per-lesion subgroup analysis based on the size of pNETs. Sensitivities for 0 to 5, 5 to 10, 10 to 20, and >20 mm were 17%, 22%, 35%, and 83%, respectively, concluding a reliable MRI detection for larger pNETs. Lewis *et al.* [28] preoperatively evaluated MRI in eight patients; with seven out of eight patients positive, sensitivity was 88%, and PPV was 100%.

All three studies had a high risk of bias in one of the four domains. No study directly compared MRI with CT as diagnostic for NF-pNETs in MEN1, so no conclusions can be made on the preferred noninvasive conventional imaging modality. Based on the reported sensitivities, quality of the included studies, and the risks associated with cumulative exposure to ionizing radiation, one could suggest MRI should be preferred above CT.

EUS

EUS sensitivity ranged from 75% to 100% [28,32–34,36,37,42–44] in most studies. One study reported a sensitivity of 50%, but this study has a high risk of bias, as no reference standard for pNETs was described (selection of patients was based on elevated biochemical markers), and histopathology was only available in a small subgroup [44]. In general, histopathology, as reference standard, could also lead to lower sensitivities of imaging modalities if very small pNETs are included in per-lesion analysis as well.

van Asselt *et al.* [36] compared four imaging modalities [CT or MRI + SRS + 11C-5-hydroxytryptophan (11C-5-HTP) PET + EUS] in 41 patients. In 35 patients, 107 pNETs were identified by combining all modalities. EUS identified 97% of the patients and 94% of the pNETs, which was significantly better than compared with the other modalities. In the subgroup analysis of pNETs >1 cm, EUS remained superior, as 97% of the pNETs were identified. Albers *et al.* [33] showed similar superiority for EUS over ^{68}Ga -DOTA PET-CT

and MRI, but this difference was only statistically significant for pNETs <1cm.

Lewis *et al.* [28] evaluated preoperative imaging in 52 individuals who underwent 56 pancreatic surgeries. EUS was performed preoperatively in 63% and had the highest sensitivity (100%) on a patient basis. Two series derived from Ann Arbor, Michigan, compared preoperative EUS with histopathology. In 13 asymptomatic patients who underwent surgery, Gauger *et al.* [43] identified pNETs on preoperative EUS in 12 (sensitivity 92%). Wamsteker *et al.* [42] found 23/28 pNETs (sensitivity 82%) on preoperative EUS in 10 asymptomatic patients.

All studies that reviewed EUS concluded that EUS is the most sensitive procedure. Therefore, EUS seems to have the highest diagnostic accuracy for the detection of small NF-pNETs [32,33]. However, some clinically relevant NF-pNETs are missed, especially in the pancreatic tail; EUS is an invasive procedure and is operator dependent. To overcome this issue, a multimodal strategy could be initiated, preferably with MRI. Concordance between MRI and EUS in tumors ≥ 10 mm was moderate (Kappa coefficient = 0.55) [32].

Functional imaging

¹¹¹In pentetreotide scan (SRS)

SRS was evaluated in seven studies [28,34–38,41]. Sensitivity varied between 20% and 84%. Morgat *et al.* [35] prospectively compared SRS with CT and ⁶⁸Ga-DOTAPET-CT for the detection of dpNETs in 31 patients and had the lowest risk of bias and the highest applicability. SRS showed a sensitivity of 20% and a specificity of 50%. Eleven pNETs were identified on SRS, whereas ⁶⁸Ga-DOTA-TOC PET/CT and CT identified 46 and 37 pNETs, respectively. All lesions depicted by SRS were positive in ⁶⁸Ga-DOTA PET-CT as well. Mean pNET size of those identified by SRS was 15 mm. Furthermore, ⁶⁸Ga-DOTA PET-CT depicted smaller lesions than SRS, leading to overall superior diagnostic performance [35]. The highest sensitivity (84%) was reported by Lewis *et al.* [28] and is possibly overestimated, as preoperative SRS was reviewed. It is possible that the average tumor size in this study was larger compared with asymptomatic MEN1 patients not undergoing surgery, but the average tumor size was not reported. The overall inferiority of SRS compared with ⁶⁸Ga-DOTA PET-CT and the insufficient sensitivity reported in studies with the lowest risk of bias assume no further indication for SRS in the screening of NF-pNETs in MEN1.

⁶⁸Ga DOTA PET-CT

⁶⁸Ga-DOTA PET-CT was evaluated in three prospective studies [33,35,45] and one study reported the diagnostic value in a retrospective case series [39]. Albers *et al.* [33] compared the diagnostic value of combined conventional imaging (EUS/MRI) with ⁶⁸Ga-DOTATOC-PET-CT for the diagnosis of dpNETs in routine follow-up in 33 MEN1 patients. Subgroup analysis for pNETs revealed a sensitivity of 78% for ⁶⁸Ga-DOTAPET-CT. Sensitivities

depended on pNET size; for pNETs <5, 5 to 10, 10 to 19, and ≥ 20 mm sensitivities were 0%, 29%, 81%, and 100%, respectively. In addition, the authors concluded that the routine use for ^{68}Ga -dodecanetetraacetic acid 1-NaI3-octreotide (DOTANOC)-PET-CT is limited for the detection of metastasis [33].

A similar sensitivity (76%) was reported by Morgat *et al.* [35], who compared ^{68}Ga -DOTA PET-CT with SRS and CT in a per-lesion analysis (75 dpNETs in 19 individuals). ^{68}Ga -DOTA PET-CT outperformed both in this study, and the reported specificity was 100%, but this was possibly overestimated, as a combination of imaging modalities, instead of histopathology, was used as a reference standard. The smallest reported lesion on ^{68}Ga -DOTA-TOC PET-CT measured 2 mm [35].

The two remaining studies on ^{68}Ga -DOTA PET-CT had small sample sizes and reported sensitivities of 100% [39,45]. Lastoria *et al.* [45] prospectively compared ^{68}Ga -DOTA PET-CT with conventional imaging for four MEN1-related tumor sites. The diagnostic value for pNETs was compared with EUS/CT or histology as reference standard [45]. Goroshi *et al.* [39] described a retrospective case series of 11 patients with 16 histopathologically proven dpNETs. Despite the maximum sensitivity in these two studies, ^{68}Ga -DOTA PET-CT should not be recommended as a first-choice imaging modality to screen MEN1 patients based on the reported results from Albers *et al.* [33] and Morgat *et al.* [35]. Both prospective studies had lower risks of bias and larger sample sizes and reported sensitivities of almost 80%. In addition, clinical management did not change after ^{68}Ga -DOTA PET-CT in 97% of all patients who also underwent conventional techniques for the complete screening of MEN1 [33]. As the diagnostic value of ^{68}Ga -DOTA PET-CT depends on tumor size, and the detecting of metastases is a major advantage, ^{68}Ga -DOTAPET-CT could be applied in patients with prevalent tumors >10 mm and not as a screening modality for detection of incident NF-pNETs.

Other imaging techniques

van Asselt *et al.* [36] reviewed ^{11}C -5-HTP PET and although superior to SRS, was of no additional value compared with standard screening (EUS), as only 54% of the patients and 32% of the pNETs were diagnosed. Kornaczewski Jackson *et al.* [46] evaluated the use of ^{18}F -fluorodeoxyglucose (^{18}F -FDG) PET in 49 patients with MEN1. Twenty-five patients had evidence of a pNET on conventional imaging, but ^{18}F -FDG PET was positive in only five (20%). ^{18}F -FDG PET avidity was positively associated with the Ki-67 index and pNET aggressiveness [46]. For risk stratification of aggressive disease, ^{18}F -FDG PET revealed a sensitivity of 86% and specificity of 95%. However, the value of ^{18}F -FDG PET for risk stratification over EUS and fine needle aspiration to determine Ki-67 needs to be determined in prospective studies. Current evidence withholds its applicability for routine screening in MEN1-related NF-pNETs.

Growth and penetrance

The systematic search identified 1369 articles (Fig. 1c). After duplicates were removed, 1038 articles were screened on title/abstract. Twenty-six studies were full-text reviewed for eligibility, of which 13 were included.

Eight studies assessed the growth of NF-pNETs [13,14,37,47–51] and six, the age-related penetrance of (NF-)pNETs in patients with MEN1 [7,50,52–55]. Of the studies assessing NF-pNET growth rate, two were derived from population-based cohorts [13,14], five assessed growth by consecutive EUS [37,47–50], and four collected data prospectively [14,37,48,50]. Studies evaluating growth rates were critically appraised (Table 7). One study had a low risk in all four domains [13]. Almost all studies have selection bias, as data were collected during patient follow-up. Patients with progressive tumors demanding surgery on short term are excluded in most studies, as two consecutive evaluations were not available. Therefore, reported growth rates are possibly underestimated, and the proportion patients with progressive tumors could be larger. Most studies did not address or report possible effect modifiers for tumor growth. Many studies did not assess current systemic treatment (e.g., somatostatin analogs), possibly influencing growth rates. Two studies on age-related penetrance were derived from a population-based cohort [7,53], whereas three other studies were multicenter studies [50,54,55].

Table 7. Risk of bias for included studies assessing growth rate in MEN1 related NF-pNETs.

Authors, years	Risk of Bias			
	Patient selection	Diagnosis	Outcome Measurement	(Statistical) Analysis
D'Souza <i>et al.</i> , 2014 (47)	–	+	+	–
Kann <i>et al.</i> , 2006 (48)	?	+	+	–
Kappelle <i>et al.</i> , 2017 (49)	–	+	+	–
Pieterman <i>et al.</i> , 2017 (13)	+	+	+	+
Sakurai <i>et al.</i> , 2007 (51)	–	–	+	?
Triponez <i>et al.</i> , 2017 (14)	+	–	–	?
Waldmann <i>et al.</i> , 2009 (37)	+	–	?	–

Growth

The annual growth rate and incidence of new lesions are summarized in Table 8. Growth rates varied between 0.1 and 1.32 mm per year. Reported factors associated with increased growth rate are scarce: MEN1 genotype [13], age [37] and number of pNETs visualized [49]. EUS and conventional imaging (CT/MRI) were used for growth assessment.

Table 8. Reported growth in NF-pNETs from included studies

Authors, year	n	No pNETs	NF-pNET size, mm	Design	Modality Used for Assessment	FU in Months	Size at First Detection (in mm Median)	Annual Growth (All lesions; mm/year)	Incidence New Lesions	Growth New Lesions (mm/year)
D'Souza <i>et al.</i> , 2014 (47)	11	18	NA	R	EUS	79 (18-134)	10.3 (5-24)	1.32	0.17	3.0
Kann <i>et al.</i> , 2006 (48)	20	84	<15	P	EUS	20 ± 12	5.9 (1.5-14.5)	1.3%±3.2%/month = 0.9±2.3	0.62	
Kappelle <i>et al.</i> , 2017* (49)	38	226	<20	R	EUS	38.4 (1.1-5.6)**	5.0	0.10	0.79	No growth
Pieterman <i>et al.</i> , 2017 (13)	99	115	<20	R	CT/MRI	156 (84-276)**	10±4	0.4 Stable: no growth Progressive: 1.6	1.04	
Triponez <i>et al.</i> , 2017 (14)	46	96	<20	P	CT/MRI/EUS	128 ±50.4	9.3± 5	Stable: <0.1 Progressive: 0.54		
Waldmann <i>et al.</i> , 2009* (37)	29	88	NA	P	EUS	72 (24-108)	9.0	11.7 ± 24.1% = 1.1 ± 2.17 mm	0.52	1.28
Sakurai <i>et al.</i> , 2007 (51)	14	26	NA	R?	CT	78±36	20±18 (5-78)	Not reported	Not reported	Not reported

FU: median (range) or means ± SD. Size: median (range) or means ± SD.
Abbreviations: NA, not applicable; P, prospective study; R, retrospective study.
*Population with NF-pNETs and (possibly) functional pNETs
**Inter quartile range

Conventional imaging

A population-based study evaluated the growth rate of 115 NF-pNETs <2 cm in 99 MEN1 patients and assessed the incidence of new NF-pNETs after a median follow-up of 13 years per patient. The growth rate was 0.4 mm/y and the incidence of new tumors was 1.04 per year [13]. An association between growth rate and tumor numbers could not be confirmed in this study. Noteworthy, subgroup analysis identified 35 tumors in 34 patients as progressive (growth rate of 1.6 mm/year), whereas the majority of pNETs (n = 80, 70%) was stable (no growth). Genotype was an important effect modifier for growth velocity, as patients with missense mutations had a significantly higher growth rate than nonsense/frameshift mutations [13]. The finding of a large proportion patients with stable disease without substantial growth is in line with Triponez *et al.* [14]. Differences in growth between those with progressive disease (increase in tumor size, number or development of a hypersecretion syndrome) and stable disease in NF-pNETs were estimated. Sixty-one percent had stable disease and showed no substantial growth [14]. Sakurai *et al.* [51] described 14 patients with MEN1 with 26 NF-pNETs and prospectively followed 13 NF-pNETs by CT. No substantial growth (increase in tumor size of >20%) was observed in 12/13 (92%), all of them smaller than 20 mm.

EUS

The fastest growth was reported by D'souza *et al.* [47] in a retrospective study, including 11 patients with a mean EUS surveillance of 79 months. Sixty-one percent of all lesions were stable during follow-up. Importantly, new lesions had a significantly faster growth rate compared with the index lesions. The authors suggested a variation in phenotypic expression of the disease [47]. The lowest growth rate was estimated in a larger EUS-based surveillance study in 226 patients [49]. Annual tumor growth was 0.10 mm in pNETs ≤2 cm, but if split for prevalent pNETs (0.21 mm/year) and incident pNETs (no growth), a significant difference was found [49]. Interestingly, the absence of growth in new lesions was in contrast with the findings of D'souza *et al.* [47]. Thomas-Marques *et al.* [56] reported outcomes of systematic follow-up with EUS in 51 MEN1 patients of whom 55% had NF-pNETs. Sixty-three percent of the patients with initial NF-pNETs had stable disease (e.g., no growth nor new lesions), whereas 25% developed new NF-pNETs, and 13% showed tumor growth only.

Penetrance

The results on age-related penetrance are described in Table 9.

The largest study on penetrance in MEN1 is derived from the French Groupe d'Etude des Tumeurs Endocrines population-based registry. Triponez *et al.* [7] reported isolated NF-pNETs penetrances of 3%, 34%, and 53% at 20, 50, and 80 years, respectively. A large

multicenter study from Germany reported lower age-related penetrances for NF-pNETs [54]. Machens *et al.* [54] also evaluated differences between penetrance and type of mutation (e.g., out-of-frame or truncating vs in-frame mutations), but no disparities were found. Gonçalves *et al.* [52] systematically screened 19 MEN1 mutation carriers in their second decade of life with EUS and/or MRI/CT. A much higher penetrance of NF-pNETs (42%) was observed in 19 patients. This difference is probably contributable to the imaging modality used for screening (mostly EUS). Fifty percent had multicentric NF-pNETs, 21% harbored a NF-pNET >2cm, and the largest pNET measured 40 mm [52]. Case reports were not included in this review, but Gonçalves *et al.* [52] also reviewed case reports of both functional and NF-pNETs in young MEN1 mutation carriers. The youngest patient with a NF-pNET >2 cm was 12 years old [57]. Goudet *et al.* [53] evaluated the penetrance and natural history of NF-pNETs in 160 young MEN1 patients from the Groupe d'Etude des Tumeurs Endocrines. By the age of 21, 23% harbored a pNET, of which NF-pNETs were present in 9%. The mean size of the largest NF-pNET was 18 mm. Five patients demanded surgical resection of the NF-pNET, on whom four were operated at the age of 13 to 15 years. In the operated patients, 43% of the NF-pNETs measured 2 cm or more, the largest being 4 cm [53]. Another German study investigated the age-

Table 9. Data on age related penetrance (NF-pNETs) from included studies

Study	No. of MEN1 Patients (pNETs)	Design	Age (Years)	Modality Used	Penetrance	Youngest Patient (Years)
Goncalves <i>et al.</i> , 2014 (52)	19 (8)	R	12-20	EUS (74%) CT/MRI	42% NF-pNETs by age 20 y	16
Goudet <i>et al.</i> , 2015 (53)	160	R	1-21	CT/MRI/EUS	9% NF-pNETs by age 21 y	13
Manoharan <i>et al.</i> , 2017 (55)	166 (8)	P	8 - 18	MRI/ EUS	1.8% NF-pNETs by age 19 y	15
Machens <i>et al.</i> , 2007 (54)	258 (126)	Cross	43 (Mean)	CT/MRI/EUS	Age related penetrance dpNETs (NF-pNETs) Mean age 14: 4% (0%) Mean age 33: 45% (18%) Mean age 48: 57% (14%) Mean age 64: 60% (13%)	NA
Triponez <i>et al.</i> , 2006 (7)	579 (108)	P		CT/MRI/EUS	Penetrance dpNET (NF-pNET) Age 20: 9% (3%) Age 50: 53% (34%) Age 80: 84% (53%)	NA
Thomas-Marques <i>et al.</i> , 2006 (50)	51	P	39 [16-71]	EUS	Frequency: 54.9% in cohort	16

Abbreviation: Cross, cross-sectional.

related penetrance in 166 MEN1 patients, 19 years derived from two centers. Twenty patients had MEN1 manifestations, of whom three had NF-pNETs at the ages of 15, 17, and 18, respectively (penetrance 1.8%) [55]. Two patients underwent pancreatic surgery for NF-pNETs <15 mm.

The reported penetrance below 10% by the age of 20 in large prospective cohorts, the sporadic need for surgical treatment below the age of 16, and the psychological burden of the screening program for young asymptomatic MEN1 children might be arguments to defend the start of screening for NF-pNETs at the age of 16. However, given the paucity of evidence, the refrainment from screening before the age of 16 cannot be advised. Results on age-related penetrance for young MEN1 patients are diverge and depend on the imaging modality used. Therefore, future studies are needed to establish the optimum timing for screening.

DISCUSSION

We conducted a thorough systematic literature search to identify studies assessing our research questions, as well as a critical appraisal to assess methodological quality and applicability of these studies. Two extensive search strings were generated, not specifically focusing on patients with MEN1, to discover studies on sporadic pNETs, also including patients with MEN1. As MEN1 is a very rare disease with heterogeneous disease manifestations and studies covering long time spans, modified risk of bias tools was composed to account for these issues as much as possible. A complete overview of currently available literature was generated, and subsequent conclusions for clinical care were drawn on current best-available evidence. In addition, important topics to assess in future research are generated.

Inherent to the rarity of the disease, only a few studies of sufficient methodological quality were included. The majority of the studies was retrospective by design, and data were collected from routine patient care, often without standardization. In line, blinding of observers (radiologists) was not done in most studies, and different reference standards were used. Most studies were conducted on populations derived from single centers, leading to a casemix of patients with MEN1. In addition, selected cohorts, such as surgically treated cohorts, were included. Sample size of most studies was limited, leading to insufficient power to detect statistically significant results and subsequent imprecise estimates. Lastly, because of the rarity of MEN1, studies included patients over a long time period. Changes in patient care, improved knowledge on MEN1, the intensive MEN1 screening program, and increased quality of imaging modalities are hard to account for in the study design and statistical analysis.

The inferior sensitivity of CT compared with EUS and ^{68}Ga -DOTA PET-CT and the

cumulative exposure to ionizing radiation, already exceeding levels deemed safe during 8 years of follow-up [58], make CT less useful as a radiologic screening modality in a life-long disease. CT seems to have advances in the preoperative assessment, but this is beyond the scope of this review. Based on the available literature, MRI turned out to be a more sensitive and convenient screening modality, as there is no radiation exposure, but reservations should be made, as currently, no direct comparison between CT and MRI has been undertaken in patients with MEN1. MRI studies evaluated 1.5 T MRI, but higher tesla imaging is currently common standard in expert centers. The use of better MRI might increase sensitivity, so the question remains as to whether these older MRI studies reflect current clinical practice. Although this is the case, the choice for the optimal screening modality to detect NF-pNETs remains ambiguous. We concluded EUS being the most sensitive imaging modality, detecting up to 2 mm. Serial assessments of tumor size to evaluate tumor growth are reliable with EUS [59], fine needle aspiration can be added, and adrenals glands can be visualized. On the other hand, EUS is limited by the operator dependence, has a decreased sensitivity in the pancreatic tail, and is an invasive procedure. Furthermore, small NF-pNETs, without therapeutic consequences, are detected but with the necessity of follow-up. This could theoretically lead to a higher psychological burden of disease. MRI has the advantage of homogenous performance throughout the pancreas, but a significant proportion of NF-pNETs >2 cm is missed. The latter also applies for EUS. To ensure maximum sensitivity, both modalities can be used alternately to detect lesions as early as possible and reduce the burden of invasive EUS (Table 10).

Regarding the high prevalence of somatostatin receptors on NETs, these tumors seem specifically interesting for somatostatin-labeled radionuclides. The recent advances ^{68}Ga -DOTA PET-CT in sporadic NETs have not been unnoticed in MEN1 research. Unfortunately, not all MEN1 studies on ^{68}Ga -DOTA PET-CT were included, as we could not extract data on (NF-)pNETs [60,61]. Contrary to Albers *et al.* [33], two studies report more promising results regarding changes in patient management based on ^{68}Ga -DOTA PET-CT. Both studies evaluated the impact of ^{68}Ga -DOTATOC PET-CT on diagnosis of MEN-associated lesions and its influence on therapeutic management [60,61]. They illustrated the advantage of “full body imaging” with ^{68}Ga -DOTA PET-CT in a disease with multiple organs involved and the risk of locoregional and distant metastases. As ^{68}Ga -DOTA PET-CT was introduced in recent years, studies on the diagnostic ability are scarce. From the current available evidence, we can conclude that although not superior in the detection of incident NF-pNETs, ^{68}Ga -DOTA PET-CT could be integrated in the follow-up program for NF-pNETs >1 cm to detect metastases in an early stage. The optimal timing and frequency of screening remain to be established. No biomarker reflects tumor behavior or predicts the course of disease over time, so imaging modalities remain the cornerstone within the surveillance program. Studies on molecular markers in MEN1 were not encountered, although the search string

Table 10. Summary of recommendations

Recommendations	Evidence (According to GRADE (21,22))
The annual use of CgA, PP, and glucagon as a tumor marker for the diagnosis of NF-pNETs is not recommended.	(1 ⊕⊕⊕○)
Radiological screening for NF-pNET should include magnetic resonance imaging (MRI) or endoscopic ultrasonography.	(2 ⊕⊕○○)
⁶⁸ Ga-DOTA PET/CT should be preferred over ¹¹¹ In single photon emission CT/CT for the diagnosis of NF-pNETs.	(1 ⊕⊕○○)
⁶⁸ Ga-DOTA PET/CT should not be routinely used for the diagnosis of NF-pNETs.	(2 ⊕○○○)
Based on the growth rate and NF-pNET size, pancreatic visualization can be extended to once per 1-2 years.	(2 ⊕⊕○○)
Screening for NF-pNETs in asymptomatic MEN1 patients should not be extended until the age of 16.	(2 ⊕○○○)

Abbreviations: GRADE, Grading of Recommendations, Assessment, Development, and Evaluation

did not specifically focus on these new biomarkers. Data on tumor growth can frame the clinical relevance of small NF-pNETs, as the malignant course of NF-pNETs seems to be correlated to tumor size [7]. We evaluated the current available data on growth to outline the average growth rate of NF-pNETs to distinguish those with more aggressive disease. Two large population-based cohorts reported very slow growth [13,14]. In addition, two recognizable phenotypes can be distinguished in patients with NF-pNETs. Most patients (60% to 70%) had stable disease with no growth at all, whereas a subgroup demonstrated tumor growth [13,14,50,51]. Pieterman *et al.* [13] speculated a multistep process of MEN1 pNET development; tumor initiation and tumor growth are two distinct steps with additional events needed for tumor growth and disease progression rather than germline mutation subtype alone. The effect of MEN1 germline mutation on menin protein may drive tumor initiation but could also be inversely correlated with tumor growth. Genetic or epigenetic events, such as mutations in DAXX or ATRX genes, might play important roles in growth-driving events [13]. This could explain the heterogeneity in incidence and annual growth among included studies. To tailor a follow-up regimen, surveillance programs should focus on identifying the course of disease in patients with NF-pNETs. Frequency of screening should be adapted for the growth rate in an individual, starting with repeated measurements every year after detection of NF-pNETs by EUS or MRI. After confirmation of stability of the tumor, surveillance could be extended to every 1 to 2 years over the course of time. In growing tumors, imaging should be repeated at least every year, and ⁶⁸Ga-DOTA PET-CT could be added in routine surveillance when tumors >10 mm are present to identify metastasis timely. Future studies should investigate the role of molecular biomarkers, such as the NETest® [62] for MEN1-related NF-pNETs, as current screening tools lack insight in the dynamics of individual tumor behavior.

The starting age of screening young mutation carriers remains controversial. The age-related penetrance for NF-pNETs is low under 20 years (1.8% to 9%) in the larger studies, but cases of large NF-pNETs requiring surgical intervention are reported. Furthermore, penetrance is underestimated in some studies because of the imaging modality used to screen included patients. One study systematically used EUS in young patients, revealing a much higher penetrance of almost 50% [52]. Included studies recommended to start screening between 10 and 16 years [53,55]. Because of the diversity in studies and outcomes, future research is needed to estimate the optimal age to start screening. In patients who elect not to have genetic testing but are at risk for MEN1, screening for pNETs should start at the same age as mutation carriers, as all manifestation can occur as first manifestation [53,63].

This study has some limitations. The inclusion criterion of more than five MEN1 patients with (NF-)pNETs in individual studies led to the exclusion of studies reporting on MEN1 patients with NF-pNETs. Regarding the high chance of selection bias for these small studies (less than six NF-pNETs) and subsequent imprecise estimations of diagnostic accuracy measures, exclusion of these studies seems reasonable regarding achievement of unbiased and precise results. Another limitation is the language restriction implemented in our search string. Based on our current understanding of MEN1, most of the available literature is published in the used languages. We assume that no studies were missed using this strategy. Some studies were not available for full text eligibility or did not report outcomes for (NF-)pNETs. No attempts were made to obtain individual patients records from studies not reporting separate outcomes. Lastly, no critical appraisal of studies assessing age-related penetrance was performed.

This systematic review collected additional evidence to substantiate and update the evidence-based approach for NF-pNET screening. Current clinical practice guidelines recommend annual surveillance, whereas recent data illustrate that less aggressive surveillance is reasonable in a substantial number of MEN1 patients. Studies on growth reported very slow rates and two distinct phenotypes in the course of disease with long-term stable disease in a large subgroup. Therefore, we promote a more individualized approach based on the observed growth tendency. Our review of recent literature offers recommendations on the use of biomarkers and imaging modalities (Table 10). Biomarkers should not play a role in the diagnostic process, as accuracies are too low. Studies evaluating the diagnostic value of imaging modalities are heterogeneous with varying risks of bias, and reported outcomes diverge for most modalities. For the detection of NF-pNETs, EUS has the highest sensitivity but also has disadvantages. A combined strategy of EUS and MRI seems to be the most useful with important advantages of MRI over CT. To estimate the growth rate of NF-pNETs, we would advise use of the same imaging modality, as concordance between EUS and MRI is moderate [32]. ⁶⁸Ga-DOTAPET-CT could be added if NF-pNETs are diagnosed in a

patient to identify metastasized disease. The superior diagnostic performance of ^{68}Ga -DOTA PET-CT over SRS makes it the preferred functional imaging modality when available [35]. The optimal age to start screening must yet be determined, as study methods and reported age-related penetrance are varying.

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SUPPLEMENTARY FILE 1 – FULL SEARCH STRING

Biomarker

Medline/Pubmed 21-12-2017

((((((((((((((biomarker[Title/Abstract]) OR biochemical marker[Title/Abstract]) OR biological marker[Title/Abstract]) OR chromogranin[Title/Abstract]) OR CgA[Title/Abstract]) OR pancreatic polypeptide[Title/Abstract]) OR PP[Title/Abstract]) OR glucagon[Title/Abstract]) OR “Biomarkers, Tumor”[MeSH Terms]) OR biomarkers[Title/Abstract]) OR biochemical markers[Title/Abstract]) OR biological markers[Title/Abstract])) AND (((((((((((((((neuroendocrine tumor[Title/Abstract]) OR neuro-endocrine tumor[Title/Abstract]) OR neuro-endocrine tumour[Title/Abstract]) OR neuroendocrine tumour[Title/Abstract]) OR endocrine tumour[Title/Abstract]) OR endocrine tumor[Title/Abstract]) OR NET[Title/Abstract]) OR NETs[Title/Abstract]) OR neuroendocrine neoplasm[Title/Abstract]) OR neuro-endocrine neoplasm[Title/Abstract]) OR carcinoid[Title/Abstract]) OR non-functioning tumor[Title/Abstract]) OR non-functioning tumour[Title/Abstract]) OR nonfunctioning tumor[Title/Abstract]) OR nonfunctioning tumour[Title/Abstract]) OR non-functional tumour[Title/Abstract]) OR non-functional tumor[Title/Abstract]) OR nonfunctional tumour[Title/Abstract]) OR nonfunctional tumor[Title/Abstract]) OR adenoma[Title/Abstract]) OR “Pancreatic Neoplasms/chemistry”[MeSH Terms]) OR “Pancreatic Neoplasms/diagnosis”[MeSH Terms])) AND (((((((((((((((pancreas[Title/Abstract]) OR pancreatic[Title/Abstract]) OR duodenopancreatic[Title/Abstract]) OR duodeno-pancreatic[Title/Abstract]) OR pancreaticoduodenal[Title/Abstract]) OR pancreatoduodenal[Title/Abstract]) OR gastroenteropancreatic[Title/Abstract]) OR gastro-enteropancreatic[Title/Abstract]) OR enteropancreatic[Title/Abstract]) OR entero-pancreatic[Title/Abstract]) OR islet cell[Title/Abstract]) OR beta cell[Title/Abstract]) OR island cell[Title/Abstract]) OR pNET[Title/Abstract]) OR foregut[Title/Abstract]))) NOT adenocarcinoma[Title]) NOT review[Publication Type] Filters: Humans; English; Dutch; French; German

Result: 2516 studies

Embase search 22-12-2017

('biomarker':ab,ti OR 'biochemical marker':ti,ab OR 'biological marker':ti,ab OR 'biomarkers':ab,ti OR 'chromogranin':ti,ab OR 'chromogranin a':ab,ti OR 'cga':ab,ti OR 'pancreatic poly peptide':ab,ti OR 'glucagon':ab,ti OR 'biochemical markers':ab,ti OR 'biological markers':ab,ti) AND ('neuro-endocrine tumour':ab,ti OR 'neuroendocrine tumour':ab,ti OR 'neuroendocrine tumor':ab,ti OR 'neuro-endocrine tumor':ab,ti OR 'endocrine tumor':ab,ti OR 'endocrine tumour':ab,ti OR net:ab,ti OR nets:ab,ti OR 'neuroendocrine neoplasm':ab,ti OR 'neuro-endocrine neoplasm':ab,ti OR 'carcinoid':ab,ti OR 'nonfunctional tumor':ab,ti OR 'non-functional tumor':ab,ti OR 'non-functioning tumor':ti,ab OR 'nonfunctioning tumor':ab,ti OR 'nonfunctional tumour':ab,ti OR 'non-functioning tumour':ti,ab OR 'nonfunctioning tumour':ab,ti OR 'adenoma':ab,ti OR 'islet cell tumor':ab,ti OR 'gastroenteropancreatic neuroendocrine tumor'/exp) AND (pancreas:ab,ti OR pancreatic:ab,ti OR duodenopancreatic:ab,ti OR 'duodeno pancreatic':ab,ti OR pancreaticoduodenal:ti,ab OR 'pancreatico-duodenal':ti,ab OR pancreatoduodenal:ab,ti OR gastroenteropancreatic:ab,ti OR enteropancreatic:ab,ti OR 'entero pancreatic':ab,ti OR 'pancreatic island cell':ab,ti OR 'pancreatic islet cell':ab,ti OR 'foregut':ab,ti) AND ([article]/lim OR [article in press]/lim OR [conference abstract]/lim OR [conference paper]/lim OR [editorial]/lim) AND ([dutch]/lim OR [english]/lim OR [french]/lim OR [german]/lim) AND [humans]/lim AND [embase]/lim

Result: 1582 studies

TI=(((((biomarker OR 'biochemical marker' OR 'biological marker' OR chromogranin OR CgA OR 'pancreatic polypeptide' OR glucagon OR biomarkers OR 'biochemical markers' OR 'biological markers') AND ('neuroendocrine tumor' OR 'neuro-endocrine tumor' OR 'neuro-endocrine tumour' OR 'neuroendocrine tumour' OR 'endocrine tumor' OR 'endocrine tumor' OR NET OR NETs OR 'neuroendocrine neoplasm' OR 'neuro-endocrine neoplasm' OR carcinoid OR 'non-functioning tumor' OR 'non-functioning tumour' OR 'nonfunctioning tumor' OR 'nonfunctioning tumour' OR 'non-functional tumour' OR 'non-functional tumor' OR 'nonfunctional tumour' OR 'nonfunctional tumor' OR adenoma)) AND (pancreas OR pancreatic OR duodenopancreatic OR 'duodeno-pancreatic' OR pancreatoduodenal OR 'pancreatico-duodenal' OR pancreatoduodenal OR gastroenteropancreatic OR 'gastro-entero-pancreatic' OR enteropancreatic OR 'entero-pancreatic' OR 'islet cell' OR 'beta cell' OR 'island cell' OR pNET OR foregut))) AND **LANGUAGE:** (English) AND **DOCUMENT TYPES:** (Article OR Abstract of Published Item OR Meeting Abstract)

Result: 183 studies

Imaging

Medline/Pubmed 21-12-2017

((((((((((((((((((((((imaging[Title/Abstract]) OR modality [Title/Abstract] OR modalities[Title/Abstract]) OR MRI[Title/Abstract]) OR magnetic resonance imaging[Title/Abstract]) OR MRI scan[Title/Abstract]) OR computed tomography[Title/Abstract]) OR CT[Title/Abstract]) OR CT-scan[Title/Abstract]) OR ultrasound[Title/Abstract]) OR ultrasonography[Title/Abstract]) OR ultrasonographic[Title/Abstract]) OR endoscopic[Title/Abstract]) OR EUS[Title/Abstract]) OR radiological[Title/Abstract]) OR Localization[Title/Abstract]) OR radiologic[Title/Abstract]) OR scintigraphy[Title/Abstract]) OR PET[Title/Abstract]) OR Gallium[Title/Abstract] OR Magnetic resonance imaging [Mesh]) OR Tomography, Emission-Computed [Mesh]) OR Positron Emission Tomography [Mesh]) OR Endosonography [Mesh]))) AND (((((((((((((((((((neuroendocrine tumor[Title/Abstract]) OR neuro-endocrine tumor[Title/Abstract]) OR neuro-endocrine tumour[Title/Abstract]) OR neuroendocrine tumour[Title/Abstract]) OR endocrine tumor[Title/Abstract]) OR endocrine tumour[Title/Abstract]) OR endocrine tumor[Title/Abstract]) OR NET[Title/Abstract]) OR NETs[Title/Abstract]) OR neuroendocrine neoplasm[Title/Abstract]) OR neuro-endocrine neoplasm[Title/Abstract]) OR carcinoid[Title/Abstract]) OR non-functioning tumor[Title/Abstract]) OR non-functioning tumour[Title/Abstract]) OR nonfunctioning tumor[Title/Abstract]) OR nonfunctioning tumour[Title/Abstract]) OR non-functional tumour[Title/Abstract]) OR non-functional tumor[Title/Abstract]) OR nonfunctional tumour[Title/Abstract]) OR nonfunctional tumor[Title/Abstract]) OR adenoma[Title/Abstract])) AND (((((((((((((((pancreas[Title/Abstract]) OR pancreatic[Title/Abstract]) OR duodenopancreatic[Title/Abstract]) OR duodeno-pancreatic[Title/Abstract]) OR pancreaticoduodenal[Title/Abstract]) OR pancreatico-duodenal[Title/Abstract]) OR pancreatoduodenal[Title/Abstract]) OR gastroenteropancreatic[Title/Abstract]) OR gastro-enteropancreatic[Title/Abstract]) OR enteropancreatic[Title/Abstract]) OR entero-pancreatic[Title/Abstract]) OR islet cell[Title/Abstract]) OR beta cell[Title/Abstract]) OR island cell[Title/Abstract]) OR pNET[Title/Abstract]) OR foregut[Title/Abstract]))) NOT adenocarcinoma[Title]) NOT review[Publication Type]))

Result: 1854 studies

Embase 22-12-2017

(imaging:ab,ti OR modality:ab,ti OR modalities:ab,ti OR mri:ab,ti OR 'magnetic resonance imaging':ab,ti OR 'mri-scan':ab,ti OR 'computed tomography':ab,ti OR ct:ab,ti OR 'ct-scan':ab,ti OR ultrasound:ab,ti

OR ultrasonography:ab,ti OR ultrasonographic:ab,ti OR endoscopic:ab,ti OR eus:ab,ti OR radiological:ab,ti OR localization:ab,ti OR radiologic:ab,ti OR scintigraphy:ab,ti OR pet:ab,ti OR 'positron emission tomography':ab,ti OR galium:ab,ti OR 'computer assisted tomography'/exp OR 'nuclear magnetic resonance imaging'/exp OR 'endoscopic ultrasonography'/exp OR 'positron emission tomography'/exp) AND ('neuro-endocrine tumour':ab,ti OR 'neuroendocrine tumour':ab,ti OR 'neuroendocrine tumor':ab,ti OR 'neuro-endocrine tumor':ab,ti OR 'endocrine tumor':ab,ti OR 'endocrine tumour':ab,ti OR net:ab,ti OR nets:ab,ti OR 'neuroendocrine neoplasm':ab,ti OR 'neuroendocrine neoplasm':ab,ti OR 'carcinoid':ab,ti OR 'nonfunctional tumor':ab,ti OR 'non-functional tumour':ab,ti OR 'non-functioning tumor':ti,ab OR 'nonfunctioning tumor':ab,ti OR 'nonfunctional tumour':ab,ti OR 'non-functional tumour':ab,ti OR 'non-functioning tumour':ti,ab OR 'nonfunctioning tumour':ab,ti OR 'adenoma':ab,ti OR 'islet cell tumor':ab,ti OR 'gastroenteropancreatic neuroendocrine tumor'/exp) AND (pancreas:ab,ti OR pancreatic:ab,ti OR duodenopancreatic:ab,ti OR 'duodeno pancreatic':ab,ti OR pancreaticoduodenal:ti,ab OR 'pancreatico-duodenal':ti,ab OR pancreatoduodenal:ab,ti OR gastroenteropancreatic:ab,ti OR enteropancreatic:ab,ti OR 'entero pancreatic':ab,ti OR 'pancreatic island cell':ab,ti OR 'pancreatic islet cell':ab,ti OR 'foregut':ab,ti) AND ([article]/lim OR [article in press]/lim OR [conference abstract]/lim OR [conference paper]/lim OR [editorial]/lim) AND ([dutch]/lim OR [english]/lim OR [french]/lim OR [german]/lim) AND [humans]/lim AND [embase]/lim

Result: 2934 studies

Web of science 23-01-2018

(TI=((imaging OR modality OR modalities OR MRI OR 'magnetic resonance imaging' OR 'MRI-scan' OR 'computed tomography' OR CT OR 'CT-scan' OR ultrasound OR ultrasonography OR ultrasonographic OR endoscopic OR EUS OR radiological OR Localization OR radiologic OR scintigraphy OR PET OR 'positron emission tomography' OR Galium) AND ('neuroendocrine tumor' OR 'neuro-endocrine tumor' OR 'neuro-endocrine tumour' OR 'neuroendocrine tumour' OR 'endocrine tumour' OR 'endocrine tumor' OR NET OR NETs OR 'neuroendocrine neoplasm' OR 'neuro-endocrine neoplasm' OR carcinoid OR 'non-functioning tumor' OR 'non-functioning tumour' OR 'nonfunctioning tumor' OR 'nonfunctioning tumour' OR 'non-functional tumor' OR 'nonfunctional tumour' OR 'nonfunctional tumor' OR adenoma)) AND (pancreas OR pancreatic OR duodenopancreatic OR 'duodeno-pancreatic' OR pancreaticoduodenal OR 'pancreatico-duodenal' OR pancreatoduodenal OR gastroenteropancreatic OR 'gastro-enteropancreatic' OR enteropancreatic OR 'entero-pancreatic' OR 'islet cell' OR 'beta cell' OR 'island cell' OR pNET OR foregut))) AND LANGUAGE: (English) AND DOCUMENT TYPES: (Article OR Abstract of Published Item OR Meeting Abstract)

Indexes=SCI-EXPANDED, SSCI, A&HCI, ESCI Timespan=All years

Result: 572 studies

Growth / penetrance

Medline/Pubmed 17-01-2018

(((((MEN1[Title/Abstract] OR MEN 1[Title/Abstract]) OR Multiple Endocrine Neoplasia Type 1[Title/Abstract]) OR Werner syndrome[Title/Abstract]) OR hereditary syndrome[Title/Abstract]) OR "multiple endocrine neoplasia type 1"[MeSH Terms]) AND (((((((((((((((neuroendocrine tumor[Title/Abstract] OR neuro-endocrine tumor[Title/Abstract]) OR neuroendocrine tumour[Title/Abstract]) OR neuro-endocrine tumour[Title/Abstract]) OR endocrine tumor[Title/Abstract]) OR endocrine tumour[Title/Abstract]) OR NET[Title/Abstract]) OR NETs[Title/Abstract]) OR neuroendocrine neoplasm[Title/Abstract]) OR (neuro-endocrine neoplasm[Title/Abstract])) OR carcinoid[Title/Abstract]) OR (nonfunctioning tumour[Title/Abstract])) OR non-functioning tumour[Title/Abstract]) OR non-functioning tumor[Title/Abstract]) OR nonfunctioning tumor[Title/

Abstract]) OR (nonfunctional tumour[Title/Abstract])) OR (non-functional tumour[Title/Abstract])) OR nonfunctional tumor[Title/Abstract]) OR non-functional tumor[Title/Abstract]) OR adenoma[Title/Abstract]) AND (((((((((((pancreas[Title/Abstract] OR pancreatic[Title/Abstract]) OR duodenopancreatic[Title/Abstract]) OR duodeno-pancreatic[Title/Abstract]) OR pancreaticoduodenal[Title/Abstract]) OR gastroenteropancreatic[Title/Abstract]) OR gastro-enteropancreatic[Title/Abstract]) OR enteropancreatic[Title/Abstract]) OR entero-pancreatic[Title/Abstract]) OR islet cell[Title/Abstract]) OR beta cell[Title/Abstract]) OR island cell[Title/Abstract]) OR pNET[Title/Abstract]) OR foregut[Title/Abstract])) OR "pancreatic neoplasms/diagnosis"[Mesh Terms]) AND ("humans"[MeSH Terms] AND English[lang])) NOT Review[Publication Type]

Results: 377 studies

Embase 17-01-2018

('men1':ab,ti OR 'men 1':ab,ti OR 'multiple endocrine neoplasia type 1':ab,ti OR 'werner syndrome':ab,ti OR 'men-1':ab,ti OR 'genetic disorder':ab,ti OR 'hereditary':ab,ti) AND ([article]/lim OR [article in press]/lim OR [conference abstract]/lim OR [conference paper]/lim OR [editorial]/lim) AND ([dutch]/lim OR [english]/lim OR [french]/lim OR [german]/lim) AND [humans]/lim AND [embase]/lim AND ('neuro-endocrine tumour':ab,ti OR 'neuroendocrine tumour':ab,ti OR 'neuroendocrine tumor':ab,ti OR 'neuro-endocrine tumor':ab,ti OR 'endocrine tumor':ab,ti OR 'endocrine tumour':ab,ti OR net:ab,ti OR nets:ab,ti OR 'neuroendocrine neoplasm':ab,ti OR 'neuro-endocrine neoplasm':ab,ti OR 'carcinoid':ab,ti OR 'nonfunctional tumor':ab,ti OR 'non-functional tumor':ab,ti OR 'non-functioning tumor':ti,ab OR 'nonfunctioning tumor':ab,ti OR 'nonfunctional tumour':ab,ti OR 'non-functional tumour':ab,ti OR 'non-functioning tumour':ti,ab OR 'nonfunctioning tumour':ab,ti OR 'adenoma':ab,ti OR 'islet cell tumor':ab,ti OR 'gastroenteropancreatic neuroendocrine tumor'/exp) AND (pancreas:ab,ti OR pancreatic:ab,ti OR duodenopancreatic:ab,ti OR 'duodeno pancreatic':ab,ti OR pancreaticoduodenal:ti,ab OR 'pancreatico-duodenal':ti,ab OR pancreatoduodenal:ab,ti OR gastroenteropancreatic:ab,ti OR enteropancreatic:ab,ti OR 'entero pancreatic':ab,ti OR 'pancreatic island cell':ab,ti OR 'pancreatic islet cell':ab,ti OR 'foregut':ab,ti) AND ([article]/lim OR [article in press]/lim OR [conference abstract]/lim OR [conference paper]/lim) AND ([dutch]/lim OR [english]/lim OR [french]/lim OR [german]/lim) AND [humans]/lim AND [embase]/lim

Results: 843 studies

Web of Science 24-01-2018

(TI=(((men1 OR 'men 1' OR 'multiple endocrine neoplasia type 1' OR 'werner syndrome' OR 'men-1' OR 'genetic disorder' OR 'hereditary') AND ('neuro-endocrine tumour' OR 'neuroendocrine tumour' OR 'neuroendocrine tumor' OR 'neuro-endocrine tumor' OR 'endocrine tumor' OR 'endocrine tumour' OR net OR nets OR 'neuroendocrine neoplasm' OR 'neuro-endocrine neoplasm' OR 'carcinoid' OR 'nonfunctional tumor' OR 'non-functional tumor' OR 'non-functioning tumor' OR 'nonfunctioning tumor' OR 'nonfunctional tumour' OR 'non-functional tumour' OR 'non-functioning tumour' OR 'nonfunctioning tumour' OR 'adenoma' OR 'islet cell tumor' OR 'gastroenteropancreatic neuroendocrine tumor')) AND (pancreas OR pancreatic OR duodenopancreatic OR 'duodeno pancreatic' OR pancreaticoduodenal OR 'pancreatico-duodenal' OR pancreatoduodenal OR gastroenteropancreatic OR enteropancreatic OR 'entero pancreatic' OR 'pancreatic island cell' OR 'pancreatic islet cell' OR 'foregut')))) AND LANGUAGE: (English) AND DOCUMENT TYPES: (Article OR Abstract of Published Item OR Meeting Abstract)

Results: 149

SUPPLEMENTARY FILE 2 – ADJUSTED QUADAS

Risk of bias

1A1: A consecutive or random sample. A subgroup (e.g. surgery cohort) is scored as 'no'.

1A2: Comparison biomarker outcomes between patients with controls (healthy controls or patients with non-endocrine tumors) is scored as 'no'.

1A3: Exclusion of patients based on medication use, chronic kidney disease (biomarker studies) tumor stage or treatment (all studies) is scored as 'no'. Exclusion of patients with concomitant lung- or gastric NETs for biomarker search is not regarded as bias and scored as 'yes'.

2A1: Imaging studies: index test must be blinded. Not applicable for biochemical biomarkers.

2A2: Biomarker studies: reference ranges must be provided in the manuscript. Not applicable for imaging studies.

3A1: Histology and imaging modalities – with exception of abdominal ultrasonography – were classified as adequate reference standards for diagnosing pNETs. Abdominal ultrasonography or biochemical criteria were classified as high risk of bias. If the index test was part of the reference standard, this was classified as high risk as well since it is possible to have incorporation bias.

3A2: It must be clear if the index test or reference standard were blinded. Biochemical markers and histopathology were classified as 'blinded' e.g. low risk.

4A1: An appropriate interval was classified as max. 6 months from each other (index test vs reference standard).

4A2: It should be clear that all patients received a reference standard.

4A3: If patients received either CT-scan, MRI or EUS as reference standard, this was classified as high risk since accuracy for diagnosis differs between these conventional imaging modalities. A low risk of biased was given when all patients underwent the same imaging modality / reference standard.

4A4: All patients should be included in the analysis

Applicability

1B: This review focus on non-functional pNET in MEN1 patients. This should be the study domain of the included study. A high concern was given when more than 33% of the population had functional NET,

if a population consisted out of patients who all underwent surgery, or a large proportion had metastasized disease (>50%).

2B: The index test should be evaluated on the diagnostic value to diagnose a pNET in MEN1 patients.

3B: Biochemical biomarkers and abdominal ultrasonography were classified as unreliable to detect the target condition defined in this review (pNET) and therefore studies with (other) biomarkers or abdominal ultrasound as reference standard were classified as high concern.

SUPPLEMENTARY FILE 3 - RISK OF BIAS TOOL WITH ADJUSTED DOMAINS FROM QUIPS-TOOL

1. Study participation and attrition

- a. Target population is adequately described:
 - i. Low risk: Baseline table or adequate description must be present. (*Adequate description of follow-up time (per patient or per NF-pNET), NF-pNET size, age, sex, patient selection (sampling frame and recruitment, period and place of recruitment)*)
 - ii. High risk: no adequate description or only some characteristics addressed.
- b. Consecutive sample: MEN1 patients in surveillance program. Were asymptomatic patients with NF-pNETs (< 2cm) included in the MEN1 surveillance and derived from an MEN1 cohort?
 - i. Low risk: 'yes' is low risk
 - ii. Unclear: not described.
 - iii. High risk: preselection of patients / no consecutive sample of MEN1 cohort.
- c. The data from an adequate proportion of the study population is available for growth analysis:
 - i. Low risk: Number of patients lost to follow-up and the reasons for loss to follow-up and drop out are adequately described or <20% drop out.
 - ii. High risk: Insufficient description of patients lost to follow-up or >20% drop out

2. Identification of NF-pNET

- a. Reliable identification and follow-up of NF-pNET: is the same tumor measured on repeated screens?
 - i. Low risk: Manner to ensure follow up of the same tumor is described.
 - ii. High risk: Manner to ensure follow up of the same tumor is not described.
- b. Valid and reliable modality for measurement of growth:
 - i. Low risk: EUS/MRI/CT
 - ii. High risk: all other

3. Outcome (growth) measurement

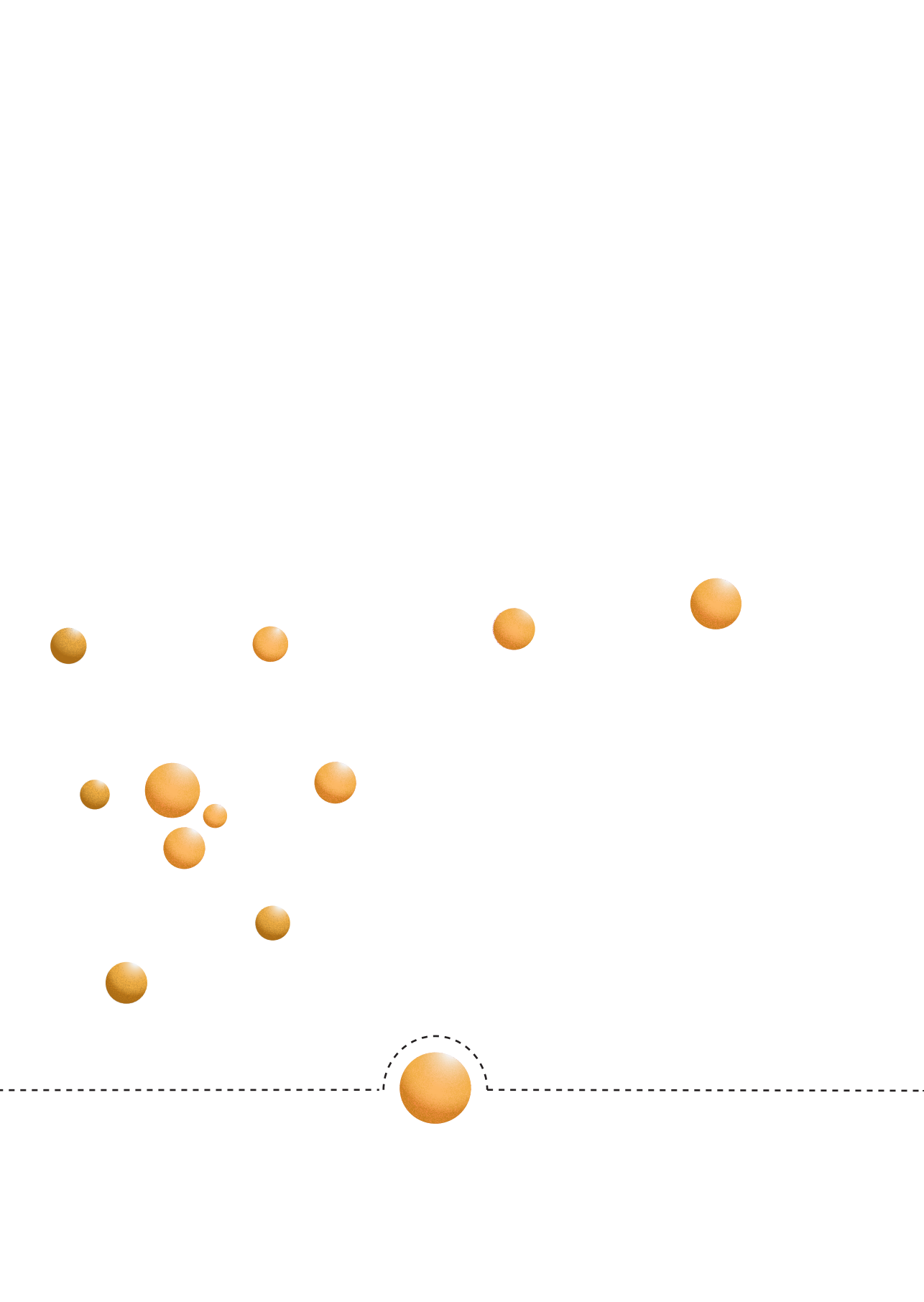
- a. The method and setting for measurement is the same for all participants (between patients):
 - i. Low risk: Only CT/MRI or EUS.
 - ii. High risk: Combination of CT/MRI and EUS and/OR histopathology or other modalities.
- b. The method and setting for measurement is the same for all participants (within patients):
 - i. Low risk: Only CT/MRI or EUS.
 - ii. High risk: Combination of CT/MRI and EUS and/or histopathology or other modalities.
- c. Total number of assessments for tumor size (median):
 - i. Low risk: > 2 measurements
 - ii. Unclear risk: Not described
 - iii. High risk: 2 measurements

4. Analysis and reporting

- a. Unit of analysis error:

Explanation: Patient characteristics and genotype might influence tumor growth. If a patient has many small NF-pNETs and all tumors are included, this could bias the results. Therefore, a per-patient analysis is preferred.

 - i. Low risk: Patient analysis
 - ii. High risk: Per lesion analysis since multiple lesions occur in MEN1.
- b. The definition of progressive and stable disease should be clear:
 - i. Low risk: Reported definition.
 - ii. High risk: No reported definitions
- c. Possible effect modifiers are addressed (treatment/intervention):
 - i. Low risk: Described.
 - ii. Unclear: Not described

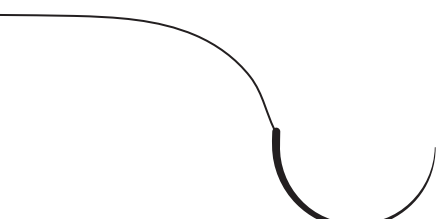


CHAPTER IV

Diagnosing pancreatic neuroendocrine tumors in patients with multiple endocrine neoplasia type 1 in daily practice

Submitted

D.J. van Beek, C.R.C. Pieterman, F.J. Wessels, A.C. van de Ven, W.W. de Herder, O.M. Dekkers,
W.T. Zandee, M.L. Drent, P.H. Bisschop, B. Havekes, I.H.M. Borel Rinkes, M.R. Vriens, G.D. Valk



ABSTRACT

Background

In multiple endocrine neoplasia type 1 (MEN1), pancreatic neuroendocrine tumors (PanNETs) have a high prevalence and represent the main cause of death. This study aimed to assess the diagnostic accuracy of the currently used conventional pancreatic imaging techniques and the added value of fine needle aspirations (FNA).

Methods

Patients with at least one imaging study were included from the population-based MEN1 database from the DutchMEN Study Group from 1990–2017. Magnetic resonance imaging (MRI), computed tomography (CT), endoscopic ultrasonography (EUS), FNA, and surgical resection specimens were obtained. The first MRI, CT or EUS was considered as the index test. For a comparison of the diagnostic accuracy of MRI versus CT, patients with their index test between 2010 and 2017 were included. The reference standard consisted of surgical histopathology or radiological follow-up.

Results

413 patients (92.8% of the database) underwent 3477 imaging studies. The number of imaging studies per patient increased, and a preference for MRI was observed in the last decade. Overall diagnostic accuracy was good with a positive (PPV) and negative predictive value (NPV) of 88.9% (95% confidence interval 76.0–95.6) and 92.8% (89.4–95.1) for PanNET in the pancreatic head and 92.0% (85.3–96.0) and 85.3% (80.5–89.1) in the body/tail. For MRI, PPV and NPV for pancreatic head tumors were 100% (76.1–100) and 87.1% (76.3–93.6) and for CT 60.0% (22.9–88.4) and 70.4% (51.3–84.3), respectively. For body/tail tumors, PPV and NPV were 91.3% (72.0–98.8) and 87.0% (75.3–93.9) for MRI and 100% (74.9–100) and 77.8% (54.3–91.5) for CT, respectively. Pathology confirmed a PanNET in 106 out of 110 (96.4%) resection specimens. FNA was performed of 34 lesions in 33 patients and was considered PanNET in 24 (all confirmed PanNET by histology (10) or follow-up (14)), normal/cyst/unrepresentative in 6 (all confirmed PanNET by follow-up), and adenocarcinoma in 4 (2 confirmed, 2 PanNET). Three patients, all older than 60 years, had a final diagnosis of pancreatic adenocarcinoma.

Conclusion

As the accuracy for diagnosing MEN1-related PanNET of MRI was higher than CT, MRI should be the preferred (non-invasive) imaging modality for PanNET screening/surveillance. The high diagnostic accuracy of pancreatic imaging and the sporadic occurrence of pancreatic adenocarcinoma question the need for routine (EUS-guided) FNA.

INTRODUCTION

Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant tumor syndrome with an estimated prevalence of 1-10 patients per 100,000 people.^{1,2} Primary hyperparathyroidism, duodenopancreatic neuroendocrine tumors (dpNETs) and pituitary adenomas are the clinical hallmarks of the syndrome.³ During their lifetime, more than 80% of patients are affected by dpNETs and metastasized dpNETs represent the leading cause of death.⁴⁻⁶

To enable timely diagnosis of pancreatic neuroendocrine tumors (PanNETs), MEN1 clinical practice guidelines suggest yearly conventional pancreatic imaging with computed tomography (CT), magnetic resonance imaging (MRI) or endoscopic ultrasonography (EUS).⁷ In a previous systematic review the diagnostic accuracy of pancreatic imaging in MEN1 was found to be relatively high.⁸ Consequently a strategy combining MRI and EUS for the diagnosis of NF-PanNETs was advised, however, with remaining uncertainty about the optimal modality.⁸ Most included studies reported on EUS or CT only, were mainly single center, and methodologically heterogeneous with varying risks of bias.⁸ More importantly, only one study compared MRI and CT; only eight patients had an MRI and all underwent operative resection.⁹ As such, firm conclusions regarding a preferred non-invasive imaging modality could not be drawn.⁸

Given the high prior probability for a PanNET in patients with MEN1, once a suspicious pancreatic lesion is identified, it is unclear if histopathological confirmation is necessary. For sporadic non-functioning PanNETs, the European Neuroendocrine Tumor Society Guidelines suggest EUS-guided biopsies, but no specific recommendations are proposed for patients with MEN1.¹⁰ A recent consensus statement lacked a conclusion regarding if and when to perform EUS-guided biopsies for the diagnosis of MEN1-related PanNETs.¹¹ However, when the diagnostic accuracy of pancreatic imaging is proven to be high, the added value of pancreatic biopsies for diagnostic purposes may be questioned.

Hence, the aims of the present study were to assess the diagnostic accuracy of conventional pancreatic imaging studies and to determine the added value of pancreatic fine needle aspiration (FNA) for the diagnosis of MEN1-related PanNETs.

METHODS

Reporting of the study was performed according to the STAndards for the Reporting of Diagnostic accuracy studies (STARD) checklist.¹²

Study design – DMSG Database

Patients with at least one conventional pancreatic imaging were included from the DutchMEN Study Group (DMSG) database, which has been described previously.¹³ Briefly, patients with MEN1 aged 16 years and older and followed in one of the eight Dutch University Medical Centers (UMC) are included. Within each center patients were identified by hospital databases of medical conditions and diseases review. The MEN1 diagnosis was established according to the Clinical Practice Guidelines for MEN1.⁷ Over 90% of the Dutch MEN1 population is included in the database. Clinical and demographic data were collected longitudinally every quarter from 1990-2014 by standardized medical record review, according to a predefined protocol. From 2014 onwards, data were captured prospectively. The protocol was approved by the Medical Ethics Committees of all UMCs.

Imaging studies and pathology in the DMSG database

All pancreatic imaging studies (CT, MRI or EUS) in the setting of screening and surveillance for MEN1-related manifestations from January 1 1990 until December 31 2017 were captured in the database. Imaging studies were captured per quarter of each year, if multiple investigations of the same modality were performed within the same quarter, only the first examination was entered. Imaging studies with other aims, e.g., to detect postoperative complications, were disregarded. Data were collected from routine patient care. The original reports were used; no review of images was performed. Radiologists, gastroenterologists, and pathologists were not blinded to previous imaging or to clinical information. According to the guidelines, outcomes were discussed in multidisciplinary tumor boards within the individual centers; conclusions could subsequently be altered based on these discussion.⁷

Index tests

I. Diagnostic accuracy of conventional pancreatic imaging, irrespective type of imaging, in MEN1 patients under surveillance from 1990-2017

For each patient the index test was defined as the first conventional screening/surveillance imaging that was performed of the pancreas. This could be either CT, MRI or EUS. The pancreatic head and pancreatic body/tail were analyzed separately. One dominant (largest) tumor from the pancreatic head and one from the pancreatic body or tail was analyzed.

II. Diagnostic accuracy of CT versus MRI

For the specific comparison of the diagnostic accuracy between MRI and CT, patients for

whom their first pancreatic screening/surveillance imaging was between 2010 and 2017 and was either an MRI or CT were analyzed. The index test was the first pancreatic screening/surveillance MRI or CT in this time period. Comparison groups were defined based on the imaging modality of the index tests (MRI or CT).

Patients with a PanNET diagnosis before 1990 were not considered for the imaging analyses.

Reference standard

The reference test was histopathology (surgical resection) or radiological follow-up. Histological verification in all patients was not possible, because not everyone underwent a resection. In the absence of histopathology, repetitive follow-up was used as reference test.^{14,15} To determine the outcome of the reference test in those with only radiological follow-up, the follow-up conventional imaging within 3 years of the index test was used, this could be either CT/MRI or EUS independent of the modality of the original index test. If the first follow-up conventional imaging after the index test was positive, but followed by two negative imaging studies within three years without operative resection the reference test was negative (i.e. the patient was deemed not to have a PanNET).¹⁶ The size of the PanNET on follow-up imaging was also taken into account; any follow-up scan within three years of the index test that showed a PanNET of 5 mm or larger was considered a positive reference test (conditional on not being followed by two negative tests as described above). Based on reported PanNET growth rates [0.1-1.32 mm/year] in this study population, it was hypothesized that a tumor of 5 mm or larger should have been visible on imaging studies within the prior 3 years.^{8,16}

Resection specimens and FNA

All resection specimens and EUS-guided pancreatic FNA after 1990 were retrieved from the database, regardless of a PanNET diagnosis before 1990. Resection specimens and FNAs were assessed for the presence of a PanNET and possible other outcomes were captured. Negative or inconclusive biopsies were classified as such.

Statistical analysis

Descriptive statistics were reported as mean \pm standard deviation (SD) or median [interquartile range (IQR) or range] for continuous variables or as counts (percentages) for categorical variables. The average number of imaging studies per 100 patients per year was calculated.

Outcomes were diagnostic accuracy measures, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR) and negative LR. Binomial Agresti-Coull 95% confidence intervals (CI) were calculated for sensitivity, specificity, PPV, and NPV.¹⁷ Likelihood ratio 95% CI were defined based on the log-method.¹⁸ Subgroup analysis according to the time period (1990-1999, 2000-2009 and 2010-2017)

and the reference standard (histopathology vs imaging follow-up) were performed.

Statistical analyses were performed by using SPSS version 25.0 (IBM Corp, Armonk, NY), and R version 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria). Figures were constructed by using Graphpad Prism version 7.02 (GraphPad Software, Inc, San Diego, CA).

RESULTS

DMSG database

A total of 445 patients were identified in the database of whom 413 patients (92.8%) underwent at least one pancreatic imaging study. In total, 3477 imaging studies were performed, of which 1818 (52.3%) were MRI, 1291 (37.1%) CT and 368 (10.6%) EUS, respectively (Supplementary Table 1). On 1845 (53.1%) studies a PanNET was reported. Median radiological follow-up time from the first scan after 1990 was 8.4 years [IQR 4.5-13.8]. Twenty-nine patients (6.5%) were lost to follow-up. The mean age at the last date of follow-up was 50.6 years (± 16.6).

Imaging over time

Since 2009 between 70 and 80 imaging studies were conducted per 100 patients in the database annually (Figure 1). The contribution of MRI increased from 38.4% of all performed imaging in 1990-1999 [range per year 28.6%-58.6%] to 61.4% in 2010-2017 [range per year 42.6%-74.0%] (Figure 1, Supplementary Table 1). The relative use of CT decreased from 60.0% to 26.9% and EUS increased from 1.6% to 11.8%.

The percentage of positive index scans was 39.4% in 1990-1999, 30.1% in 2000-2009 and 37.0% in 2010-2017. Of the patients with MRI as index test, 33.7% had a positive index MRI in 2010-2017, 19.8% in 2000-2009 and 38.7% in 1990-1999 (Supplementary Table 1). For CT, these percentages were 40.0%, 37.0% and 44.4%, respectively. When stratifying by age (10-year strata), increase of the percentage of positive studies increased with age at the index scan (Supplementary Table 2).

Diagnostic accuracy of all conventional imaging

Of the 413 patients with imaging studies, 17 patients (4.1%) had a PanNET diagnosis before 1990 and 19 patients (4.6%) had only one imaging study without resection. Subsequently, 377 patients were available for analysis, their mean age at the index study was 38.6 (± 15.8) years. A total of 131 patients (34.7%) had a positive index test, of whom 26 had both a PanNET of the pancreatic head and body/tail. 45 index studies documented a PanNET of the pancreatic head and 112 a PanNET of the body/tail. MRI and CT were the index study

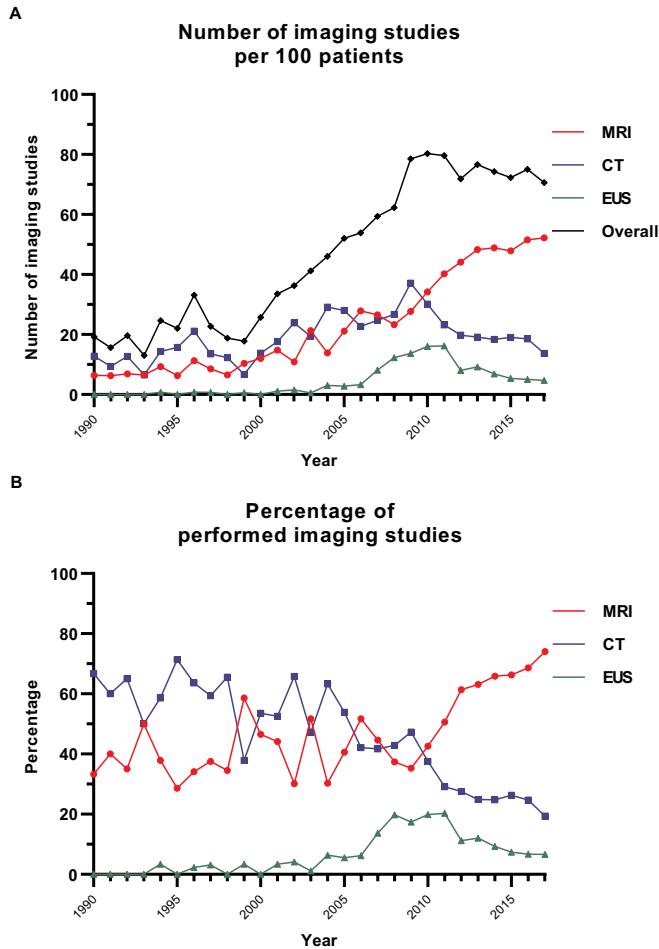


Figure 1. Imaging studies over time. A) The number of imaging studies over time per 100 patients. B) The percentage of imaging studies for each modality per year. Abbreviations: *CT* computed tomography, *EUS* endoscopic ultrasonography, *MRI* magnetic resonance imaging

in 192 (50.9%) and 184 (48.8%) patients, respectively, whereas only one patient had EUS as index test.

Contingency tables are presented in Table 1 and diagnostic accuracy is reported in Table 2. The diagnostic accuracy of all conventional imaging, irrespective type of imaging, for tumors in the pancreatic head was sensitivity 62.5% (50.2-73.4), specificity 98.4% (96.2-99.4), PPV 88.9% (76.0-95.6) and NPV 92.8% (89.4-95.1), respectively. For pancreatic body/tail tumors, sensitivity was 72.5% (64.6-79.2), specificity 96.2% (92.8-98.1), PPV 92.0% (85.3-96.0), and NPV 85.3% (80.5-89.1), respectively.

Table 1. Contingency tables of all imaging studies 1990 – 2017.

Pancreatic head			
	Reference standard		
	PanNET (no, %)	No PanNET (no, %)	
All imaging (n = 377)			Total
Index PanNET	40 (62.5)	5 (1.6)	45 (11.9)
Index no PanNET	24 (37.5)	308 (98.4)	332 (88.1)
Total	64 (17.0)	313 (83.0)	377 (100)
2010 – 2017 (n = 109)			Total
Index PanNET	18 (52.9)	2 (2.7)	20 (18.3)
Index no PanNET	16 (47.1)	73 (97.3)	89 (81.7)
Total	34 (31.2)	75 (68.8)	109 (100)
2000 – 2009 (n = 199)			Total
Index PanNET	17 (70.8)	1 (0.6)	18 (9.0)
Index no PanNET	7 (29.2)	174 (99.4)	181 (91.0)
Total	24 (12.1)	175 (87.9)	199 (100)
1990 – 1999 (n = 69)			Total
Index PanNET	5 (83.3)	2 (3.2)	7 (10.1)
Index no PanNET	1 (16.7)	61 (96.8)	62 (89.9)
Total	6 (8.7)	63 (91.3)	69 (100)
Pancreatic body/tail			
	Reference standard		
	PanNET (no, %)	No PanNET (no, %)	
All imaging (n = 377)			Total
Index PanNET	103 (72.5)	9 (3.8)	112 (29.7)
Index no PanNET	39 (27.5)	226 (96.2)	265 (70.3)
Total	142 (37.7)	235 (62.3)	377 (100)
2010 – 2017 (n = 109)			Total
Index PanNET	35 (76.1)	2 (3.2)	37 (33.9)
Index no PanNET	11 (23.9)	61 (96.8)	72 (66.1)
Total	46 (42.2)	63 (57.8)	109 (100)
2000 – 2009 (n = 199)			Total
Index PanNET	51 (71.8)	1 (0.8)	52 (26.1)
Index no PanNET	20 (28.6)	127 (99.2)	147 (73.9)
Total	71 (35.7)	128 (64.3)	199 (100)
1990 – 1999 (n = 69)			Total
Index PanNET	17 (68.0)	6 (13.6)	23 (33.3)
Index no PanNET	8 (32.0)	38 (86.4)	46 (66.7)
Total	25 (36.2)	44 (63.8)	69 (100)

In the 2x2 tables percentages are column percentages. The row “total” has row percentages, the column “total” has column percentages.

Abbreviations: *PanNET* pancreatic neuroendocrine tumor

In total, 90 potential PanNETs had histopathology as reference standard of which 20 (22.2%) were not reported on the index test (Supplementary Table 3). Of the 15 patients who underwent operative resection within 2 years of the index test the median histological tumor size was 12 mm [range 6–29]. Sensitivity analyses according to the applied reference standard are reported in Supplementary Table 4.

Diagnostic accuracy of CT and MRI

109 patients (26.4%) had their first CT or MRI between 2010 and 2017, of whom 77 (70.6%) had MRI and 32 (29.4%) CT as initial imaging study. The median age at the first imaging study was 39.0 years [IQR 19.2–54.1]. The index test was positive for a PanNET in 43 patients (39.4%) and 56 patients (51.4%) had a PanNET according to the reference standard (Table 3). Median radiological size for pancreatic head and body/tail tumors were 9 mm [IQR 7–11] and 12 mm [IQR 9–17.5], respectively. Histopathology was part of the reference standard in 6 patients (5.5%) with a PanNET in the pancreatic head and 16 patients (14.7%) with a PanNET in body/tail.

Contingency tables (Table 3) show that MRI was true positive for 15 out of 15 pancreatic head tumors and 21 out of 23 pancreatic body/tail tumors, indicating a PPV of 100% (76.1–100) for tumors in the head and 91.3% (72.0–98.8) for tumors in the body/tail (Table 4). For CT, 3 of 5 were positive in the pancreatic head and 14 out of 14 for the pancreatic body/tail. The corresponding PPV for CT was 60% (22.9–88.4) and 100% (74.9–100), respectively. For the pancreatic head, 8 of 27 patients with a negative CT were considered to have a PanNET (NPV 70.4% [51.3–84.3]), compared with 8 of 62 patients with a negative MRI (NPV 87.1% [76.3–93.6]), respectively. For the pancreatic body/tail, 4 of 14 patients with a negative CT were deemed to have a PanNET (NPV 77.8% [54.3–91.5]) versus 7 of 54 patients with a negative MRI (NPV 87.0% [75.3–93.9]).

For both modalities, diagnostic accuracy measures were generally similar for the pancreatic head and body/tail (Table 4). For CT, lower sensitivity and PPV for pancreatic head tumors, albeit with wide confidence intervals, were observed. For tumors in the pancreatic head, point estimates of sensitivity, PPV, and NPV were higher for MRI, but 95% CIs overlapped. For the pancreatic body/tail, diagnostic accuracy measures were similar between both modalities.

Resection specimens

A total of 110 surgical resection specimens were available, of which 106 (96.4%) had a final diagnosis of PanNET (Table 5). Two patients were considered to have a pancreatic ductal adenocarcinoma (PDAC); one of those underwent operative resection of a collision tumor consisting of a PDAC and surrounding PanNETs. Two patients had normal pancreatic tissue after resection. The first patient underwent an enucleation of the pancreatic body

Table 2. Diagnostic accuracy of conventional imaging 1990 – 2017.

	Sensitivity	Specificity	PPV	NPV	Positive LR	Negative LR
Pancreatic head						
Conventional imaging						
1990 – 2017	62.5 (50.2 – 73.4)	98.4 (96.2 – 99.4)	88.9 (76.0 – 95.6)	92.8 (89.4 – 95.1)	39.1 (16.1 – 95.3)	0.38 (0.28 – 0.52)
2010 – 2017	52.9 (36.7 – 68.6)	97.3 (90.2 – 99.8)	90.0 (68.7 – 98.4)	82.0 (72.7 – 88.7)	19.9 (4.9 – 80.8)	0.48 (0.34 – 0.70)
2000 – 2009	70.8 (50.6 – 85.3)	99.4 (96.5 – 100)	94.4 (72.4 – 100)	96.1 (92.1 – 98.3)	124.0 (17.3 – 889.9)	0.29 (0.16 – 0.55)
1990 – 1999	83.3 (41.8 – 98.9)	96.8 (88.5 – 99.8)	71.4 (35.2 – 92.4)	98.4 (90.6 – 100)	26.3 (6.4 – 107.5)	0.17 (0.03 – 1.03)
Pancreatic body/tail						
Conventional imaging						
1990 – 2017	72.5 (64.6 – 79.2)	96.2 (92.8 – 98.1)	92.0 (85.3 – 96.0)	85.3 (80.5 – 89.1)	18.9 (9.9 – 36.2)	0.29 (0.22 – 0.37)
2010 – 2017	76.1 (61.9 – 86.2)	96.8 (88.5 – 99.8)	94.6 (81.4 – 99.4)	84.7 (74.5 – 91.4)	24.0 (6.1 – 94.6)	0.25 (0.15 – 0.41)
2000 – 2009	71.8 (60.4 – 81.0)	99.2 (95.3 – 100)	98.1 (88.9 – 100)	86.4 (79.8 – 91.1)	91.9 (13.0 – 651.3)	0.28 (0.20 – 0.41)
1990 – 1999	68.0 (48.3 – 82.9)	86.4 (72.9 – 94.0)	73.9 (53.2 – 87.7)	82.6 (69.0 – 91.2)	5.0 (2.3 – 11.0)	0.37 (0.21 – 0.66)

95% CIs are given in parentheses.

Abbreviations: *LR* likelihood ratio, *NPV* negative predictive value, *PPV* positive predictive value

Table 3. Contingency tables of MRI and CT as first imaging study in 2010 - 2017

Pancreatic head			
	Reference standard		
	PanNET (no, %)	No PanNET (no, %)	
MRI (n = 77)			Total
Index positive	15 (65.2)	0 (0)	15 (19.5)
Index negative	8 (34.8)	54 (100)	62 (80.5)
Total	23 (29.9)	54 (70.1)	77 (100)
CT (n = 32)			Total
Index positive	3 (27.3)	2 (9.5)	5 (15.6)
Index negative	8 (72.7)	19 (90.5)	27 (84.4)
Total	11 (34.4)	21 (65.6)	32 (100)
Pancreatic body/tail			
	Reference standard		
	PanNET (no, %)	No PanNET (no, %)	
MRI (n = 77)			Total
Index positive	21 (75.0)	2 (4.1)	23 (29.9)
Index negative	7 (25.0)	47 (95.9)	54 (70.1)
Total	28 (36.4)	49 (63.6)	77 (100)
CT (n = 32)			Total
Index positive	14 (77.8)	0 (0)	14 (43.8)
Index negative	4 (22.2)	14 (100)	18 (56.3)
Total	18 (56.3)	14 (43.8)	32 (100)

In the 2x2 tables percentages are column percentages. The row “total” has row percentages, the column “total” has column percentages.

Abbreviations: *CT* computed tomography, *MRI* magnetic resonance imaging, *PanNET* pancreatic neuroendocrine tumor

showing normal pancreatic tissue, follow-up imaging studies showed the 14mm tumor. The other patient underwent an unsuccessful distal pancreatectomy without PanNET followed by a successful resection of the tumor during follow-up.

Pancreatic FNA

In total, FNA was performed on 34 pancreatic lesions in 33 patients, of 3 lesions FNA was performed twice. This means a total of 37 FNA results were available for 33 patients (Table 5). FNA was obtained once in 29 patients and twice in 4 patients. In the 3 patients with FNA of the same lesion more than once, in one patient the FNA was positive for a PanNET twice, in one normal pancreatic tissue was followed by PanNET and in the last patient the biopsy was positive for PDAC twice. One patient had a biopsy of two separate lesions and was therefore studied twice.

In 24 patients FNA diagnosis was a PanNET; one patient had two FNA's of a different PanNET. In 10 this was subsequently confirmed after operative resection and 14 had imaging follow-up confirming the presence of a PanNET. Six patients had either normal tissue, a cyst or an unrepresentative FNA; all were considered to have a PanNET at follow-up with the FNA most likely being sampling error – three underwent resection and three had imaging follow-up. Of the four patients with PDAC according to the FNA, two patients finally had a PDAC and two a PanNET. In one patient, a FNA and the following surgical resection specimen showed a PDAC. In one patient two biopsies of the same lesion were positive for PDAC, which was the cause of death shortly after. In one patient with FNA positive for PDAC, the tumor in the operative resection specimen appeared to be a PanNET. In one patient the FNA was initially positive for PDAC; revision of the specimen during follow-up changed the conclusion into a PanNET. The median age of the three patients with a final diagnosis of a PDAC was 68.8 years [range 61.9-75.5].

Table 4. Diagnostic accuracy measures of MRI and CT as first imaging study in 2010 – 2017.

	Sensitivity	Specificity	PPV	NPV	Positive LR	Negative LR
Pancreatic head						
MRI	65.2 (44.8 – 81.3)	100 (92.1 – 100)	100 (76.1 – 100)	87.1 (76.3 – 93.6)	Inf	0.35 (0.20 – 0.61)
CT	27.3 (9.2 – 57.1)	90.5 (69.9 – 98.6)	60.0 (22.9 – 88.4)	70.4 (51.3 – 84.3)	2.86 (0.56 – 14.7)	0.80 (0.55 – 1.18)
Pancreatic body/tail						
MRI	75.0 (56.4 – 87.6)	95.9 (85.5 – 99.6)	91.3 (72.0 – 98.8)	87.0 (75.3 – 93.9)	18.4 (4.7 – 72.6)	0.26 (0.14 – 0.50)
CT	77.8 (54.3 – 91.5)	100 (74.9 – 100)	100 (74.9 – 100)	77.8 (54.3 – 91.5)	Inf	0.22 (0.09 – 0.53)

95% CIs are given in parentheses.

Abbreviations: CT computed tomography, LR likelihood ratio, MRI magnetic resonance imaging, NPV negative predictive value, PPV positive predictive value

Table 5. Histopathological outcomes of all specimens

	All specimens	Resection specimen	FNA
PanNET	131 (89.1%) [*]	106 (96.4%) [*]	25 (67.6%)
Normal pancreas	6 (4.1%)	2 (1.8%)	4 (10.8%)
PDAC	7 (4.8%) ^{†^}	2 (1.8%) [*]	5 (13.5%) ^{†^}
Other or not representative	3 (2.0%)	0 (0%)	3 (8.1%)

All tissues were collected indicating that patients could contribute multiple specimens; With regards to FNA: three patients had two biopsies of the same lesion: In one patient the FNA was positive for a PanNET twice, in one normal pancreatic tissue was followed by PanNET and in the last patient the biopsy was positive for PDAC twice. One patient had FNA of two lesions, so the 37 FNA results represent 34 lesions from 33 patients.

^{*}In one patient the tumor was a collision tumor consisting of a PDAC surrounded by multiple PanNETs.

[†]In one patient a biopsy was initially positive for PDAC; revision of the specimen during follow-up changed the conclusion into a PanNET.

[^]In one patient with a PDAC positive biopsy, the surgical resection specimen revealed a PanNET and no PDAC.

Abbreviations: FNA fine needle aspiration, PDAC pancreatic ductal adenocarcinoma, PanNET pancreatic neuroendocrine tumor

DISCUSSION

The present study shows an increase in the use of pancreatic imaging in patients with MEN1 over the past three decades, as well as a shift in imaging modality towards MRI. The diagnostic accuracy of the pancreatic imaging in this population-based cohort of patients with MEN1 was high. The diagnostic accuracy of MRI is excellent, and exceeds that of CT, particularly for NETs of the pancreatic head. Routine histopathological confirmation of PanNETs can be considered to have limited added value over repeat imaging for the diagnosis of MEN1-related PanNETs.

The imaging-based screening program is quintessential to enable timely diagnosis of MEN1-related PanNETs. The present study shows the tremendous cumulative burden of pancreatic imaging in MEN1 as 70-80 scans per 100 patients with MEN1 are annually performed which is not including imaging of the pituitary gland and lungs. In addition, these observed numbers are an underestimation of all pancreatic imaging, since each modality was only captured once per quarter. The number of imaging studies has substantially increased especially after the first MEN1 clinical practice guidelines in 2001.³ A substantial shift towards MRI was observed. Over one third of the index scans was documented as positive in the most recent decade. After the DMSG MEN1 database initiation in 2008 the Dutch collaboration also led to nationwide protocols aiming to standardize clinical practice processes. The high number of scans showing a PanNET could be attributed to the increased diagnostic accuracy of new scanners, increased experience of endosonographers, the duration of follow-up and patients' age, and the adjustments of imaging protocols.

It is currently unclear whether histopathological confirmation of PanNET is needed in MEN1 after a radiological diagnosis. This study shows that the overall PPV of MRI, CT

and EUS combined were 88.9% (76.0-95.6) for the head and 92.0% (85.3-96.0) for the body/tail, indicating that routine histopathology or FNA could potentially add a maximum of 11.1% (4.4-24.0) and 8.0% (4.0-14.7) on top of imaging studies for the head and body/tail respectively. To be used as an add-on, the diagnostic accuracy must improve the existing diagnostic pathway.²⁰ Of the 10 biopsies which were not considered as a PanNET by FNA, eight were considered as PanNET after surgical resection or follow-up. Two of the four patients with PDAC in the FNA were incorrectly considered as PDAC. These numbers indicate that routine histopathological or FNA confirmation is not necessary to accurately diagnose a pancreatic lesion in MEN1 as a PanNET. However, it was unknown in how many patients FNA changed clinical management. For newly diagnosed lesions, biopsies could be considered selectively based on age and radiological characteristics to identify the rare cases of PDAC. In this study three patients were considered to have a PDAC, of whom all were older than 60 years. PDAC in MEN1 has been rarely reported in the literature including one case report²¹, a prevalence and cause of death in 0-0.3% in multicenter^{6,22} and single center²³⁻²⁵ studies, and 0% in a literature review²³ including 1613 patients.

The sensitivity in previous studies ranged from 54-81% for CT and 74-88% for MRI.⁸ Within our cohort with index imaging between 2010 and 2017, the sensitivity of the pancreatic body/tail was on the high end of the percentages reported in the literature. These higher percentages are likely a consequence of including imaging studies from recent years compared to other studies. However, for the pancreatic head, sensitivity of especially CT was lower. In the present study index scans were analyzed, whereas in studies included in the systematic review, not necessarily index scans were used or surgical cohorts were analyzed which could influence the sensitivity.⁸ In addition, the outcomes of our cohort indicate that the diagnostic accuracy of MRI is high, irrespective the location of the lesion.

The choice for an imaging modality is not solely guided by the diagnostic accuracy to detect PanNETs, but also based on its ability to detect lymph node and liver metastasis, the precision of tumor size estimation, invasiveness, local availability, side-effects and costs. EUS lacks the ability to detect distant metastases. Somatostatin receptor imaging and CT in MEN1 are associated with ionizing radiation in a dose which is related with an increased risk of developing secondary solid tumors or leukemia; however, a direct relation between ionizing radiation and the development of these tumors in patients with MEN1 has not been proven *in vivo*.^{11,26} Basal studies provide theoretical mechanisms which postulate that loss of the protein menin – involved in DNA repair, cell cycle control and transcriptional regulation – could lead to cells becoming more sensitive to the effects of ionizing radiation.²⁷ Nevertheless, whether repeated ionization could induce PanNETs development or tumor aggressiveness in patients with MEN1 and its subsequent mechanism needs to be established.²⁷ Somatostatin receptor or glucagon-like peptide-1 receptor positron emission tomography (PET)/CT should not be regarded as first line screening modality, but could be subsequently

used to detect metastases or to localize insulinomas.^{8,30} The added value of somatostatin receptor PET/CT was recently shown, however, in screened patients without a previous PanNET, PET/CT was positive in 90.9% of patients and MRI in 92.3%.³¹ MRI can accurately estimate tumor size, the main prognostic factor.^{22,28,29} Although no preference is reported within the guidelines, this study shows that clinical decision making tends towards preferring MRI in the Netherlands. Considering the diagnostic accuracy, patterns in daily practice, and the consequences of undesirable radiation exposure in a hereditary disease with necessary lifelong radiological follow-up, MRI should be considered the preferred non-invasive imaging modality.

In this paper we focus on the diagnostic value of FNA, however, it is important to consider that FNA could potentially contribute to risk stratification and thereby to personalized follow-up and treatment. This is currently not part of standard clinical practice in MEN1. One might consider the use of Ki-67, although studies show that FNA-based Ki-67 could lead to undergrading.¹¹ Additionally, tissue-based prognostic factors such as DAXX/ATRX and alternative lengthening of telomeres may become of use, although more evidence is needed before implementation in clinical practice.^{32,33} There is a need for novel non-invasive markers for risk stratification such as liquid biopsies or imaging-based risk stratification.³⁴

The major strength of our study is the population-based cohort comprising patients from 8 academic institutions taking care of more than 90% of all Dutch MEN1 patients, which subsequently reduces case-mix issues and provides an accurate disease prevalence. Previous studies mainly were single center studies, with selected patient cohorts (e.g., surgically treated patients), and non-invasive imaging modalities were only compared in less than ten patients.⁸ Appropriate methods to deal with differential verification such as an alternative reference standard with stratified analyses were performed.¹⁵ By adapting an alternative reference standard, the study population included all potential patients with a PanNET and not specifically those who underwent operative resection, leading to a representative cohort. This allowed representative contingency tables and calculation of all corresponding diagnostic accuracy measures, whereas former studies mainly reported sensitivity. In addition, this study was the first to compare MRI and CT within a recent cohort. Subgroup analyses according to time periods were performed to account for the increased diagnostic accuracy over time. The main study limitations are adherent to the database design, including its retrospective nature, including only one tumor from the pancreatic head and body/tail, the data collection by multiple investigators, and the data collection from daily practice including different scanners and different scan protocols. Nevertheless, a stringent protocol was adhered to in order to realize uniform data collection and the number of data collectors was minimized and low taking the number of imaging studies into account.¹³ Only a prospective comparative study including MRI, CT, and EUS

plus FNA in all patients and standardized and blinded reading and reporting of imaging reports would overcome some of these limitations. In this respect, The European Neuroendocrine Tumor Society has only just published protocols for standardized reporting of imaging for PanNETs to improve uniformity and potentially reduce underreporting.¹⁹ Nevertheless, the present analysis, including multiple readers from each participating hospital, without centralized review by a dedicated MEN1/NET team, reflects current daily clinical practice. In addition, at the time of reading imaging studies, radiologists, gastroenterologists, and pathologists were not aware that their contributions were used for scientific research, making a Hawthorne effect unlikely. Furthermore, all participating centers were academic hospitals. Since the imaging studies were captured from clinical practice, the time between the index test and reference standard could vary and was not adjusted for in the analysis. The number of FNAs was relatively low, nevertheless the number of patients undergoing surgical resection was substantial and therefore histopathology was obtained in more than 25% of the cohort. The impact of EUS-guided FNA on clinical decision making in individual patients was unknown due to the retrospective nature of the database. For radiological follow-up as reference standard no adjustment was done for which scan was performed first or whether follow-up was performed by CT or MRI. Nevertheless, we believe that the decision for the choice of the first scan was influenced by the treating physician and hospital and not necessarily by other factors affecting the chance of having a PanNET. In addition, a cut-off of 5 mm within 3 years was applied to consider which increased the number of false negative studies.

In conclusion, the use of pancreatic imaging studies (CT, MRI, EUS) in MEN1 has substantially increased over the past decades and a shift towards MRI was observed. The overall diagnostic accuracy of imaging for PanNET in MEN1 is high and the incidence for PDAC was nil in patients younger than 60, so routine pancreatic biopsies will likely add little additional information for the differential diagnosis of pancreatic cancer. The potential use of FNA in prognostication and treatment planning is beyond the scope of this work. Diagnostic accuracy measures of MRI are excellent. Considering the disadvantages of CT, the high diagnostic accuracy of MRI and its predominant use, MRI should be advocated as the preferred (non-invasive) modality for the detection of MEN1-related PanNETs.

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Supplementary Table 1. Characteristics of imaging studies.

	All imaging	MRI	CT	EUS
Total no of scans (%)	3477	1818 (52.3%)	1291 (37.1%)	368 (10.6%)
No of scans per patient, median [IQR, range]	7 [3-11, 0-32]	4 [2-7, 0-19]	2 [0-4, 0-29]	0 [0-1, 0-14]
PanNET, no (%)	1845 (53.1%)	959 (52.8%)	560 (43.4%)	326 (88.6%)
PanNET head, no (%)	932 (26.8%)	452 (48.5%)	251 (19.4%)	229 (62.2%)
PanNET body/tail, no (%)	1471 (42.3%)	773 (42.5%)	408 (31.6%)	290 (78.8%)
Total scans performed, no (%)				
1990-1999	255 (7.3%)	98 (38.4%)	153 (60.0%)	4 (1.6%)
2000-2009	1237 (35.6%)	502 (40.6%)	605 (48.9%)	130 (10.5%)
2010-2017	1985 (57.1%)	1218 (61.4%)	533 (26.9%)	234 (11.8%)
Index scans positive (%)				
1990-1999	28/71 (39.4%)	12/31 (38.7%)	16/40 (40.0%)	-
2000-2009	62/206 (30.1%)	17/86 (19.8%)	44/119 (37.0%)	1/1 (100%)
2010-2017	44/119 (37.0%)	28/83 (33.7%)	16/36 (44.4%)	-

All scans performed per patient were included.

Abbreviations: *CT* computed tomography, *EUS* endoscopic ultrasound, *IQR* interquartile range, *MRI* magnetic resonance imaging, *PanNET* pancreatic neuroendocrine tumor

Supplementary Table 2. Percentage positive index scans stratified by age category.

	Age category (years)							All (n = 396)
	<20 (n = 61)	20 – 29 (n = 69)	30 – 39 (n = 75)	40 – 49 (n = 81)	50 – 59 (n = 59)	60 – 69 (n = 44)	>70 (n = 7)	
1990 – 1999	25.0% (1/4)	23.1% (3/13)	38.9% (7/18)	50.0% (9/18)	55.6% (5/9)	25.0% (2/8)	100% (1/1)	39.4% (28/71)
2000 – 2009	29.6% (8/27)	17.5% (7/40)	34.1% (15/44)	32.6% (15/46)	36.4% (8/22)	33.3% (8/24)	33.3% (1/3)	30.1% (62/206)
2010 – 2017	23.3% (7/30)	43.8% (7/16)	30.8% (4/13)	35.3% (6/17)	35.7% (10/28)	66.7% (8/12)	66.7% (2/3)	37.0% (44/119)
All	26.2% (16/61)	24.6% (17/69)	34.7% (26/75)	37.0% (30/81)	39.0% (23/59)	40.9% (18/44)	57.1% (4/7)	33.8% (134/396)

Data denote the percentage of positive scans, number of positive scans and total number of scans.

For each patient their exact age at the time of the imaging study was calculated. Patients were subsequently categorized into age groups at the time of the index scan.

Supplementary Table 3. Contingency tables stratified by reference standard.

Reference standard = pathology				Reference standard = imaging follow-up			
	PanNET (no, %)	No PanNET (no, %)			PanNET (no, %)	No PanNET (no, %)	
Pancreatic head			Total	Pancreatic head			Total
Index PanNET	14 (70)	0 (0)	14 (70.0)	Index PanNET	26 (59.1)	5 (1.6)	31 (8.7)
Index no PanNET	6 (30)	0 (0)	6 (30.0)	Index no PanNET	18 (40.9)	309 (98.4)	327 (91.6)
Total	20 (100)	0 (0)	20 (100)	Total	44 (12.3)	313 (87.7)	357 (100)
Pancreatic body/tail			Total	Pancreatic body/tail			Total
Index PanNET	56 (80.0)	0 (0)	56 (80.0)	Index PanNET	47 (65.3)	9 (3.8)	56 (18.2)
Index no PanNET	14 (20.0)	0 (0)	14 (20.0)	Index no PanNET	25 (34.7)	226 (96.2)	251 (81.8)
Total	70 (100)	0 (0)	70 (100)	Total	72 (23.5)	235 (76.5)	307 (100)

In the 2x2 tables percentages are column percentages. The row “total” has row percentages, the column “total” has column percentages.

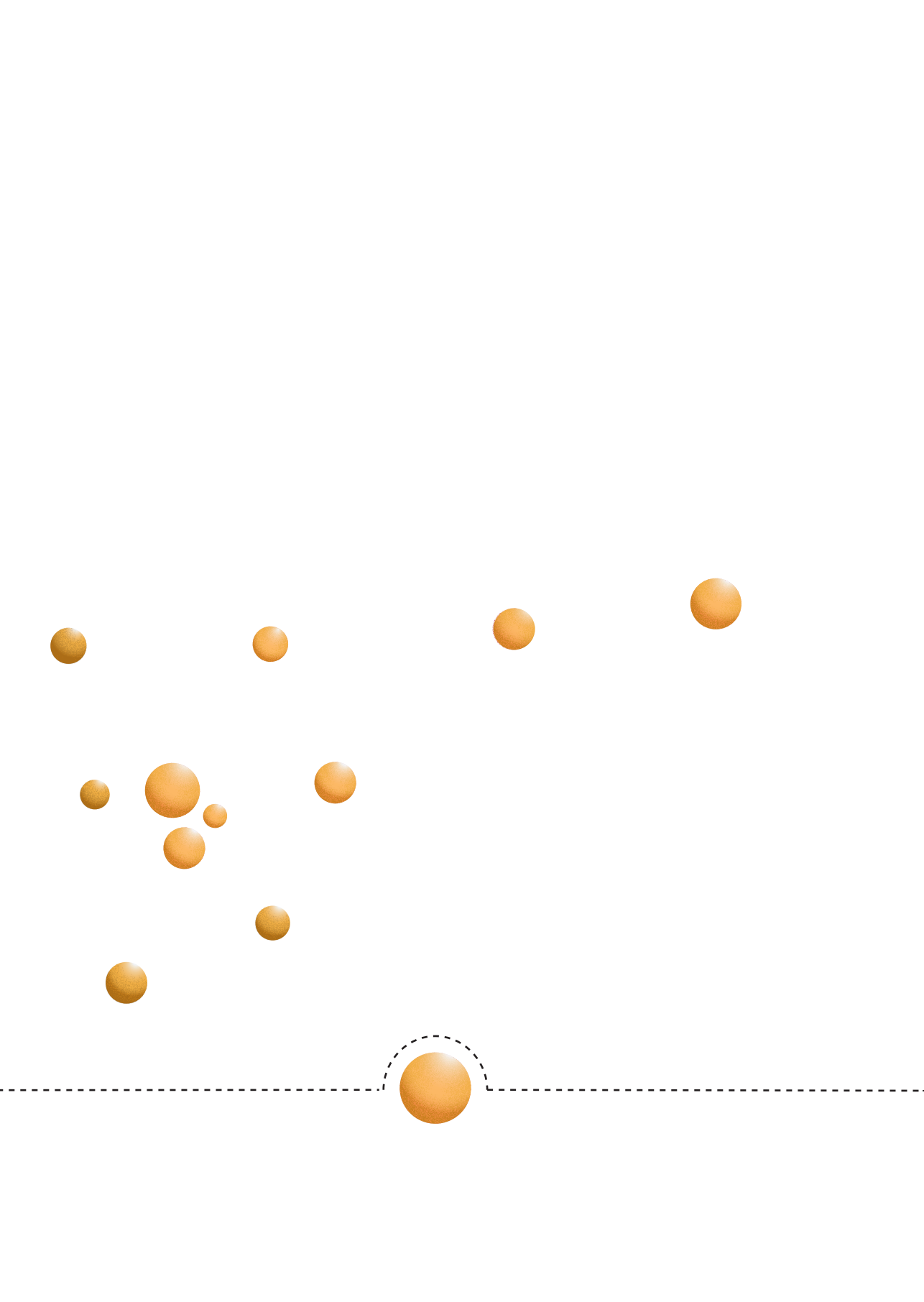
Abbreviations: *PanNET* pancreatic neuroendocrine tumor

Supplementary Table 4. Diagnostic accuracy measures stratified by reference standard.

	Sensitivity	Specificity	PPV	NPV	Positive LR	Negative LR
Pancreatic head						
Pathology	70.0 (47.9 – 85.7)	NA	100 (74.9 – 100)	0 (0.0 – 44.3)	NA	NA
Imaging	59.1 (44.4 – 72.3)	98.4 (96.2 – 99.4)	83.4 (66.9 – 93.4)	94.5 (91.4 – 96.5)	37.1 (15.0 – 91.6)	0.42 (0.29 – 0.59)
Pancreatic body/tail						
Pathology	80.0 (69.1 – 87.8)	NA	100 (92.3 – 100)	0 (0.0 – 25.1)	NA	NA
Imaging	65.3 (53.7 – 75.3)	96.2 (92.8 – 98.1)	83.9 (72.0 – 91.5)	90.0 (85.7 – 93.2)	17.0 (8.8 – 33.1)	0.36 (0.26 – 0.50)

95% CIs are given in parentheses.

Abbreviations: *LR* likelihood ratio, *NPV* negative predictive value, *PPV* positive predictive value

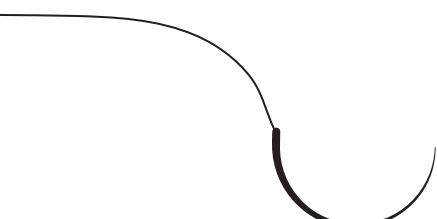


CHAPTER V

Reliability and agreement of radiological and pathological tumor size in patients with MEN1-related pancreatic neuroendocrine tumors: results from a population-based cohort

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D.J. van Beek, H.M. Verkooijen, S. Nell, B.A. Bonsing, C.H. van Eijck, H. van Goor, E.J.H. Hoogwater, E.J. Nieveen van Dijkum, G. Kazemier, C.H.C. Dejong, L.A. Brosens, F.J. Wessels, I.H.M. Borel Rinkes, G.D. Valk, M.R. Vriens



ABSTRACT

Background

Pancreatic neuroendocrine tumors (pNETs) have a high prevalence in patients with multiple endocrine neoplasia type 1 (MEN1) and are the leading cause of death. Tumor size is still regarded as the main prognostic factor and therefore used for surgical decision-making. We assessed reliability and agreement of radiological and pathological tumor size in a population-based cohort of patients with MEN1-related pNETs.

Methods

Patients were selected from the Dutch MEN1 database if they had undergone a resection for a pNET between 2003 and 2018. Radiological (MRI, CT, and endoscopic ultrasonography [EUS]) and pathological tumor size were collected from patient records. Measures of agreement (Bland-Altman plots with limits of agreement [LoA] and absolute agreement) and reliability (intraclass correlation coefficients [ICC] and unweighted kappa) were calculated for continuous and categorized ($<$ or ≥ 2 cm) pNET size.

Results

In 73 included patients, the median radiological and pathological tumor sizes measured were 22 (3–160) and 21 (4–200) mm, respectively. Mean bias between radiological and pathological tumor size was -0.2 mm and LoA ranged from -12.9 to 12.6 mm. For the subgroups of MRI, CT, and EUS, LoA of radiological and pathological tumor size ranged from -9.6 to 10.9 , -15.9 to 15.8 , and -13.9 to 11.0 , respectively. ICCs for the overall cohort, MRI, CT, and EUS were 0.80, 0.86, 0.75, and 0.76, respectively. Based on the 2 cm criterion, agreement was 81.5%; hence, 12 patients (18.5%) were classified differently between imaging and pathology. Absolute agreement and kappa values of MRI, CT, and EUS were 88.6, 85.7, and 75.0%, and 0.77, 0.71, and 0.50, respectively. Conclusion:

Conclusion

Within a population-based cohort, MEN1-related pNET size was not systematically over- or underestimated on preoperative imaging. Based on agreement and reliability measures, MRI is the preferred imaging modality.

INTRODUCTION

Duodenopancreatic neuroendocrine tumors (dpNETs) affect over 80% of patients with multiple endocrine neoplasia type 1 (MEN1) by the age of 80 years [1,2]. The trait occurs in 2–3 per 100,000 people and leads to multiple tumors in endocrine and non-endocrine organs [3]. MEN1-related dpNETs represent 2 distinct groups of tumors regarding hormone production: functioning dpNETs, which excessively produce hormones leading to a distinct clinical syndrome, and non-functioning pancreatic neuroendocrine tumors (NF-pNETs), which do not induce any secretory disorder. Duodenal gastrinomas and pancreatic insulinomas are the most frequently encountered functioning dpNETs [4]. Since dpNETs are the leading cause of death in MEN1, guidelines recommend intensive radiological screening programs starting in childhood to diagnose dpNETs at an early stage, enabling timely initiation of treatment [1,5–7].

Nowadays, World Health Organization tumor grade and tumor size of NF-pNETs and gastrinomas in patients with MEN1 are still the main predictors of metastases and survival, and treatment indications are primarily based on tumor size [2,7,8]. Most NF-pNETs <2 cm have an indolent natural disease course and can be safely managed without surgery [9–12]. European Neuroendocrine Tumor Society (ENETS) guidelines recommend operative resection of pancreatic neuroendocrine tumors (pNETs) and gastrinomas >2 cm [8]. Underestimation of tumor size may lead to a prolonged period of watchful waiting, whereas overestimation may hypothetically lead to unnecessary surgery. Since surgery for MEN1-related NF-pNETs is associated with a high risk of severe complications, the decision to operate should be carefully balanced against the risk of complications [13].

Considering that pNET size is used for individual patient risk stratification and subsequent clinical decision-making, accurate tumor size estimation is important. Since surgical decision-making is currently guided by the 2 cm criterion, adequate measurements of tumor size are of utmost importance. Although previous studies have focused on diagnosing pNETs on conventional imaging, guidelines lack evidence-based recommendations regarding a preferred imaging modality [7,14–19]. In the debate on the optimal modality, the accuracy of tumor size estimation by MRI, CT, or endoscopic ultrasonography (EUS) should be taken into account.

One single-center study from an ENETS center of excellence investigated the correlation between preoperative radiological tumor size and the pathological tumor size in 44 patients with MEN1-related pNETs and concluded that tumor size is frequently overestimated [20]. Since patients with MEN1 are generally treated in tertiary referral centers by dedicated multidisciplinary teams, data from a population-based cohort are necessary to estimate the outcomes in general MEN1 care [7]. In addition, a large multicenter study makes subgroup analyses for imaging modalities more robust. Therefore, the present study assessed the

reproducibility, in terms of reliability and agreement, of radiological and pathological tumor size of resected pNETs in patients with MEN1 from a population-based cohort representing daily clinical practice.

PATIENTS AND METHODS

Reporting of the study was performed according to the Guidelines for Reporting Reliability and Agreement Studies (GRRAS) recommendations [21].

Study Design and Patient Selection

Patients were selected from the DutchMEN Study Group (DMSG) database [22]. In brief, the database includes MEN1 patients aged 16 years and older and under treatment in one of the 8 University Medical Centers (UMCs) in the Netherlands. In each center, patients were identified by reviewing hospital databases of medical conditions and diseases. MEN1 diagnosis was established according to the clinical practice guidelines [7]. Over 90% of the Dutch MEN1 population is included in the database [23]. Clinical and demographic data were collected longitudinally every quarter from 1990 to 2017 by standardized medical record review, according to a predefined protocol. From 2016 onward, data were captured prospectively. The protocol was approved by the Medical Ethics Committees of all UMCs. All consecutive patients who underwent resection of a MEN1-related pNET from 2003 up to and including 2017 with information on preoperative radiological tumor size within 9 months before surgery and histopathological tumor size were identified. By including patients after 2003, the population is more representative for current practice, where patients are screened according to clinical practice guidelines [7,24]. In addition, considering the screening program, reproducibility analyses were performed in patients with pNETs <5 cm on radiology or pathology.

Clinical Definitions

In the absence of excessive hormone production leading to a clinical syndrome, a pNET was considered as an NF-pNET [9,10]. Insulinomas were diagnosed based on a 72-hour fasting test [9,25,26]. Gastrinomas were diagnosed based on hypergastrinemia and a gastrin positive (duodenal) neuroendocrine tumor [27]. In patients with both a pNET and (duodenal) gastrinoma the resection was considered for an NF-pNET and gastrinoma.

Data Collection

Data were collected from routine patient care, that is, no prospective study protocol existed for radiologists, gastroenterologists, and pathologists to determine tumor size, so size

reflected the tumor size used in clinical practice for decision-making. In line, preoperative imaging was generally examined once by 1 local senior radiologist (CT or MRI) or gastroenterologist (EUS) and the surgical specimen was assessed by 1 local senior pathologist. Surgical resection specimens were processed in each center's pathology department according to local practice; tumor size was generally measured after formalin fixation. According to the guidelines, outcomes were discussed in the multidisciplinary tumor boards within the individual tertiary centers ensuring reliable outcomes [7]. Over the years, multiple radiologists, gastroenterologists, and pathologists have performed the observations within the UMCs in the Netherlands; the exact number of observers is unknown. Radiologists, gastroenterologists, and pathologists were not blinded to previous imaging or to clinical information, but at the time of examination, they were not aware of their observations being used for scientific research, so the Hawthorne effect (i.e., an alteration in observers' behavior due to awareness of being observed) is unlikely. At the time of radiological assessment, radiologists were by definition unaware of histopathological tumor size. MRI, CT, and EUS reports up to 9 months before surgery were collected. Imaging and histopathological reports were reviewed for the location and size of the pNETs. For each patient, the diameter of the largest tumor in the pancreatic head and/or body/tail was obtained from the imaging (MRI, CT, and/or EUS) and pathology reports. For patients with multiple resected tumors (i.e., from both the pancreatic head and body/tail), the size of the largest tumor (either from the pancreatic head or body/tail) on imaging was used for the overall analysis. The tumor size measured on the modality closest to the date of surgery was used for the overall analysis. For patients with multiple modalities, these data were used for subgroup analyses of the respective modality.

Statistical Analysis

Baseline characteristics were described as mean (\pm standard deviation [SD]), median (range), or counts (percentages), as appropriate. Reproducibility of tumor size between imaging and pathology was assessed in terms of agreement and reliability [28]. Agreement indicates the degree to which measurements are identical and is particularly relevant when assessing the absolute closeness of repeated measures [21,28]. Reliability implies the ability to distinguish patients with different tumor sizes from each other despite biological variability between study objects [21,28]. Agreement between imaging and histology of continuous tumor size was assessed using the Bland-Altman plots and their limits of agreement (LoA) with 95% confidence intervals (95% CI) [29]. In brief, for every patient, the difference between the radiological and pathological tumor size (y axis) was plotted against the mean of the 2 measurements (x axis). The mean and SD of these differences were used to calculate the LoA (mean difference \pm 1.96 \times SD). The LoA represent the maximum range by which repeated measurements would be expected to differ in 95% of repetitions and indicate the

range of observer variation; differences beyond the LoA are not accounted for by observer variation alone [29]. Reliability of tumor size was assessed by the intraclass correlation coefficient (ICC), which was calculated by a 2-way model with absolute agreement and single measures (ICC(2,1)) [30]. The ICC is interpreted as the percentage of variability between the measurements, which is not caused by measurement error. ICC values should generally be at least 0.90 to guide important clinical decisions [21].

Contingency tables were tabulated for pNET size categorized to <2 or ≥ 2 cm on imaging and pathology, according to current clinical insights [8,10,11]. These were additionally performed for <3 and ≥ 3 cm [10]. Agreement was assessed using percentages of absolute agreement and specific agreement. Reliability was assessed using unweighted Cohen's kappa. Kappa's are rated as "fair" for values between 0.21 and 0.40, "moderate" for 0.41–0.60, "substantial" for 0.61–0.80, and "almost perfect" for values above 0.80 [31]. 95% CIs for reliability measures (kappa and ICC) were generated by drawing 10,000 bias-corrected and accelerated bootstrap replications [32–34].

All analyses were performed for all imaging modalities (MRI/ CT/EUS) together and separately. Furthermore, subgroup analyses were performed including patients with an NF-pNET, with or without concurrent gastrinoma, as surgical indication, since tumor size guides clinical management in these patients [8,10,11]. To investigate the overall effect of time and the effect of different modalities over time, subgroup analyses were additionally performed for patients undergoing surgery before 2011 (2003–2010) and in 2011 or later (2011–2017). Statistical analyses were conducted using R version 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria) using the "IRR," "blandr," and "boot" packages.

RESULTS

Of the 445 patients in the DMSG database, 275 (61.8%) had a pNET on imaging (Fig. 1). Seventy-three patients underwent surgery for a pNET between 2003 and 2018 and were eligible for inclusion; reasons for exclusion are listed in Figure 1. Patients had undergone surgery at a mean age of 44.6 years (± 14.5), and 39 (53.4%) were females (Table 1). Median age at diagnosis was 36.6 years (range 18.8–81.8) in patients with an insulinoma and 42.5 years (range 14.5–73.3) in the other patients. An NF-pNET was the most frequent surgical indication in 47 patients (64.4%), and 15 (20.5%) patients underwent surgery for a functioning pNET. Twenty-five patients had a resected pNET of the head, and 57 patients had a resected pNET of the body/tail. Almost half of the patients (47.9%, $n = 35$) underwent a distal pancreatectomy; 12 (16.4%) underwent 1 or more enucleation(s); 10 (13.7%), a total/completion pancreatectomy; 7 (9.6%), a Whipple/PPPD; and 1 (1.4%), a central pancreatectomy, and in 8 patients (11.0%), a combined resection was performed. Most recent imaging

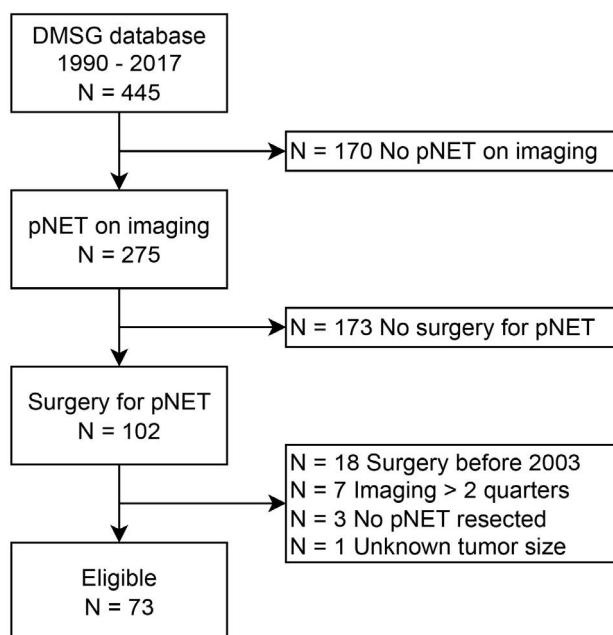


Figure 1. Flow-chart of patient selection. Sixty-five of the 73 patients had a pNET smaller than 5 cm. Abbreviations: DMSG DutchMEN Study Group, pNET pancreatic neuroendocrine tumor.

before surgery was MRI in 23 (31.5%), CT in 28 (38.4%), and EUS in 22 (30.1%). Overall, MRI, CT, and EUS were available for analysis in 36 (49.3%), 43 (58.9%), and 29 (39.7%) patients, respectively. Thirty-three patients (45.2%) had multiple imaging modalities for analysis. Thirty-nine patients (53.4%) underwent surgery before 2011 and 34 (46.6%) in 2011 or later. Before 2011, MRI, CT, and EUS were available for 11 (28.2%), 28 (71.8%), and 18 (46.2%) patients, and after 2011, these were available for 25 (73.5%), 15 (44.1%), and 11 (32.4%) patients, respectively.

Radiological and Pathological Tumor Size

Radiological and pathological tumor sizes of the largest tumor of individual patients are presented in Figure 2. The median radiological and pathological tumor sizes were 22 mm (range 3–160) and 21 mm (range 4–200), respectively (Table 2). In 28 patients (38.4%), radiological size was larger than pathological size; in 40 patients (54.8%), radiological size was smaller than pathological size; and in 5 (6.8%), both were exactly similar. Twelve patients (16.4%) had a difference of >10 mm between radiological and pathological size, of whom 5 had a pNET of ≥ 5 cm. Nine of these 12 patients underwent surgical resection before 2011; all 8 patients with a difference of >15 mm were operated on before 2011.

For the subgroups of MRI, CT, and EUS, radiological size was larger than pathological size in 38.9% (14/36), 37.2% (16/43), and 48.3% (14/29) patients, and smaller than pathological size in 55.6% (20/36), 48.8% (21/43), and 51.7% (15/29), respectively. Radiological and pathological sizes were exactly similar in 2/36 patients (5.6%) with an MRI and 6/14 (14.0%) with a CT. Median radiological and pathological size of pNETs of the head were 19 mm

Table 1. Baseline table

Variable	N = 73 (%)
Age at surgery in years, mean (\pm SD)	44.6 (\pm 14.5)
Sex	
Male	34 (46.6%)
Female	39 (53.4%)
Surgery	
Primary surgery	64 (87.7%)
Reoperation	9 (12.3%)
Surgical indication	
NF-pNET	47 (64.4%)
Insulinoma	14 (19.2%)
Gastrinoma	4 (5.5%)
NF-pNET and gastrinoma	7 (9.6%)
Other functioning pNET	1 (1.4%)
Type of resection	
Enucleation	12 (16.4%)
Enucleation head	4 (5.5%)
Enucleation body/tail	7 (9.6%)
Enucleation head and body/tail	1 (1.4%)
Distal pancreatectomy	35 (47.9%)
Whipple/PPPD	7 (9.6%)
Distal pancreatectomy and enucleation	3 (4.1%)
Whipple/PPPD and distal pancreatectomy	5 (6.8%)
Pancreatic body resection	1 (1.4%)
Total/completion pancreatectomy	10 (13.7%)
Type of imaging available for size analysis	
MRI	36 (49.3%)
CT	43 (58.9%)
EUS	29 (39.7%)
Multiple imaging strategies available for size analysis	33 (45.2%)
MRI and CT	11 (15.1%)
MRI and EUS	7 (9.6%)
CT and EUS	13 (17.8%)
MRI, CT and EUS	2 (2.7%)
Time from imaging to surgery	
0 quarters	18 (24.7%)
1 quarter	41 (56.2%)
2 quarters	14 (19.2%)
Period of surgery	
2003 – 2010	39 (53.4%)
2011 – 2017	34 (46.6%)

Abbreviations: *CT* computed tomography, *EUS* endoscopic ultrasonography, *MRI* magnetic resonance imaging, *N* number of, *NF-pNET* non-functioning pancreatic neuroendocrine tumor, *pNET* pancreatic neuroendocrine tumor, *PPPD* pylorus-preserving pancreatoduodenectomy, *SD* standard deviation

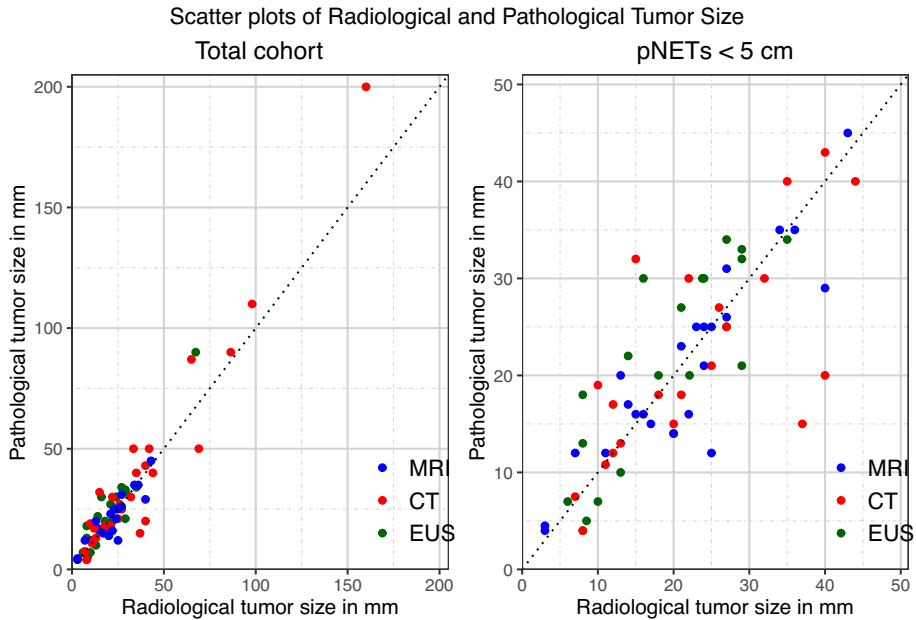


Figure 2. Scatterplots of radiological and pathological tumor size of the total cohort and for pNETs smaller than 5 cm. Abbreviations: CT computed tomography, EUS endoscopic ultrasonography, MRI magnetic resonance imaging.

(range 3–43) and 15 mm (range 4–50), and 22 mm (range 5–160), and 21 mm (range 4–200) of pNETs of the body/tail. Median radiological and pathological sizes of the NF-pNET subgroup ($n = 54$, 74.0%) were 25 mm (range 3–160) and 25 mm (range 4–200), respectively. Seven patients (9.6%) had a pNET > 5 cm on imaging or pathology, and therefore, reproducibility analyses were performed using 65 patients.

Tumor Size

Agreement between radiological and pathological tumor size is shown in Figure 3 and Table 3. Overall, imaging underestimated pathological tumor size with -0.2 mm (95% CI: -1.8 to 1.4). LoA ranged from -12.9 mm (95% CI: -15.6 to -10.1) to 12.6 mm (95% CI: 9.8 – 15.3), respectively. This indicates that differences in size of up to 13 mm between imaging and pathology can be contributed to observer or measurement error, so a tumor measured on imaging or pathology can be up to 13 mm smaller or larger. No systematic over- or underestimation of radiological tumor size was observed for any of the modalities. LoA of MRI and EUS were generally smaller than those of CT (Fig. 3; Table 3). For MRI, LoA ranged from -9.6 mm (95% CI: -12.7 to -6.5) to 10.9 mm (95% CI: 7.8 – 14.0), and for EUS, from -13.9 mm (95% CI: -18.2 to -9.7) to 11.0 mm (95% CI: 6.7 – 15.3). LoA did

Table 2. Radiological and pathological tumor size of the total cohort and stratified by imaging modality and pNET localization

Overall				pNETs head				pNETs body/tail				
Imaging modality	N (%)	Radiological size (mm)	Pathological size (mm)	Difference (mm)	N (%)	Radiological size (mm)	Pathological size (mm)	Difference (mm)	N (%)	Radiological size (mm)	Pathological size (mm)	Difference (mm)
All	73 (100%)	22 [3 – 160]	21 [4 – 200]	-1 [-40 – 22]	26 (35.6%)	19 [3 – 43]	15 [4 – 50]	-1 [-17 – 22]	58 (79.5%)	22 [5 – 160]	21 [4 – 200]	0 [-40 – 20]
MRI	36 (49.3%)	23 [3 – 43]	21 [4 – 50]	-1 [-9 – 17]	14 (19.2%)	16 [3 – 43]	14 [4 – 45]	-1 [-6 – 15]	29 (39.7%)	21 [3 – 41]	20 [4 – 50]	0 [-15 – 17]
CT	43 (58.9%)	25 [8 – 160]	25 [4 – 200]	0 [-40 – 22]	14 (19.2%)	20 [7 – 42]	17 [4 – 50]	0.3 [-23 – 22]	33 (45.2%)	25 [9 – 160]	25 [4 – 200]	0 [-40 – 20]
EUS	29 (39.7%)	20 [6 – 67]	21 [4 – 90]	-1 [-23 – 8]	10 (13.7%)	19 [5 – 29]	16 [5 – 33]	0.8 [-5 – 6]	22 (30.1%)	17 [5 – 67]	22 [4 – 90]	-1 [-23 – 8]

All values are given as median [range]. The differences were calculated by subtracting the pathological size from the radiological size. Abbreviations: CT computed tomography, EUS endoscopic ultrasonography, mm millimeter, MRI magnetic resonance imaging, N number of, pNET pancreatic neuroendocrine tumor

not increase with increasing tumor size (Fig. 3). Within the NF-pNET subgroup, similar results were observed (Table 3). For pNETs in the pancreatic head, mean bias was 0.9 mm (95% CI: -1.4 to -3.1) and LoA ranged from -9.6 mm (95% CI: -13.5 to -5.8) to 11.4 mm (95% CI 7.5-15.2). For pancreatic body/tail tumors, mean bias, lower LoA, and upper LoA were -0.3 mm (95% CI: -2.1 to 1.5), -13.0 mm (95% CI: -16.2 to -9.9), and 12.4 mm (95% CI: 9.3-15.6), respectively. LoA of MRI and EUS seemed narrower for pancreatic head tumors compared to those in the body/tail, whereas patients with a CT had narrower LoA for pancreatic body/tail tumors, but these subgroups were small.

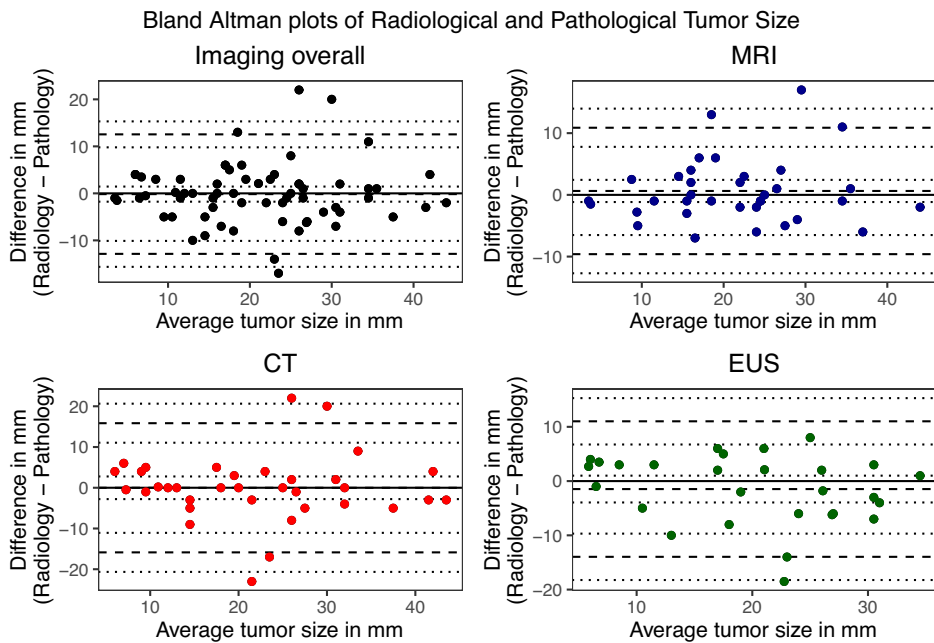


Figure 3. Bland-Altman plots of radiological and pathological tumor size of MEN1-related pNETs. For every patient the difference between the radiological and pathological tumor size (y-axis) is plotted against the mean of the two measurements (x-axis). The mean and standard deviation (SD) of these differences are used to calculate the limits of agreement (LoA); (mean difference $\pm 1.96 \times \text{SD}$). The LoA indicate the range of observer variation; differences beyond the LoA are not accounted for by observer variation alone [29]. The solid line shows a mean bias of 0. The middle dashed line represents the mean difference between radiological and pathological tumor size with dotted lines indicating 95% confidence intervals (95% CI) of the mean bias. The upper and lower dashed lines represent the upper and lower LoA with subsequent upper and lower bounds of the 95% CI of the LoA shown in dotted lines. Abbreviations: CT computed tomography, EUS endoscopic ultrasonography, MRI magnetic resonance imaging.

Table 3. Bland-Altman analysis showing mean bias and LoA.

Imaging modality	Overall				NF-pNETs			
	N	Mean bias (95% CI)	LoA (95% CI)		N	Mean bias (95% CI)	LoA (95% CI)	
			Lower	Upper			Lower	Upper
All	65	-0.2 (-1.8 – 1.4)	-12.9 (-15.6 – -10.1)	12.6 (9.8 – 15.3)	49	0.6 (-1.3 – 2.5)	-12.6 (-16.0 – -9.3)	13.8 (10.5 – 17.2)
MRI	35	0.6 (-1.2 – 2.4)	-9.6 (-12.7 – -6.5)	10.9 (7.8 – 14.0)	27	1.5 (-0.7 – 3.7)	-9.4 (-13.2 – -5.6)	12.5 (8.7 – 16.3)
CT	35	0.0 (-2.7 – 2.8)	-15.9 (-20.7 – -11.1)	15.8 (11.1 – 20.6)	26	0.1 (-3.6 – 3.8)	-17.8 (-24.2 – -11.4)	18.1 (11.7 – 24.5)
EUS	28	-1.8 (-3.9 – 1.0)	-13.9 (-18.2 – -9.7)	11.0 (6.7 – 15.3)	19	-1.1 (-4.2 – 1.9)	-13.5 (-18.8 – -8.2)	11.2 (5.9 – 16.5)

Imaging modality	2003 – 2010				2011 - 2017			
	N	Mean bias (95% CI)	LoA (95% CI)		N	Mean bias (95% CI)	LoA (95% CI)	
			Lower	Upper			Lower	Upper
Overall	32	-0.2 (-3.0 – 2.6)	-15.5 (-20.3 – -10.6)	15.1 (10.2 – 19.9)	33	-0.1 (-1.9 – 1.7)	-10.0 (-13.0 – -6.9)	9.7 (6.6 – 12.8)
MRI	10	-1.9 (-5.6 – 1.8)	-12.2 (-18.8 – -5.5)	8.4 (1.7 – 15.0)	25	0.7 (-1.4 – 2.9)	-9.6 (-13.3 – -5.8)	11.0 (7.3 – 14.8)
CT	21	1.0 (-2.8 – 4.9)	-15.4 (-22.1 – -8.8)	17.5 (10.9 – 24.1)	14	-1.0 (-3.8 – 1.9)	-10.5 (-15.4 – -5.6)	8.6 (3.7 – 13.5)
EUS	17	-2.7 (-6.2 – 0.9)	-16.2 (-22.4 – -10.0)	10.9 (4.7 – 17.1)	11	0.4 (-3.2 – 4.0)	-10.1 (-16.5 – -3.8)	10.9 (4.6 – 17.3)

Abbreviations: *CI* confidence interval, *CT* computed tomography, *EUS* endoscopic ultrasonography, *LoA* limits of agreement, *mm* millimeter, *MRI* magnetic resonance imaging, *N* number of, *NF-pNET* non-functioning pancreatic neuroendocrine tumor

LoA of patients who underwent surgery before 2011 and after 2011 are shown in Table 3. Before 2011, LoA ranged from –15.5 mm (95% CI: –20.3 to –10.6) to 15.1 mm (95% CI 10.2–19.9), and after 2011, ranged from –10.0 mm (95% CI: –13.0 to –6.9) to 9.7 mm (95% CI 6.6–12.8).

The overall ICC for tumor size was 0.80 (95% CI: 0.65– 0.89), indicating moderate reliability of continuous tumor size measurement between imaging and pathology (Table 4). The ICCs of MRI, CT, and EUS were 0.86 (95% CI: 0.69–0.94), 0.75 (95% CI: 0.48–0.89), and 0.76 (95% CI: 0.50–0.88), respectively. These ICCs are probably due to the relatively wide range of observed radiological and pathological tumor sizes and therefore can adequately distinguish between patients.

Table 4. Reliability of tumor size and pNETs categorized as < or \geq 2 cm

	Overall cohort		NF-pNET subgroup	
	pNET \geq 2 cm	Tumor size	pNET \geq 2 cm	Tumor size
Type	Kappa (95% CI)*	ICC (95% CI)*	Kappa (95% CI)*	ICC (95% CI)*
All	0.63 (0.40 – 0.78)	0.80 (0.65 – 0.89)	0.55 (0.27 – 0.77)	0.79 (0.58 – 0.90)
MRI	0.77 (0.46 – 0.94)	0.86 (0.69 – 0.94)	0.68 (0.29 – 0.92)	0.84 (0.62 – 0.94)
CT	0.71 (0.40 – 0.89)	0.75 (0.48 – 0.89)	0.67 (0.28 – 0.92)	0.70 (0.35 – 0.89)
EUS	0.50 (0.08 – 0.77)	0.76 (0.50 – 0.88)	0.36 (-0.16 – 0.77)	0.76 (0.28 – 0.91)

	2003 – 2010		2011 – 2017	
	pNET \geq 2 cm	Tumor size	pNET \geq 2 cm	Tumor size
Type	Kappa (95% CI)*	ICC (95% CI)*	Kappa (95% CI)*	ICC (95% CI)*
All	0.55 (0.19 – 0.80)	0.72 (0.43 – 0.89)	0.70 (0.38 – 0.88)	0.89 (0.78 – 0.95)
MRI	1.00 (NA)	0.93 (0.71 – 0.99)	0.68 (0.33 – 0.92)	0.84 (0.64 – 0.94)
CT	0.62 (0.17 – 0.90)	0.69 (0.28 – 0.92)	0.86 (0.34 – 1.00)	0.82 (0.25 – 0.96)
EUS	0.29 (-0.21 – 0.66)	0.67 (0.25 – 0.87)	0.81 (NA)	0.91 (0.74 – 0.96)

* 95% CI were generated by drawing 10,000 bias-corrected and accelerated bootstrap replications.

Abbreviations: CT computed tomography, EUS endoscopic ultrasonography, ICC Intraclass Correlation Coefficient, MRI magnetic resonance imaging, NF-pNET non-functioning pancreatic neuroendocrine tumor, pNET pancreatic neuroendocrine tumor

Clinical implications

Forty-two patients (64.6%) were classified as having a pNET \geq 2 cm by imaging or pathology. For pNETs categorized as < and \geq 2 cm, agreement was 81.5%, indicating that imaging and pathology classified patients differently in 18.5% ($n = 12/65$) of the patients (Table 5). Of these patients, 7 (10.8%) were operated on with a radiological pNET \geq 2 cm but had a pNET < 2 cm according to pathology (Table 6). On the contrary, 5 patients (7.7%) underwent surgery for a histopathological pNET \geq 2 cm, while they would have been refrained from surgery based on preoperative imaging. Agreement between MRI, CT, and EUS, and pathology was 88.6, 85.7, and 75.0%, respectively. In terms of percentage, MRI and CT would lead least often to under- or overtreatment. Agreement was similar for the patients with NF-pNETs, except for the negative agreement of EUS. Before and after 2011, agreement was 78.1 and 84.8%, respectively. In the subgroup of patients with a preoperative MRI and EUS ($n = 8$), MRI and pathology were concordant in 8/8 (100%), whereas EUS and pathology were concordant in 6/8 (75.0%). In the subgroup of patients with an NF-pNET, 12 (24.5%) were classified as radiology and pathology < 2 cm, 4 (8.2%) as radiology < 2 cm and pathology \geq 2 cm, 6 (12.2%) as radiology \geq 2 cm and pathology < 2 cm, and 27 (55.1%) as radiology and pathology \geq 2 cm. For pNETs categorized as < and \geq 3 cm, agreement was 81.5%. Radiology was considered as \geq 3 cm and pathology as < 3 cm in 3 patients (6.3%) and radiology < 3 cm and pathology \geq 3 cm in 9 patients (16.7%).

Table 5. Agreement of pNETs categorized as < or \geq 2 cm

Overall cohort					NF-pNET subgroup			
Type	Absolute Agreement (%)		Specific Agreement (%)		Absolute Agreement (%)		Specific Agreement (%)	
	N		Negative	Positive	N		Negative	Positive
All	65	81.5%	79.3%	83.3%	49	79.6%	70.6%	84.4%
MRI	35	88.6%	86.7%	90.0%	27	85.2%	80.0%	88.2%
CT	35	85.7%	83.9%	87.2%	26	84.6%	80.0%	87.5%
EUS	28	75.0%	72.0%	77.4%	19	73.7%	54.5%	81.5%

2003 - 2010					2011 - 2017			
Type	Absolute Agreement (%)		Specific Agreement (%)		Absolute Agreement (%)		Specific Agreement (%)	
	N		Negative	Positive	N		Negative	Positive
All	32	78.1%	74.1%	81.0%	33	84.8%	83.9%	85.7%
MRI	10	100%	100%	100%	25	84.0%	83.3%	84.6%
CT	21	81.0%	77.8%	83.3%	14	92.9%	92.3%	93.3%
EUS	17	64.7%	62.5%	66.7%	11	90.9%	88.9%	92.3%

Abbreviations: CT computed tomography, EUS endoscopic ultrasonography, MRI magnetic resonance imaging, NF-pNET non-functioning pancreatic neuroendocrine tumor, pNET pancreatic neuroendocrine tumor

Table 6. Contingency tables of pNETs categorized as < or \geq 2 cm

Imaging overall vs pathology			MRI vs pathology		
	PA < 2 cm	PA \geq 2 cm		PA < 2 cm	PA \geq 2 cm
Imaging < 2 cm	23 (35.4%)	5 (7.7%)	MRI < 2 cm	13 (37.1%)	1 (2.9%)
Imaging \geq 2 cm	7 (10.8%)	30 (46.1%)	MRI \geq 2 cm	3 (8.6%)	18 (51.4%)

CT vs pathology			EUS vs pathology		
	PA < 2 cm	PA \geq 2 cm		PA < 2 cm	PA \geq 2 cm
CT < 2 cm	13 (37.1%)	2 (5.7%)	EUS < 2 cm	9 (32.1%)	4 (14.3%)
CT \geq 2 cm	3 (8.6%)	17 (48.6%)	EUS \geq 2 cm	3 (10.7%)	12 (42.9%)

Abbreviations: CT computed tomography, EUS endoscopic ultrasonography, MRI magnetic resonance imaging, PA pathology, pNET pancreatic neuroendocrine tumor

Reliability was substantial, with a kappa of 0.63 (95% CI: 0.40–0.78), indicating that 63% extra agreement was observed beyond the chance agreement (Table 4). Stratified for each modality, the kappa values ranged from moderate to substantial (EUS 0.50 [95% CI: 0.11–0.78], CT 0.71 [95% CI: 0.48–0.89], and MRI 0.77 [95% CI: 0.47–0.94]). Within NF-pNETs, reliability was slightly lower, but still substantial for MRI and CT, whereas it was considered fair for EUS (Table 4).

DISCUSSION

The present study shows that preoperative tumor size of MEN1-related pNETs was not systematically over- or underestimated in a population-based cohort, reflecting daily clinical practice. Mean bias was -0.2 mm and LoA ranged from -12.9 to 12.6 mm, indicating that 95% of the repetitions; the difference between radiological and pathological size will be less than 13 mm. Radiology and pathology were in agreement for a pNET of 2 cm or larger in 81.5%. Seven patients underwent surgery, while the tumor was <2 cm, whereas 5 would be refrained from an operation while they would be considered potential surgical candidates according to current insights. MRI appeared to be superior to other modalities, but this was not formally tested. Furthermore, the data indicate that agreement and reliability have increased in more recent years.

An overestimation of MEN1-related pNET size on imaging was previously reported [20]. In that study, pathological size was 21 versus 13 mm in the present study, while radiological size was similar [20]. Multiple differences exist between both studies. First, Polenta et al. [20] measured histopathological size from fresh specimens, whereas the histopathological report, including formalin fixation, was leading in the current study. Although no data are available regarding effect of formalin fixation for pNETs, others have assessed this in breast, lung, and renal tumors [35–37]. A decrease in tumor size after formalin fixation was reported in 4% (breast cancer) and 46.8% (lung cancer), respectively [35,36]. Park et al. [36] observed a decrease of 4.06% and 0.66 mm between fresh and formalin-fixed specimens, which is similar to the 4.6% reported in renal tumors by Tran et al. [37]. Therefore, it is unlikely that formalin fixation has contributed to the observed differences, especially considering that major shrinkage will occur during the processing after formalin fixation [35–38]. Second, data were collected from daily clinical practice, indicating that no measurement criteria existed. In addition, the transverse diameter, which was used to determine radiological and pathological size by Polenta et al. [20], is not necessarily the largest diameter used for clinical decision-making, and determining the transverse diameter could be challenging in histopathological examination. Third, patients were included from population-based cohort, thereby increasing the sample size (73 vs. 44) and making subgroup analyses more robust. MRI and CT data were available in a larger proportion of the present cohort, 36 (49.3%) and 41 (58.9%) patients, respectively, compared to 18 (40.9%) and 16 (36.4%) patients in the study by Polenta et al. [20], especially EUS overestimated tumor size – which tended to be similar in this cohort – while the differences between MRI and pathology were smallest. Last, data were analyzed according to current standards for reproducibility research, which are endorsed by guidelines for reproducibility research [29].

Another single-center study has investigated MRI and CT measurements with pathology in 292 patients with sporadic pNETs [39]. Tumors were larger on imaging and pathology (26 and 25 mm), reflecting differences between sporadic and MEN1-related pNETs. The mean bias of MRI and CT was 0.2 and 1 mm, which is similar to that of the present analysis. Nevertheless, compared to our study, LoA were wider –15.4 to 15.8 and –15.7 to 17.7 [39]. Agreement coefficients are population specific, and differences could be partially caused by the sporadic pNETs, since size estimation might be more difficult in very large (symptomatic) tumors. In line, we also observed large differences in some patients with pNETs larger than 5 cm. These results underscore that preoperative imaging in MEN1 gives reliable estimates of tumor size. EUS was not studied in the sporadic pNETs, but EUS data are demanded considering its frequent use in patients with MEN1.

The relation between tumor size and risk of metastases was described in 2006 and has been translated into the 2 cm cutoff for (NF-)pNET surgical decision-making [2,10,11]. In 12 patients (18.5%), imaging and pathology were discordant regarding the 2 cm criterion. Similar results were observed for a potential 3 cm cutoff. Radiology >2 cm and pathology <2 cm were observed in 5/27 patients (19%) with an NF-pNET by Polenta et al. [20], compared to 6/49 patients (12.2%) with an NF-pNET in the present cohort. Other studies investigated the concordance between MRI and EUS for pNETs ≥ 2 cm. Daskalakis et al. [40] observed a concordance of 97% in 31 patients with a kappa of 0.912. Barbe et al. [14] identified 20 patients with a pNET ≥ 2 cm on EUS or MRI, but EUS classified 7 (35%) as <2 cm and MRI only 3 (15%). In the current study, 8 patients had an MRI and EUS, showing concordance between MRI and pathology in all cases and between EUS and pathology in 6 out of 8. Hence, EUS seems to classify pNETs as <2 cm more frequently than MRI. Given the variability in tumor size in some individual patients, relying solely on tumor size as indicator for operative resection will be outdated in the near future. JunD and CHES1, interacting domains of Menin, have been reported as risk factors in MEN1-related pNETs, but those have not been validated [9,12,41,42]. Recent insight in the existence of alpha and beta subtypes of MEN1-related pNETs, which determine prognosis, might cause a paradigm shift in patient selection for surgery [43,44]. Subtype-specific cutoffs could be established, which would underscore the need for accurate tumor size estimation by using the most reliable modality.

In the debate on the most suitable imaging for MEN1-related pNETs, tumor size estimation should be considered. Although CT is widely available and frequently used for anatomical planning preoperatively, repeated exposure to ionizing radiation makes this modality less suitable for the lifelong screening for (NF-)pNETs in MEN1 [45,46]. LoA were relatively wide, showing that CT is less accurate for tumor size estimation. For adequate size measurements on CT, a contrast difference is demanded to adequately distinguish the tumor from surrounding (unaffected) pancreatic parenchyma, which depends on the

(hyper)vascularity of a pNET. This is even more challenging for iso- and hypovascular pNETs and MRI might overcome these limitations. After 2011, LoA of CT were more similar to those of MRI, and moreover, agreement and reliability of pNETs categorized as 2 cm or larger between CT and pathology were comparable to MRI. CT and MRI visualize surrounding organs including peri-pancreatic lymph nodes and the liver, which is important for pNET staging, but MRI is superior in the detection of (p)NET liver metastases, and diffusion-weighted MRI increases the sensitivity and specificity over conventional MRI [47–50]. MRI and EUS are generally recommended for the detection of pNETs [14,46]. Both MRI and pathology classified pNETs more often as 2 cm or larger than EUS and pathology. In addition, EUS is operator dependent and detects many very small NF-pNETs that are not of clinical significance but often misses clinically relevant pNETs in the pancreatic tail and has a complication risk [9,14,46]. On the other hand, EUS offers the possibility of concurrent biopsies of suspected pNETs or lymph nodes with subsequent grading of tumors and growth can be assessed [51,52]. Nevertheless, the need for diagnostic biopsies is uncertain in these patients, considering the very high a priori chance [53]. Notwithstanding, a cost reduction of 0–67% for the diagnosis of MEN1-related pNETs by MRI over EUS was recently reported [40]. The latter should be investigated in a population-based cohort of MEN1 patients with pNETs, taking all costs into account.

The major strengths of the present study are the patient inclusion from a population-based cohort including multiple MEN1 centers and the data collection from routine care. Thus, reliability and agreement were investigated in daily clinical practice [21]. The study also has several limitations. Only patients undergoing surgery were included. Since the decision to perform surgery has been center dependent, small(er) tumors were included, thereby increasing the generalizability of the results [10]. Size was only measured once, and the workup was center- and physician-dependent, so imaging might have been chosen selectively [7]. No standardized protocol for measurements of size was available; in clinical practice, size measurements on MRI or CT are generally performed in the axial plane and acquisition of MRI sequences is often performed in the axial plane. A potential predominance of transverse measurements – instead of 3D size measurements – is therefore a limitation of the study. However, the latter probably has had no influence on the observed outcomes, since no systematic over- or underestimation of radiological size was noted. Nevertheless, future studies should aim to identify the optimal measurement methodology – by using radiological acquisitions, which are suitable for 3D measurements, and taking intra- and interobserver variation into account – to prospectively establish a protocol for size measurements of pNETs which will improve patient care. Numbers of patients were too small to investigate the effect of center on agreement and reliability. The time from imaging until surgery differed between patients, but still the growth rate of NF-pNETs in MEN1 is generally low [9,46]. Due to the rarity of MEN1, patients were included over a relatively

long time, so the quality, experience, and techniques of CT, MRI and EUS might have increased. Subgroup analyses, based on the period of surgery, showed that the LoA were narrower in patients operated on in 2011 and later compared to those operated on before 2011. In addition, all 8 patients with a difference of 15 mm or more between radiological and pathological size were operated on before 2011.

In conclusion, preoperative tumor size is not systematically over- or underestimated. Nevertheless, in 18.5% of patients, radiology and pathology would classify patients differently regarding the 2 cm cutoff, having direct clinical implications. Agreement and reliability of MRI seemed to be superior to CT and EUS for estimating tumor size. In the debate on the preferred imaging modality for radiological pancreas screening programs in MEN1, combining the diagnostic performance, several other advantages, and reproducibility of radiological size, we would suggest performing MRI.

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P.H. Bisschop, MD, PhD, Department of Endocrinology and Metabolism, Amsterdam UMC location Academic Medical Center, Amsterdam, The Netherlands.

O.M. Dekkers, MD, PhD, Departments of Endocrinology and Metabolism and Clinical Epidemiology, Leiden University Medical Center, Leiden, The Netherlands.

M.L. Drent, MD, PhD, Department of Internal Medicine, Section of Endocrinology, Amsterdam UMC location, VU University Medical Center, Amsterdam, The Netherlands

B. Havekes, MD, PhD, Department of Internal Medicine, Division of Endocrinology, Maastricht University Medical Center, Maastricht, The Netherlands.

W.W. de Herder, MD, PhD, Department of Internal Medicine, Erasmus Medical Center, Rotterdam, The Netherlands.

C.R.C. Pieterman, MD, PhD, Department of Endocrine Oncology, University Medical Center Utrecht, Utrecht, The Netherlands.

A.N.A. van der Horst-Schrivers, MD, PhD, Department of Endocrinology, University Medical Center Groningen, Groningen, The Netherlands.

A.C. van de Ven, MD, PhD, Department of Endocrinology, Radboud University Medical Center, Nijmegen, The Netherlands.

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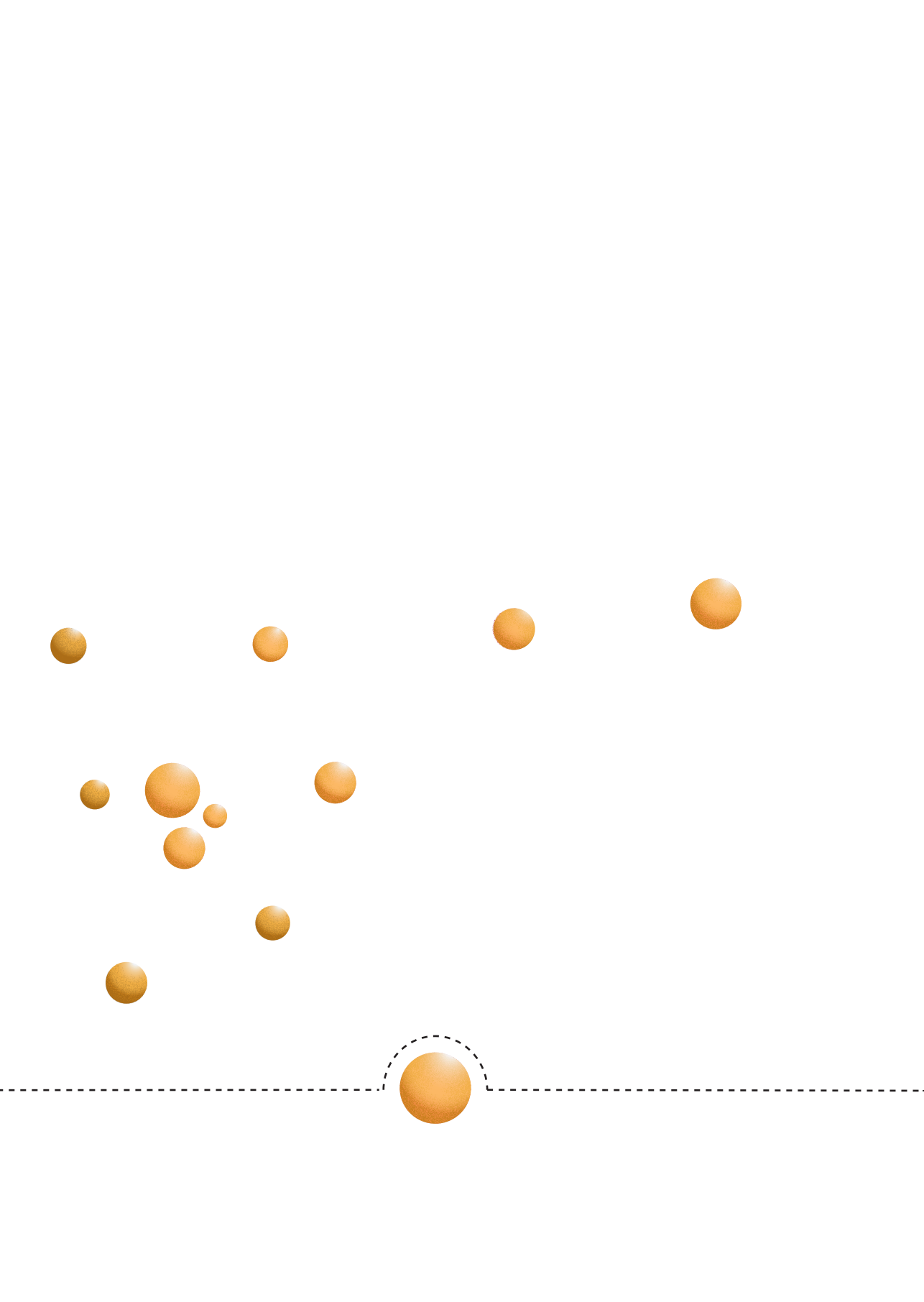
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PART III

PROGNOSIS

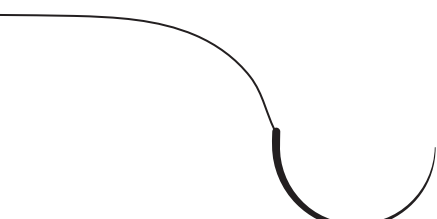


CHAPTER VI

Prognostic factors and survival in MEN1 patients with gastrinomas: results from the DutchMEN Study Group (DMSG)

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D.J. van Beek, S. Nell, C.R.C. Pieterman, W.W. de Herder, A.C. van de Ven,
O.M. Dekkers, A.N. van der Horst-Schrivers, M.L. Drent, P.H. Bisschop,
B. Havekes, I.H.M. Borel Rinkes, M.R. Vriens, G.D. Valk



ABSTRACT

Background and objectives

Gastrinomas are the most prevalent functioning neuroendocrine tumors (NET) in multiple endocrine neoplasia type 1 (MEN1). Guidelines suggest medical therapy in most patients, but surgery may be considered in a subgroup. Currently, factors to guide management are necessary. This population-based cohort study assessed prognostic factors of survival in patients with MEN1-related gastrinomas.

Methods

Patients with MEN1 having gastrinomas were identified in the Dutch MEN1 database from 1990 to 2014 based on fasting serum gastrin (FSG) levels and/or pathology. Predictors of overall survival were assessed using Cox regression.

Results

Sixty-three patients with gastrinoma (16% of the MEN1 population) were identified. Five- and 10-year overall survival rates were 83% and 65%, respectively. Prognostic factors associated with overall survival were initial FSG levels $\geq 20\times$ upper limit of normal (ULN) (hazard ratio [HR] 6.2 [95% Confidence Interval, 1.7–23.0]), pancreatic NET $\geq 2\text{cm}$ (HR 4.5; [1.5–13.1]), synchronous liver metastases (HR 8.9; [2.1–36.7]), gastroduodenoscopy suspicious for gastric NETs (HR 12.7; [1.4–115.6]), and multiple concurrent NETs (HR 5.9 [1.2–27.7]).

Conclusion

Life expectancy of patients with MEN1 gastrinoma is reduced. FSG levels and pancreatic NETs $\geq 2\text{ cm}$ are prognostic factors. FSG levels might guide surveillance intensity, step-up to additional diagnostics, or provide arguments in selecting patients who might benefit from surgery.

INTRODUCTION

Multiple endocrine neoplasia type 1 (MEN1) is a rare autosomal dominant disorder caused by a mutation in the *MEN1* gene leading to a combination of endocrine and nonendocrine tumors.[1] Duodenopancreatic neuroendocrine tumors (dpNETs) are a common manifestation and have a prevalence of 56% in the Dutch MEN1 population.[2] Gastrinomas are the most frequently encountered functioning dpNETs and occur in approximately 30% of patients with MEN1.[3] These tumors produce gastrin which induces gastric acid hypersecretion and subsequently leads to ulcerative peptic disease and gastrointestinal bleeding, known as the Zollinger-Ellison syndrome.[4] MEN1-related gastrinomas are generally located in the duodenal submucosa and are rarely found in the pancreas.[5,6] Duodenal gastrinomas are often small (<1 cm), multiple and accompanied by pancreatic neuroendocrine tumors (pNETs).[6] Approximately 70 to 80% of surgically treated patients have lymph node metastases, and 10% present with synchronous liver metastases.[7,8]

Gastric acid hypersecretion-related complications used to be the leading cause of death in patients with MEN1 having gastrinomas before the widespread use of proton pump inhibitors (PPIs).[9,10] Nowadays, compared with the general population, patients with MEN1 have a seriously decreased life expectancy mainly caused by malignant dpNETs.[2] However, the reported prognosis of patients with MEN1 gastrinoma varies widely.[8,11–13] In the French cohort, studied from 1956 to 2005, gastrinomas have been reported as independent risk factor for death.[9] Actual data on MEN1 gastrinoma survival are scarce and survival rates are difficult to interpret since patients are diagnosed and treated differently among studies. In addition, the understanding that MEN1 gastrinomas mostly originate in the duodenum instead of the formerly assumed pancreatic origin, emphasizes the need for new studies. Besides, data regarding the long-term natural history are important, because guidelines suggest symptomatic management using PPIs in the majority of patients.[14,15] Nevertheless, the only potentially curative oncological treatment remains surgery. Pancreatico-duodenectomy offers the possibility to achieve biochemical cure for MEN1-related duodenal gastrinomas.[8] However, controversies exist regarding the timing and the extent of surgery, considering the unpredictable tumor course and morbidity associated with extensive surgery.[14,15] Therefore, the necessity of prognostic factors to guide therapy has recently been underscored.[16] Since gastrinomas are hormone producing tumors, we hypothesized that, besides known dpNET-related prognostic factors such pNET size and liver metastases, gastrin levels might predict survival in this population.[17] Therefore the present study aims to assess prognostic factors and survival in patients with MEN1 having gastrinomas.

MATERIALS AND METHODS

Study design

Patients were selected from the national Dutch MEN1 database from the DutchMEN Study Group (DMSG).[18] Patients with MEN1 aged 16 years and older and under treatment in one of the eight University Medical Centers (UMCs) are included. In each center, patients were identified by reviewing hospital databases of medical conditions and diseases. MEN1 diagnosis was established according to the guidelines.[14] Over 90% of the Dutch MEN1 population is included. Clinical and demographic data were collected longitudinally every quarter from 1990 to 2014 by standardized medical record review, according to a predefined protocol. The protocol was approved by the Medical Ethics Committees of all UMCs.

Patient selection

The diagnosis of gastrinomas in patients with MEN1 was challenging, since the reference standard, provocative tests using secretin, is not widely available and routine measurements of basal acid output at gastroduodenoscopy were not routinely performed. Therefore, based on stringent criteria we aimed to identify those patients of whom we were confident of having gastrinomas, also using subsequently elevated fasting serum gastrin (FSG) levels. Gastrinoma diagnosis was based on (a) pathology reports of gastrin immunohistochemistry positive tumors, or (b) elevated FSG levels, or (c) gastroduodenoscopy suspicious for gastrinoma, or a combination of these. Serum gastrin reference values were obtained from all UMCs over the study period. FSG levels were calculated as a factor of the upper limit of normal (ULN) of the reference values. Gastrinoma diagnosis was considered certain when FSG levels were increased more than a 10-fold of ULN (regardless of PPI use) or probable when FSG measurements were elevated consecutively (a) more than a two-fold of ULN (without PPI) without consecutive FSG levels $<2\times$ ULN during follow-up or (b) more than a five-fold (under PPI) without consecutive FSG levels $<5\times$ ULN during follow-up, without surgery or the start of systemic anti-tumor therapy (Supplementary Table 1). Pathological gastrinoma diagnosis was established if immunohistochemistry of tumor tissue stained positive for gastrin, in the presence of hypergastrinemia.

Clinical definitions

The date of gastrinoma diagnosis was based on the date of the pathology report or on the date of the first FSG measurement fulfilling one of the diagnostic criteria. For nongastrinoma patients the date of the first FSG measurement was used. FSG levels at the time of gastrinoma diagnosis were regarded as initial FSG levels for further analysis.

Patients were categorized as a pathological or a biochemical diagnosis in line with whichever diagnosis came first.

Conventional imaging reports of computed tomography (CT), magnetic resonance imaging (MRI), endoscopic ultrasonography (EUS), or gastroduodenoscopy, were reviewed for lesions suspicious for duodenal NETs and suspicious abdominal lymph nodes. Data from imaging reports up to 1 year before or after the gastrinoma diagnosis were extracted. Gastroduodenoscopies were also interpreted for gastric NETs. Lesions considered suspicious included visible tumor, polyposis, and small nodules. Gastritis and hypergastrinemia-related complications were not considered as NET if there was no lesion suspicious for gastrinoma.

Liver metastases were defined as (a) pathologically proven or (b) radiologically confirmed liver metastases. Radiology was considered positive if consecutive CT or MRI reports described suspicious liver lesions. An MEN1 expert panel, blinded to patient identity, decided most likely origin of the liver metastases. The presence of synchronous liver metastases was studied as prognostic factor, regardless of origin.

Deaths caused by MEN1 manifestations and MEN1-related therapy were considered as MEN1-related. Other causes of death were regarded as non-MEN1-related.[2] MEN1-related NETs at the moment of gastrinoma diagnosis were diagnosed according to pathology reports.[2] If no pathology reports were available, imaging results were used for diagnosis as previously described.[19] Gastric NETs were also diagnosed using gastro-duodenoscopies. The size of the largest pNET on conventional imaging was used for further analysis.

Treatment

Patients were treated medically by PPIs to prevent acid-related complications, by somatostatin analogues or surgically to prevent metastatic disease. Treatment regimen was decided by the treating physician together with the patient after multidisciplinary team discussion.

Outcome measures

The primary outcomes were 5- and 10-year overall survival (OS). Possible prognostic factors at gastrinoma diagnosis were analyzed for influence on OS. The date of death or date of last follow-up was used for analysis.

Statistical analysis

Descriptive statistics were reported as mean (Standard Deviation [SD]) or median (range), as appropriate, or as numbers (percentages). Differences in means were tested using t tests. Survival curves were plotted according to the Kaplan-Meier method and survival probabilities were obtained.[20] Follow-up time started at the moment of gastrinoma diagnosis. Kaplan-Meier curves were plotted for MEN1 gastrinoma patients against nongastrinoma patients from the database. Concerning the difference age, gastrinoma patients were 1:1 age and gender matched with a nongastrinoma patient. The log-rank test was used for Kaplan-Meier curve comparison.

Prognostic factors for OS were assessed using uni- and multivariable Cox proportional hazard regression providing Hazard Ratios (HR's) with 95% Confidence Intervals (95% CIs), ties were handled using the exact method. Cox proportional hazard assumptions were formally tested and graphically assessed using scaled Schoenfeld residual plots; the assumptions were not violated. Prognostic factors were adjusted for age, since age is associated with OS.[11] Continuous variables were dichotomized based on previous literature; pNET size on conventional imaging to <2.0 cm and ≥ 2.0 cm, and FSG levels at gastrinoma diagnosis to $<20\times$ ULN and $\geq 20\times$ ULN.[10,11]

Since surgery might reduce FSG levels, subgroup analysis for the prognostic value of FSG levels was performed in nonsurgically managed patients. Furthermore, subgroup analysis was conducted in patients without liver metastases at gastrinoma diagnosis. All test were performed two-tailed. P-values $<.05$ were considered statistically significant. Statistical analysis was performed using SPSS version 25.0 (IBM Corp, New York, USA), RStudio version 1.0.143 (RStudio, Inc., Boston, MA); figures were constructed using Graphpad Prism version 7.02 (GraphPad Software Inc, California, USA).

RESULTS

DMSG database

A total of 396 patients were identified, of whom 357 (90%) had FSG measurements at least once between 1990 and 2014. The median number of measurements was 7 (1–54) per patient. Hypergastrinemia was observed in 193 patients (54%), regardless of PPI use. In 114 patients (32%), FSG levels were $>1\times$ ULN in the absence of PPI. One hundred patients (28%) had FSG levels $>2\times$ ULN under PPI. Ten-fold increased FSG levels were longitudinally observed in 45 patients (13%).

Patient characteristics

Demographic and clinical characteristics are described in Table 1. Sixty-three patients with gastrinoma (16%) were identified in the DMSG database with a mean age of 51 years (± 13). Fifty-four percent were female. Most patients were diagnosed biochemically (64%) and 15 patients (24%) had a biochemical diagnosis with histopathological gastrinoma confirmation. In 22 patients (35%) gastrinomas were histopathologically proven. On the basis of biochemical criteria, 45 patients (71%) were diagnosed as certain and 10 (16%) as probable. Median FSG levels at diagnosis were $9.5\times$ ULN (0.5–412).

Thirty-five patients (56%) harbored a concurrent NET at the time of gastrinoma diagnosis. Thirty-three patients had a concurrent pNET (52%). Eight patients had a pNET and concurrent lung or gastric NET. One patient with a pathologically confirmed gastric

Table 1. Patient and disease characteristics at moment of gastrinoma diagnosis

	Overall patients (n = 63)
Age, mean [SD]	51 [13]
Gender	
Male (%)	29 (46%)
Female (%)	34 (54%)
MEN1-associated tumors at the moment of gastrinoma diagnosis	
Pancreatic NET	33 (52%)
Gastric NET	7 (11%)
Lung NET	5 (8%)
Thymic NET	0
Gastrinoma diagnosis	
Pathological only	7 (11%)
Biochemical and pathological confirmation	15 (24%)
Biochemical only	40 (64%)
Imaging suspect for gastrinoma with elevated FSG levels	1 (2%)
Basis of biochemical gastrinoma diagnosis	
1x > 10x ULN	45 (71%)
2x > 2x ULN without PPI or > 5x ULN with PPI	10 (16%)
FSG levels not fulfilling above criteria	8 (13%)
Fasting serum gastrin factor of ULN at diagnosis, median [range]*	
Overall (n=61)	9.5 [0.5 - 412.3]
No PPI, no somatostatin analogues (n=21)	7.2 [1.4 - 137.1]
Under PPI (n=37)	9.64 [1.1 - 412.3]
Under somatostatin analogues (n=2)	45.7 [0.5 - 90.9]
Under PPI and somatostatin analogues (n=1)	19.2
Fasting serum gastrin factor of ULN at diagnosis, median [range]	
Biochemical diagnosis (n=53)	11.0 [2.0 - 412.3]
Pathological diagnosis (n=7)	2.1 [0.5 - 3.4]
Year of diagnosis	
Before 2007	31 (49%)
2007 and after	32 (51%)
Imaging suspicious for NET duodenum**	
Yes	15/5 (26%)
No	41/57 (74%)
Gastroduodenoscopy suspicious for NET duodenum***	
Yes	13/25 (52%)
No	12/25 (48%)
Patients with positive gastroduodenoscopy suspicious for NET duodenum	
Solitary lesion	5/13 (38%)
Multiple lesions	8/13 (62%)
Size duodenal abnormalities in mm, median [range] (n=9)	7.5 [3-20]
Gastroduodenoscopy suspicious for NET stomach	
Yes	7/25 (28%)
No	18/25 (72%)
Suspicious lymph nodes on imaging at gastrinoma diagnosis	
Yes	12 (19%)
No	51 (81%)
Liver metastases at diagnosis****	5 (8%)
Gastrinoma	3
NF-pNET	1
Gastrinoma or NF-pNET	1

Abbreviations: SD Standard deviation, NET Neuroendocrine tumor, ULN Upper limit of normal of the reference value, PPI Proton pump inhibitor, NF-pNET Non-functioning pancreatic neuroendocrine tumor.

* FSG levels are reported for subgroups on medical therapy (PPI, somatostatin analogues or both) at the moment of FSG measurement.

** Imaging suspect for gastrinoma duodenum: abnormalities on computed tomography (CT), magnetic resonance imaging (MRI), gastroduodenoscopy or endoscopic ultrasonography.

*** Gastroduodenoscopy suspicious for NET: visible tumor, polyposis without another diagnosis and small nodules which could be biopsied. Possible Zollinger Ellison Syndrome related complications such as peptic ulcers were not considered as suspected for NET. Gastritis was not documented as suspect for NET.

**** Origin of liver metastases according to the expert panel.

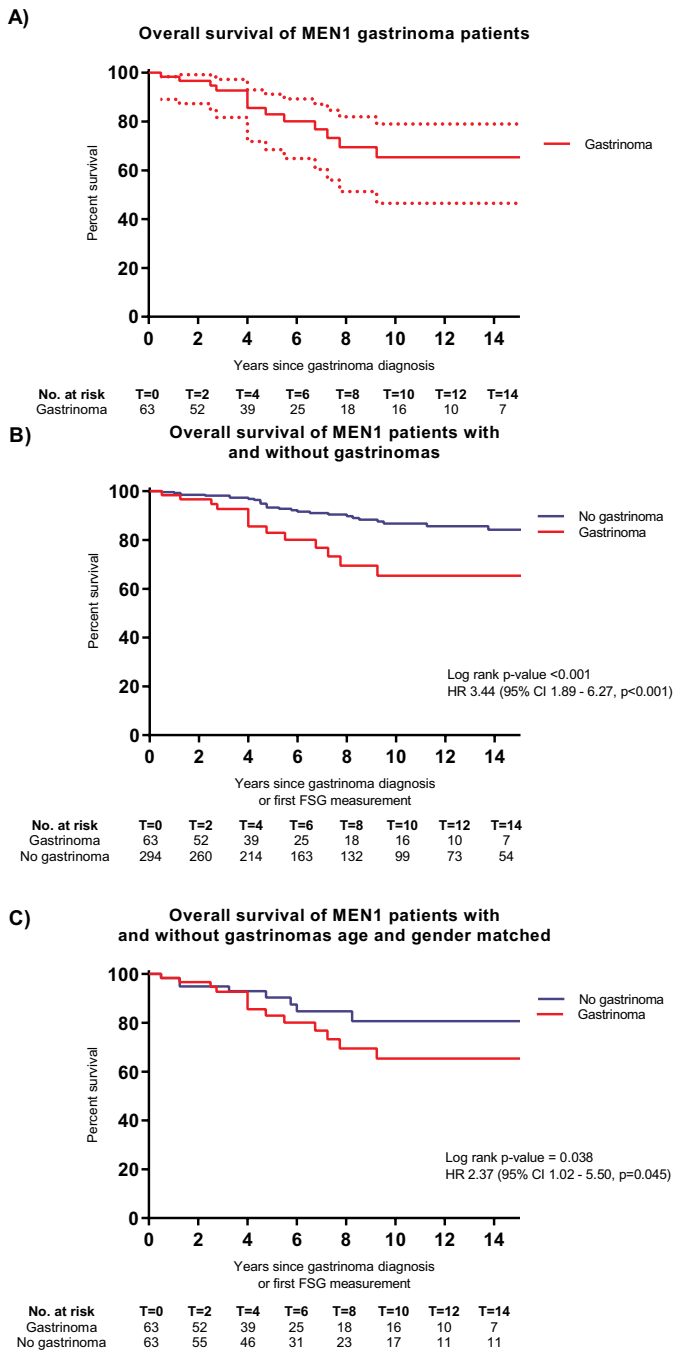


Figure 1. Overall survival (OS) of MEN1 gastrinoma patients (A). OS of MEN1 patients with and without gastrinomas (B). OS of MEN1 patients with and without gastrinomas (age and gender matched) (C).

gastrinoma also had a concurrent gastric NET. Five patients (8%) harbored synchronous liver metastases. For two patients the liver metastases were pathologically proven; non-functioning pNET (NF-pNET) liver metastases in one and gastrinoma-related in another.

Forty-seven patients (75%) received medical treatment and 16 patients (25%) underwent surgery (Supplementary Figure 1). Two patients additionally received peptide receptor radionuclide therapy, of whom one died during follow-up.

Long-term outcomes

Patient outcomes are reported in Table 2. Eight patients (14%) developed liver metastases after a median follow-up of 4.5 years (0.3–23.5 years). Liver metastases of any MEN1-related manifestation were confirmed by pathology in three patients, whereas in five patients the diagnosis and origin were established by the expert panel. Gastrinoma liver metastases were pathologically confirmed in one patient. After a follow-up of 4.7 years, 17 patients (27%) had died; at a median age of 58 years (33–81 years). Eleven deaths (65%) were regarded as MEN1 related. Most MEN1-related deaths (73%) resulted from dpNET progression. Two patients were lost during follow-up.

Survival of patients with MEN1 gastrinoma

OS rates of MEN1 gastrinoma patients after 5 and 10 years were 83% and 65%, respectively (Table 2, Figure 1A). Five and 10-year OS rates for patients with MEN1 having FSG measurements not indicative for a gastrinoma were 93% and 87%, respectively (Figure 1B). Patient with gastrinomas were older than patients without gastrinomas (51 versus 39 years, $p < .001$). Ten-year OS rates were 65% versus 81% for age and gender matched MEN1 patients without gastrinomas (Figure 1C).

Prognostic factors for OS

Prognostic factors for OS are shown in Table 3. Factors significantly associated with OS were FSG levels $>20\times$ ULN (HR, 6.16; [95% CI 1.65–23.02]), pNET ≥ 2.0 cm on conventional imaging (HR, 4.46; [1.52–13.06]), synchronous liver metastases of any origin (HR, 8.86; [2.14–36.7]), multiple concurrent NETs (HR, 5.86; [1.24–27.65]), and gastroduodenoscopy suspicious for gastric NET (HR, 12.74; [1.40–115.6]). After adjusting for age, these factors were significantly associated with OS.

Initial FSG levels determined prognosis in patients with MEN1 gastrinoma. Ten-year OS was more favorable for patients with FSG $<10\times$ ULN compared with patients with FSG ranging from 10 to $20\times$ ULN, and FSG $\geq 20\times$ ULN (83%, 66% and 33%, respectively) (Figure 3). Corresponding HR's were 2.66 ([0.57–12.27]; $P = .214$) for FSG ranging from 10– $20\times$ ULN and 6.16 ([1.65–23.02]; $P = .007$) for FSG $\geq 20\times$ ULN. Comparable results were observed in nonsurgically managed patients and patients without synchronous liver metastases (Table 4).

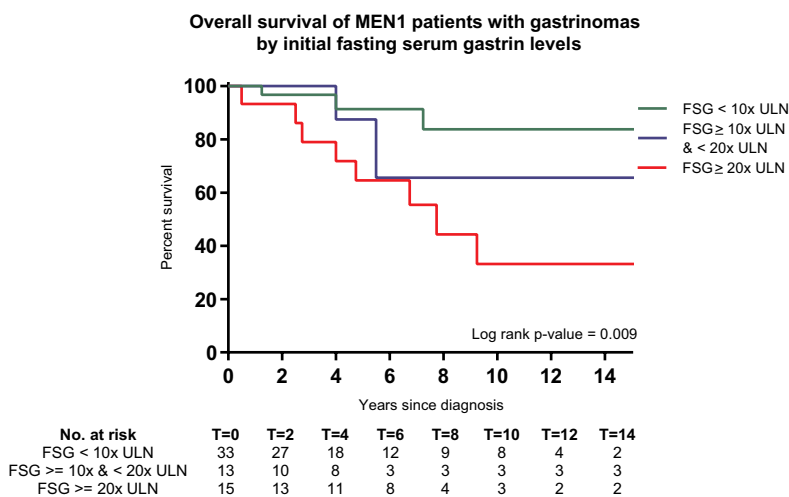


Figure 2. Overall survival of MEN1 patients according to initial fasting serum gastrin levels.

Table 2. Survival and long-term outcomes of MEN1 gastrinoma patients

	Overall patients (n = 63)
Follow-up in years, median [range]*	4.7 [0.25 - 23.5]
Overall survival	
5-year, % (95% CI)	83% (68 - 92%)
10-year, % (95% CI)	65% (47 - 79%)
Liver metastases**	8 (14%)
Gastrinoma	3
NF-pNET	2
Gastrinoma or NF-pNET	1
Thymic NET	1
Unknown origin/unknown if MEN1 dpNET related	1
Death	17 (27%)
MEN1-related	11 (65%)
Duodenopancreatic NET related	8
Thymic NET	1
Renal insufficiency caused by pHPT	1
Complication MEN1 pancreatic surgery	1
Non-MEN1-related	5 (29%)
Unknown	1 (6%)

Abbreviations: 95% CI 95% Confidence interval, *dpNET* duodenopancreatic neuroendocrine tumor, *MEN1* multiple endocrine neoplasia type 1, *NF-pNET* Non-functioning pancreatic neuroendocrine tumor, *NET* Neuroendocrine tumor, *pHPT* primary hyperparathyroidism.

* Follow-up until death or end of follow-up.

** Origin of liver metastases is based on the expert panel. Percentage is based on the group of patients without liver metastases at diagnosis (n=58).

Table 3. Cox proportional Hazards regression for prognostic factors for overall survival

	N=	Deaths	Overall survival probabilities		Unadjusted HR (95% CI), p-value	Age-adjusted HR (95% CI), p-value
			5-year	10-year		
Age	63	17	NA	NA	1.04 (0.99 – 1.09), p=0.116	NA
Gender						
Male	29	8	81%	60%	1 (Ref. cat.)	1 (Ref. cat.)
Female	34	9	85%	71%	0.60 (0.21 – 1.70), p=0.335	0.55 (0.19 – 1.62), p=0.283
MEN1-associated NETs at gastrinoma diagnosis						
No concurrent NET	27	6	94%	72%	1 (Ref. cat.)	1 (Ref. cat.)
Pancreatic NET	25	7	79%	69%	1.46 (0.49 – 4.38), p=0.499	1.93 (0.61 – 6.13), p=0.263
Gastric NET	3	1	67%	67%	2.38 (0.27 – 20.83), p=0.433	7.20 (0.67 – 77.46), p=0.103
Multiple (pNET, gastric NET and/or lung NET)	8	3	56%	0%	5.86 (1.24 – 27.65), p=0.026	9.73 (1.77 – 53.44), p=0.009
Initial gastrinoma diagnosis						
Histopathological diagnosis	9	1	89%	89%	1 (Ref. cat.)	1 (Ref. cat.)
Biochemical diagnosis	53	16	82%	63%	1.54 (0.20 – 11.99), p=0.68	1.04 (0.12 – 8.50), p=0.971
Basis of gastrinoma diagnosis						
No 1x >10x ULN	18	1	94%	94%	1 (Ref. cat.)	1 (Ref. cat.)
1x >10x ULN	45	16	80%	61%	3.09 (0.40 – 24.03), p=0.282	3.11 (0.40 – 24.31), p=0.280
Basis of gastrinoma diagnosis						
No 2x > 2x ULN without PPI or > 5x ULN with PPI	53	16	83%	64%	1 (Ref. cat.)	1 (Ref. cat.)
2x > 2x ULN without PPI or > 5x ULN with PPI	10	1	88%	88%	0.67 (0.09 – 5.21), p=0.701	0.62 (0.08 – 4.80), p=0.645
Fasting serum gastrin levels at diagnosis						
<10x ULN	33	3	91%	84%	1 (Ref. cat.)	1 (Ref. cat.)
≥ 10x ULN & < 20x ULN	13	4	88%	66%	2.66 (0.57 – 12.27), p=0.214	2.40 (0.51 – 11.33), p=0.266
≥ 20x ULN	15	10	65%	33%	6.16 (1.65 – 23.02), p=0.007	5.56 (1.45 – 21.27), p=0.012
Conventional imaging suspicious for NET duodenum*						
No	42	9	85%	69%	1 (Ref. cat.)	1 (Ref. cat.)
Yes	15	5	79%	26%	2.82 (0.87 – 9.15), p=0.084	2.81 (0.86 – 9.13), p=0.087

Table 3 continues on page 152

Table 3 continued from page 151

	N=	Deaths	Overall survival probabilities		Unadjusted HR (95% CI), p-value	Age-adjusted HR (95% CI), p-value
			5-year	10-year		
Gastroduodenoscopy suspicious for NET duodenum						
No	12	2	89%	71%	1 (Ref. cat.)	1 (Ref. cat.)
Yes	13	4	85%	28%	2.11 (0.38 – 11.62), p=0.391	2.61 (0.46 – 14.87), p=0.281
Gastroduodenoscopy suspicious for NET stomach						
No	18	2	100%	69%	1 (Ref. cat.)	1 (Ref. cat.)
Yes	7	4	57%	0%	12.74 (1.40 – 116), p=0.024	18.97 (1.62 – 222), p=0.019
Pancreatic NET at gastrinoma diagnosis						
No	30	7	92%	72%	1 (Ref. cat.)	1 (Ref. cat.)
Yes	33	10	74%	59%	1.70 (0.64 – 4.50), p=0.288	1.97 (0.71 – 5.45), p=0.190
Pancreatic NET ≥ 2.0 cm on imaging at gastrinoma diagnosis						
No	51	11	89%	71%	1 (Ref. cat.)	1 (Ref. cat.)
Yes	12	6	56%	42%	4.46 (1.52 – 13.06), p=0.006	6.71 (2.02 – 22.4), p=0.002
Suspicious lymph nodes on imaging at gastrinoma diagnosis						
No	51	15	80%	64%	1 (Ref. cat.)	1 (Ref. cat.)
Yes	12	2	100%	67%	0.88 (0.20 – 3.95), p=0.868	1.16 (0.24 – 5.52), p=0.852
Liver metastases at gastrinoma diagnosis						
No	58	14	88%	69%	1 (Ref. cat.)	1 (Ref. cat.)
Yes	5	3	25%	-	8.86 (2.14 – 36.7), p=0.003	6.56 (1.42 – 30.38), p=0.016

Abbreviations: HR Hazard ratio. NA Not applicable. NET Neuroendocrine tumor, PPI Proton pump inhibitor, ULN Upper limit of the normal of the reference value.

* Conventional imaging: magnetic resonance imaging, computed tomography, endoscopic ultrasonography or gastroduodenoscopy suspicious for duodenal gastrinoma. In one case the gastroduodenoscopy was suspicious for a gastric gastrinoma.

Table 4. Prognostic value of initial fasting gastrin levels on overall survival (OS)

	Overall cohort (n = 63)		Non-surgically managed patients (n = 47)		Patients without liver metastases (n = 58)	
	10- year OS	HR (95% CI), p-value	10- year OS	HR (95% CI), p-value	10- year OS	HR (95% CI), p-value
Fasting serum gastrin levels						
< 10x ULN	84%	1 (Ref. cat.)	88%	1 (Ref. cat.)	92%	1 (Ref. cat.)
≥ 10x ULN & < 20x ULN	66%	2.66 (0.57 – 12.27), p=0.214	53%	3.67 (0.58 – 23.07), p=0.165	66%	7.53 (0.82 – 69.46), p=0.075
≥ 20x ULN	33%	6.16 (1.65 – 23.02), p=0.007	25%	8.40 (1.76 – 40.03), p=0.008	33%	17.34 (2.15 – 140.21), p=0.007

Abbreviations: *CI* Confidence interval, *HR* Hazard ratio, *OS* Overall survival, *ULN* Upper limit of the normal of the reference value.

DISCUSSION

Gastrinomas in patients with MEN1 lead to a decreased life expectancy with 5 and 10-year OS rates of 83% and 65%, respectively. Factors associated with decreased OS were initial FSG levels $\geq 20x$ ULN, a pNET ≥ 2.0 cm on conventional imaging, synchronous liver metastases, multiple concurrent NETs, and gastroduodenoscopy suspicious for gastric NET. These factors may guide clinical decision making in daily practice.

The 10-year OS rate in our cohort of gastrinoma patients was 65% with a median age at death of 58 years. Overall, MEN1 patients in the Netherlands have a life expectancy of 73 years.[2] We performed subgroup analysis in age and gender matched controls, also showing a significantly decreased OS of MEN1 gastrinomas. This underscores that age and gender did not influence this outcome. Previous studies on patients with MEN1 gastrinoma reported 10-year survival rates of 88% to 100% regardless of therapy.[7,8,11–13] Several factors could account for the different survival rates. First, disease specific survival is generally higher than OS.[10–12] Second, in the other cohorts also nongastrinoma patients might have been included because of the method of identifying gastrinomas. Finally, patient prognosis might be influenced by treatment regimen. In our cohort, only 25% of the patients underwent surgery and in a substantial part the duodenum was not removed, which is deemed necessary for achieving biochemical cure.[8] In addition, patients with synchronous liver metastases were included in our study. Liver metastases, either NF-pNET or gastrinoma-related, are associated with survival.[7,11,13,17,21] Although the OS rate was lower, the age of death (58 years) was slightly higher than in previous series (55–56 years).[10–12]

Initial FSG levels were associated OS, also after adjusting for age. More specifically, OS decreased as FSG levels increased. Ito et al. described very high FSG levels (>20-fold elevated) more often in deceased patients.[10] We observed a significantly increased HR for death in patients with FSG levels higher than 20x ULN. In line, we observed a HR of 2.66 and a 10-year OS of 66% for patients with FSG levels between 10 and 20x ULN. We believe that the outcomes of this analysis were not statistically significant due to the low number of patients and events in this subgroup. NIH series observed higher FSG levels in patients with an aggressive disease course and in patients with liver metastases, although no survival analysis was conducted.[7,13] The only study focusing on initial FSG levels and survival in patients with MEN1 from the NIH, did not find a significant correlation in MEN1 gastrinoma patients (log rank $P=.068$).[22] In this study of 53 patients with MEN1 gastrinoma, only four deaths were observed.[22] Compared with our study, in this NIH series other diagnostic criteria were applied probably influencing the case mix and, in addition, the study period was a decade earlier.

Pancreatic NET ≥ 2.0 cm on imaging and liver metastases at gastrinoma diagnosis were associated with decreased OS. Formerly, gastrinomas were generally regarded as pNETs causing liver metastases and death. However, studies including gastrin immunohistochemistry and pathological series report the predominant duodenal origin.[5,8,23] Therefore, it can be hypothesized that these patients have decreased OS because of a concurrent NF-pNET ≥ 2.0 cm instead of a pancreatic gastrinoma. Recently, the acceptable prognosis of NF-pNETs <2 cm has been highlighted.[24–26] The decreased survival of patients with MEN1 having liver metastases is in line with other studies.[11,13] Nevertheless, the exact cause of death in patients with MEN1 gastrinoma with concurrent large (NF)-pNETs remains challenging.

This study is limited by the challenges of gastrinoma diagnosis in patients with MEN1. Current guidelines recommend the combination of hypergastrinemia and basal gastric acid hypersecretion ($\text{pH} < 2$).[14,15] Gastrinoma diagnosis frequently differs from these criteria, because of the lack of gastric pH measurement, the unavailability of secretin testing, and the use of immunohistochemistry and imaging as alternatives.[27] In daily clinical practice of the DMSG, gastrinoma diagnosis was complicated by the unavailability of stimulation tests, no routine measurements of gastric pH, and widespread use of PPI. Because we aimed to study OS and predictors of OS, we wanted to be sure to select gastrinoma patients only. Therefore, strict selection criteria were formulated beforehand. Only 11% did not have 10-fold increased FSG levels nor pathologically proven gastrinoma. To identify this subgroup of gastrinoma cases, we reasoned that gastrinomas lead to gradual FSG increases, therefore, longitudinally collected FSG values were analyzed and patients with spontaneously decreasing values over time were not regarded as patients with gastrinoma. FSG measurements in light of the annual MEN1 screening were performed regardless of PPI use. Although PPIs are preferably discontinued before FSG measurement, serious adverse events can occur during

sudden interruption.[28] Thus, we believe that for identifying patients with MEN1 gastrinoma including serial FSG measurements provide a more pragmatic, but still reliable approach. Other limitations include the retrospective design and the number of events. Due to the low number of events, extensive multivariable analysis was impossible and relatively wide confidence intervals were observed.

The major strength of this study is the population-based cohort including >90% of patients with MEN1, with standardized data collection and long-term follow-up. In addition, patients are included from 1990 onwards, providing more actual survival rates, since patients with MEN1 are a biochemically screened population and gastric acid hypersecretion-related deaths have been rarely reported over the last two decades.[9,10] In the present study, OS was used as outcome, because OS is more informative and establishing gastrinoma-related deaths (disease-specific survival) is challenging in the presence of multiple MEN1 manifestations. Furthermore, this is the first study to assess prognostic factors, including FSG levels, on OS in patients with MEN1 using time-to-event analysis.

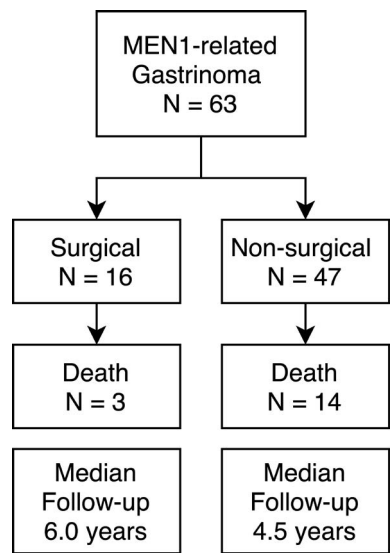
The observed prognostic factors might aid clinicians in selecting patients with MEN1 having gastrinomas for more intensive follow-up regimens or extended localization imaging. Furthermore, knowledge of prognostic factors and survival can help in selecting those who might benefit from surgery. MEN1 and ENETS guidelines recommend surgery for patients with MEN1 having pancreatic gastrinomas >2.0 cm.[14,15] Although 52% had a pNET at the moment of gastrinoma diagnosis, only 19% of all patients in this cohort had pNETs >2.0 cm on cross-sectional imaging. Especially, in the coexistence of hypergastrinemia and pNETs ≥ 2.0 cm, the optimal surgical strategy is hard to establish. Merging the need for pancreaticoduodenal resections to achieve biochemical cure on the one hand and the scarcity of data regarding postoperative complications and long-term oncological outcomes after pancreaticoduodenal resections on the other, future studies should address these topics to come to meaningful advice.[29]

In conclusion, life expectancy in patients with MEN1 having gastrinomas is reduced compared with other studies. OS was associated with initial FSG levels ≥ 20 x ULN, a pNET ≥ 2.0 cm on conventional imaging, synchronous liver metastases, multiple concurrent NETs, and gastroduodenoscopy suspicious for gastric NET in patients with MEN1. OS decreases as FSG levels increase, starting from ≥ 10 x ULN. Therefore, FSG levels might provide a valuable tool to guide surveillance intensity, step-up to additional diagnostic modalities or provide arguments in selecting those patients who might benefit from surgery.

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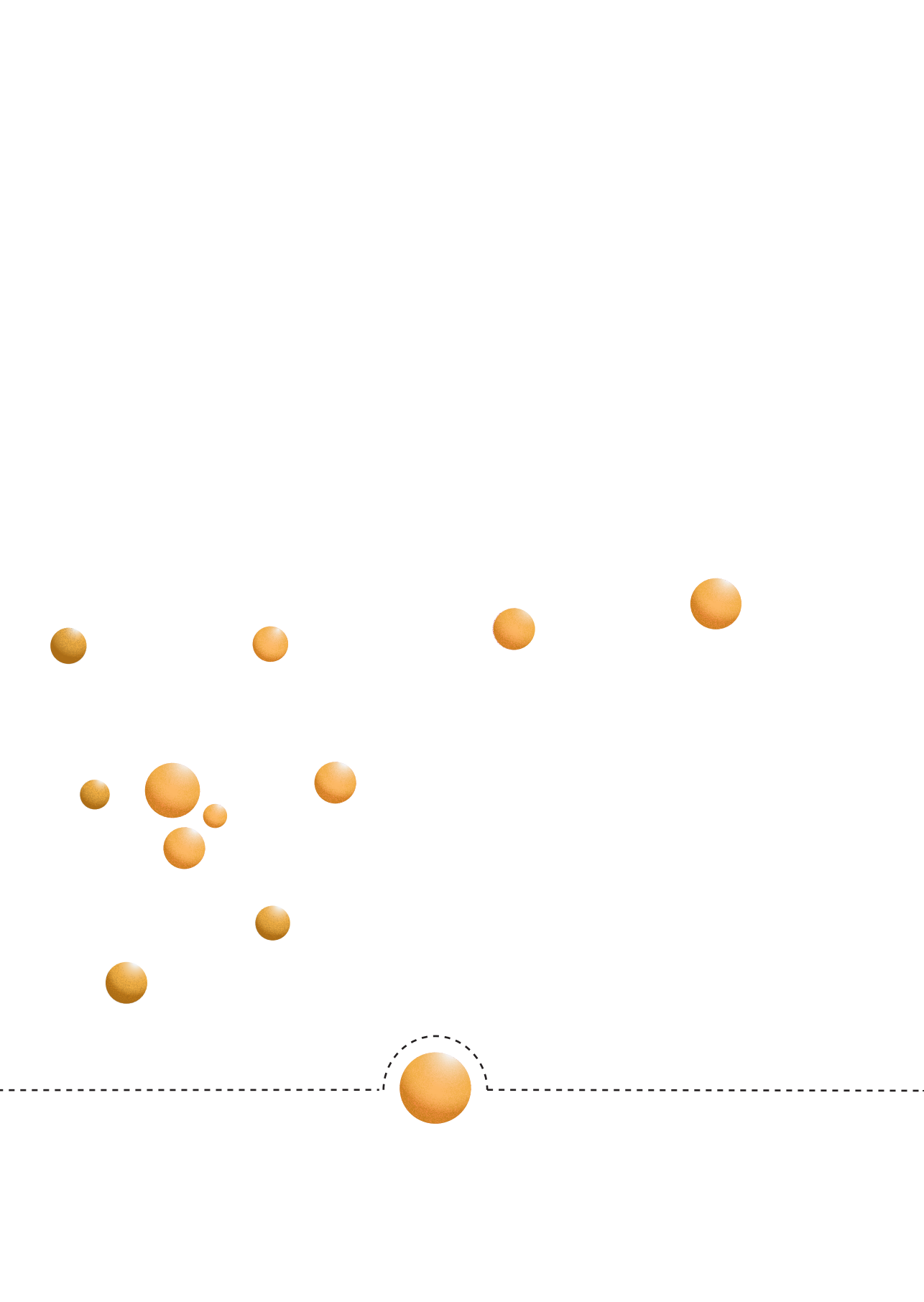


Supplemental Figure 1. Flow-chart of MEN1-related gastrinomas treatment.

Supplementary Table 1. Biochemical gastrinoma diagnosis DutchMEN Study Group database¹⁹

Gastrinoma	FSG >10 times the upper limit of normal (ULN)
Certain	
Gastrinoma	FSG >2 times twice consecutive in the absence of proton pump inhibitor use (no value <2 ULN allowed in between) and not followed by two consecutive measurements <2 ULN without surgery or start of systemic anti-tumor therapy
Probable	FSG >5 times twice consecutive in the presence of proton pump inhibitor use (no value <5 ULN allowed in between) and not followed by two consecutive measurements <5 ULN without surgery or start of systemic anti-tumor therapy

Abbreviations: FSG Fasting serum gastrin, ULN Upper limit of normal.



CHAPTER VII

Metastatic patterns of duodenopancreatic neuroendocrine tumors in patients with multiple endocrine neoplasia type 1

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W.M. Hackeng, D.J. van Beek, A.S.M. Kok, M. van Ernst, F.H.M. Morsink, M.J.C. van Treijen,
I.H.M. Borel Rinkes, K.M.A. Dreijerink, G.J.A. Offerhaus, G.D. Valk, M.R. Vriens, L.A.A. Brosens



ABSTRACT

Patients with multiple endocrine neoplasia 1 syndrome (MEN1) often develop multifocal duodenopancreatic neuroendocrine tumors (dpNET). Nonfunctional pancreatic NETs (PanNETs) and duodenal gastrinomas are the most frequent origins of metastasis. Current guidelines recommend surgery based on tumor functionality, size ≥ 2 cm, grade, or presence of lymph node metastases. However, in case of multiple primary tumors it is often unknown which specific tumor metastasized. This study aims to unravel the relationship between primary duodenopancreatic NETs and metastases in patients with MEN1 by studying endocrine differentiation.

First, it was shown that expression of the endocrine differentiation markers ARX and PDX1 was concordant in 18 unifocal sporadic neuroendocrine tumors (NETs) and matched metastases. Thereafter, ARX, PDX1, Ki67 and gastrin expression, and the presence of alternative lengthening of telomeres (ALT) were determined in 137 microscopic and macroscopic dpNETs and 36 matched metastases in 10 patients with MEN1. ARX and PDX1 H-score clustering was performed to infer relatedness. For patients with multiple metastases, similar intrametastases transcription factor expression suggests that most metastases (29/32) originated from a single NET of origin, while few patients may have multiple metastatic primary NETs. In 6 patients with MEN1 and hypergastrinemia, periduodenopancreatic lymph node metastases expressed gastrin, and clustered with minute duodenal gastrinomas, not with larger pancreatic NETs. PanNET metastases often clustered with high grade or alternative lengthening of telomeres-positive primary tumors.

In conclusion, for patients with MEN1-related hypergastrinemia and PanNETs, a duodenal origin of periduodenopancreatic lymph node metastasis should be considered, even when current conventional and functional imaging studies do not reveal duodenal tumors preoperatively.

INTRODUCTION

Metastatic duodenopancreatic neuroendocrine tumors (dpNETs) are the main cause of death in patients with multiple endocrine neoplasia type 1 syndrome (MEN1).¹ The syndrome is caused by germline mutations in the tumor suppressor gene *MEN1* and is characterized by the occurrence of parathyroid adenomas, pituitary tumors, pancreatic neuroendocrine tumors (PanNETs) – insulinomas or nonfunctional PanNETs – and duodenal gastrinomas. For MEN1-associated PanNETs, operative resection is the cornerstone of curative therapy and guidelines recommend surgery based on size (≥ 2 cm), growth rate, hormone production, and radiological signs suspicious of malignancy like lymph node metastases (LNM).^{2–4} Indications for surgical resection of MEN1-associated gastrinoma are more controversial, but include size and the presence of LNM.^{2–4}

The high diagnostic accuracy of ⁶⁸Gallium-labelled somatostatin analog positron emission tomography has increased the detection rate of LNM and distant metastases in patients with MEN1.⁵ As it is often unclear whether locoregional LNM originate from PanNETs or gastrinomas, choosing the surgical procedure is challenging, especially since major duodenopancreatic resections in patients with MEN1 are associated with severe complications and a remarkable cumulative burden of morbidity.^{6,7}

Histopathological duodenopancreatic resection specimens in patients with MEN1 typically reveal multiple PanNETs, many microadenomas (PanNETs <0.5cm) and minute duodenal gastrinomas. For this reason, also the postoperative determination of the primary origin of metastases is challenging, which hampers the appropriate use and applicability of tumor, node, and metastasis (TNM) staging systems, limiting prognostication of individual patients. Better understanding of the origins of metastases may help to refine the criteria for surgery and tailor duodenopancreatic resections to the individual patient's disease.

Recently, histone modification patterns and DNA methylation profiles revealed that PanNETs come in a range of distinct endocrine-cell differentiations, which show similarities to normal endocrine alpha and beta cells.^{8–11} Transcription factors ARX and PDX1, which are surrogate immunohistochemical markers for endocrine differentiation⁹, are expressed in similar proportions of primary nonfunctional PanNETs and a separate cohort of distant PanNET metastases.¹² Although yet to be confirmed in matched primary PanNETs and metastases, this suggests that endocrine differentiation is preserved in metastases.

Therefore, we questioned if transcription factor expression could serve to match locoregional and distant metastases to a specific primary tumor in patients with multifocal MEN1-associated dpNETs. Furthermore, in case of multiple (locoregional) metastases, it is unknown if multiple primary tumors of origin should be considered. An in-depth understanding of the metastatic patterns of MEN1-associated dpNETs is key to justify major duodenopancreatic surgery.

Here, we aim to unravel the relatedness of multifocal primary dpNETs and metastases in patients with MEN1. First, the preservation of immunohistochemical ARX and PDX1 expression in 18 sporadic unifocal neuroendocrine tumors (NETs) and 50 matched metastases was confirmed. Thereafter, a detailed analysis was performed to assess the relation between multifocal primary dpNETs and microadenomas ($n = 137$) and matched metastases ($n = 36$) in 10 patients with MEN1.

MATERIALS AND METHODS

Selection

The use of archival material was approved by the University Medical Center Utrecht Biobank Research Ethics Committee. The University Medical Center Utrecht pathology archives were searched for NETs within duodenopancreatic resection specimens from 1990-2017. Autopsies were excluded. The identified specimens were cross referenced with the Dutch MEN1 database from the DutchMEN Study Group to identify patients with MEN1.¹³ Mutation negative patients fulfilling clinical MEN1 criteria were excluded. Patients with sporadic disease were eligible for further investigation if formalin-fixed, paraffin-embedded material (FFPE) was available of a unifocal primary PanNET and at least 1 metastasis. Patients with MEN1 were eligible for further investigation if FFPE material was reported of at least 2 dpNETs or microadenomas and at least 1 metastasis. Metastases were defined as tumor positive lymph nodes or distant metastases, at any time during follow-up.

Data Collection

Pathological data were collected from the pathology reports (tumor size, lymph node involvement, and distant metastases) and clinical data were obtained from the DutchMEN Study Group database.¹³ Clinical data included demographic data (age and sex), laboratory values (fasting serum gastrin levels), preoperative conventional (computed tomography, magnetic resonance imaging, or endoscopic ultrasonography) or functional imaging (⁶⁸Gallium-labeled somatostatin analog positron emission tomography) and multidisciplinary tumor board discussion. Tumor size was determined on conventional imaging as previously described.¹⁴ The presence and number of duodenal NETs, PanNETs in the pancreatic head or body/tail, and presence of LNM was based on conventional and functional imaging derived up to six months before surgery. Gastroduodenoscopies were evaluated for lesions suspicious for duodenal NETs.

Histologic slides were reviewed and PanNETs (i.e., 0.5cm), pancreatic microadenomas (i.e., <0.5cm), duodenal NETs and metastases were annotated. All sections containing dpNETs or metastases were selected for further analysis. In addition, several slides containing

only pancreatic microadenomas were included. Macroscopic gross tumor size was extracted from the pathology reports if available, and was otherwise measured on the microscopic slides. Periduodenopancreatic LNM were defined as LNM within pancreatoduodenectomy, but not distal pancreatectomy resection specimens, for example, pancreaticoduodenal and infrapyloric lymph node stations.

Immunohistochemistry and Telomere Fluorescence In Situ Hybridization

4µm sections of the selected tissue blocks were cut, cleared at 60°C and deparaffinized. Immunohistochemistry was performed on consecutive sections as previously described¹¹ with antibodies against ARX (1:2000, MABN102, Millipore, Burlington, MA) and PDX1 (1:2000, ab134150, Abcam, Cambridge, United Kingdom). Immunohistochemistry for Gastrin and Ki67 was similarly performed with the following conditions: 20 minutes antigen retrieval in 10mM citrate (pH 6) at 100°C, 1-hour incubation of gastrin (1:3000, A0568, Agilent, Santa Clara, CA) and Ki67 (1:100, SP6, Thermo Fisher, Waltham MA) antibodies with DAB or bright-DAB as chromogen (Immunologic, VWRKBS04-110), respectively. Immunohistochemistry for ATRX (1:100, HPA0001906, Sigma, St. Louis, MO) and DAXX (1:400, HPA008736, Atlas antibodies, Bromma, Sweden) was performed only for cases with ultrabright telomeric foci determined by telomere fluorescence in situ hybridization (FISH). Telomere FISH was performed as previously described¹¹ using telomere specific Cy3 labeled probes (100 nM, F1002 Lot no. 180723PL-01, Panagene, Yuseong-gu, Republic of Korea) and centromere specific probes (100 nM, F3013 Lot no. 172865, Panagene) as hybridization control.

Scoring

ARX, PDX1, and gastrin expression were scored in consensus by two observers (L.A.A.B., W.M.H., M.E., A.S.M.K.). ARX and PDX1 percentages of nuclear staining were scored on a continuous scale, and intensities were scored as absent, weak, intermediate, or strong. Before consensus scoring, ARX and PDX1 stained sporadic tumors were scored independently (L.A.A.B., W.M.H.) for categorical percentages of staining cells (0%, <10%, <50%, <70%, <100%) and intensities for the determination of interobserver agreement.¹¹ If patchy expression was present, a representative average was determined reflecting the whole slide. Dichotomous expression of transcription factors was determined using previously described cut-offs for expression.¹¹ An estimated H-score (range: 0 to 300) for ARX and PDX1 was calculated per lesion by multiplying representative staining nuclear intensity (0 to 3) and continuous percentage (0 to 100). Ki67 nuclear expression was digitally scored in the 2 highest labeling tumor areas, in 2000 cells if present, using a previously validated digital counting method on scanned slides.¹⁵ Tumor grade was assigned following the World Health Organization (WHO) 2017 classification (Grade 1 [G1] <3%, G2 3 to 20%, G3 >20% Ki67 labeling-index).¹⁶ Cytoplasmic gastrin expression was scored as absent or present. Alternative

lengthening of telomeres (ALT) was defined as >1% of cells containing ultrabright telomeric signals >10x the cumulative signal intensity of all telomeric signals in a normal stromal cell. The percentage of ultrabright telomeric signals was visually assessed at x20 magnification (W.M.H.), foci were confirmed at x100 magnification, and signal intensities were quantified using Telometer as previously described.¹¹

Statistical Analysis

Non-normally distributed continuous variables were presented as median (interquartile range [IQR] or range). The Mann-Whitney *U* test was used to assess differences in continuous variables between 2 groups. The Chi-squared test was used for comparisons of categorical variables. Two-sided *p*-values <0.05 were considered as statistically significant. The Euclidean distance was used to calculate similarities between inpatient and outpatient lesions and for hierarchical clustering. In interobserver analysis, the weighted kappa with equal spacing was calculated as measure of reliability, and interpreted as previously described.¹⁷ Data transformations, statistical tests, and data visualization were performed using R version 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

ARX and PDX1 Expression and ALT Are Preserved in Sporadic PanNET Metastases

To establish the preservation of immunohistochemical ARX and PDX1 expression as surrogate clonal markers of differentiation, we identified 18 patients with sporadic unifocal primary PanNETs and 50 matched LNM or distant metastases. The cohort consisted of 8 females and 10 males with a median age of 59 years (range: 42 to 76 y). The median primary tumor size measured 5.0 cm (IQR: 2.7 to 9.0 cm). Twelve PanNETs were nonfunctional and 6 were functional (2 insulinomas, 2 glucagonomas, 1 gastrinoma and 1 VIPoma).

Analysis of the Euclidean distance between H-scores of the primary and matched metastatic tumors revealed close inpatient proximity (median distance 20, IQR: 3 to 65). Indeed, visual inspection of H-scores also showed a highly similar ARX and PDX1 expression between primary tumors and matched metastases (Figure 1). Notably, in matched primary tumors and metastases, ARX expression was either consistently present or absent/near absent. However, for PDX1, 2 metastases showed a change in expression (patients no. S1 and S4), whereas the vast majority of metastases (48/50) showed a similar pattern to primary tumors and/or other metastases. Transcription factors were scored highly similar between 2 observers, substantiated by almost perfect reliability of ARX scoring (percentage kappa 0.94, intensity kappa 0.89), and almost perfect and substantial reliability of PDX1 scoring (percentage kappa 0.86, intensity kappa 0.74).

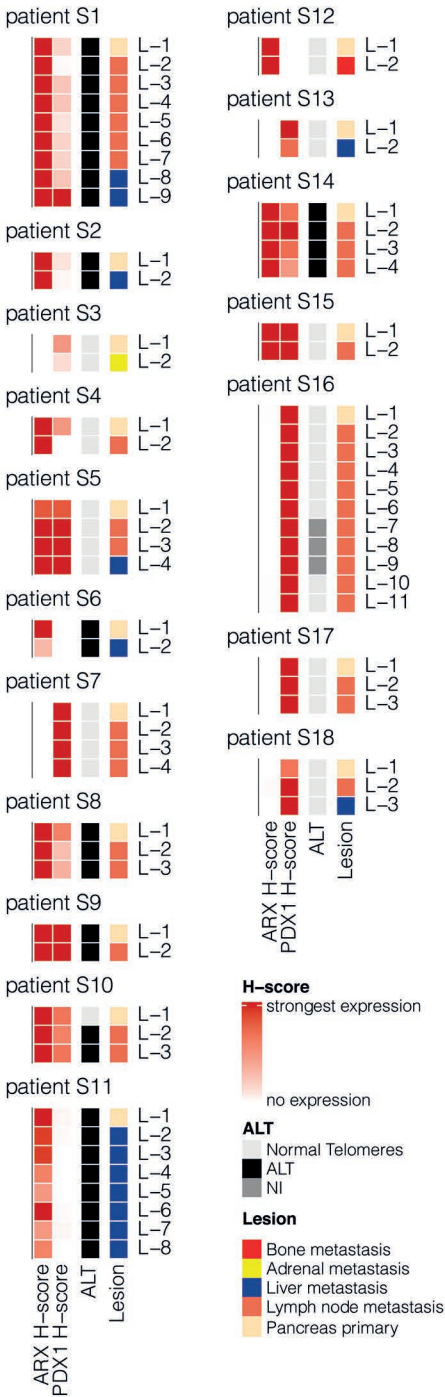


Figure 1. Sporadic PanNET metastases. Consensus ARX and PDX1 H-scores (immunohistochemical nuclear staining intensity*percentage of staining cells) and ALT status of 18 patients with sporadic unifocal primary PanNETs and synchronous or metachronous metastases. In most patients, highly similar patterns can be seen for ARX and PDX1 expression and ALT for matched primary tumors and lymph node or distant metastases. NI indicates not interpretable.

Table 1. Surgical indications and pre- and postoperative characteristics

#	Surgical procedure	Surgical indication	Hyper- gastrinemia FSG (Factor ULN)	Radiology		
				Duodenal NET conventional imaging (max size in mm)/ duodenoscopy	Duodenal NET Gallium-68 labeled PET/CT	Tumors head no. (any size)
M1	Whipple/PPPD + DP	NF-PanNET + Gastrinoma	+ (3.1)	- / -	NA	3
M2	DP	Insulinoma	- (0.6)	- / NA	-	3
M3	Whipple/PPPD + DP	NF-PanNET + Gastrinoma	+ (18.0)	+ (11) / +	+	2
M4	TP	Insulinoma	- (0.5)	- / NA	NA	2
M5	Whipple/PPPD	Gastrinoma	+ (42.9)	+ (8) / NA	+	2
M6	DP	NF-PanNET	- (0.7)	- / NA	NA	0
M7	DP	NF-PanNET	- (0.8)	- / NA	NA	1
M8	TP	Gastrinoma	+ (4.8)	- / +	-	2
M9	Whipple/PPPD	Gastrinoma ^{''}	+ (2.3)	- / +	-	1
M10	Whipple/PPPD + DP	NF-PanNET + Gastrinoma	+ (15.0)	+ / NA	+	1
	NA	NA	6 (60%) (2.7 [0.5-42.9])	3 (30%) / 3 (75%)	3 (50%)	2 [0 – 3]

Continuous values reported as median [range].

^{*}Magnetic resonance imaging/Computed Tomography/Endoscopic Ultrasound

^{''}Suspected pancreatic gastrinoma

^{'''}Primary lung NET after revision

Abbreviations: *DP* distal pancreatectomy, *TP* total pancreatectomy, *PPPD* pylorus-preserving pancreatoduodenectomy, *LNM* lymph nodes metastasis, *NF-PanNET* non-functioning pancreatic neuroendocrine tumor, *PanNET* pancreatic neuroendocrine tumor, *DuoNET* duodenal neuroendocrine tumor, *Microad* microadenoma, *FSG* fasting serum gastrin, *ULN* upper limit of normal, *PET* positron emission tomography, *NR* not reported, *NA* not applicable, *mm* millimeter

In the same cohort, telomere FISH showed that ALT was clonally present in 7 patients, in which both primary tumors and all 23 matched metastases were ALT positive. In one additional patient (patient no. S10), the primary tumor was ALT negative, while 2 metastases were ALT positive. This high degree of similarity indicates that ARX and PDX1 expression profiles and ALT are preserved during progression, and thus can be used as surrogate markers for clonal relatedness between primary tumors and metastases.

MEN1 Cohort

Within our institution, 27 patients with genetically proven MEN1 underwent duodenopancreatic resections of whom 20 had multifocal dpNETs with or without metastasis (flowchart in Supplementary Figure 1). Ten patients with FFPE tissue specimens of multiple dpNETs and metastasis were eligible for inclusion: 6 had locoregional LNM during the

	Tumor head size max (mm)	Tumors Body-Tail no. (any size)	Tumor Body-Tail size max (mm)	LNM	Pathology						
					no. Duodenal NET studied	no. Duodenal NET Max Size (mm)	no. PanNET (≥5mm) studied	no. Microad studied	PanNET and Microad size max (mm)	LNM	Distant metastases
	29	1	20	+	1	10	7	16	33	+	-
	7	2	11	-	NA	NA	2	6	11	+	-
	43	1	15	+	3	2	3	4	45	+	-
	17	2	21	-	NA	NA	11	16	18	-	Lung***
	7	4	14	+	7	7	1	9	8	+	-
	NA	1	48	+	NA	NA	1	0	45	NR	Liver/other
	8	6	24	-	NA	NA	1	19	30	NR	Retro-peritoneal
	3	5	7.2	+	1	4	0	10	4	-	Liver
	8	5	-	+	4	1	1	4	15	+	-
	20	1	18	+	1	1	5	4	15	+	-
	8 [3 – 43]	2 [1 – 6]	18 [7.2 – 48]	7 (70%)	2 [1-7]	3 [1-10]	1.5 [0 – 11]	7.5 [0 – 19]	16.5 [4 – 45]	6 (60%)	4 (40%)

primary resection, and 4 patients had lymph node, retroperitoneal, liver or lung metastases removed during other procedures. Of note, the lung metastases showed TTF1 expression at initial histopathological analysis, suggesting primary pulmonary NET. Of the 10 patients, 50% were males, median age at surgery was 50 years (range: 31 to 63 y), and surgical indications included nonfunctional PanNET (n = 2), insulinoma (n = 2), gastrinoma (n = 3), and nonfunctional PanNET plus gastrinoma (n = 3) (Table 1, Supplementary Table 1). Most (n = 9) had radiological evidence of multiple PanNETs. The median radiological size of the largest PanNET or microadenoma measured 2 cm (range: 0.3 to 4.8 cm). Duodenal NETs were reported preoperatively in 5 out of 6 patients with suspected gastrinoma: conventional imaging was positive in 3 of 6, gastroduodenoscopy in 3 out of 4 and PET/CT in 3 of 5, respectively.

Histopathological Characteristics of MEN1-associated dpNETs

In total, 32 PanNETs, 88 pancreatic microadenomas (<0.5cm), and 17 duodenal NETs and 36 metastases were scored for ARX and PDX1 expression (Table 2, Figure 2, Supplementary Figure 2). Two patients did not have multifocal dpNETs or distant metastases after extensive histopathological analysis. In patient no. M4, the resected lung lesions were negative for ARX and PDX1 expression and positive for TTF1 and therefore considered primary pulmonary NETs.¹² In patient no. M6, a microadenoma that was initially observed was not present in deeper sections, and only a single PanNET (the largest) remained. These 2 patients were partially included in further analyses: patient no. M4 for the descriptive statistics of primary tumor transcription factor expression, and patient no. M6 for the analysis of relatedness between primary tumors and metastases.

Median size measured 1.04 cm (range: 0.50 to 4.50 cm) for PanNETs, 0.10 cm (range: 0.05 to 0.40 cm) for microadenomas and 0.10 cm (range: 0.05 to 1.00 cm) for duodenal NETs. In 4 patients (patients no. M3, M8-M10), the largest duodenal tumor was <0.5 cm. Sole PDX1 expression (ARX-/PDX1+) was exclusively seen in PanNETs (Table 2). Both pancreatic and duodenal tumors showed cases with sole ARX (ARX+/PDX1-) or double positive expression (ARX+/PDX1+). Compared to PanNETs, microadenomas were significantly more often positive for ARX only (ARX+/PDX1-). No transcription factor subtype was specifically associated with metastases (Table 2). Examples of the immunohistochemical staining are shown in Figure 3. ALT was only seen in a single patient (patient no. M7).

Metastatic Patterns of MEN1-associated dpNETs

Intrapatient Euclidean distance hierarchical clustering on the ARX and PDX1 H-scores was performed to discover relations between primary tumors and metastases. Metastases within individual patients clustered together and showed close proximity to each other (Figure 2). In line, the transcription factor proximity between multiple intrapatient (i.e., in patients with multiple metastases) metastases in MEN1 (n = 32) was similar to the distance observed between matched metastases in the sporadic setting (median Euclidean distance 0 [IQR: 0 to 42] versus 3, [IQR: 0 to 41], respectively, p=0.07; Supplementary Figure 3). Three out of 32 metastases (in patients no. M1, M3, M8) clustered apart and had a different transcription factor profile compared to other intra-patient metastases. This indicates that in the presence of multiple metastases, the majority of the metastases likely derived from one single primary tumor, but synchronous metastatic events may occur. In contrast, no relatedness was observed between different primary tumors in patients with MEN1, and a significantly greater Euclidean distance was seen for intrapatient primaries as compared with intrapatient metastases (median 145 [IQR: 30 to 297] versus 0 [IQR: 0 to 42], respectively, p<0.001; Supplementary Figure 3). In every patient, 1 or more primary tumors clustered with the metastases, identifying them as possible primary tumor of origin.

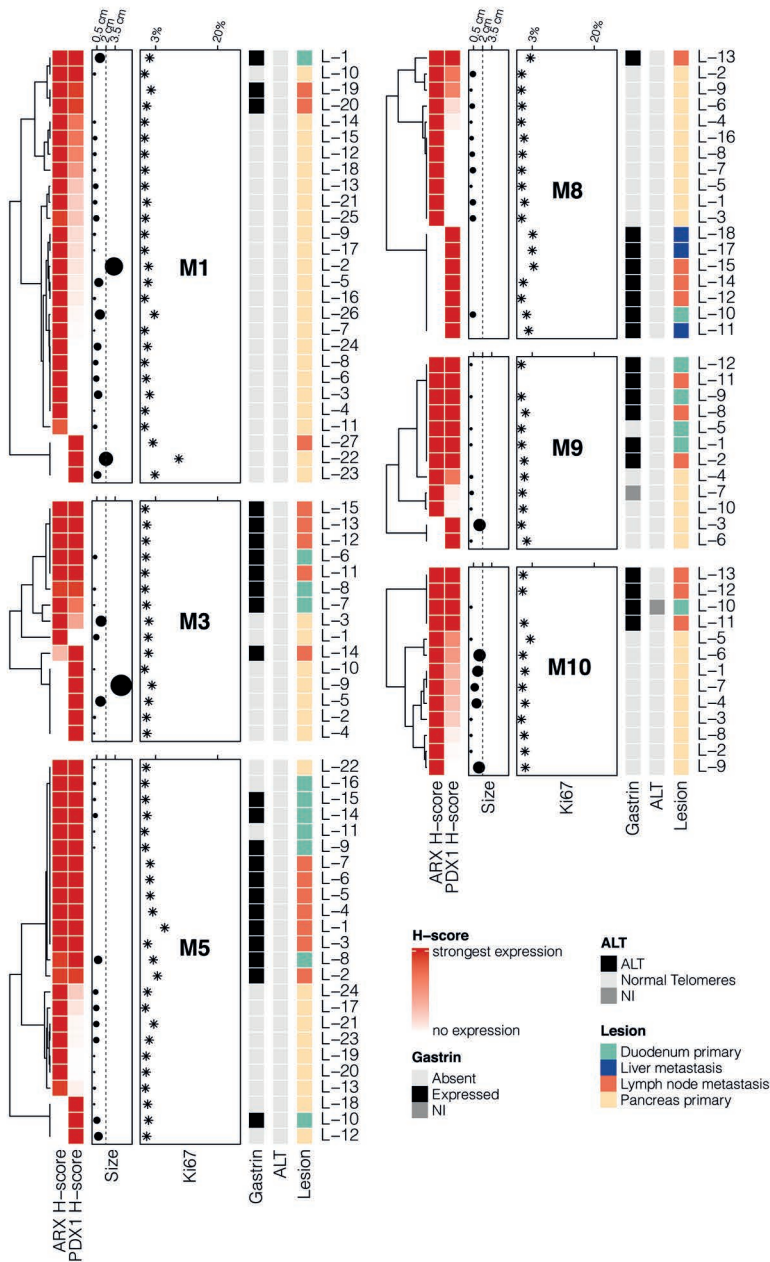


Figure 2. Metastatic patterns of dpNETs in patients with MEN1. Metastatic patterns in patients no. M1, M3, M5, M8, M9, and M10. Unsupervised hierarchical clustering is performed on the ARX and PDX1 H-score heatmap. An H-score is the immunohistochemical nuclear staining intensity multiplied by the percentage of staining cells. Lesions are annotated with size (except metastases), Ki67 labeling index, gastrin expression, the presence of ALT, and anatomic location. *Continuous Ki67 scale.

Table 2. Transcription factor expression in primary dpNETs and metastases

	ARX+/PDX1-	ARX-/PDX1+	ARX+/PDX1+	ARX-/PDX1-	WHO G1	WHO > G1	ALT	Total
Pancreatic microadenomas	48/88 (55%)	18/88 (20%)	22/88 (25%)	-	87/88 (99%)	1/88 (1%)	1/88 (1%)	88
Pancreatic NETs [*]	9/32 (28%)	12/32 (38%)	11/32 (34%)	-	26/32 (81%)	6/32 (19%)	1/32 (3%)	32
Duodenal NETs	0/17 (0%)	2/17 (12%)	15/17 (88%)	-	16/16 ^{**} (100%)	0/16 ^{**} (0%)	0/16 ^{**} (0%)	17
Gastrin + metastases	0/27 (0%)	6/27 (22%)	21/27 (78%)	-	21/26 ^{**} (81%)	5/26 ^{**} (19%)	0/27 (0%)	27
Gastrin - metastases	4	1	2	-	2	5	1	7
Other ^{***}	-	-	-	2	2	-	-	2

^{*} Contingency table transcription factors ARX/PDX1: pancreatic microadenomas and pancreatic NETs. Chi-square $p = 0.03$

^{**} Grading or ALT determination was not interpretable for few duodenal tumors/metastases due to low cell count

^{***} primary lung NETs mistaken as pancreatic NET metastases

Abbreviations: NET, neuroendocrine tumor. WHO G1/2, World Health Organization grade 1/2, ALT Alternative Lengthening of Telomeres

Origin of Duodenopancreatic LNM With Suspected Gastrinoma

Six patients (patients no. M1, M3, M5, M8-M10) underwent a pancreatoduodenectomy or total pancreatectomy and had LNM (Figure 2). All periduodenopancreatic LNMs clustered tightly to duodenal NETs and few pancreatic microadenomas, but not to the (larger) PanNETs. Of note, patient no. M1 (pancreatoduodenectomy plus distal pancreatectomy) also had a single LNM (L-27) located around the pancreatic tail which clustered with 2 PanNETs in the tail (Figure 2, Figure 3). All duodenal NETs and most associated LNM were WHO G1. In 2 patients with suspected gastrinoma (patients no. M5 and M8), a Ki67 labeling index of $\geq 3\%$ (G2) was seen in LNMs. To rule out the presence of (microscopic) pancreatic gastrinoma, and subsequently confirm the suspected clonal relations based on ARX and PDX1 expression, we performed gastrin immunohistochemistry. Indeed, not one PanNET or microadenoma, but most primary duodenal NETs (14/17), and all periduodenopancreatic LNM expressed gastrin. In 2 patients with gastrin positive lymph nodes, a single gastrin positive LNM (patient no. M3 L-14 and patient no. M8 L-13) showed aberrant ARX expression compared to other LNM, while all other metastases showed relatively uniform ARX and PDX1 expression. Furthermore, these two aberrantly staining LNM did not cluster with any dpNET, but as complete duodenal embedding was not performed, it is possible that several microscopic primary tumors were not embedded as FFPE block.¹⁸

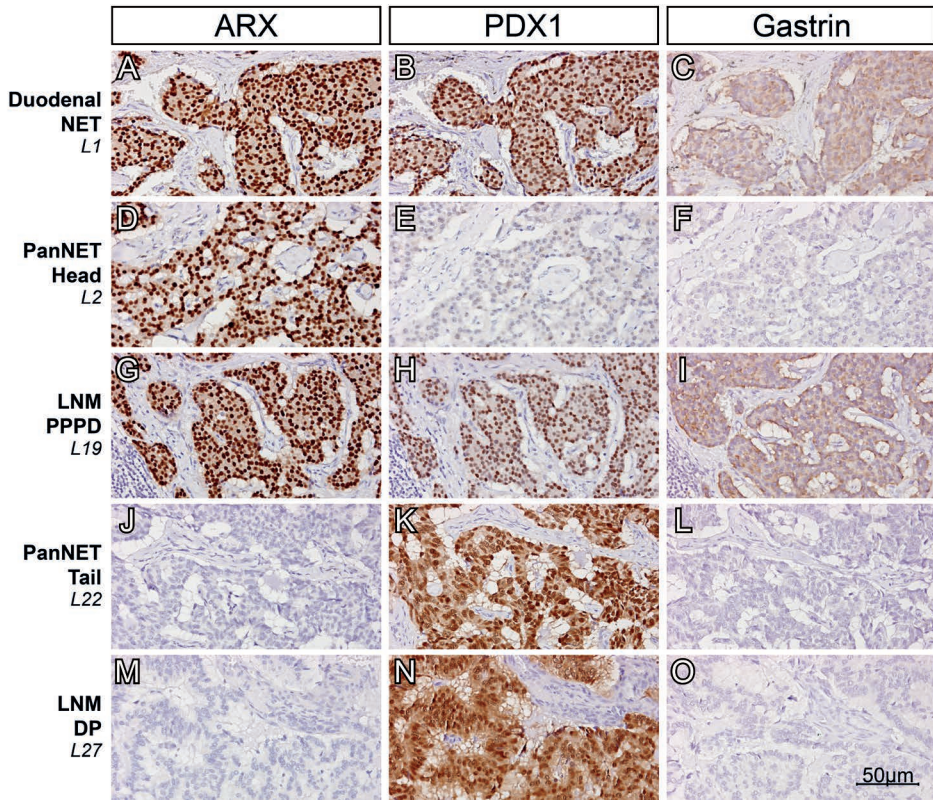


Figure 3. ARX, PDX1, gastrin immunohistochemistry in single patient with MEN1. Representative images of ARX and PDX1 and gastrin expression from patient no. M1, with L-numbers linking to Figure 2. A-C, lesion L-1, the largest primary duodenum NET (1 cm). D-F, lesion L-2, the largest primary PanNET (3.3 cm) in the pancreatic head. G-I, lesion L-19, LNM in pylorus-preserving pancreatoduodenectomy specimen. J-L, lesion L-22, primary PanNET (2cm) in the pancreatic tail. M-O, lesion L-27, LNM in distal pancreatectomy specimen. Abbreviations: NET neuroendocrine tumor, PanNET pancreatic neuroendocrine tumor, LNM lymph node metastasis, PPPD pylorus-preserving pancreatoduodenectomy, DP distal pancreatectomy.

Origin of Metastases Without Suspected Gastrinoma

In 4 patients (patients no. M1, M2, M6, M7) metastases clustered with primary PanNETs (Figure 2, Supplementary Figure 2). In patient no. M1, a G1 LNM showed clustering with 2 primary PanNETs, located in the distal pancreatectomy specimen (L-22, 2 cm, G2 and L-23, 0.6 cm, G1). In patient no. M2, the LNM (G1) did not cluster with the largest PanNET (L-8, 1.1 cm, G2), but instead showed greater proximity to a smaller PanNET (L-1, 0.9cm, G1) and 2 microadenomas. Of note, the original pathology report noted insulin expression in L-8, while L-1 and the LNM were insulin negative. Patient 6 and 7 both had a ≥ 2 cm G2 primary PanNET with G2/G3 metastases. In patient 7 the primary

tumor (L-21) and retroperitoneal metastasis (L-1), and a 1 mm microadenoma were ALT positive (Supplementary Figure 4). All 3 ALT positive lesions had retained ATRX and DAXX expression.

DISCUSSION

In patients with MEN1, the expected oncological benefits of operative resection and the anticipated risk of complications must be weighed for every single dpNET. However, predicting tumor behavior is challenging in the preoperative setting. In this histopathological study, we inferred relations of primary dpNETs, locoregional and distant metastases by ARX and PDX1 transcription factor clustering in 10 patients with MEN1 and in 18 patients with sporadic PanNETs. In patients with MEN1 and hypergastrinemia, none of the periduodenopancreatic LNM had a pancreatic origin. Instead, most showed similar transcription factor expression to duodenal gastrinomas and all expressed gastrin. In patients with multiple metastases, intrametastases transcription factor similarity indicated that most metastases originated from a single dpNET. One of the exceptions included a patient with 2 metastasized tumors; a gastrinoma with periduodenopancreatic LNM and a PanNET within the pancreatic tail with a local peripancreatic LNM.

This study provides detailed insight in the metastatic patterns of MEN1-associated dpNETs, in particular for patients with hypergastrinemia and radiological LNM. In 6 patients with MEN1 and hypergastrinemia, all 27 periduodenopancreatic LNM – and not a single PanNET – expressed gastrin. Previous histopathological studies have found similar associations. Pipeleers-Marichal et al¹⁹ initially reported 8 patients with MEN1 and hypergastrinemia, of which 4 had periduodenopancreatic LNM. All periduodenopancreatic LNM expressed gastrin, as did all 29 primary duodenal NETs. In contrast, none of 12 PanNETs, and only 1 of 115 microadenomas expressed gastrin. Anlauf et al²⁰ reported 18 patients with MEN1 and hypergastrinemia, including the 8 previously reported patients by Pipeleers-Marichal and colleagues. In 10 patients with LNM, all LNM were gastrin positive. Most patients (15/18) had primary duodenal gastrinomas and none had pancreatic gastrinomas. These data indicate that gastrinomas in MEN1 have a duodenal origin and are associated with gastrin expressing locoregional lymph node metastases. In the present series, we report a detailed analysis for all individual LNM. In addition to gastrin, virtually all periduodenopancreatic LNM (25/27) showed similar ARX and PDX1 transcription factor expression to the duodenal gastrinomas and not a single LNM showed a similar expression pattern to a PanNET. This is important novel evidence of relatedness, as it has been described that hormonal production of metastases can change as compared to the primary NET.^{19,21} Furthermore, 2 individual gastrin positive LNM showed different

transcription factor expression compared to other gastrin positive metastases of the same patients. This suggests multiclonal LNM, which has been reported in MEN1 before.²² The present study provides firm evidence for a common duodenal origin of periduodenopancreatic LNM in patients with MEN1 and hypergastrinemia, which will contribute to more accurate, and tumor-specific staging in case of multifocal duodenopancreatic disease in MEN1. Furthermore, as 4 of 6 patients with hypergastrinemia had primary gastrinomas <0.5cm, a duodenal origin of periduodenopancreatic LNM should be considered, even if no duodenal NETs can be found on imaging or during gastroduodenoscopy.

Management of gastrinomas in MEN1 is challenging for endocrinologists and surgeons. The widespread use of proton pump inhibitors has reversed the hypersecretion state and its consequences, and has shifted the therapeutic aim to preventing metastasis-related morbidity and mortality. The benefit of major surgery for MEN1-associated gastrinoma is controversial, because of the variation in diagnosis, treatment strategies, and prognosis presented in different studies. A universal consensus regarding surgical indication, timing, type of procedure and extent of surgery is lacking.²³

In the first place, several studies have shown that the prognosis of the majority of patients with MEN1 and gastrinomas is excellent if PanNETs are <2 cm,^{3,24} regardless of therapy or the presence of periduodenopancreatic LNM.²⁵ Although a recent large multicenter MEN1 cohort study showed the presence of gastrinomas and PanNETs >2 cm were both independently associated with distant metastases, only metastasized PanNETs >2 cm subsequently increased the risk of death.²⁶ Given the good prognosis, conservative symptomatic treatment with proton pump inhibitors might be favored, especially as major duodenopancreatic surgery – necessary to achieve long-term cure – is associated with severe morbidity.^{6,27–29} On the other hand, liver metastases can also cause significant morbidity. Furthermore, several other studies describe a subgroup of aggressive MEN1-associated gastrinomas (10 to 25% of patients) with worse survival.^{30,31} Aggressive disease was associated with higher gastrin levels, liver metastases, concurrent PanNET size and fast tumor growth, but not with the presence of LNM.^{30,31} Those with aggressive disease can potentially benefit from a major duodenopancreatic resection that can cure gastrinoma disease. Patients undergoing limited resections, that is, duodenotomy with local resection, often have persistent or recurrent hypergastrinemia either due to missed or new gastrinomas in the remnant duodenum.^{3,32} The high probability of persistent disease is underscored by the very small gastrinomas (0.05 to 0.20 cm) in the present study.³³

With the current findings, MEN1 patients with hypergastrinemia, a NET in the head of the pancreas and radiological signs of periduodenopancreatic LNM, a duodenal origin of the LNM should be considered, which is consistent with previous literature.^{19,20} Since periduodenopancreatic LNM in patients with MEN1-related hypergastrinemia are most likely of duodenal origin, a major duodenopancreatic resection is necessary to cure both

duodenal and pancreatic disease. Nevertheless, in patients with nonaggressive duodenal gastrinoma LNM (i.e., with mild hypergastrinemia and slow growth rate), preventing postoperative morbidity by non-surgical treatment versus a major duodenopancreatic resection might outweigh the risk of distant gastrinoma metastases – estimated at 10% – and gastrinoma-related death.^{24,26,27,31}

Only few nonfunctional PanNETs with metastases were included, so it was not possible to study the association of ARX/PDX1 expression and metastatic behavior, which was previously shown in MEN1-associated NF-PanNETs.⁹ The relative abundance of sole ARX expressing microadenomas as compared with PanNETs is consistent with the higher proportion of glucagon (alpha cell, ARX+/PDX1–^{34,35}) expressing microadenomas seen in patients with MEN1.²⁰ Nevertheless, the unexpected large proportion of low-grade PanNETs and microadenomas without PDX1 expression (Table 2), previously associated with the development of liver metastases⁹, could hamper the preoperative applicability of these biomarkers. In this respect, WHO grade more accurately separated indolent from malignant PanNETs. Three G2 PanNETs clustered with presumed pancreatic metastases (patients no. M1, M6, and M7), and only 4 other dpNETs were G2. ALT was seen in a single PanNET with metastases, and in none of the duodenal NETs and gastrin positive periduodenopancreatic LNMs. Interestingly, in the same patient a G1 microadenoma showed the ALT phenotype.³⁶ A microadenoma with ALT has not been reported in MEN1 before, although an intrapancreatic metastasis cannot be excluded. The retained ATRX/DAXX expression can be explained by point mutations, translocations, or non-ATRX/DAXX mutations causing the ALT phenotype.³⁷

The major strength of the present study includes the extensive histopathological analysis of individual dpNETs and metastases in a well-defined MEN1 cohort. Despite the rarity of MEN1, 10 patients undergoing pancreatoduodenectomies or total pancreatectomies were included leading to a high number of dpNETs to enable robust and comprehensive (histopathological) analysis. Limitations include the fact that only highly selected patients undergo major duodenopancreatic surgery or metastasectomies. Surgical indications are ambiguous and particularly for MEN1-associated gastrinomas surgical indications are equivocal. This limits generalizability of these results to all other MEN1 patients. Moreover, most patients have remaining pancreatic, duodenal or pyloric tissue, often harboring additional dpNETs (Table 1). However, it is assumed that dominant and clinically relevant disease was removed during surgery. Finally, only 2 markers were used as surrogate markers for clonal relations, therefore, a coincidental similar expression cannot not be excluded. Indeed, often > 1 primary dpNET clustered with the metastases. Therefore, assigning a single tumor of origin (or the sequence of events) without taking known risk factors, such as tumor size and grade, into consideration remains challenging. Nevertheless, even without knowing the exact tumor of origin, the results show clear transcription factor proximity of periduodenopancreatic LNM to duodenal gastrinomas

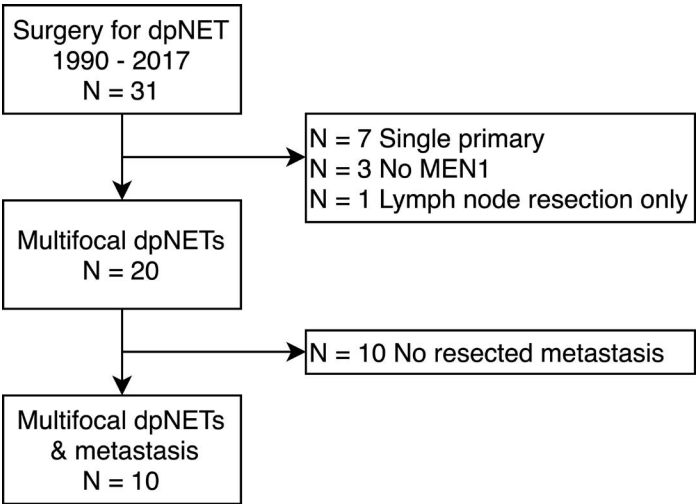
and not a single PanNET and are backed by the expression of gastrin.

In conclusion, this study provides detailed insight in the metastatic spread of dpNETs in a group of patients with resected multifocal MEN1-associated dpNETs. In 6 patients with MEN1-related hypergastrinemia, all periduodenopancreatic LNM originated from duodenal gastrinomas based on ARX, PDX1, and gastrin expression. Duodenal gastrinomas were often small, and not always detected by imaging preoperatively. Therefore, this study underlines that a duodenal origin of periduodenopancreatic LNM should be considered in all patients with MEN1-associated hypergastrinemia, also when clinically relevant NETs are present in the pancreatic head.

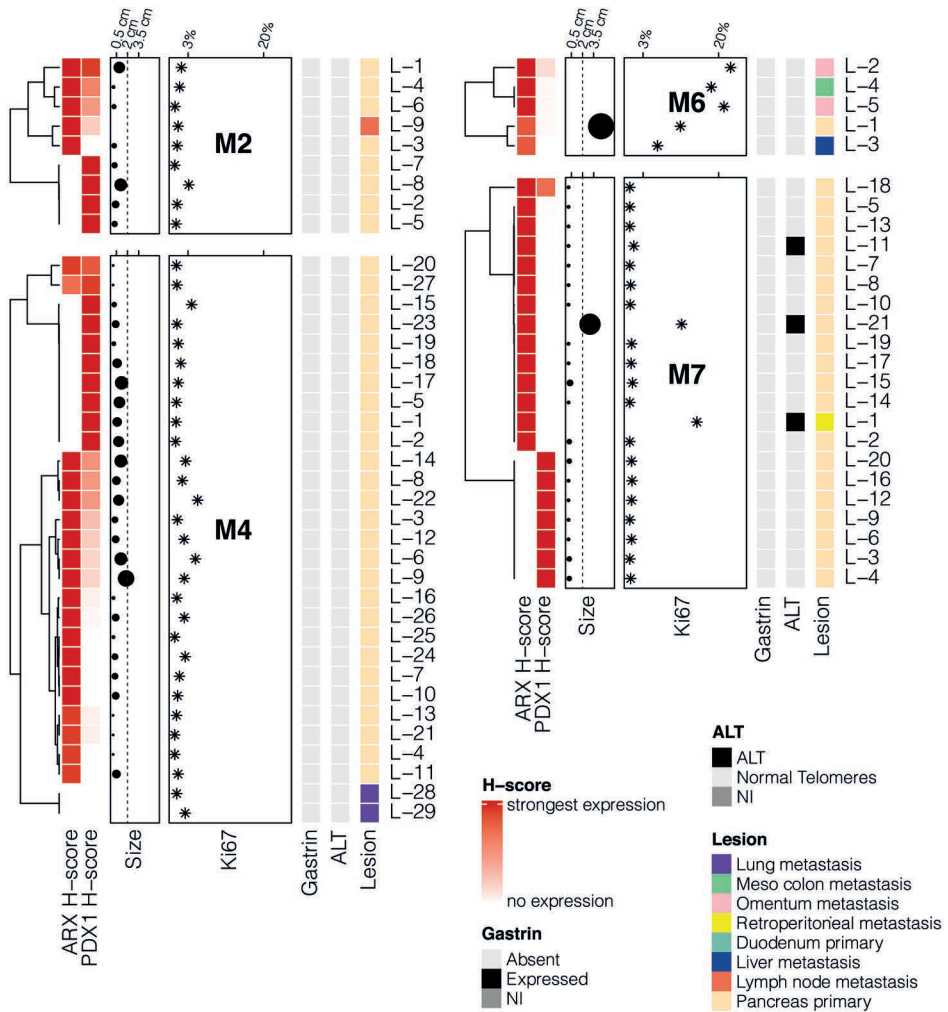
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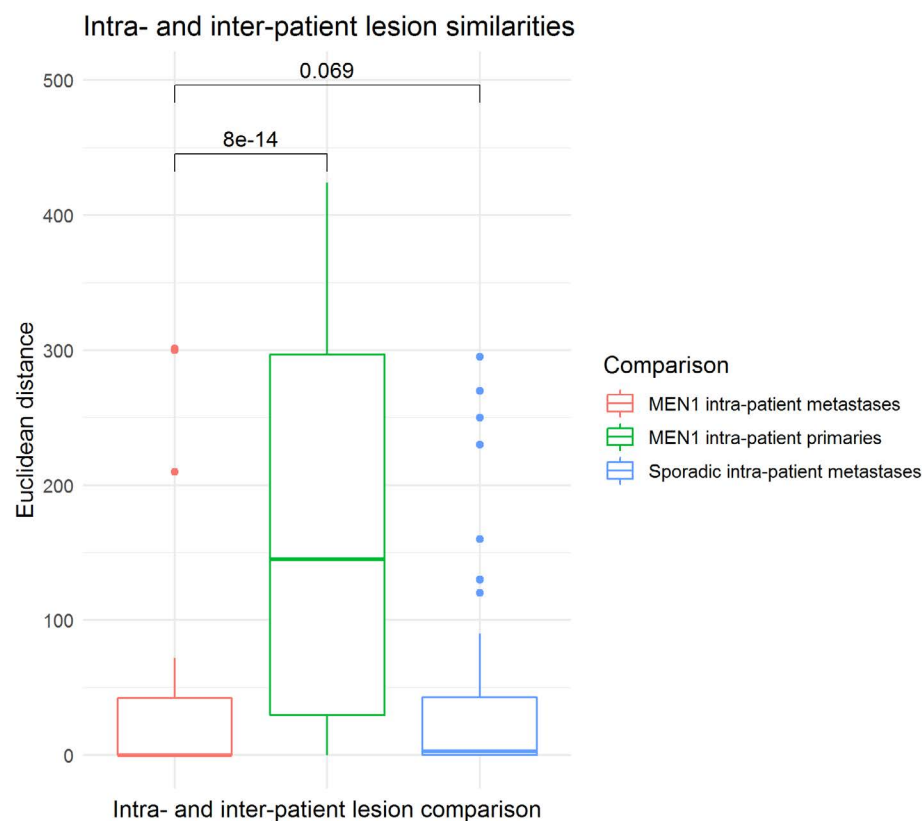
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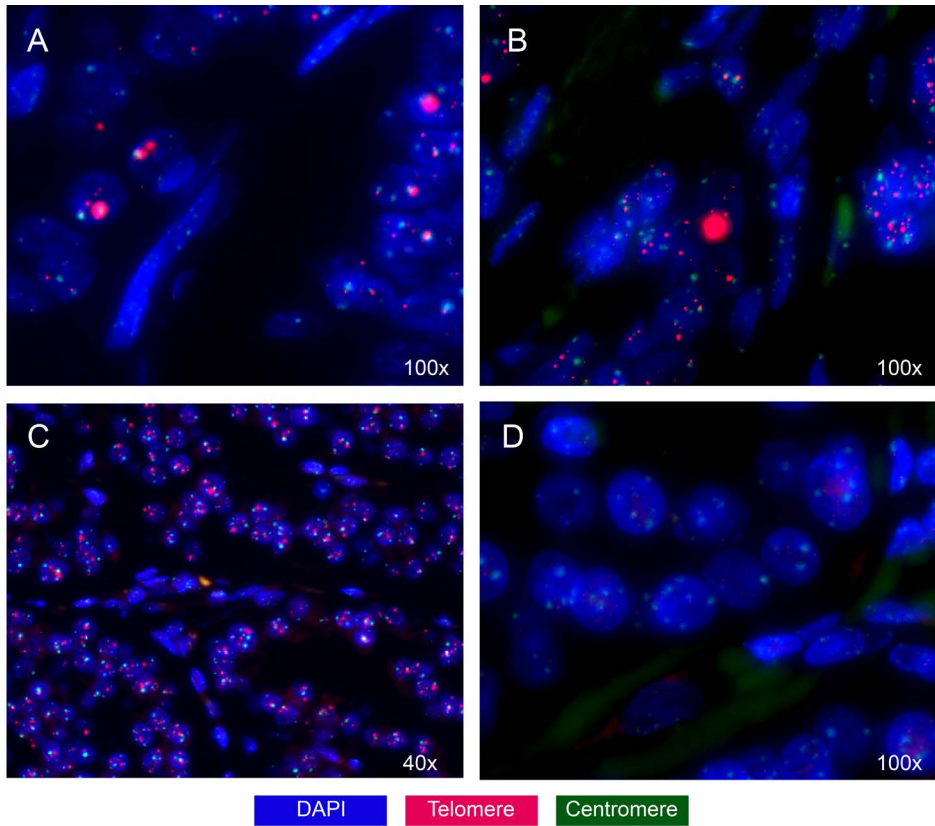
Supplementary Figure 1. Flowchart. Abbreviations: dpNET, duodenopancreatic neuroendocrine tumor.



Supplementary Figure 2. Metastatic dpNET pattern in additional patients with MEN1. Metastatic patterns of patients M2, M4, M6, and M7. Unsupervised hierarchical clustering is performed on the ARX and PDX1 H-score heatmap. An H-score is the immunohistochemical nuclear staining intensity multiplied with the percentage of staining cells. Lesions are annotated with size (except metastases), Ki-67 labeling index, gastrin expression, the presence of Alternative Lengthening of Telomeres (ALT), and anatomic location.



Supplementary Figure 3. Intra-patient Euclidean distance comparison. Intra-patient Euclidean distance comparison for sporadic metastases, MEN1-associated primary tumors, and MEN1-associated metastases

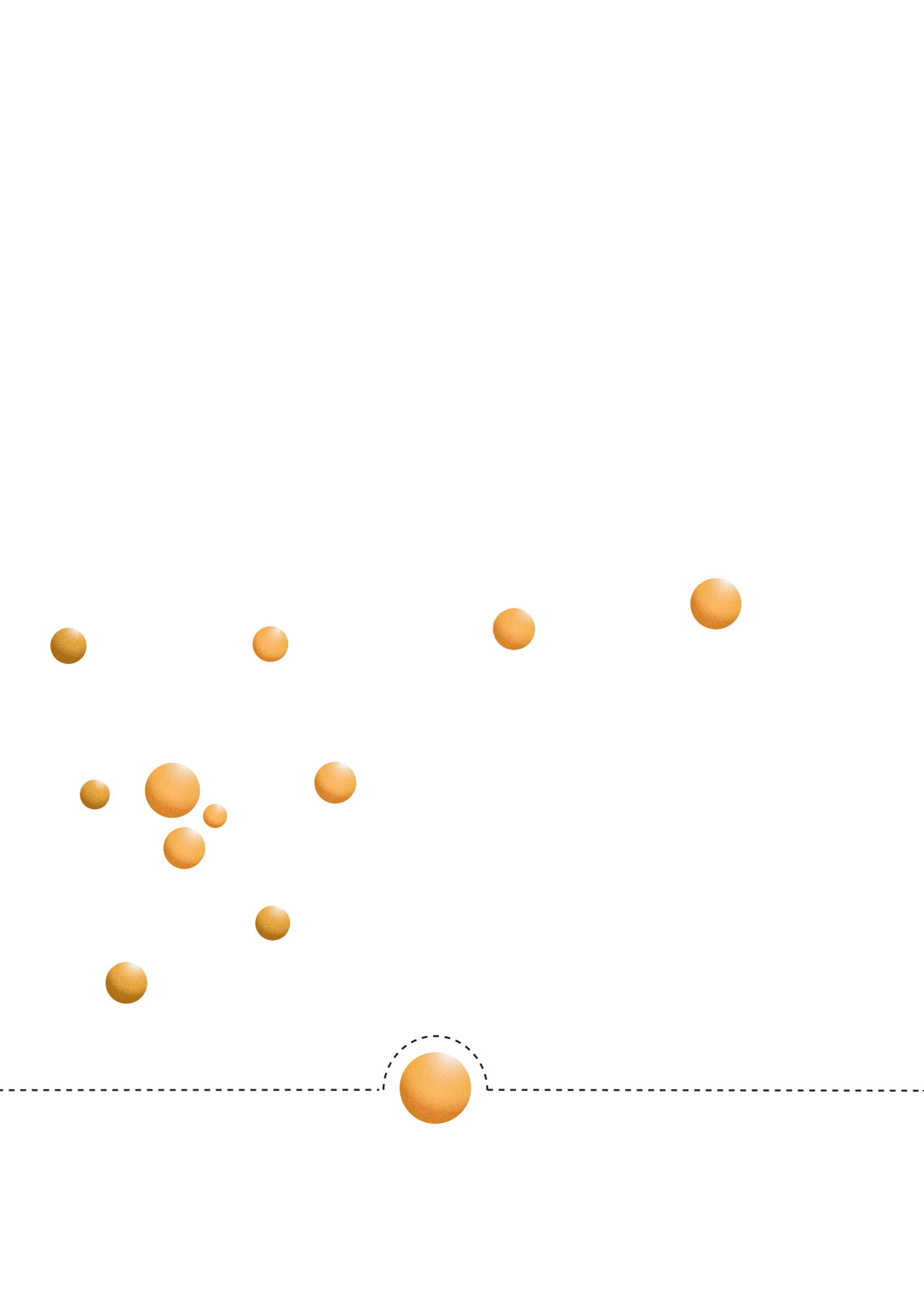


Supplementary Figure 4. Telomere fluorescence in situ hybridization in patients with MEN1. A,B,D 100x magnification. C 40x magnification. A, alternative lengthening of telomeres (ALT) in NET (patient no. M7, L-21). Ultrabright telomeric signals are >10x the signal intensity of the cumulative signal intensities of nearby stromal cells (spindle shaped). B, ALT in retroperitoneal NET metastasis (patient no. M7, L-1). C, ALT in microadenoma (patient no. M7, L-11). D, normal telomeres in pancreatic NET (patient no. M2, L-8).

Supplementary Table 1. Baseline characteristics

Characteristic	Overall n = 10
Age at surgery in years, median [range]	50 [31 – 63]
Sex	
Male	5 (50%)
Female	5 (50%)
Surgical indication	
NF-PanNET	2 (20%)
Insulinoma	2 (20%)
Gastrinoma	3 (30%)
NF-PanNET and gastrinoma	3 (30%)
Size largest PanNET on conventional imaging in mm, median [range]	20 [3 – 48]
PanNET ≥2 cm on preoperative imaging	5 (50%)
Pancreatic head	1 (10%)
Pancreatic body/tail	3 (30%)
Pancreatic head and body/tail	1 (10%)
Number of PanNETs on preoperative imaging	
1	1 (10%)
2	1 (10%)
≥3	8 (80%)
Number of PanNETs head on preoperative imaging	
0	1 (10%)
1	3 (30%)
2	4 (40%)
≥3	2 (20%)
Number of PanNETs body/tail on preoperative imaging	
1	4 (40%)
2	2 (20%)
≥3	4 (40%)
Suspected lymph node metastases on imaging	7 (70%)
Fasting serum gastrin levels (Factor ULN)	2.7 [0.5 – 42.9]
Procedures	
Distal pancreatectomy	3 (30%)
Whipple/PPPD	2 (20%)
Whipple/PPPD + distal pancreatectomy	3 (30%)
Total or completion pancreatectomy	2 (20%)

Abbreviations: *ULN* upper limit of normal, *N* number of, *NF-PanNET* non-functioning pancreatic neuroendocrine tumor, *PanNET* pancreatic neuroendocrine tumor, *PPPD* pylorus-preserving pancreatoduodenectomy



CHAPTER VIII

Prognosis after surgery for multiple endocrine neoplasia type 1-related pancreatic neuroendocrine tumors: functionality matters

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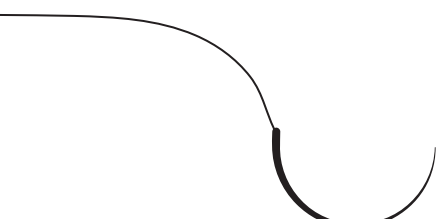
D.J. van Beek, S. Nell, H.M. Verkooijen, I.H.M. Borel Rinkes,
G.D. Valk (on behalf of the DutchMEN Study Group (DMSG)), M.R. Vriens

International MEN1 Insulinoma Study Group

P. Goudet (on behalf of the French Endocrine Tumor Study Group (GTE)), N. Santucci,
D.K. Bartsch, J. Manoharan, N.D. Perrier, J. Zagzag, M.L. Brandi, F. Giusti, N. Nilubol,
L. Brunaud, J.D. Pasternak, R. Hsiao, C. Sturgeon, S. Giri, E.B. Conemans, L.A. Brosens

DutchMEN Surgery Study Group

B.A. Bonsing, C.H. van Eijck, H. van Goor, R.H.J. de Kleine,
E.J. Nieveen van Dijkum, G. Kazemier, C.H.C. Dejong



ABSTRACT

Background

Metastasized pancreatic neuroendocrine tumors are the leading cause of death in patients with multiple endocrine neoplasia type 1. Aside from tumor size, prognostic factors of pancreatic neuroendocrine tumors are largely unknown. The present study aimed to assess whether the prognosis of patients with resected multiple endocrine neoplasia type 1-related non-functioning pancreatic neuroendocrine tumors differs from those with resected multiple endocrine neoplasia type 1-related insulinomas and assessed factors associated with prognosis.

Methods

Patients who underwent resection of a multiple endocrine neoplasia type 1-related pancreatic neuroendocrine tumor between 1990 and 2016 were identified in two databases: the DutchMEN Study Group and International MEN1 Insulinoma Study Group databases. Cox regression was performed to compare liver metastases-free survival of patients with a non-functioning pancreatic neuroendocrine tumor versus those with an insulinoma and to identify factors associated with liver metastases-free survival.

Results

Out of 153 patients with multiple endocrine neoplasia type 1, 61 underwent resection for a non-functioning pancreatic neuroendocrine tumor and 92 for an insulinoma. Of the patients with resected lymph nodes, 56% (18/32) of non-functioning pancreatic neuroendocrine tumors had lymph node metastases compared to 10% (4/41) of insulinomas ($P = .001$). Estimated 10-year liver metastases-free survival was 63% (95% confidence interval 42%–76%) for non-functioning pancreatic neuroendocrine tumors and 87% (72%–91%) for insulinomas. After adjustment for size, World Health Organization tumor grade, and age, non-functioning pancreatic neuroendocrine tumors had an increased risk for liver metastases or death (Hazard Ratio 3.04 [1.47–6.30]). In pancreatic neuroendocrine tumors ≥ 2 cm, non-functioning pancreatic neuroendocrine tumors (2.99 [1.22–7.33]) and World Health Organization grade 2 (2.95 [1.02–8.50]) were associated with liver metastases-free survival.

Conclusion

Patients with resected multiple endocrine neoplasia type 1-related non-functioning pancreatic neuroendocrine tumors had a significantly lower liver metastases-free survival than patients with insulinomas. Postoperative counseling and follow-up regimens should be tumor type specific and at least consider size and World Health Organization grade.

INTRODUCTION

Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant inherited cancer syndrome caused by a germline mutation in the MEN1 tumor suppressor gene located on chromosome 11q13 encoding for the protein menin.^{1,2} The trait occurs in 2 to 3 per 100,000 persons.¹ One of the hallmark manifestations of the disease is pancreatic neuroendocrine tumors (pNETs), which have a prevalence of 56%.³ Moreover, the age-related penetrance of pNETs gradually increases to over 80% by the age of 80 years.^{3,4} Metastasized pNETs are the leading cause of death in patients with MEN1 and significantly reduce life expectancy.^{3,5,6}

Clinically, pNETs are classified as non-functioning or functioning tumors, depending on the presence of a distinct clinical syndrome caused by excessive hormone production. Non-functioning pancreatic neuroendocrine tumors (NF-pNETs) are the most prevalent pNETs, whereas insulinomas are the most frequently encountered functioning pNETs.^{7–10} Tumor formation occurs as a result of independent clonal events leading to loss of heterozygosity of the wild-type MEN1 allele, which is observed in pNETs, microadenomas and mono-hormonal endocrine cell clusters.¹¹ Despite the assumed shared origin of pNETs, varying survival rates have been reported for patients with MEN1-related NF-pNETs and functioning pNETs.^{5,12,13}

Although the majority of pNETs in MEN1 follow a relatively indolent natural course, a subgroup of pNETs metastasizes to the liver and subsequently leads to decreased survival.^{6,12,14–16} Therapy should be aimed at maintaining a good quality of life by relieving symptoms associated with excessive hormone production as well as preventing liver metastases.¹⁷ Besides tumor size as a predictor of liver metastases, prognostic factors of MEN1-related pNETs are largely unknown. It is generally assumed that insulinomas in MEN1 have a more favorable prognosis compared with NF-pNETs because of a relatively small tumor size, early symptomatology with subsequent treatment, or because of differences in grade.^{5,8,18} Patient counseling is inevitable in MEN1 daily clinical care considering the high, age-related prevalence of pNETs, intensive lifelong screening programs, and considerable operative morbidity in those referred for surgery.^{17,19} Nevertheless, the major unmet need for adequate patient counseling is insight in prognosis of MEN1-related pNETs.^{20,21} Knowledge of differences in prognostic factors will contribute to tailoring of surgical indications, timing and extent of surgery, and postoperative follow-up regimens in individual patients. Therefore, this study aimed to assess if patients with a resected MEN1-related NF-pNET have a different prognosis than those with a resected MEN1-related insulinoma. Furthermore, survival and factors associated with liver metastases-free survival were assessed to come to meaningful advice regarding postoperative counseling and follow-up specifically for NF-pNETs and insulinomas.

METHODS

Study design and patient selection

For this observational study, patients with NF-pNETs and insulinomas were selected from the DutchMEN Study Group (DMSG) cohort.²² Considering the rarity of MEN1-related insulinomas, patients with a MEN1-related insulinoma were additionally identified from a MEN1 collaboration including European and North American hospitals. Patients were eligible if they had a resection for a NF-pNET or an insulinoma between 1990 and 2016 with histopathological neuroendocrine tumor confirmation and were followed for at least one year after surgery. MEN1 diagnosis was established according to most recent practice guidelines.¹⁷ Patients operated on for a pNET before 1990 or with distant metastases at diagnosis were excluded. Patients with glucagonomas, vasoactive intestinal peptidomas, and somatostatinomas were not included considering their rarity (<2%).²³ The study protocol was approved by the medical ethics committees or institutional review boards of all participating centers.

*The DMSG database*²²

The DMSG database includes patients with MEN1 aged 16 years and older under treatment in 1 of the Dutch University Medical Centers. Patients were identified by review of the hospital diagnosis databases. Over 90% of the total Dutch MEN1 population is included in the database. Clinical and demographic data were collected every 3 months by standardized medical record review, according to a predefined protocol.

*International MEN1 Insulinoma Study Group*²⁴

The collaboration includes the population-based database from the DMSG, the national database from the Groupe d'étude des Tumeurs Endocrines (GTE) from France and 7 MEN1 expert centers including European and North American hospitals. Patients with a MEN1-related insulinoma were identified by hospital database review using International Classification of Diseases codes.²⁴ Clinical and demographic data were gathered by investigators from every hospital according to the same predefined protocol.

Clinical definitions

A pNET was considered as NF-pNET in case of positive histopathology or computed tomography (CT), magnetic resonance imaging (MRI), and/or endoscopic ultrasonography (EUS) diagnostic of a pNET in combination with the absence of excessive hormone production provoking a distinct clinical tumor syndrome.¹⁶ The date of diagnosis was recorded as the date of the first positive imaging or the date of pathology.^{16,25}

The presence of an insulinoma was based on a positive 48-hour to 72-hour supervised fast test.^{26,27} If no 72-hour supervised fast test was performed, the insulinoma diagnosis was based on clinical criteria: symptoms or signs of hypoglycemia with concomitant biochemical endogenous hyperinsulinemic hypoglycemia according to clinical practice guidelines.^{26,27} The date of diagnosis was based on the date of the supervised fast test or the date of symptoms accompanied by endogenous hyperinsulinemic hypoglycemia. Patients with an insulinoma were analyzed in the insulinoma group, also in the presence of co-existing NF-pNETs.

Gastrinomas in MEN1 have a predominant duodenal origin and rarely occur in the pancreas.^{10,28,29} Therefore, patients with hypergastrinemia and a pNET were regarded as patients with a co-existing duodenal gastrinoma.

Multiple enucleations, a distal pancreatectomy plus enucleation, Whipple plus enucleation, and Whipple plus distal pancreatectomy were considered as combined resections.

Pathology

Pancreatic specimens were examined for the number of pNETs and size of the largest pNET. The size of the largest pNET was used for analysis. In patients with a resection for an insulinoma, positive immunohistochemistry for insulin classified the tumor as insulinoma. If insulin staining was negative or if detailed information on immunohistochemical staining was missing, the size of the largest pNET was used for analysis. Specimens were examined for lymph node metastases. Any peripancreatic lymph node harboring neuroendocrine tumor cells was considered as lymph node metastasis, regardless of hormone expression. If no lymph nodes were observed during the examination, it was assumed that no lymph nodes were resected. Tumors were examined for mitotic rate and Ki67 labelling. Tumor grade was classified according to the World Health Organization (WHO) 2017 classification: Grade 1 (G1): Ki67 labelling index (LI) <3 and mitosis <2 per 10 high power fields (HPF); G2: Ki67 LI 3-20 and/or mitoses 2-20/10 HPF; G3 Ki67 LI >20 and/or mitosis >20/10 HPF.³⁰ In case of a contradiction between mitotic rate and Ki67 labelling, WHO grade was determined by the highest of both.^{31,32}

For patients with multiple pancreatic resections for pNETs, tumor characteristics from the first resection were used for analysis. If the time between two resections was less than three months, characteristics of the largest tumor was obtained, since it is to be expected that the largest tumor was present at the time of the first operation and likely determines prognosis.

Outcomes

Primary outcomes were the occurrence of pNET-related liver metastases during follow-up and overall survival. A composite endpoint (pNET liver metastases and/or overall survival) was computed. Liver metastases were defined as (1) pathologically proven or (2) radiologically

confirmed. If at least two consecutive CT/MRI reports described lesions suspicious for liver metastases, radiology was documented as positive. Pre- and postoperative assessment of the liver was performed according to local availability of imaging modalities and considered conventional imaging (CT or MRI) during the study period. Intraoperative assessment of the liver was guided by individual surgeon's preference and might have included bimanual palpation or intraoperative ultrasonography. The most likely cause of liver metastases was determined by multidisciplinary team discussion. Causes of death were captured from medical records. Deaths caused by MEN1 manifestations and MEN1-related therapy were considered as MEN1-related. Other causes of death were regarded as non-MEN1-related.³

Statistical analysis

Continuous variables were reported as median (range or interquartile range [IQR]) and categorical variables as counts (proportions). Mann-Whitney *U* tests were used for comparison of continuous variables, and categorical variables were compared using Chi-square or Fisher's exact tests. Follow-up time started at the date of surgery and ended at the date of (1) diagnosis of pNET-related liver metastases or (2) death or (3) last follow-up (ie, date of last visit or January 1st 2018). Kaplan-Meier curves were plotted and survival probability estimates were obtained.³³ The log-rank test was used for univariable survival comparison. Pancreatic neuroendocrine tumor size was dichotomized to <2 cm and ≥ 2 cm.^{25,34}

Univariable and multivariable Cox proportional hazard regression analyses were performed with the time to pNET-related liver metastases or death as outcome. Considering the relatively low number of outcomes, four covariates could be included in the multivariable analysis, which were selected based on clinical reasoning and previous literature.^{4,25,31} Besides pNET functionality (NF-pNET versus insulinoma), pNET size in mm, WHO grade (G2/G3 versus G1), and age in years were included in the model.^{4,25,31} A stratified cox model was performed for pNETs <2 cm and ≥ 2 cm. Crude and adjusted hazard ratios (HR) and corresponding 95% confidence intervals (95% CI) were calculated. Cox proportional hazard regression assumptions were formally tested and graphically assessed using scaled Schoenfeld residuals; the assumptions were not violated. Tied events were handled using the exact method. A sensitivity analysis was performed including pNET functionality (NF-pNET versus insulinoma), pNET size in mm, and lymph node status (metastases versus none resected versus no metastases).

In addition, univariable Kaplan-Meier and/or Cox proportional hazard regression were performed to assess the influence of age at surgery (in years), sex (female versus male), pNET size in mm, pNET size (≥ 2 cm versus <2 cm), pNET functionality (NF-pNET versus insulinoma), WHO grade (G2/G3 versus G1), lymph node status (lymph node metastases versus no lymph node metastases versus no lymph nodes resected), and time from diagnosis until surgery (in years) on time to liver metastases or death. The latter analyses were

additionally performed for the subgroups of NF-pNETs and insulinomas. In addition, these analyses were performed with pNET size arbitrarily categorized into <2 cm, 2-3 cm and ≥ 3 cm.

Missing data were encountered for variables used in the Cox regression, and these were considered as missing at random and, therefore, imputed using multiple imputation with the iterative Markov chain Monte Carlo method creating 40 datasets.^{35,36} Variables listed in Table 1 were used as predictor variables for multiple imputation, together with the primary outcome (known in all patients) and the Nelson-Aalen estimator.^{37,38} For multiple imputation of time-to-event data, the event and the censoring time should be taken into account. The cumulative baseline hazard at the time of the event or censoring is often unknown but can be approximated by the Nelson-Aalen estimator.³⁸ Hazard ratios with corresponding 95% CIs were pooled using Rubin's rules.³⁹

P-values of < .05 were considered statistically significant. Statistical analysis was performed using SPSS version 25.0 (IBM Corp, NY, USA), R version 3.4.1 (R Foundation for Statistical Computing, Vienna, Austria) with 'survival' and 'Mice' packages and Graphpad Prism version 7.02 (GraphPad Software, Inc, San Diego, CA, USA).

RESULTS

A total of 153 patients underwent resection for a pNET, 61 for a NF-pNET and 92 for an insulinoma (Figure 1). Baseline characteristics are shown in Table 1. Patients with NF-pNETs were older at diagnosis, older at surgery, more often male, had larger tumors on imaging, more often with suspected of lymph node metastases on imaging, and a longer time between diagnosis and surgery. Twenty-six patients (41%) in the NF-pNET group and 15 patients (16%) in the insulinoma group were operated on more than 1 year after diagnosis. Combined resections were more often performed for insulinomas. All insulinoma patients, except 1, were cured from hyperinsulinemic hypoglycemia immediately postoperative.

Pathology

Median size of the largest pNET in the surgical specimen was larger for NF-pNETs compared with insulinomas (median 25 mm [IQR 15 – 35 mm] versus 20 mm [IQR 15 – 25 mm], respectively; Table 1). Tumor size, lymph node status, and WHO grade were missing in 8%, 12%, and 27%, respectively. Thirty-eight NF-pNETs (64%; 38/59) and 47 insulinomas (57%; 47/82) were larger than 2 cm. Multiple insulin immunopositive pNETs were observed in 24 (30%) patients with insulinomas. Three patients in the insulinoma group had negative insulin immunohistochemistry, all of whom were cured.

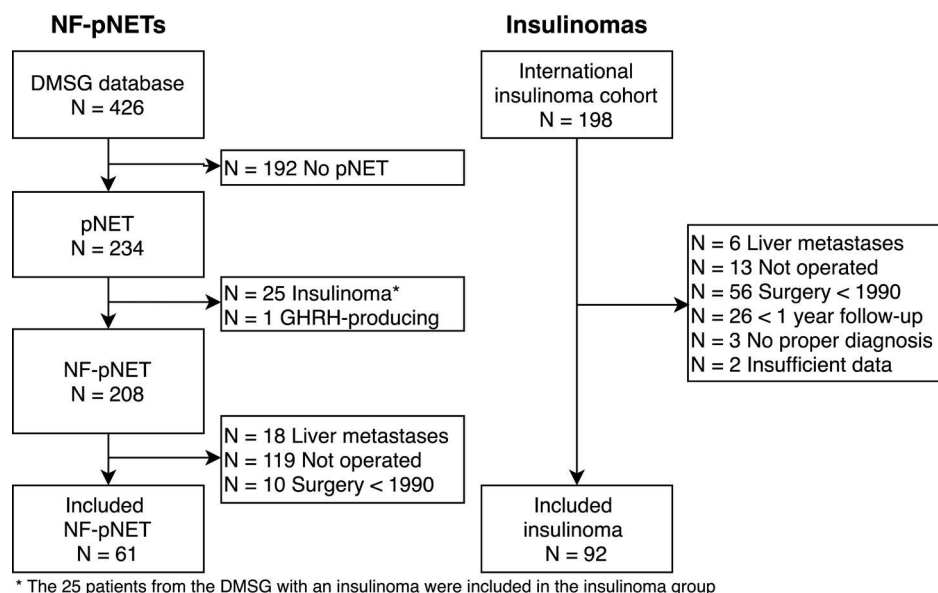


Figure 1. Flow-chart of patient inclusion. Abbreviations: *DMSG* DutchMEN Study Group, *GHRH* Growth hormone-releasing hormone, *NF-pNET* non-functioning pancreatic neuroendocrine tumor, *pNET* pancreatic neuroendocrine tumor

Of the 73 patients with lymph nodes resected, lymph nodes were tumor positive in 18 patients with NF-pNETs (56%; 18/32) compared to 4 (10%; 4/41) with insulinomas ($P < 0.001$; Table 1). Lymph node metastases were more often observed in patients with a pNET ≥ 2 cm (17/44, 39%) compared with pNET < 2 cm (4/25, 16%) ($P = .050$); 3 patients with lymph node metastases had missing tumor size. Of the 44 patients with resected lymph nodes and a pNET ≥ 2 cm, metastatic lymph nodes were observed in 15 patients (63%; 15/24) with NF-pNETs compared with 2 patients (10%; 2/20) with insulinomas ($P = .001$). No differences in WHO grade (Ki-67 and/or mitosis) were observed between NF-pNETs and insulinomas. In 1 patient the NF-pNET considered a well-differentiated WHO G3 tumor.

Long-term outcomes

Long-term outcomes are summarized in Supplementary Table 1. After a median follow-up of 8.8 years (range 0.3–25.3 years), 37 patients (24%) had developed liver metastases or died, which occurred more often in patients with NF-pNETs compared to insulinomas (22/61 (36%) versus 15/92 (16%), $P = .005$). No differences were observed regarding follow-up time between the NF-pNETs and insulinoma group. Liver metastases were observed in

Table 1. Demographic, preoperative, surgical and histopathological characteristics.

Characteristic	Overall (n = 153)	NF-pNET [†] (n = 61)	Insulinoma (n = 92)	p-value
Age at pNET [†] diagnosis in years, median [range]	36 [6 – 82]	40 [15 – 73]	32 [6 – 82]	0.002
Missing data (%)	1 (1%)	0 (0%)	1 (1%)	
Age at surgery in years, median [range]	38 [6 – 82]	41 [20 – 73]	34 [6 – 82]	0.002
Time from diagnosis until surgery in years, median [range]	0.5 [0 – 15]	0.5 [0 – 15]	0.3 [0 – 15]	0.006
Missing data (%)	1 (1%)	0	1 (1%)	
Sex (%)				
Male	66 (43%)	34 (56%)	32 (35%)	0.010
Female	87 (57%)	27 (44%)	60 (65%)	
Size largest pNET [†] on preoperative imaging in mm, median [range]	20 [4 – 98]	27 [8 – 86]	20 [4 – 98]	0.009
Missing data (%)	18 (12%)	5 (8%)	13 (14%)	
Number of pNETs [†] on preoperative imaging (%)				
0	5 (3%)	4 (7%)	1 (1%)	0.221
1	71 (46%)	29 (48%)	42 (48%)	
2	29 (19%)	9 (15%)	20 (23%)	
≥3	42 (28%)	18 (30%)	24 (28%)	
Missing data (%)	6 (4%)	1 (1%)	5 (5%)	
Suspected lymph node metastases on preoperative conventional imaging (%)	5 (3%)	5 (8%)	0 (0%)	0.009
Missing data (%)	1 (1%)	0	1 (1%)	
Type of resection (%)				
Enucleation	23 (15%)	9 (15%)	14 (15%)	-
Multiple enucleations	6 (4%)	2 (3%)	4 (4%)	
Distal pancreatectomy	71 (46%)	33 (54%)	38 (41%)	
Distal pancreatectomy and enucleation	27 (18%)	2 (3%)	25 (27%)	
Whipple or PPPD [‡]	10 (7%)	5 (8%)	5 (5%)	
Whipple/PPPD [‡] and enucleation	2 (1%)	0 (0%)	2 (2%)	
Whipple/PPPD [‡] and distal pancreatectomy	5 (3%)	3 (5%)	2 (2%)	
Pancreatic body resection	1 (1%)	1 (2%)	0 (0%)	
Total pancreatectomy	8 (5%)	6 (10%)	2 (2%)	
Combined resection (%)	40 (26.1%)	7 (12%)	33 (36%)	0.001
Number of pNETs in the resection specimen (%)				
1	39 (26%)	18 (30%)	21 (24%)	0.100
2	27 (18%)	15 (25%)	12 (14%)	
≥3	83 (56%)	28 (46%)	55 (63%)	
Missing data (%)	3 (2%)	0 (0%)	4 (4%)	
Size largest pNET [†] pathology in mm, median [range]	20 [3 – 120]	25 [3 – 120]	20 [5 – 110]	0.045
Missing data (%)	13 (8%)	3 (5%)	10 (11%)	
Lymph node metastases (%)				
Lymph node metastases	22 (14%)	18 (30%)	4 (4%)	<0.001
No lymph node metastases	51 (33%)	14 (23%)	37 (40%)	
No lymph nodes resected	61 (40%)	29 (48%)	32 (35%)	
Missing data (%)	19 (12%)	0 (0%)	19 (21%)	
WHO [§] grade				
G1	87 (78%)	45 (79%)	42 (76%)	0.539
G2	24 (21%)	11 (19%)	13 (24%)	
G3	1 (1%)	1 (2%)	0	
Missing data (%)	41 (27%)	4 (7%)	37 (40%)	

Abbreviations: [†]NF-pNET non-functioning pancreatic neuroendocrine tumor, [†]pNET pancreatic neuroendocrine tumor, [‡]PPPD pylorus-preserving pancreatoduodenectomy, [§]WHO World Health Organization

15 patients (25%) with a NF-pNET and 6 (7%) with an insulinoma. Two of the 6 patients with a resected insulinoma had recurrent hypoglycemia at the time of liver metastases diagnosis. Median time from surgery until the development of liver metastases or death

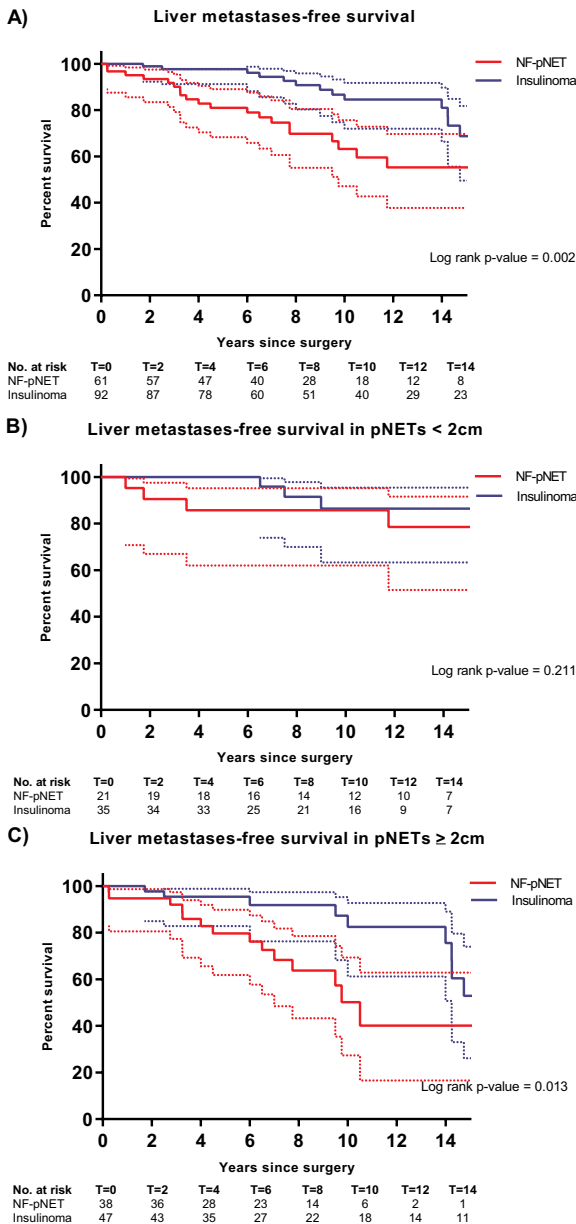


Figure 2.
A) Liver metastases-free survival of patients with resected MEN1-related pancreatic neuroendocrine tumors. B) Liver metastases-free survival of patients with resected MEN1-related pancreatic neuroendocrine tumors <2 cm. C) Liver metastases-free survival of patients with resected MEN1-related pancreatic neuroendocrine tumors ≥2 cm

was significantly shorter for NF-pNETs (5.3 versus 9.5 years, $P = .036$). The development of subsequent liver metastases or death occurred after 5 and 10 years in 24 (65%) and 11 patients (30%), respectively. Causes of death are listed in Supplementary Table 2. The percentage of liver metastases, death, or both correlated with tumor size (Figure 2). The proportion of patients with NF-pNETs developing liver metastases was higher for all tumor sizes (<2, 2–3 and ≥ 3 cm) compared to patients with an insulinoma, whereas patients with a resected insulinoma tended to die more often without pNET-related liver metastases.

Liver metastases-free survival after resection of NF-pNETs versus insulinomas

Patients with a resected NF-pNET had a significantly reduced liver metastases-free survival (LMFS) compared to those with a resected insulinoma (log rank P value .002) (Figure 3). Ten-year LMFS probability estimates were 63% (95% CI 42%–76%) for NF-pNETs versus 87% (95% CI 72%–91%) for insulinomas. Of the 60 patients with pNETs <2 cm, 9 (15%) developed liver metastases or died; no survival differences were observed between patients with NF-pNETs and insulinomas (Figure 3B). In contrast, 28 of the 93 patients (30%) with a pNET ≥ 2 cm developed liver metastases or died, and LMFS was significantly lower for patients with NF-pNETs ≥ 2 cm compared to insulinomas ≥ 2 cm ($P = .011$; Figure 3C).

After adjusting for age at surgery, pNET size, and WHO grade, patients with a resected NF-pNET had a significantly increased risk for liver metastases or death compared to patients with a resected insulinoma (HR 3.04 [95% CI 1.47–6.30]; Table 3). In addition, pNET size per mm increase (HR 1.01 [95% CI 1.001–1.02]) was independent of pNET type, WHO grade, and age at surgery associated with LMFS. Sensitivity analysis showed similar HRs for pNET functionality and size when adjusted for lymph node status (Supplementary Table 3).

Table 2. Multivariable analysis for factors associated with liver metastases or death.

Characteristic	Multivariable analysis (Adjusted Hazard Ratio)	
	Hazard ratio	95% CI [†]
Age at surgery (per year)	1.02	0.99 – 1.05
Size largest pNET [‡] (per mm)	1.01	1.001 – 1.02
Tumor functionality		
Insulinoma	1	Ref. cat.
NF-pNET [†]	3.04	1.47 – 6.30
WHO§ grade		
G1	1	Ref. cat.
G2/G3	2.09	0.89 – 4.90

Data reported after multiple imputation.

Multivariable analysis included all factors listed above.

Abbreviations: [†]CI Confidence Interval, [‡]NF-pNET non-functioning pancreatic neuroendocrine tumor, [§]pNET pancreatic neuroendocrine tumor, [§]WHO World Health Organization

Stratified by pNET size <2 and ≥ 2 cm, no factors were associated with LMFS in pNETs <2 cm (Table 4). For pNETs ≥ 2 cm, NF-pNETs were associated with LMFS after adjusting for age at surgery and WHO grade (HR 2.95 [95% CI 1.18–6.67]). In addition, patients with WHO G2 tumors had an increased risk for liver metastases or death (HR 2.52 [95% CI 1.16 - 5.47]).

Prognostic factors for LMFS

Estimated LMFS probabilities and factors associated with LMFS are summarized in Table 5. Patients who were older at surgery, had a NF-pNETs versus an insulinoma, had larger pNETs, a pNET ≥ 2 cm versus <2 cm, a WHO G2/G3 versus G1 tumor, lymph node metastases versus no lymph node metastases, and a longer delay from diagnosis until surgery had higher probabilities of liver metastases or death. Ten-year LMFS probability estimates were more than 80% for patients with an insulinoma (87%), pNET <2 cm (87%), G1 tumor (80%), and no lymph node metastases (81%). By contrast, for patients with a NF-pNET

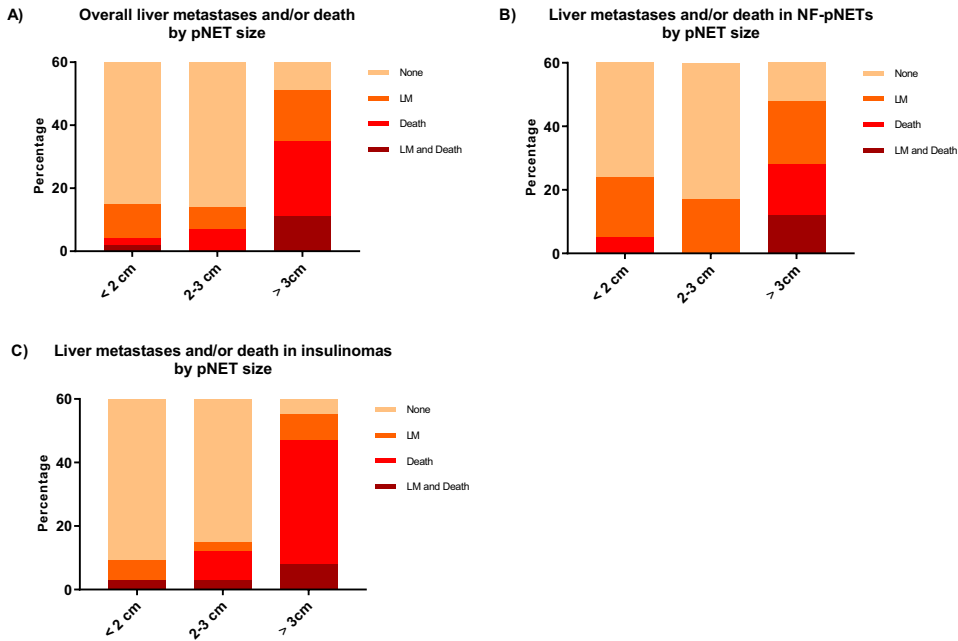


Figure 3. A) Occurrence of liver metastases or death stratified by pNET size and pNET functionality in total cohort. B) Occurrence of liver metastases or death stratified by pNET size for patients with a resected NF-pNET. C) Occurrence of liver metastases or death stratified by pNET size for patients with a resected insulinoma. Abbreviations: LM liver metastases, NF-pNET non-functioning pancreatic neuroendocrine tumor, pNET pancreatic neuroendocrine tumor

Table 3. Univariable and multivariable analysis for factors associated with liver metastases or death stratified for pNETs <2 and ≥ 2 cm.

	Characteristic	No. of patients	LM [†] /death	Univariable analysis (Crude HR [†])		Multivariable analysis (Adjusted HR [†])	
				HR [†]	95% CI [†]	HR [†]	95% CI [†]
pNET [‡] <2 cm	Age at surgery (per year)	60	9	1.03	0.98 – 1.08	1.03	0.98 – 1.08
	Tumor functionality						
	Insulinoma	38	3	1	Ref. cat.	1	Ref. cat.
	NF-pNET [§]	22	6	2.44	0.58 – 10.30	2.19	0.52 – 9.28
pNET [‡] ≥ 2 cm	Age at surgery (per year)	93	28	1.02	0.99 – 1.06	1.01	0.98 – 1.05
	Tumor functionality						
	Insulinoma	54	12	1	Ref. cat.	1	Ref. cat.
	NF-pNET [§]	39	16	3.11	1.39 – 6.98	2.92	1.28 – 6.67
	WHO [¶] grade						
	G1	64	15	1	Ref. cat.	1	Ref. cat.
	G2/G3	29	13	2.13	0.998 – 4.55	2.52	1.16 – 5.47

Data reported after multiple imputation.

Multivariable analysis includes age and tumor functionality; for pNETs ≥ 2 cm WHO grade was additionally included.

Abbreviations: [†]CI Confidence Interval, [†]HR Hazard Ratio, [†]LM liver metastases, [§]NF-pNET non-functioning pancreatic neuroendocrine tumor, [‡]pNET pancreatic neuroendocrine tumor, [¶]WHO World Health Organization

(63%), pNET ≥ 2 cm (42%) and G2/G3 tumor (42%), and lymph node metastases (51%), LMFS probability estimates were lower. Additional analysis revealed that patients with a resected pNET ≥ 3 cm as compared to those with a resected pNET <2 cm had a significantly decreased LMFS (HR 4.80 [95% CI 2.09–11.02]; Figure 4, Table 5). No differences were observed for those with a pNET 2 to 3 cm compared to those with a pNET <2 cm. Estimated 5- and 10-year LMFS probabilities were 92% and 80% for patients operated on before 2003 and 90% and 75% for patients operated on from 2003 onwards.

Prognostic factors for LMFS in NF-pNETs

Within the patients with a resected NF-pNET, 10-year LMFS probability estimates were 50% for those with a NF-pNET ≥ 2 cm, 24% for those with a WHO G2/G3 tumor, and 44% for those with lymph node metastases. Size in mm (HR 1.02 [95% CI 1.01–1.04]) and WHO G2/G3 versus G1 (HR 2.99 [95% CI 1.19–7.54]) were associated with LMFS. Of the patients with a NF-pNET ≥ 2 cm graded as G2/G3, estimated 10-year LMFS was 23% compared with 84% for patients with a G1 NF-pNET <2 cm (Figure 5).

Prognostic factors for LMFS in insulinomas

A longer time from diagnosis until surgery was associated with liver metastases or death in patients with a resected insulinoma. The CIs of other factors, such as age at surgery, size in mm, presence of a pNET ≥ 2 cm, and WHO G2/G3 crossed unity. Point estimates and

Table 4. Liver metastases-free survival and factors associated with liver metastases-free survival.

	Characteristic		LMFS [*]		Univariable Cox regression [†]		
			5-yr (%)	10-yr (%)	Log rank p-value	LM /death	Crude HR [§] 95% CI [‡]
Overall	Age at surgery (per year)		-	-	NA [§]	37 of 153	1.03 1.01 – 1.06
	Sex	Male	87	77	0.491	18 of 66	1 Ref. cat.
		Female	94	77		19 of 87	0.80 0.42 – 1.52
	pNET functionality	Insulinoma	98	87	0.002	15 of 92	1 Ref. cat.
		NF-pNET [*]	81	63		22 of 61	2.72 1.41 – 5.28
	Size continuous (per mm)		-	-	NA [§]	37 of 153	1.02 1.01 – 1.03
	pNET ^{***} ≥ 2 cm	<2 cm	95	87	0.003	9 of 60	1 Ref. cat.
		≥2 cm	88	70		28 of 93	2.97 1.36 – 6.48
	WHO ^{††} grade	G1	93	80	0.002	21 of 109	1 Ref. cat.
		G2/G3	83	42		16 of 44	2.20 1.02 – 4.76
NF-pNET [*]	Lymph node status	No metastases	91	81	0.144	12 of 60	1 Ref. cat.
		None resected	90	76		16 of 69	1.35 0.61 – 2.99
		Metastases	86	51		9 of 24	2.77 1.12 – 6.85
	Years from diagnosis until surgery		-	-	NA [§]	37 of 153	1.12 1.01 – 1.24
	Age at surgery (per year)		-	-	NA [§]	22 of 61	1.04 0.995 – 1.08
	Sex	Male	80	68	0.752	12 of 34	1 Ref. cat.
		Female	84	58		10 of 27	1.15 0.85 – 1.51
	Size continuous (per mm)		-	-	NA [§]	22 of 61	1.02 1.01 – 1.04
	pNET ^{***} ≥ 2 cm	<2 cm	86	86	0.058	6 of 22	1 Ref. cat.
		≥2 cm	80	50		16 of 39	2.37 0.89 – 6.34
Insulinoma	WHO ^{††} grade	G1	86	73	0.046	14 of 47	1 Ref. cat.
		G2/G3	74	24		8 of 14	2.99 1.19 – 7.54
	Lymph node status	No metastases	77	77	0.360	5 of 14	1 Ref. cat.
		None resected	83	70		8 of 29	0.63 0.21 – 1.96
		Metastases	82	44		9 of 18	1.28 0.42 – 3.94
	Years from diagnosis until surgery		-	-	NA [§]	22 of 61	1.01 0.88 – 1.16
	Age at surgery (per year)		-	-	NA [§]	15 of 92	1.02 0.99 – 1.06
	Sex	Male	97	90	0.784	6 of 32	1 Ref. cat.
		Female	98	85		9 of 60	0.83 0.60 – 1.18
	Size continuous (per mm)		-	-	NA [§]	15 of 92	1.01 0.998 – 1.03
Insulinoma	pNET ^{***} ≥ 2 cm	<2 cm	100	86	0.068	3 of 40	1 Ref. cat.
		≥2 cm	95	87		12 of 52	3.43 0.98 – 12.01
	WHO ^{††} grade	G1	100	90	0.003	7 of 62	1 Ref. cat.
		G2/G3	91	68		8 of 30	2.60 0.92 – 7.29
	Lymph node status	No metastases	97	83	0.385	7 of 46	1 Ref. cat.
		None resected	97	82		8 of 40	1.91 0.66 – 5.54
		Metastases	100	-		0 of 6	NA [§] NA [§]
	Years from diagnosis until surgery		-	-	NA [§]	15 of 92	1.25 1.05 – 1.49

^{*}Estimated survival percentages are based on the patients with complete data.

[†]Data presented after multiple imputation.

Abbreviations: ^{*}Liver metastases-free survival, [‡]CI Confidence Interval, [§]HR Hazard Ratio, ^{||}LM liver metastases, [§]NA not applicable, ^{*}NF-pNET non-functioning pancreatic neuroendocrine tumor, ^{***}pNET pancreatic neuroendocrine tumor, ^{††}WHO World Health Organization

95% CI of these factors had similar direction and magnitude as within the NF-pNET group. Of the patients with complete data, those with an insulinoma ≥ 2 cm, which was G2/G3, had an estimated 10-year LMFS of 57%.

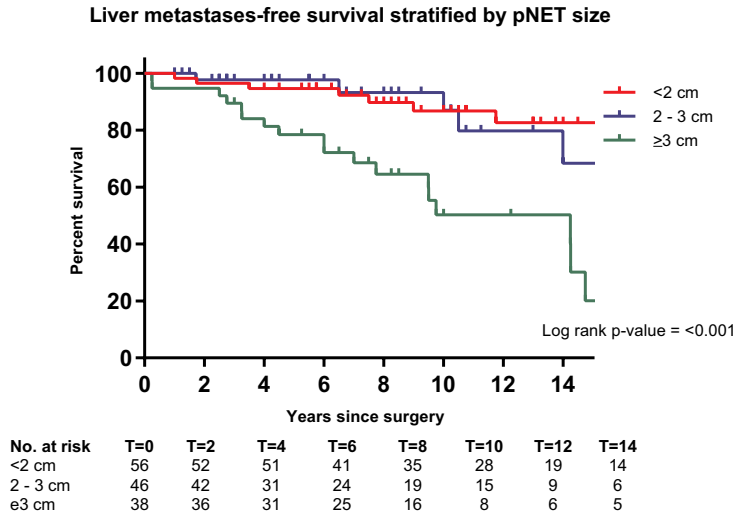


Figure 4. Liver metastases-free survival of patients with resected MEN1-related pancreatic neuroendocrine tumors stratified by tumor size (<2 cm, 2-3 cm and ≥ 3 cm).

Table 5. Association between tumor size and liver metastases-free survival.

Size	LMFS [*]			Univariable Cox regression [†]		
	5-yr (%)	10-yr (%)	Log rank p-value	LM /death	Crude HR [‡]	95% CI [‡]
Overall						
<2 cm	95	87	<0.001	9 of 62	1	Ref. cat.
2-3 cm	98	93		7 of 50	1.46	0.50 – 4.27
≥3 cm	78	50		21 of 41	4.80	2.09 – 11.02
NF-pNET subgroup						
<2 cm	86	86	0.012	5 of 21	1	Ref. cat.
2-3 cm	100	86		3 of 13	1.36	0.29 – 6.28
≥3 cm	71	33		14 of 27	3.21	1.14 – 9.06
Insulinoma subgroup						
<2 cm	100	86	0.046	3 of 40	1	Ref. cat.
2-3 cm	97	97		4 of 37	1.95	0.44 – 8.68
≥3 cm	92	73		8 of 15	5.47	1.45 – 20.63

^{*}Estimated survival percentages are based on the patients with complete data.

[†]Data presented after multiple imputation.

Abbreviations: ^{*}Liver metastases-free survival, [§]CI Confidence Interval, [‡]HR Hazard Ratio, ^{||}LM liver metastases

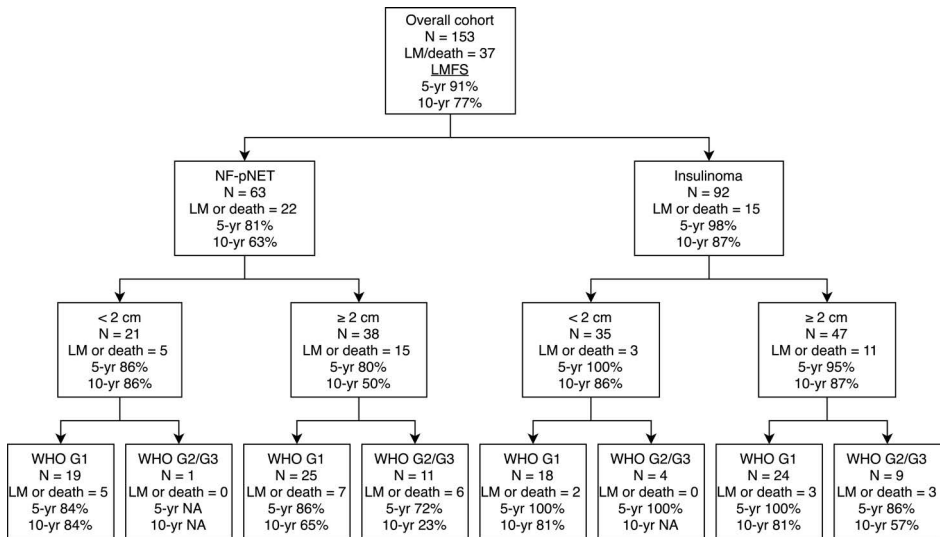


Figure 5. Liver metastases-free survival according to pathology. Abbreviations: LM liver metastases, LMFS liver metastases-free survival, n number of, pNET pancreatic neuroendocrine tumors, NF-pNET non-functioning pancreatic neuroendocrine tumor, WHO World Health Organization.

DISCUSSION

This study shows that patients with a resected MEN1-related NF-pNET had a reduced LMFS compared to those with a resected MEN1-related insulinoma, irrespective the age of surgery and the size and WHO grade of the tumor. These observations suggest differences in underlying tumor origin, development, or biology of MEN1-related pNETs. Postoperative counseling and monitoring of patients during follow-up should, therefore, be tumor type specific and at least include tumor size and WHO grade.

Previous studies hypothesized that patients with MEN1-related insulinomas have favorable prognosis because of small tumor size, early symptomatology with subsequent treatment, or because of differences in grade.^{5,8,18} Indeed, in this study, patients with insulinomas were younger, had a shorter time from diagnosis to surgery, and had smaller pNETs than patients with NF-pNETs. No differences in WHO grade were observed between NF-pNETs and insulinomas. Nevertheless, when adjusted for age at surgery, size, and WHO grade, the risk of liver metastases or death was tripled for patients with a resected NF-pNET compared to those with a resected insulinoma. This indicates that the pathology of NF-pNETs likely is more aggressive.

Tumor size and WHO grade were associated with LMFS, also after adjusting for pNET type and age. Although size has been extensively studied and translated in clinical decision making, the present study observed that size – on a continuous scale – was associated with long-term outcomes and might therefore be used for postoperative counseling.^{12,25,34,40} In line with tumor size on a continuous scale being associated with LMFS, subsequent analyses revealed that especially patients with a resected pNET of at least 3 cm had the highest chance of subsequent liver metastases or death, regardless of pNET functionality. Although a randomized controlled trial is ideally demanded to determine whether surgery has added value over watchful waiting, based on these observations one might hypothesize that a MEN1-related pNET should ideally be resected before the 3cm cutoff is reached. These observations further underscore the importance of accurate size estimations.⁴¹ It has previously been observed within the DMSG database that patients with a resected WHO G2 NF-pNET larger than 2 cm had the highest risk of developing liver metastases.³¹ In the present study, WHO G2 or G3 tumors posed a 2.5 times increased risk for LMFS compared to G1 tumors in pNETs of 2 cm or larger. Although a number of patients with NF-pNETs was included in the previous DMSG study³¹, the present analysis showed that WHO grade is associated with postoperative LMFS also in patients with resected MEN1-related insulinomas, irrespective of age and size. In line, patients with a resected WHO G2 of 2 cm had a reduced 10-year LMFS compared to those with G1 tumor of at least 2 cm (23% versus 65% for NF-pNETs and 57 versus 81% for insulinomas, respectively). Considering the relatively low number of outcomes, multivariable analysis was restricted to age at surgery, size, functionality, and WHO grade. Nevertheless, in univariable analysis, time from diagnosis until surgery was associated with LMFS, specifically in patients with a resected insulinoma. Despite that some CIs (barely) crossed unity, within the subgroups of patients with a resected NF-pNET and insulinoma, higher age at surgery, larger tumors, a pNET of 2 cm or larger, and WHO G2 or G3 increased the risk of liver metastases or death and could, therefore, be used for postoperative counseling.

Mutations in the interacting domains of menin, which affect transcriptional regulation–JunD and checkpoint kinase 1 (CHES1), have been reported to be associated with the prognosis of patients with MEN1-related pNETs, but have not been validated successfully.^{12,16,42,43} More recently, tumor-based transcription factors ARX and PDX1 have been identified as enhancer signatures resembling a distinct alpha (ARX positive) or beta cell (PDX1 positive) subtype differentiation in MEN1-related pNETs. These subtypes subsequently affect prognosis and imply differences in cell lineages of origin responsible for the development of distinct subtypes of pNETs, which justify the present clinical observations.^{44,45} Liver metastases were reported almost exclusively in patients with ARX positive tumors, whereas patients with PDX1 positive tumors had a generally low risk.⁴⁴ Although these immuno-histochemical markers were not studied in the present study, one might reason that a higher

proportion of NF-pNETs will harbor a true alpha cell differentiation, whereas insulinomas will generally resemble a beta cell differentiation. A small subgroup of insulinomas – which developed liver metastases – possibly harbors an alpha cell differentiation. This should be investigated in future studies in MEN1-related pNETs within a large and international cohort with surgical specimen collection and adequate follow-up. In addition, unraveling these differences in tumor biology might lead to subtype specific size cutoffs for operative resection.

Apart from long-term outcomes, pathological characteristics might reflect the more aggressive behavior of NF-pNETs, because of early spread to regional lymph nodes. Within the 73 patients with lymph nodes removed, metastatic lymph nodes were more often observed in NF-pNETs compared to insulinomas (56% versus 10%) and in those with a pNET of 2 cm or larger (39% versus 16%). Patients with lymph node metastases had an almost three times higher risk of liver metastases or death than patients without lymph node metastases, which is supported by 10-year LMFS probabilities of 51% in the entire cohort and 44% in the subgroup of NF-pNETs. Although lymph nodes were resected in only 73 patients, it is unlikely that this has influenced the observations, since LMFS was similar between those with tumor negative lymph nodes and those without lymphadenectomy. In at least 40% of patients, no lymph nodes were resected, which might reflect the absence of guideline recommendations regarding lymph node resections in MEN1.^{17,40} European Neuroendocrine Tumor Society guidelines recommend routine dissection of lymph nodes in noninsulinoma pNETs.⁴⁰ Current data might substantiate these recommendations also for patients with MEN1, since only 3 patients with insulinomas had positive lymph nodes compared to 18 patients with NF-pNETs. Nevertheless, only 5 patients (3%) – all in the NF-pNET group – had a suspicion of lymph node metastases on preoperative conventional imaging. ⁶⁸Ga labelled positron emission tomography (PET)/CT might overcome the limitations of conventional imaging in this matter.^{46,47} Nevertheless, the diagnostic, prognostic, and therapeutic implications of pNET-related lymph node metastases in patients with MEN1 should be investigated in future studies.

The major strength of the present study is that it represents the largest cohort of patients with resected MEN1-related pNETs to date. Histopathological data were available by including surgically treated patients, which has provided the unique opportunity to adjust for and study tumor size and grade. Patients were included over a recent period where MEN1 patients are screened and followed according to guidelines.¹⁷ Missing data were retrieved as far as possible and otherwise handled using multiple imputation – generating a sufficient number of datasets – which is currently considered as the best available statistical method.^{35,48,49} In addition, several statistical analyses, including Kaplan-Meier and Cox proportional hazard regression, were conducted to derive statistically sound conclusions. Despite the low prevalence of MEN1 and relatively low event rate, even multivariable

analyses were performed. Nevertheless, a larger study population would enable more extensive multivariable analyses. Data from patients undergoing surgery for NF-pNETs in centers not included in the DMSG were not available, which is the main limitation of the present study. Inclusion of those patients could have led to a more homogeneous cohort. In addition, by including only patients undergoing surgery, the question remains as to whether the results are generalizable for patients not being exposed to operative resection. Patients with other MEN1-related duodenopancreatic neuroendocrine tumors (dpNETs), such as gastrinomas or rare functioning pNETs were not included. Furthermore, determining the origin of liver metastases is challenging considering the multifocality of dpNETs in MEN1, eg, only 2 of the 6 patients with a resected insulinoma had recurrent hypoglycemia at the time of liver metastases. The exact number of resected and metastatic lymph nodes was unknown, and therefore, patients were grouped stratified into metastases, no metastases or no lymph nodes resected regardless of the number of lymph nodes analyzed. Imaging (ie, presence of liver metastases) and histopathological specimens (ie, WHO grade) were not centrally collected and reassessed for the purpose of this study.

Pre and postoperative localization of insulinomas is challenging in the presence of diffuse background adenomatosis in MEN1. In the preoperative setting, ^{68}Ga -DOTA-Exendin-4 PET/CT can successfully localize insulinomas in MEN1.⁵⁰ Postoperatively, immunohistochemistry is still the most widely available and used method to identify the insulinoma. Most sporadically occurring insulinomas express insulin.⁵¹ Nevertheless, in MEN1, multiple pNETs might show immunoreactivity for insulin, and insulinomas might be negative for insulin.^{51,52} Insulin negative insulinomas were observed in 3, all of whom were biochemically cured, and multiple insulin immunopositive pNETs were encountered in 24. Insulinomas show positive immunohistochemistry signals specifically for PDX1.⁴⁴ Therefore, PDX1 might be additionally used or replace insulin immunohistochemistry to overcome limitations encountered in clinical practice.

The differences in prognosis after surgery for MEN1-related pNETs of direct clinical importance for postoperative patient counseling and monitoring during follow-up, regarding intensity and use of diagnostic modalities, to optimize care in MEN1. Based on the present data, at least tumor functionality, tumor size, and grade should be taken into account during postoperative MEN1 care. Patients with resected insulinomas – especially if small and WHO G1 – can be counseled about the low likelihood of metastases, and the aim of the follow-up should be the detection and follow-up of new (NF)-pNETs. The follow-up of MEN1-related NF-pNETs should focus on identifying metastatic disease. Regardless of functionality, those with WHO G2 or G3 or large tumors have an increased risk. In patients with an increased risk of liver metastases, ^{68}Ga labelled PET/CT might be used to identify metastatic disease to enable timely initiation of adjuvant therapy. Furthermore, regardless of tumor origin, patients should be counseled about the risk of recurrence after 5 or 10

years after surgery, which additionally underscores long-term follow-up of patients with resected MEN1-related pNETs. Whether these observations will alter the currently accepted 2 cm criterion should be investigated in future studies. Additional studies should investigate the optimal surgical strategy and determine the added value of routine lymphadenectomy for the individual MEN1 patient, taking long-term oncological outcomes, survival, future occurrence of clinically relevant dpNETs, and postoperative complications as well as pancreatic function and quality of life into account.

In conclusion, patients with resected MEN1-related NF-pNETs have a lower LMFS than those with insulinomas. These tumors should therefore be regarded as distinct entities of MEN1-related pNETs. Postoperative counseling and follow-up regimens should be subtype specific and, additionally at least, be guided by size and WHO grade.

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Supplementary Table 1. Long-term outcomes after surgery for MEN1-related pNETs.

Characteristic	Overall (n = 153)	NF-pNET [§] (n = 61)	Insulinoma (n = 92)	p-value
Follow-up in years, median [range]	8.8 [0.3 – 25.3]	8.5 [0.3 – 21.8]	9.4 [1 – 25.3]	0.999
Overall survival probability estimates				
5-year, % (95% CI [†])	96% (91% - 99%)	92% (80% - 96%)	99% (92% - 100%)	0.241 [*]
10-year, % (95% CI [†])	91% (84% - 95%)	87% (75% - 93%)	93% (83% - 96%)	
Liver metastases-free survival probability estimates				
5-year, % (95% CI [†])	91% (84% - 95%)	81% (68% - 88%)	98% (91% - 100%)	0.002 [*]
10-year, % (95% CI [†])	77% (66% - 83%)	63% (42% - 76%)	87% (72% - 91%)	
Development of pNET -related LM [‡] (%)	21 (14%)	15 (25%)	6 (7%)	0.001
Death (%)	22 (14%)	11 (18%)	11 (12%)	0.294
pNET -related LM [‡] or death (%)	37 (24%)	22 (36%)	15 (16%)	0.005
Age at death, median [range]	52 [21 – 77]	52 [33 – 77]	51 [21 – 76]	0.653
Age at pNET -related LM [‡] or death, median [range]	51 [21 – 77]	53 [33 – 77]	50 [21 – 76]	0.614
Time from surgery until pNET -related LM [‡] or death, median [range]	7.5 [0.3 – 21.8]	5.3 [0.3 – 21.8]	9.5 [1.7 – 20.8]	0.036

^{*}log rank p-value for comparison of Kaplan Meier curves.

Abbreviations: [†]CI Confidence interval, [‡]LM Liver metastases, [§]NF-pNET non-functioning pancreatic neuroendocrine tumor, ^{||}pNET pancreatic neuroendocrine tumor

Supplementary Table 2. Causes of death.

NF-pNET [‡] (n = 11, 18%)		Insulinoma (n = 11, 12%)	
Cause of death	n (%)	Cause of death	n (%)
MEN1-related	8 (73%)	MEN1-related	5 (45%)
• Metastasized pNET [§]	3 (38%)	• Metastasized pNET [§]	3 (60%)
• Metastasized dpNET [*]	1 (13%)	- NF-pNET [‡]	2 (67%)
• Complications pNET [§] surgery	2 (25%)	- Glucagonoma	1 (33%)
• Metastasized thymicNET	2 (25%)	• Metastasized thymicNET [†]	1 (20%)
		• Radio-induced pituitary sarcoma	1 (20%)
Non MEN1-related	3 (38%)	Non MEN1-related	4 (36%)
• Cerebrovascular accident	1 (33%)	• Metastasized pancreatic adenocarcinoma	1 (25%)
• Physical decline	1 (33%)	• Metastasized adenocarcinoma unknown origin	1 (25%)
• Elderly	1 (33%)	• Pulmonary embolism	1 (25%)
		• Pneumonia (had metastatic pNET [§])	1 (25%)
		Unknown	2 (18%)

Abbreviations: ^{*}dpNET duodenopancreatic neuroendocrine tumor, [†]NET neuroendocrine tumor, [‡]NF-pNET non-functioning pancreatic neuroendocrine tumor, [§]pNET pancreatic neuroendocrine tumor

Supplementary Table 3. Sensitivity analysis.

Characteristic	Multivariable analysis (Adjusted Hazard Ratio)	
	Hazard ratio	95% CI*
Size largest pNET [‡] (per mm)	1.02	1.01 – 1.03
Tumor functionality		
Insulinoma	1	Ref. cat.
NF-pNET [†]	2.56	1.18 – 5.53
Lymph node status		
No metastases	1	Ref. cat.
None resected	1.14	0.49 – 2.64
Metastases	1.39	0.50 – 3.89

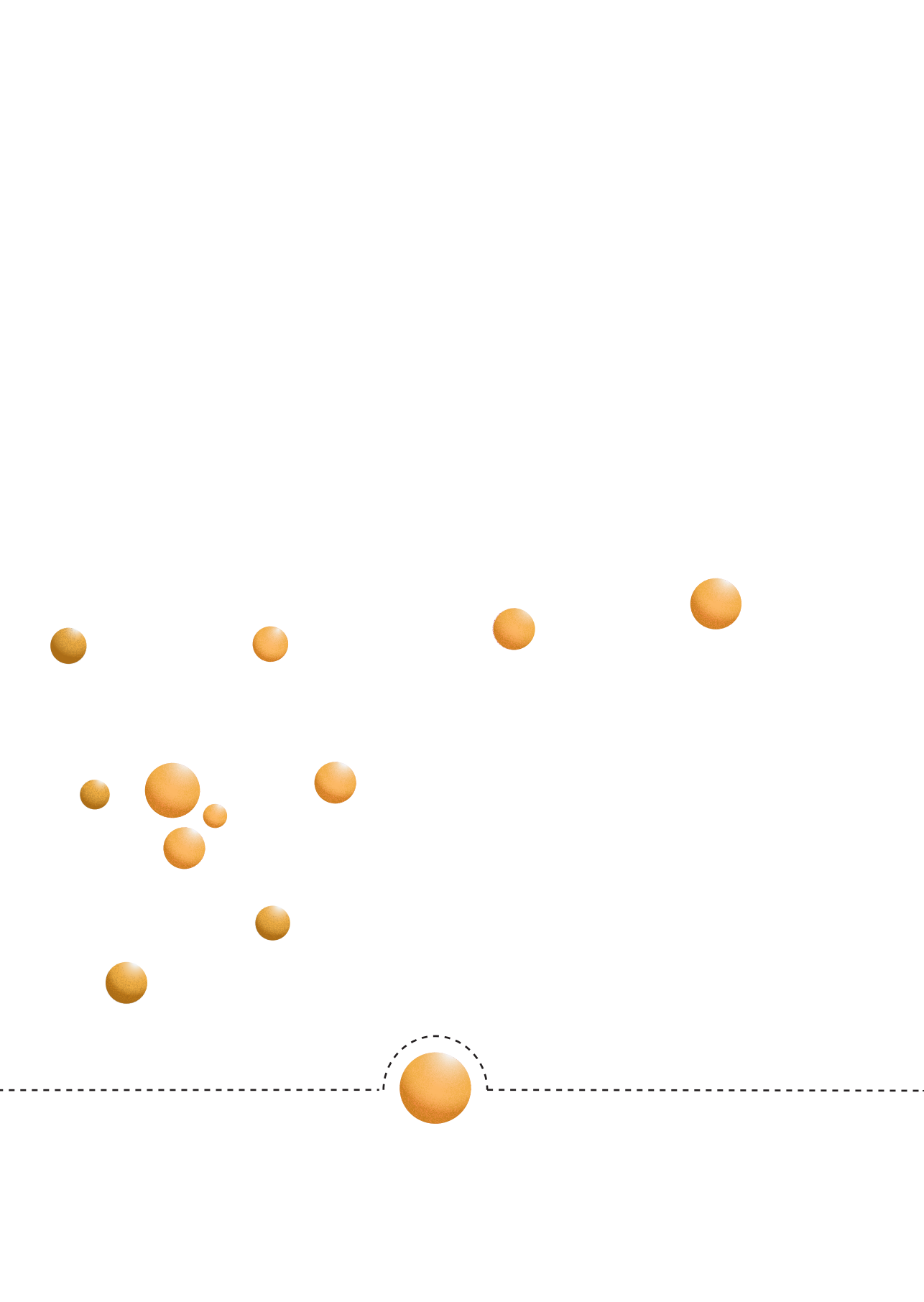
Data reported after multiple imputation.

Multivariable analysis included all factors listed above.

Abbreviations: *CI Confidence Interval, [†]NF-pNET non-functioning pancreatic neuroendocrine tumor, [‡]pNET pancreatic neuroendocrine tumor

PART IV

SURGICAL THERAPY



CHAPTER IX

Surgery for multiple endocrine neoplasia type 1-related insulinoma: long-term outcomes in a large international cohort

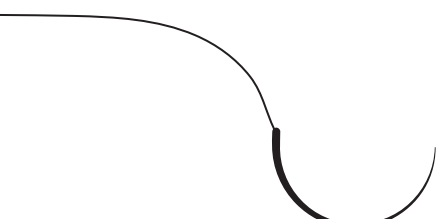
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D.J. van Beek*, S. Nell*, H.M. Verkooijen, I.H.M. Borel Rinkes, G.D. Valk, M.R. Vriens

* Both authors contributed equally to this manuscript

International MEN1 Insulinoma Study Group

P. Goudet (on behalf of the French Endocrine Tumor Study Group (GTE)), A. Vella, D. Donegan,
D.K. Bartsch, J. Manoharan, N.D. Perrier, I. Christakis, M.L. Brandi, R. Zarnegar, E.L. Postma,
E. Kebebew, P. Nockel, L. Brunaud, J.D. Pasternak, W.P. Kluijfhout, C. Sturgeon, S. Giri,
B.A. Bonsing, C.H. van Eijck, H. van Goor, R.H.J. de Kleine, E.J. Nieveen van Dijkum, C.H.C. Dejong



ABSTRACT

Background

Insulinomas are found in 10–15 per cent of patients with multiple endocrine neoplasia type 1 (MEN1) and lead to life-threatening hypoglycaemia. Surgical outcome and the optimal surgical strategy for MEN1-related insulinoma are unknown.

Methods

Patients with MEN1-related insulinomas were identified in 46 centres in Europe and North America between 1990 and 2016. Insulinomas were considered localized if the lesion was in the pancreatic head or body/tail. Patients with pancreatic neuroendocrine tumours throughout the pancreas were suspected of having multifocal insulinoma. The primary outcome was postoperative hypoglycaemia, defined as persistent hypoglycaemia, or recurrent hypoglycaemia caused by a new insulinoma or insulin-producing liver metastases. Hypoglycaemia-free survival was estimated by the Kaplan–Meier method.

Results

Ninety-six patients underwent resection for MEN1-related insulinoma. Sixty-three and 33 patients had localized and multifocal insulinomas respectively. After a median follow-up of 8 (range 1–22) years, one patient (1 per cent) had persistent disease and six (6 per cent) had developed recurrent disease, of whom four had a new insulinoma. The 10-year hypoglycaemia-free survival rate was 91 (95 per cent c.i. 80 to 96) per cent. Of those with localized disease, 46 patients underwent pancreatic resection and 17 enucleation. One of these patients had persistent disease and one developed recurrent insulinoma. Among patients with multifocal disease, three developed new insulinomas and two developed insulin-producing liver metastases.

Conclusion

Surgery for MEN1-related insulinomas is more successful than previously thought.

INTRODUCTION

Insulinoma is a pancreatic neuroendocrine tumour (pNET) that produces insulin and leads to symptomatic and life-threatening hypoglycaemia¹. Currently, surgical resection is the only curative treatment^{2,3}. Some 4–8 per cent of insulinomas are associated with multiple endocrine neoplasia type 1 (MEN1), a rare hereditary disorder occurring in two to three per 100 000 people^{4,5}. Patients with MEN1 develop pNETs with a very high and age-related penetrance. These pNETs are insulin-producing in 10–15 per cent of patients^{6–8}. Patients with MEN1-related insulinoma are often young and have multiple pNETs, making the decision regarding the extent of surgery complicated⁹.

Localization of the insulin-producing pNET is a major challenge in the presence of a diffuse background of non-functioning pNETs (NF-pNETs) in MEN1. Extensive resections were initially proposed, such as 80 per cent resection of the pancreas left of the superior mesenteric/portal vein, with subsequent enucleations of pNETs in the pancreatic head^{8,10}. Although persistent and recurrent hypoglycaemia seem uncommon after this aggressive approach, the procedure is associated with pancreatic insufficiency^{8,10,11}. The sensitivity of CT and MRI has improved, and the availability of endoscopic ultrasonography (EUS) has increased. In addition, glucagon-like peptide-1 receptor (GLP-1R) imaging using ⁶⁸Ga-DOTA-exendin-4 ([Nle¹⁴,Lys⁴⁰(Ahx-DOTA-⁶⁸Ga)NH₂] exendin-4) PET/CT, an emerging localization technique, might overcome the difficulties of localizing MEN1-related insulinomas¹². These advances in preoperative localization raise the question of the optimal surgical procedure for MEN1-related insulinomas, taking both short-term cure of hypoglycaemia and long-term risk of recurrence into account.

The European Neuroendocrine Tumour Society (ENETS) and the MEN1 clinical practice guidelines^{2,3} lack well grounded recommendations regarding the optimal surgical strategy because there is limited evidence. In the case of a single pNET on CT, MRI or EUS, the ENETS guidelines^{2,3} recommend pancreas-sparing surgery, based on two single-centre studies^{13,14} that included only 13 and eight patients with MEN1 respectively. Persistence or recurrence of hypoglycaemia has been reported in 25–50 per cent after enucleation and 2.6–20 per cent after extensive resection^{11,13,15}. Most data are, however, based on old and small single-centre series^{9–11,13,15,16}, hampering comparisons between surgical strategies. In the only population-based cohort study¹⁷, a higher risk of recurrent hypoglycaemia was observed after enucleation (33.3 per cent) than after distal pancreatectomy (8.7 per cent), but 42 per cent of the patients had surgery before 1990. Studies often also failed to differentiate between recurrent hypoglycaemia because of insulin-producing liver metastases and new insulin-producing pNETs.

The aim of this study was to provide evidence for surgical decision-making in patients with MEN1-related insulinoma. The risk of recurrence after pancreatic surgery was investigated in a comprehensive international cohort of patients with MEN1-related insulinoma.

METHODS

Study design and patient selection

This study was an international collaboration between 40 hospitals in Europe and six in North America. Patients with MEN1 were identified in the hospital databases using ICD-9/10 codes for MEN1 and insulinoma. Eligible patients underwent surgery for insulinoma between 1990 and 2016, had a pNET tumour confirmed histologically, and follow-up for at least 1 year after surgery. The MEN1 diagnosis was established either by genetic testing, family history or clinically, according to most recent practice guidelines². Patients who underwent total pancreatectomy and those with distant metastases at diagnosis were excluded. Clinical and demographic data were collected by review of medical records in a standardized manner using predefined definitions. The medical records were examined by an investigator at the collaborating institution and discussed with the coordinating investigators. The study protocol was approved by the medical ethics committees or institutional review boards of all participating centres.

Insulinoma diagnosis

The presence of an insulinoma was based on a positive 72-h supervised fasting test^{18,19}. If no test was performed, the diagnosis was based on symptoms or signs of hypoglycaemia with concomitant biochemical endogenous hyperinsulinaemic hypoglycaemia, according to clinical practice guidelines^{18,19}. The date of diagnosis was based on the date of the supervised 72-h fasting test or the date of symptoms accompanied by endogenous hyperinsulinaemic hypoglycaemia.

Insulinoma localization

The evaluation for MEN1-related insulinoma was dependent on the surgeon's preference and availability of localization techniques in each centre at the time of surgery. Generally, conventional imaging (CT, MRI, EUS) was undertaken before surgery, eventually followed by more invasive techniques, such as arterial (calcium) stimulation venous sampling or GLP-1R receptor imaging. Most importantly, intraoperative findings, based on intraoperative ultrasonography and/or bimanual palpation, were used to localize the insulinoma and subsequently guide intraoperative surgical decision-making.

Based on all preoperative and intraoperative findings, the insulinoma was localized to the pancreatic head or body/tail, or the surgeon suspected multifocal insulinomas in both the head and body/tail. Subsequent surgical decisions were based on whether during surgery the surgeon considered the insulinoma to be localized (suspected to be located in pancreatic head or body/tail) or possibly multifocal (insulinomas in both pancreatic head and body/tail).

Surgical strategy

Patients with localized insulinoma underwent enucleation, Whipple/pylorus-preserving pancreatoduodenectomy (PPPD) or distal pancreatectomy. In these patients, enucleation was compared with resection (distal pancreatectomy or Whipple/PPPD).

Patients with multifocal insulinomas underwent combined procedures involving the pancreatic head and body/tail, including multiple enucleations, distal pancreatectomy plus enucleation of a pNET in the pancreatic head, Whipple/PPPD plus enucleation and Whipple/PPPD plus distal pancreatectomy. In such patients, distal pancreatectomy combined with enucleation of tumour in the head of the pancreas was compared with multiple enucleations, Whipple/PPPD plus enucleation or Whipple/PPPD plus distal pancreatectomy. In addition, patients who underwent one or multiple enucleations were compared with those who had other resections.

Pathologic findings

The total number of pNETs, number of immunohistochemically insulin-positive pNETs in the head and body/tail, size of the largest insulin-positive pNET of the head and body/tail, and number of tumour-positive locoregional lymph nodes were registered.

Postoperative and long-term outcomes

The primary outcome was hypoglycaemia at 3 months after surgery. Patients with hypoglycaemia within 3 months of surgery were considered to have persistent disease. Insulinoma recurrence was defined as recurrence of hypoglycaemia more than 3 months after surgery owing to a new insulinoma in the remaining pancreas. Patients with recurrence of hypoglycaemia and newly diagnosed liver metastases were considered to have insulin-producing liver metastases.

Secondary outcomes were early and late complications after surgery. Postoperative pancreatic fistula (POPF), postpancreatectomy haemorrhage (PPH), bile leak and delayed gastric emptying (DGE) were graded according to the International Study Group of Pancreatic Surgery (ISGPS) classification^{20–23}. Clavien–Dindo grade III–IV postoperative complications within 30 days after surgery, or during the hospital stay, were recorded²⁴. Postoperative mortality was defined as death within 30 days of surgery. Exocrine pancreatic insufficiency

was defined by use of pancreatic enzyme supplementation for at least 6 months. New-onset diabetes was defined as the use of antidiabetic medication for 6 months or more after surgery.

Statistical analysis

Data are presented as median (range) or count (percentage). The time to recurrence of hypoglycaemia and insulinoma was assessed using Kaplan–Meier analysis²⁵, and hypoglycaemia- and insulinoma-free survival probabilities were estimated. Follow-up started on the date of insulinoma surgery and ended on the date of hypoglycaemia or insulinoma recurrence, last follow-up or death. Analyses were done separately for patients with localized *versus* multifocal disease, and for patients with one or more enucleation(s) and those who had other resections (all resections other than one or more enucleation(s)). Statistical analyses were undertaken using SPSS® version 25.0 (IBM, Armonk, New York, USA).

RESULTS

A total of 159 patients with MEN1 were identified, of whom 96 met the inclusion criteria. Sixty-three patients did not meet the inclusion criteria for the following reasons: no surgery (15), liver metastases at diagnosis (6), no proper diagnosis (3), follow-up less than 1 year (34), total pancreatectomy (4) or no details on surgical procedure (1). Demographics and clinical characteristics of the cohort are shown in Table 1. Median age at diagnosis was 30 (range 5–81) years and 15 of the 96 patients (16 per cent) were younger than 21 years at diagnosis. There were 58 female patients (60 per cent). The insulinoma diagnosis was confirmed following a 72-h fasting test in 64 patients (67 per cent).

Preoperative imaging and type of surgery

Preoperative imaging results and surgical strategies are shown in Table 1 and Fig. 1. The insulinoma was localized in 63 patients (66 per cent), and these patients underwent typical resections: single enucleation (17), distal pancreatectomy (41) or Whipple/PPPD (5). The disease was considered multifocal in 33 patients (34 per cent), leading to combined pancreatic resections, of which the majority (26 of 33) were distal pancreatectomies with enucleation of tumour in the pancreatic head. Clinical characteristics of patients undergoing one or multiple enucleations and other resections are shown in Supplementary Table 1. Twenty-nine of 48 patients operated before 2006 were considered to have localized insulinoma compared with 34 of 48 who had surgery from 2006 onwards (Supplementary Table 2). Of 92 patients with preoperative imaging, 45 (49 per cent) had a solitary pNET on imaging. Despite having a solitary pNET on imaging, 14 of these patients underwent resection for

multifocal disease, based on invasive imaging or intraoperative findings. Ten had distal pancreatectomy with enucleation, one had Whipple/PPPD with enucleation and three had multiple enucleations.

Table 1. Baseline characteristics of multiple endocrine neoplasia type 1 study cohort undergoing insulinoma surgery

	Overall (n = 96)	Localized insulinoma (n = 63)	Multifocal insulinoma (n = 33)
Age at surgery (years)*	32 (6–82)	32 (6–82)	31 (13–62)
Aged less than 21 years at insulinoma surgery			
Yes	15 (16)	8 (13)	7 (21)
No	81 (84)	55 (87)	26 (79)
Sex ratio (M:F)	38:58	24:39	14:19
Diagnosis			
Fasting test	64 (67)	43 (68)	21 (64)
Clinical and biochemical	32 (33)	20 (32)	12 (36)
No. of pNETs on conventional imaging (n = 91)			
0	4 (4)	3 (5)	1 (3)
1	45 (49)	31 (52)	14 (45)
2	19 (21)	12 (20)	7 (23)
≥3	23 (25)	14 (23)	9 (29)
Distribution of pNETs on conventional imaging (n = 92)			
None	4 (4)	3 (5)	1 (3)
Head only	11 (12)	6 (10)	5 (16)
Body/tail only	51 (55)	39 (65)	12 (38)
Multifocal (head and body/tail)	26 (28)	12 (20)	14 (44)
Size of largest pNET on preoperative imaging (mm)* (n = 79)	20 (4–90)	20 (4–60)	22.5 (10–90)
pNET ≥ 2 cm on preoperative imaging (n = 81)			
None	36 (44)	26 (50)	10 (34)
Head	13 (16)	6 (12)	7 (24)
Body/tail	29 (36)	19 (37)	10 (34)
Head and body/tail	3 (4)	1 (2)	2 (7)
Suspected lymph node metastases on preoperative imaging (n = 92)	1 (1)	1 (2)	0 (0)
Time interval of surgery			
1990–2006	48 (50)	29 (46)	19 (58)
2006–2016	48 (50)	34 (54)	14 (42)
Type of resection			
Enucleation	17 (18)	17 (27)	0 (0)
Multiple enucleations	3 (3)	0 (0)	3 (9)
Distal pancreatectomy	41 (43)	41 (65)	0 (0)
Distal pancreatectomy and enucleation	26 (27)	0 (0)	26 (79)
Whipple/PPPD	5 (5)	5 (8)	0 (0)
Whipple/PPPD and enucleation	2 (2)	0 (0)	2 (6)
Whipple/PPPD and distal pancreatectomy	2 (2)	0 (0)	2 (6)

Values in parentheses are percentages unless indicated otherwise; *values are median (range). pNET, pancreatic neuroendocrine tumour; PPPD, pylorus-preserving pancreatoduodenectomy.

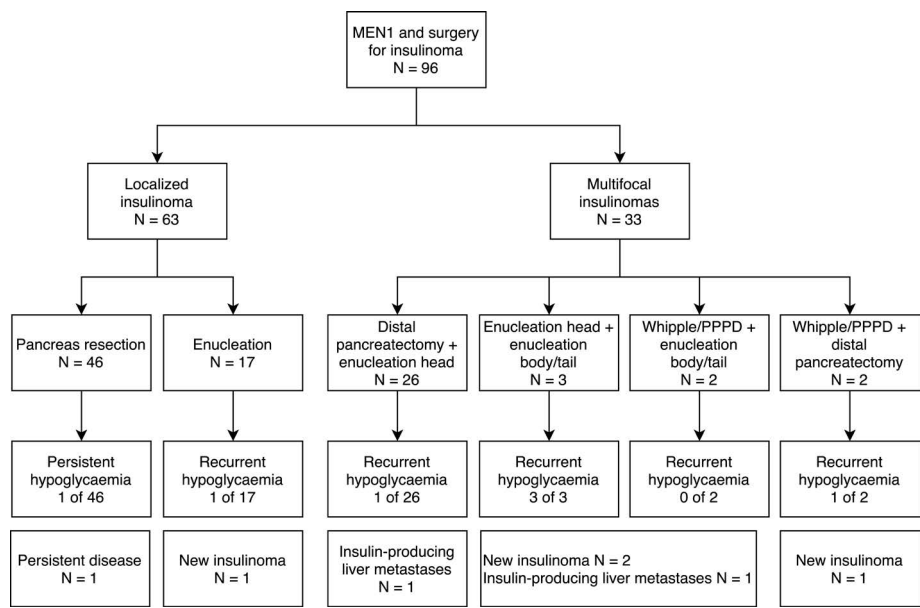


Figure 1. Study flow chart showing insulinoma location, surgical procedures and postoperative hypoglycaemia. Abbreviations: *MEN1* Multiple endocrine neoplasia type 1, *N* number of, *PPPD* pylorus-preserving pancreaticoduodenectomy.

Pathology

The median number of pNETs resected was 3 (range 1–74), of which a median of 1 (0–4) was insulin-positive. Histopathological reports for four patients could not be retrieved, and detailed information regarding the exact number of insulin-positive pNETs was not available for another 17 patients. Three patients had no insulin-positive pNETs, all of whom were cured by surgery. A single insulin-positive pNET was observed in 40 of 50 patients with a localized insulinoma. Preoperative conventional imaging and outcomes of histology in patients with localized disease are summarized in Supplementary Table 3. Among 25 patients with multifocal insulinomas, one, two and at least three insulin-positive pNETs were observed in 12, eight and four respectively, and one patient had no insulin-positive pNET. Eight of these patients had an insulin-positive pNET in the pancreatic head and in the body/tail (Supplementary Table 4). In the remaining eight patients with multifocal disease, no detailed information regarding insulin staining was available. Among the patients with a solitary pNET on imaging but the finding of multifocal disease during surgery, five of 11 had multifocal insulin-positive pNETs. Lymph nodes were resected in 45 patients and were tumour-positive in seven, but as insulin immunohistochemistry of metastatic lymph nodes was not undertaken routinely, the exact source could not be assessed.

Table 2. Procedure-specific outcomes after insulinoma surgery in patients with multiple endocrine neoplasia type 1

	Localized		Multifocal			
	Enucleation (n = 17)	Whipple/PPPD (n = 5)	Distal pancreatectomy (n = 41)	Distal pancreatectomy + enucleation head (n = 26)	Enucleation body/tail + enucleation head (n = 3)	Whipple/PPPD + Enucleation body/tail (n = 2) + Distal pancreatectomy (n = 2)
Early postoperative outcomes						
ISGPS grade B/C complications						
POPF	3 of 16	0 of 4	6 of 36	5 of 24	1 of 3	1 of 2
B	3	-	4	2	1	1
C	0	-	2	3	0	0
DGE	0 of 16	0 of 4	3 of 36	1 of 24	1 of 3	0 of 2
PPH	0 of 16	0 of 4	0 of 36	0 of 24	0 of 3	0 of 2
Bile leak	-	0 of 4	-	-	-	0 of 2
Other Clavien-Dindo grade III-IV complication	0 of 13	1 of 4	2 of 27	1 of 24	0 of 2	0 of 2
Death	0 of 17	0 of 5	0 of 41	0 of 26	0 of 3	0 of 2
Duration of hospital stay (days)* (n = 57)	8.5 (4 – 12)	19 (14 – 23)	9 (3 – 25)	15 (7 – 53)	-	15 (15 – 15)
Readmission	3 of 14	0 of 4	3 of 36	2 of 25	1 of 3	0 of 2
Long-term outcomes						
Endocrine or exocrine insufficiency	0 of 17	1 of 5	14 of 41	7 of 26	0 of 3	2 of 2
Exocrine insufficiency	0 of 17	1 of 5	1 of 41	2 of 26	0 of 3	2 of 2
New-onset diabetes	0 of 17	0 of 5	13 of 41	5 of 26	0 of 3	0 of 2
Development of liver metastases	0 of 17	0 of 5	3 of 41	2 of 26	1 of 3	0 of 2
Death	0 of 17	1 of 5	3 of 41	4 of 26	1 of 3	0 of 2
Follow-up (years)	5 (1 – 22)	9 (7 – 13)	8 (1 – 22)	8 (1 – 21)	18 (10 – 20)	3 (1 – 5)
						11 (8 – 14)

* Values are median (range). PPPD, pylorus-preserving pancreatoduodenectomy; ISGPS, International Study Group of Pancreatic Surgery; POPF, postoperative pancreatic fistula; DGE, delayed gastric emptying; PPH, postpancreatectomy haemorrhage.

Intraoperative data, postoperative complications and hospital stay

Median duration of operation was 225 (range 43–440) min, and median blood loss was 200 (0–4150) ml. Median operating time was shorter (133 versus 244 min) and there was less blood loss (50 versus 250 ml) in enucleations compared with other procedures. Postoperative outcomes are shown in Table 2. There was no postoperative death. ISGPS grade B/C POPF, DGE, PPH and bile leak developed in 16 of 87 (18 per cent), five of 87 (6 per cent), none of 87 and none of nine patients respectively. No relevant differences were observed between the procedures. Median hospital stay was 10 (range 3–53) days, and ten of 85 patients (12 per cent) were readmitted. Patients who underwent Whipple/PPPD alone (4) or distal pancreatectomy with enucleation (15) had a relatively long hospital stay: median 19 (14–23) and 15 (7–53) days respectively.

Recurrence of hypoglycaemia and insulinoma

The distribution of patients with postoperative hypoglycaemia is shown in Fig. 1. After a median follow-up of 8 (range 1–22) years, seven patients (7 per cent) had hypoglycaemia. One patient (1 per cent) had persistent hypoglycaemia and six (6 per cent) had recurrent hypoglycaemia. Of those with recurrent hypoglycaemia, four had a new insulinoma and two developed insulin-producing liver metastases. The patient with persistent disease underwent distal pancreatectomy to remove a 5-mm insulinoma with immunohistochemistry positive for insulin. Postoperative EUS showed two lesions in the pancreatic body that were resected 10 months later, resulting in biochemical cure. Estimated 10-year hypoglycaemia-free and insulinoma-free survival rates were 91 (95 per cent c.i. 80 to 96) and 93 (83 to 97) per cent respectively (Fig. 2). The estimated 10-year hypoglycaemia-free survival rate was 96 (84 to 98) per cent for patients with localized insulinoma and 81 (58 to 92) per cent among those with multifocal insulinomas (Fig. 3). Ten patients (10 per cent) had follow-up of less than 2 years, 34 (35 per cent) less than 5 years, 63 (66 per cent) less than 10 years, 81 (84 per cent) less than 15 years, and the remaining 15 patients (16 per cent) had follow-up of 15 years or more. Five of 64 patients (8 per cent) who were diagnosed according to a 72-h fasting test developed recurrent hypoglycaemia and two of 32 who were diagnosed based on clinical criteria. Outcomes for patients who had one or more enucleations versus those with other resections are summarized in Supplementary Table 1 and Supplementary Figure 1. Of the 15 patients aged less than 21 years at the time of surgery, two developed recurrent insulinoma.

Pancreatic insufficiency, liver metastases, and death

Twenty-five patients (26 per cent) developed pancreatic insufficiency (Table 2). Seven patients developed exocrine pancreatic insufficiency, and 18 had new-onset diabetes. None of the patients who underwent enucleation developed exocrine pancreatic insufficiency or

new-onset diabetes. New-onset diabetes was only observed after distal pancreatectomy with or without enucleation, and occurred in 27 per cent of patients. Six patients developed liver metastases, of whom two had insulin-producing liver metastases. Nine patients died during follow-up but no deaths were insulinoma-related.

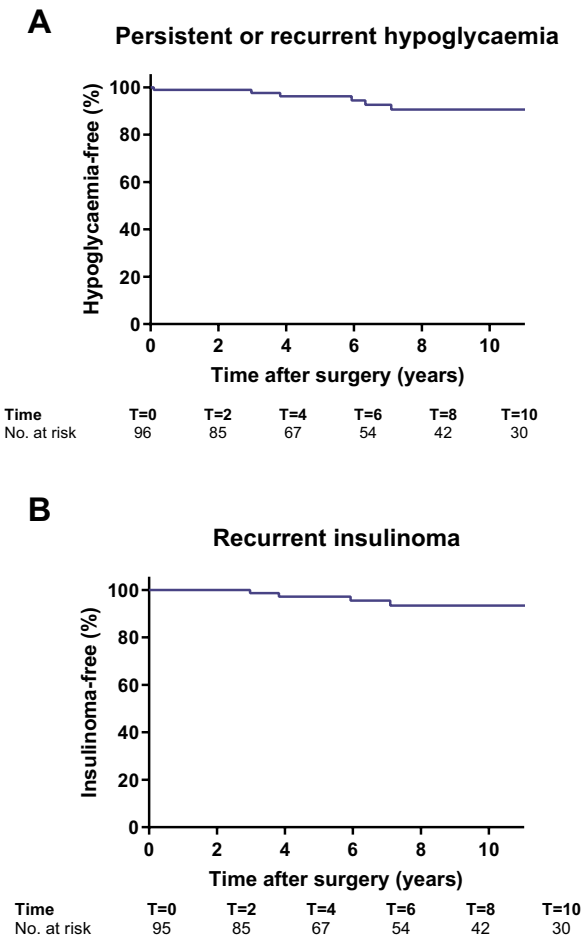


Figure 2. Persistent or recurrent hypoglycaemia- and recurrent insulinoma-free survival after surgery for multiple endocrine neoplasia type 1-related insulinoma (A). Recurrent insulinoma after surgery for MEN1-related insulinoma (B). The dotted lines indicate the 95% confidence interval.

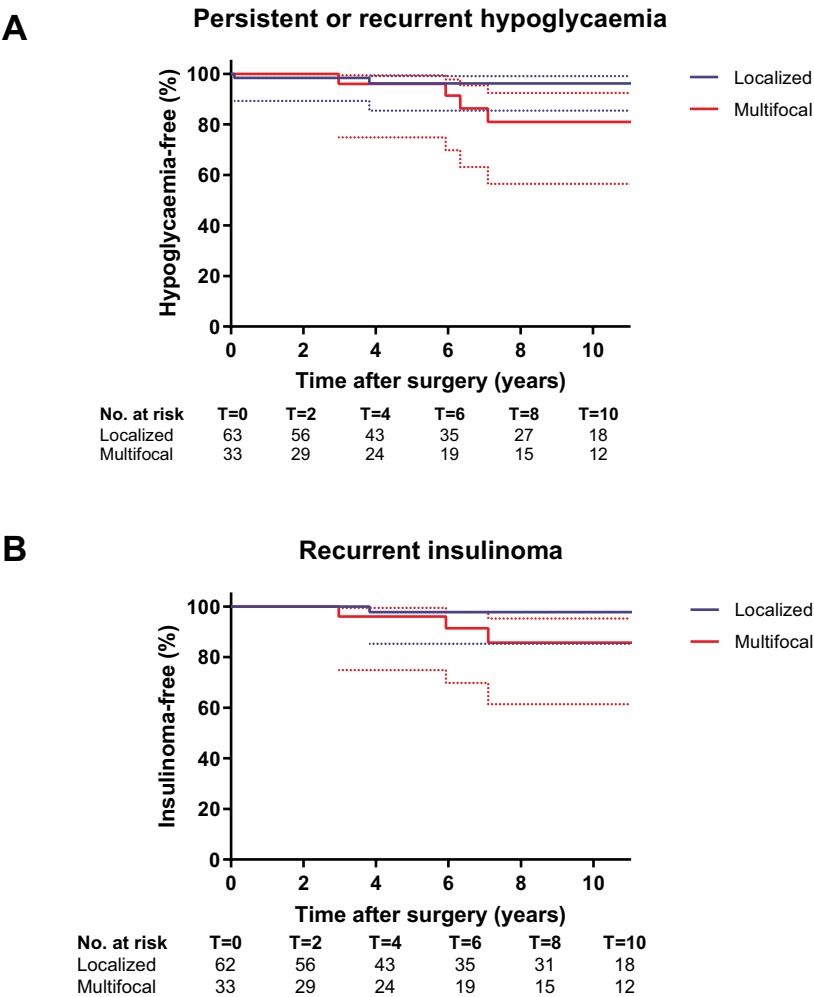


Figure 3. Persistent or recurrent hypoglycaemia- and recurrent insulinoma-free survival after surgery for multiple endocrine neoplasia type 1-related insulinoma, stratified by location of insulinoma (A). Recurrent insulinoma after surgery for MEN1-related insulinoma stratified for patients with likely localized and possibly multifocal insulinoma (B). The dotted lines indicate the 95% confidence interval.

DISCUSSION

These data from a large international cohort showed that surgery for MEN1-related insulinoma was effective as only one patient (1 per cent) had persistent disease. In addition, only four patients (4 per cent) developed a new insulinoma after a median follow-up of

8 years, leading to an estimated 10-year insulinoma-free survival rate of 93 per cent. Enucleation appeared to be the favourable surgical strategy for treatment of localized MEN1-related insulinoma, owing to the absence of pancreatic insufficiency and high rate of symptom resolution. In patients with multifocal disease, distal pancreatectomy combined with enucleation of tumours in the head led to cure of hypoglycaemia, but less extensive resections were also effective in some patients.

In a series¹⁷ from the French Endocrine Tumour Study Group (GTE), 73 patients with MEN1-associated insulinomas were analysed, including a large group of patients who underwent surgery before 1990. After a median follow-up of 9 years, the rate of persistent postoperative hypoglycaemia was 4 per cent and the rate of late recurrence of hypoglycaemia was 14 per cent. Interestingly, the rate of overall persistent or recurrent hypoglycaemia (14 per cent) was much higher than that in the present study (7 per cent). This is likely explained by an improvement in perioperative imaging and operative techniques over the past three decades. More than 40 per cent of patients in the GTE cohort were treated before 1990, whereas the present study only included patients who had surgery from 1990 onwards. A more recent German publication¹³ supports this as late recurrent hypoglycaemia was shown in only one of 13 patients with MEN1 who underwent surgery between 1997 and 2013, and the authors concluded that enucleation and limited resection can provide long-term cure in patients with solitary or dominant tumours.

Current ENETS guidelines advise using pancreas-sparing surgery (enucleation or limited resection) in patients with a solitary insulinoma on MRI, CT or EUS. Although this is reasonable for sporadically occurring insulinomas, patients with MEN1 are often affected by multiple concurrent NF-pNETs which might be missed on conventional imaging²⁶. A solitary pNET was observed on imaging in 45 of 92 patients in the present cohort, but 14 of these patients underwent combined resections. In only five of these patients were insulin-positive pNETs found throughout the pancreas. In addition, the only patient with persistent disease underwent resection of an insulin-positive pNET, but postoperative EUS revealed multiple pNETs, which were not observed on preoperative CT. This underscores that the decision to resect a single lesion after findings on conventional imaging should be made with caution, as limited resections are performed using minimally invasive techniques without the opportunity for intraoperative bimanual palpation^{27,28}. In addition, patients with a solitary lesion on conventional imaging should be counselled about the substantial risk of a complex procedure.

For patients with equivocal preoperative imaging, ⁶⁸Ga-DOTA-exendin-4 PET/CT could improve preoperative insulinoma localization, facilitate minimally invasive surgery and offer better surgical outcomes^{12,29}. Outcomes of arterial stimulation venous sampling, an invasive localization technique, have been reported for only a small number of patients with MEN1; this technique could not adequately localize the insulinoma in all of the patients,

questioning its value in the evaluation of MEN1-related insulinoma^{13,29}. Almost all insulinomas express GLP-1R, offering the opportunity to target these receptors and visualize insulinomas using PET/CT with ⁶⁸Ga-labelled tracer and exendin-4.

In the present study, 33 patients were deemed to have multifocal insulinomas by the operating surgeons, leading to combined resections of which the majority were distal pancreatectomy with enucleation. Patients undergoing this classical approach for MEN1-related insulinomas had a median hospital stay of 15 days. Although all patients who had combined resections were cured of hypoglycaemia, 17 of 25 patients only had an insulin-positive pNET in the head or body/tail. Some of these patients had a pNET of 2 cm or larger, which is nowadays considered as an indication for surgery, but at least a subgroup of the patients with multifocal disease might have undergone resections that were too extensive^{3,30,31}. Prevention of long-term complications is important, because pancreatic insufficiency decreases quality of life in this young and otherwise unaffected population. Insulinoma is often the first manifestation of MEN1 and a common surgical indication in children and adolescents with MEN1, which underscores the importance of long-term pancreatic function^{32–35}. None of the patients in this cohort who underwent enucleation developed endocrine or exocrine insufficiency, which is in line with other studies^{36,37}. New-onset diabetes, on the contrary, was observed after distal pancreatectomies with or without enucleations in 26 per cent of patients. Adequate preoperative localization of the insulinoma can lead to preservation of pancreatic tissue and function.

Enucleation for patients with MEN1 and a localized insulinoma seems preferable if surgically feasible, as it is associated with a high rate of cure of hypoglycaemia, low risk of recurrent disease and absence of long-term complications. The feasibility of enucleation depends on the insulinoma size, location and relation to the main pancreatic duct. In patients with multifocal disease, a more aggressive approach seems advisable based on the present findings, but localization of the insulinoma(s) in these patients is particularly important. Surgical decision-making in patients with multiple pNETs on preoperative imaging should be tailored to the individual patient's needs and guided by the location of the insulinoma. Concurrent large NF-pNETs carry a substantial risk of malignancy and ultimately determine the life expectancy of patients with MEN1^{38–40}. Furthermore, surgeons must be aware of the risk of new-onset diabetes or exocrine pancreatic insufficiency after distal pancreatectomy with or without enucleations of tumours in the head, which was 31 per cent in the present series.

Although curative resection is the recommended therapy for MEN1-related insulinoma, radiofrequency ablation (RFA) has been reported as a successful treatment^{2,3}. The feasibility, efficacy and safety of percutaneous, intraoperative and EUS-guided RFA for pNETs was described in ten patients in 2014⁴¹. Although all patients had a complete ablation, severe complications requiring reintervention were observed in three. More recently, a retrospective

study⁴² from two tertiary referral centres showed that EUS-guided RFA led to complete relief of the symptoms of hypoglycaemia in all of seven patients with insulinomas, of whom one had a MEN1-related insulinoma. Another nine patients with insulinomas and symptom improvement or resolution after EUS-guided RFA were described in the literature review⁴². Complications after EUS-guided RFA have been reported in two of 18 and two of 12 patients with pNETs^{42,43}. Considering the short follow-up (less than 1 year), relatively small pNET size (under 30 mm), selection of patients (those who had either refused surgery or were ineligible for surgery), and the limitations of RFA for pNETs close to surrounding structures or to the pancreatic duct, further comparative studies, ideally RCTs, are needed to clarify the role of EUS-guided RFA for MEN1-related insulinomas. For patients ineligible for surgery, EUS-guided RFA seems a viable alternative.

There are limitations to this study. The retrospective design has known disadvantages, and it was not possible to correct for possible confounding factors influencing surgical strategy, such as age, tumour size, treatment period, centre and localization of the insulinoma. A large prospective observational study or RCT comparing different surgical strategies could overcome this issue, but would be unrealistic owing to the rarity of the disease. Furthermore, identification of insulinoma on pathology is challenging because NF-pNETs might also express insulin and some insulinomas might not express insulin⁴⁴, as also observed here. Future use of enhancer signatures might differentiate more accurately between pNET subtypes in MEN1⁴⁵.

Surgery for MEN1 insulinoma is associated with higher cure rates than previously reported. Enucleation is recommended for MEN1 with a suspected solitary insulinoma if feasible surgically. Distal pancreatectomy combined with enucleation of pancreatic head lesions seems favourable for patients with MEN1 and multiple insulinomas, but this type of resection is probably too extensive for some patients.

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Supplementary Table 1. Patients undergoing enucleation(s) versus other resections

Characteristic	Enucleation(s) (n = 20)	Other resections (n = 76)
Age at surgery in years, median [range]	35 [16 – 74]	31 [6 – 82]
Sex (%)		
Male	9	29 (38.2%)
Female	11	47 (61.8%)
Diagnosis (%)		
Fasting test	15	49 (64.5%)
Clinical and biochemical	5	27 (35.5%)
Number of pNETs on conventional imaging (%), n=91		
0	2	2 (2.8%)
1	14	31 (43.1%)
2	1	18 (25.0%)
≥3	2	21 (29.2%)
Distribution of pNETs on conventional imaging (%), n=92		
None	2	2 (2.7%)
Head only	4	7 (9.6%)
Body/tail only	13	38 (52.1%)
Multifocal (head and body/tail)	0	26 (35.6%)
Size largest pNET on preoperative imaging in mm, median [range], n=79	19 [4 – 90]	20 [7 – 60]
pNET ≥ 2 cm on preoperative imaging (%), n=79	3	30 (46.2%)
Localization (%)		
Likely localized insulinoma	17	46 (60.5%)
Possibly multifocal insulinoma	3	30 (39.5%)
Intra-operative outcomes and complications		
POPF grade B/C (%)	4	12 (15.8%)
DGE grade B/C (%)	1	4 (5.3%)
Other Clavien-Dindo grade III-IV complication (%), n=74	0	4 (6.8%)
Postoperative mortality (%)	0	0 (0%)
Hospital stay in days, median [range], n=57	9 [4 – 14]	13 [3 – 53]
Readmission (%), n=85	4	6 (8.8%)
Pancreatic insufficiency (%)	0	25 (32.9%)
Long-term outcomes		
Follow-up time from surgery until last follow-up in years, median [range]	6 [1 – 22]	8 [1 – 22]
Persistent or recurrent hypoglycaemia (%)	4	3 (3.9%)
Time to persistence or recurrence of hypoglycaemia in years, median [range]	7 [4 – 14]	3 [0 – 6]
Outcomes (%)		
Persistent disease	0	1 (1.3%)
Recurrent insulinoma	3	1 (1.3%)
Insulin-producing liver metastases	1	1 (1.3%)

Supplementary Table 1 continues on page 235

Supplementary Table 1 continued from page 234

Characteristic	Enucleation(s) (n = 20)		Other resections (n = 76)	
Subgroup analysis likely localized versus possibly multifocal				
	Localized (N = 17)*	Multifocal (N = 3)**	Localized (N = 46)	Multifocal (N = 30)
Persistent or recurrent hypoglycaemia	1/17	3/3	1/46	2/30
Time to recurrence of hypoglycaemia in years, median [range]	4	7 [6 – 14]	0	5 [3 – 6]
Outcomes				
Persistent disease	0/17	0/17	1/46	0/30
Recurrent insulinoma	1/17	2/17	0/46	1/30
Insulin-producing liver metastases	0/17	1/17	0/46	1/30

*All of these patients underwent a single enucleation.
**All of these patients underwent two enucleations.
Abbreviations: *DGE* delayed gastric emptying, *pNET* pancreatic neuroendocrine tumour, *POPF* postoperative pancreatic fistula

Supplementary Table 2. Patients undergoing resections before and after 2006.

Characteristic	Before 2006 (n = 48)	2006 and later (n = 48)
Age at insulinoma diagnosis in years, median [range]	35 [5 – 71]	28 [4 – 81]
Age at surgery in years, median [range]	35 [6 – 74]	29 [5 – 82]
Under 21 at insulinoma surgery		
Yes	10	8
No	38	40
Sex		
Male	19	19
Female	29	29
Diagnosis		
Fasting test	35	29
Clinical and biochemical	13	19
Number of pNETs on conventional imaging, n=91		
0	4	0
1	21	24
2	9	10
≥3	9	14
Distribution of pNETs on conventional imaging, n=92		
None	4	0
Head only	6	5
Body/tail only	26	25
Multifocal (head and body/tail)	8	18
Size largest pNET on preoperative imaging in mm, median [range], n=79	20 [7 – 90]	20 [4 – 60]
pNET ≥ 2 cm on preoperative imaging, n=79		
Yes	16	17
No	18	28
Localization		
Likely localized insulinoma	29	34
Possibly multifocal insulinoma	19	14
Type of resection		
Enucleation	6	11
Multiple enucleations	3	0
Distal pancreatectomy	20	21
Distal pancreatectomy and enucleation	14	12
Whipple/PPPD	3	2
Whipple/PPPD and enucleation	0	2
Whipple/PPPD and distal pancreatectomy	2	0
Follow-up time since surgery in years, median [range]	13 [1 – 22]	4 [1 – 9]
Persistent or recurrent hypoglycaemia	6	1
Outcomes		
Persistent disease	1	0
Recurrent insulinoma	4	0
Insulin producing liver metastases	1	1

Abbreviations: ASA American Society of Anaesthesiologists, pNET pancreatic neuroendocrine tumour, PPPD pylorus-preserving pancreatoduodenectomy

Supplementary Table 3. Preoperative imaging and histopathological outcomes of patients with localized insulinomas

	Patients with localized insulinoma in the pancreatic head				
	Total number of pNETs pathology			Number of insulin positive pNETs	
		1	> 1	1	> 1
Number of pNETs in the pancreatic head on imaging	1	5	1	5	-
	> 1	1	1	2	-
	Patients with localized insulinoma in the pancreatic body/tail				
	Total number of pNETs pathology			Number of insulin positive pNETs	
		1	> 1	1	> 1
Number of pNETs in the pancreatic body/tail on imaging	0	2	1	1	-
	1	12	17	22	4
	> 1	2	17	11	2

Of the nine patients who had pancreatic head surgery, 1 had a missing pathology report and 1 had missing data regarding number of insulin positive pNETs.

One of the eight patients with pancreatic head surgery also had a pNET in the pancreatic body/tail on imaging.

Of the 54 patients who had pancreatic body/tail surgery, 2 had no preoperative imaging, 1 had a missing pathology report and 11 had missing data regarding number of insulin positive pNETs.

Eleven of the 51 patients with pancreatic body/tail surgery also had a pNET in the pancreatic head on preoperative imaging.

Abbreviations: pNETs pancreatic neuroendocrine tumours

Supplementary Table 4. Immunohistochemistry outcomes in patients with multifocal insulinomas.

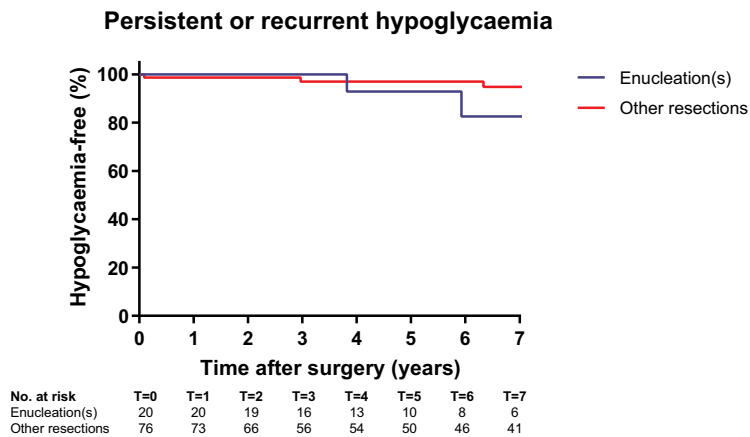
	Number of insulin positive pNETs in the pancreatic body or tail					Multifocal insulin positive
		0	1	2	Total	
Number of insulin positive pNETs in the pancreatic head	0	1	9	3	13	N
	1	3	4	1	8	Y
	2	1*	0	3	4	Y
	Total	5	13	7	25	-
Multifocal insulin positive		N	Y	Y	-	9/25

Abbreviations: N no, pNETs, pancreatic neuroendocrine tumours, Y yes

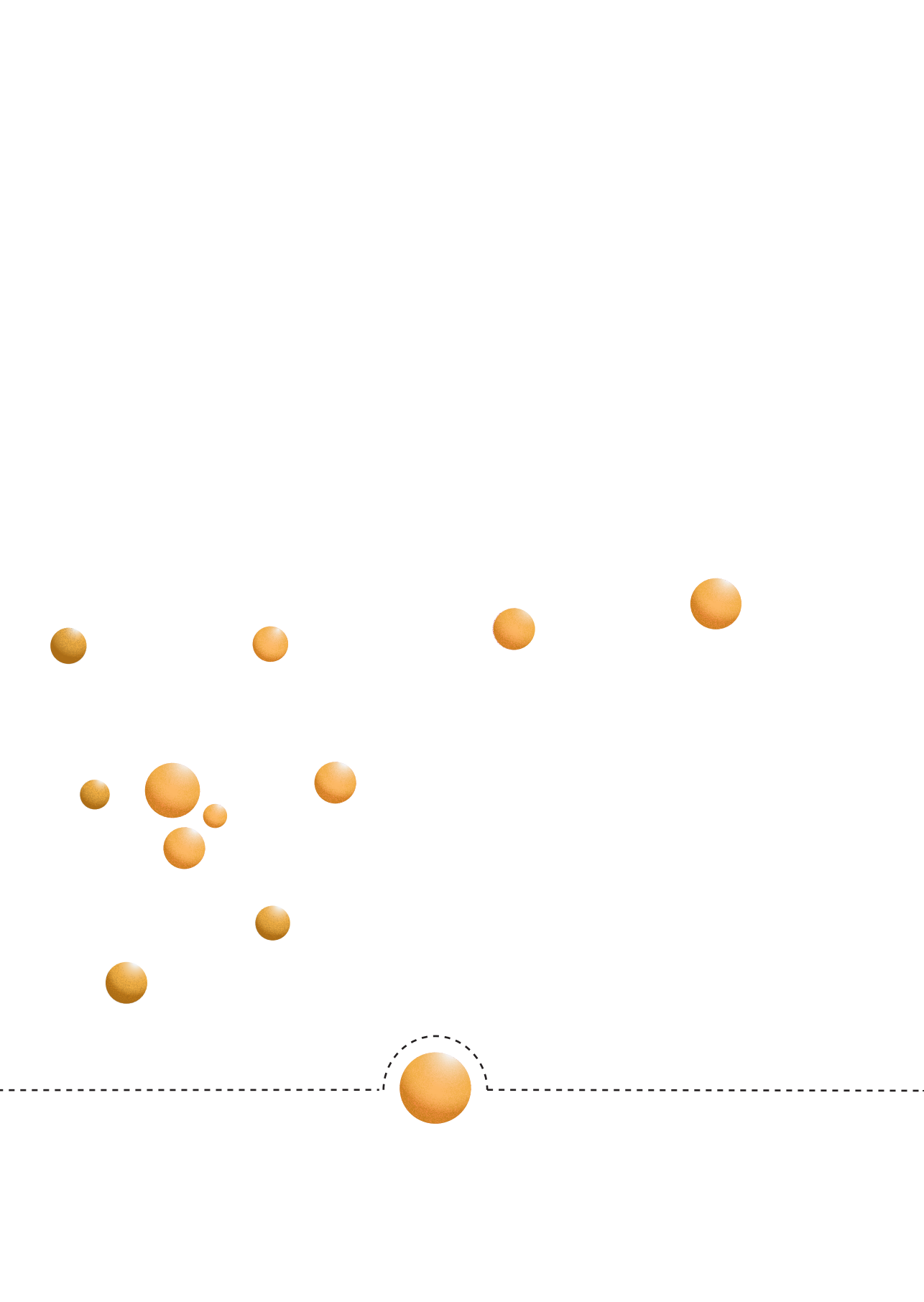
*This patient had 2 enucleations with 2 insulin positive pNETs and is therefore considered as multifocal insulin positive. The group total thus becomes 9.

Eight patients with multifocal disease were excluded from the analysis, because of missing histopathological reports or missing data regarding number of insulin positive pNETs.

Of the patients with 17 patients with only an insulin positive pNET in the pancreatic head or body/tail, 3 had a pNET ≥ 2 cm on preoperative imaging.



Supplementary Figure 1. Persistent and recurrent hypoglycaemia after surgery for MEN1-related insulinoma stratified by extent of surgical resection

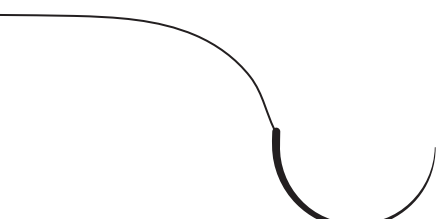


CHAPTER X

Complications after major surgery for duodenopancreatic neuroendocrine tumors in patients with MEN1: results from a nationwide cohort

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D.J. van Beek, S. Nell, W.M.C.M. Vorselaars, B.A. Bonsing, H. van Goor, E.J. Nieveen van Dijkum, C.H.C. Dejong, G.D. Valk (on behalf of the DutchMEN Study Group (DMSG)), I.H.M. Borel Rinkes, M.R. Vriens



ABSTRACT

Background

Little is known about complications after major duodenopancreatic surgery for duodenopancreatic neuroendocrine tumors (dpNETs) in multiple endocrine neoplasia type 1 (MEN1). Therefore, the incidence and severity of complications after major surgery for MEN1-related dpNETs were assessed.

Methods

Patients were selected from the population-based Dutch MEN1 database if they had undergone a Whipple procedure or total pancreatectomy from 2003 to 2017. Complications were graded according to the Clavien-Dindo classification (grade \geq III or higher complications were considered a severe complication) and definitions from the International Study Group of Pancreatic Surgery. The Cumulative Complication Index (CCI) was calculated as the sum of all complications weighted for their severity. Univariable logistic regression was performed to assess potential associations between predictor candidates and a severe complication.

Results

Twenty-seven patients (median age 43 years) underwent a major duodenopancreatic resection, including 14 Whipple procedures and 13 total pancreatectomies. Morbidity and mortality were 100% (27/27) and 4% (1/27), respectively. A severe complication occurred in 17/27 (63%) patients. The median CCI was 47.8 [range 8.7 – 100]. Grade B/C pancreatic fistulas, delayed gastric emptying, bile leakage, hemorrhage and chyle leakage occurred in 7/14 (50%), 10/27 (37%), 1/27 (4%), 7/27 (26%), 3/27 (11%) patients, respectively. Patients with a severe complication had longer operative time and higher blood loss. After Whipple, new-onset endocrine and exocrine insufficiency occurred in 1/13 and 9/14 patients, respectively.

Conclusions

Major duodenopancreatic surgery in MEN1 is associated with a very high risk of severe complications and cumulative burden of complications and should therefore be reserved for a select subgroup of patients with MEN1-related dpNETs.

INTRODUCTION

Metastasized duodenopancreatic neuroendocrine tumors (dpNETs) are the leading cause of death in patients with multiple endocrine neoplasia type 1 (MEN1).^{1,2} Over 80% of patients are diagnosed with a dpNET by the age of 80.^{3,4} The majority of pancreatic tumors are clinically silent and are therefore considered non-functioning pancreatic neuroendocrine tumors (NF-pNETs). Duodenal gastrinomas and pancreatic insulinomas are the most frequently encountered hormone-producing dpNETs in MEN1.⁵ Surgical resection is the only potentially curative therapy. Nevertheless, surgery is not recommended for all patients with MEN1-related dpNETs, because of the low oncological risk of small NF-pNETs and the equivocal surgical indications for duodenal gastrinomas.⁶⁻⁹

The decision to proceed to surgery is a risk-benefit balance analysis guided by the oncological benefits against the risks of potential complications and adverse effects. Disease-related factors as well as the young age and postoperative life expectancy of patients with MEN1-related dpNETs influence the timing and extent of surgery. For those patients with duodenal gastrinomas, multifocal dpNETs, or pancreatic head pNETs unsuitable for enucleation, major duodenopancreatic surgery is demanded. A severe complication (Clavien-Dindo grade III or higher) affects one in three patients undergoing pancreatic surgery for MEN1-related NF-pNETs in The Netherlands.¹⁰ This reflects severe morbidity considering that 80% of the surgical procedures in this cohort included distal pancreatectomies and enucleations.¹⁰ It is to be expected that the most severe complications occur after more extensive duodenopancreatic resections (i.e., Whipple procedure or total pancreatectomy), resulting in an overall morbidity that is even higher.¹⁰

Especially if major duodenopancreatic surgery is demanded, the pros and cons should be carefully weighted, but currently clinicians are confronted by a paucity of data regarding postoperative complications and long-term pancreatic function after major duodenopancreatic surgery in MEN1. In addition to the single most severe complication, complications of lesser severity might be clinically relevant and no studies have assessed the cumulative burden of complications in MEN1. Studies on complications after major duodenopancreatic surgery in patients with MEN1 are limited by the low number of pancreatoduodenectomies, reflecting the rarity of the disease, the single-center design, the non-reporting of complications, or the lack of uniform assessment and grading of complications according to currently appraised definitions and grading systems.¹⁰⁻²³ Therefore, this study aimed to assess the incidence, severity, and cumulative burden of postoperative complications and pancreatic function after major duodenopancreatic surgery in a population-based cohort of patients with MEN1. In addition, we aimed to identify potential pre- and intra-operative factors associated with a severe complication.

PATIENTS AND METHODS

Study Design and Patient Selection

Patients were selected from the Dutch MEN1 database which is owned by the DutchMEN Study Group (DMSG) and has been described in detail before.²⁴ Briefly, patients with MEN1 diagnosed according to clinical practice guidelines and aged 16 years and over were included.⁶ Patients were identified in each center based on hospital databases of medical conditions and diseases. More than 90% of MEN1 patients in the Netherlands are included in the database. Clinical and demographic data were collected longitudinally from 1990-2017 by standardized medical record review, according to a predefined protocol. The protocol was approved by the Medical Ethics Committees of all University Medical Centers.

Patients undergoing an elective major duodenopancreatic resection from 2003 to 2017 were identified. During the study period, major duodenopancreatic resections were performed in six of eight referral centers in The Netherlands by experienced teams consisting of endocrine and hepato-pancreato-biliary (HPB) surgeons.

A total pancreatectomy is considered a total (duodeno)pancreatic resection. Completion (total) pancreatectomies were defined as Whipple or pylorus-preserving pancreato-duodenectomy (PPPD) after previous distal pancreatectomy or enucleation(s), thus all remaining pancreatic tissue was removed. Whipple/PPPD procedures were performed with or without a distal pancreatectomy. To be classified as Whipple/PPPD plus distal pancreatectomy, preservation of at least a part of the pancreatic body or tail was demanded.

Clinical Definitions

Patients were operated on for a NF-pNET in case of a pNET on imaging in the absence of excessive hormone production.⁸ Insulinomas were diagnosed based on a 72-hour fasting test. Gastrinomas were diagnosed based on hypergastrinemia and a gastrin positive (duodenal or lymph node) neuroendocrine tumor.²⁵ In patients with a gastrinoma and pNET on imaging, the resection was considered for a NF-pNET and gastrinoma.

Data regarding preoperative imaging were collected from conventional imaging, i.e. magnetic resonance imaging (MRI), computed tomography (CT), and endoscopic ultrasonography (EUS). From 2014 onwards, data from gallium-68-labeled imaging were obtained. Tumor size was based on conventional imaging with the shortest time before surgery.²⁶ The number of pNETs and presence of lymph node metastases was assessed from both conventional and functional imaging.

Preoperative clinical condition was determined based on the American Society of Anesthesiology (ASA) fitness grade. The duration of surgery was calculated from skin incision until skin closure and the length of stay (LOS) was computed from the day of surgery until the day of discharge. A readmission was defined as a hospital admission for

any surgical complication after discharge. Unplanned intensive care unit (ICU) admission was documented during the initial hospital stay as well as during any readmissions. The number of days on the ICU was calculated from the day of admission until the day of discharge from the ICU. Center volume was defined as high (more than 5 major resections) or low volume (fewer than 5 major resections). Period of surgery was stratified into 2003–2010 and in 2011–2017.

Outcomes

The primary outcome of the study was the occurrence of a severe postoperative complication (Clavien-Dindo grade III or higher), since this indicates the need for surgical, radiological or endoscopic reinterventions.²⁷ All complications (i.e. general and pancreatic surgery-specific complications) were graded according to the Clavien-Dindo classification. Morbidity was defined as any complication during the postoperative course (Clavien-Dindo grade I or higher, i.e. any deviation from the normal postoperative course without requiring interventions or pharmacological treatment other than antiemetics, antipyretics, analgesics, diuretics and electrolytes).²⁷ Mortality included deaths within 90 days after surgery (Clavien-Dindo grade V).²⁷ For every patient, the Comprehensive Complication Index (CCI) score was determined.²⁸ The CCI is calculated as the cumulative sum of all complications weighted for their respective severity and expressed on a continuous scale as a value between 0 (no complication) and 100; patients who die automatically receive a CCI score of 100.²⁸

Secondary outcomes included the presence and severity of pancreatic surgery-specific complications such as postoperative pancreatic fistula (POPF), delayed gastric emptying (DGE), post-pancreatectomy hemorrhage (PPH), bile leak and chyle leak. These were assessed and graded according to definitions and criteria formulated by the International Study Group of Pancreatic Surgery (ISGPS).^{29–33}

Patients had pancreatic insufficiency in case of postoperative new-onset diabetes mellitus (endocrine insufficiency) or if patients demanded treatment with pancreatic enzymes (exocrine insufficiency) for at least 6 months. Patients diagnosed with endocrine or exocrine insufficiency preoperatively were excluded from the analysis of new-onset insufficiency. The duration of medication use for pancreatic insufficiency was calculated from the date of the first prescription until the date of withdrawal or date of last follow-up.

Statistical Analysis

Baseline characteristics are presented as median [range] or counts (percentages). Outcomes are presented for the total cohort and separately for the total pancreatectomy and Whipple/PPPD subgroups. Patients undergoing a total pancreatectomy or completion pancreatectomy were analyzed as total pancreatectomy and patients undergoing a Whipple/PPPD with or without distal pancreatectomy were studied as Whipple/PPPD. Additionally, the Whipple/

PPPD plus distal pancreatectomy subgroup was separately analyzed. Differences in patient, disease, surgical, and intraoperative characteristics were compared between patients with and without a severe complication using Chi-square, Fisher's exact, or Mann–Whitney U tests. Univariable logistic regression was performed to identify factors associated with a severe complication. Variables were selected based on clinical reasoning and included age at surgery (years), sex (female vs. male), center volume (high vs. low), ASA score (1 and 2 vs. 3), tumor size (mm), pNET size ≥ 2 cm (present vs. absent), type of resection (Whipple/PPPD vs. total pancreatectomy), period of surgery (2003–2010 vs. 2011–2017), operative time (minutes), and intraoperative blood loss (mL). Odds ratios (OR) with corresponding 95% confidence intervals (CIs) were calculated. Considering the low number of included patients and low absolute number of patients with a severe complication, multivariable analysis was deemed inappropriate. No missing data were observed for variables assessed by logistic regression. Two-tailed p-values <0.05 were considered statistically significant. Analyses were performed using SPSS version 25.0 (IBM Corporation, Armonk, NY, USA) and R version 3.5.1 (The R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Baseline and Surgical Characteristics

A total of 445 patients were identified in the DMSG database of whom 106 underwent 118 surgical procedures for a dpNET between 1990 and 2018. Twenty-nine patients underwent a major duodenopancreatic resection, of whom two were operated on before 2003 (Fig. 1). Fourteen patients underwent a Whipple/PPPD, and in five of those patients a concurrent distal pancreatectomy was performed, thus leaving a part of the pancreas in situ. Thirteen patients underwent a total pancreatectomy; in four patients, this was considered a completion pancreatectomy after distal pancreatectomy ($n = 2$), enucleation ($n = 1$) or distal pancreatectomy and enucleation ($n = 1$). Two patients underwent a duodenum-preserving total pancreatectomy and one patient underwent a robot-assisted Whipple/PPPD. Twenty-one patients (78%) were operated within two centers and the remaining six patients were treated in four other hospitals.

Patients underwent surgery at a median age of 43 years [range 28 – 75] (Table 1). Ten patients (37%) were 40 years or younger at the time of surgery and the majority of patients (85%; 23/27) had an ASA score of 1 or 2. At the time of surgery, 12 patients (44%) had a suspicion of lymph node metastases, 9 (33%) had a NF-pNET ≥ 2 cm, 3 (11%) had a functioning dpNET, and 3 (11%) had multiple NF-pNETs <2 cm, respectively (Supplementary Table 1). Multiple pNETs on preoperative imaging were observed in 96% (26/27) of patients. The indications for a concomitant distal pancreatectomy were a pNET of ≥ 2 cm on preoperative imaging in two patients, an intraoperatively detected pNET of 18 mm, a 15

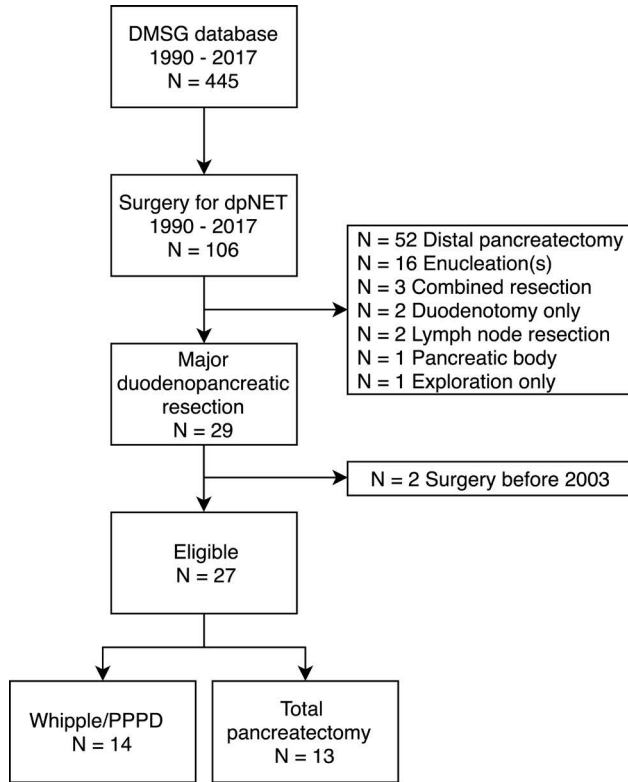


Figure 1. Patient inclusion process. Abbreviations: *DMSG* DutchMEN Study Group, *dpNET* duodenopancreatic neuroendocrine tumor, *N* number of, *PPPD* pylorus-preserving pancreatoduodenectomy.

mm pNET with suspected lymph node metastases on $^{68}\text{Gallium}$ labeled PET/CT, and multiple small pNETs with suspected lymph node metastases.

More procedures were performed from 2011 to 2017 than from 2003 to 2010 (18/27 [67%] vs. 9/27 [33%], respectively). Seventy-one percent (10/14) of the Whipples/PPPD procedures and 62% (8/13) of the total pancreatectomies, of which four were completion pancreatectomies, were performed from 2011 to 2017.

Occurrence of Complications

Intraoperative outcomes and characteristics of hospital stay are shown in Table 2. Morbidity and mortality were 100% (27/27) and 4% (1/27), respectively (Table 3). The only death occurred in a patient 30 days after total pancreatectomy. Although complicated by ascites, thrombocytosis and a magnesium calcium electrolyte disorder, the exact cause of death remained unknown after autopsy.

Table 1. Baseline characteristics

Variable	Overall (n = 27)	Whipple/ PPPD** (n = 14)	TP†† (n = 13)
Age at surgery in years, median [range]	43.2 [27.5 – 75.3]	45.4 [29.5 – 62.5]	42.3 [27.5 – 75.3]
Sex			
Male	14 (52%)	6 (43%)	8 (62%)
Female	13 (48%)	8 (57%)	5 (38%)
Surgery			
Primary surgery	22 (81%)	13 (93%)	9 (69%)
Reoperation	5 (19%)	1 (7%)	4 (31%)
Surgical indication			
NF-pNET	13 (48%)	5 (36%)	8 (62%)
Insulinoma	2 (7%)	0 (0%)	2 (15%)
Gastrinoma	4 (15%)	3 (21%)	1 (8%)
NF-pNET and gastrinoma	8 (30%)	6 (43%)	2 (15%)
ASA [*]			
1	2 (7%)	1 (7%)	1 (8%)
2	21 (78%)	12 (86%)	9 (69%)
3	4 (15%)	1 (7%)	3 (23%)
Number of pNETs* on preoperative imaging (CT [‡] , MRI [§] , EUS [§] , PET [¶])			
0	1 (4%)	1 (8%)	0 (0%)
1	9 (33%)	4 (31%)	5 (39%)
2	16 (60%)	8 (62%)	8 (62%)
≥ 3			
Size of the largest pNET pancreatic head, mm, median [range]	16.5 [3 – 42]	20 [3 – 42]	12 [3 – 40]
Size of the largest pNET pancreatic body/tail, mm, median [range]	15.5 [3 – 35]	14.5 [3 – 35]	18 [5 – 30]
Suspected lymph node metastases on imaging (CT, MRI, EUS, PET)	12 (44%)	9 (64%)	3 (23%)
Type of resection			
Whipple/PPPD	14 (52%)	NA	NA
Whipple/PPPD plus distal pancreatectomy ^{††}	5 (36%)		
Total pancreatectomy	13 (48%)		
Completion pancreatectomy	5 (38%)		
Period of surgery			
2003 – 2010	9 (33%)	4 (29%)	5 (38%)
2011 – 2017	18 (66%)	10 (71%)	8 (62%)
Lymph node resection	22 (81%)	13 (93%)	9 (69%)
Approach			
Conventional	26 (96%)	13 (93%)	13 (100%)
Robot-assisted	1 (4%)	1 (7%)	0 (0%)

^{††} In order to be classified as Whipple/PPPD plus distal pancreatectomy, a part of the pancreatic body or tail had to be left in situ. Abbreviations: ^{*}ASA American Society of Anesthesiology, [‡]CT computed tomography, [§]EUS endoscopic ultrasonography, [§]MRI magnetic resonance imaging, ^{||}NF-pNET non-functioning pancreatic neuroendocrine tumor, [¶]PET positron emission tomography, ^{*}pNET pancreatic neuroendocrine tumor, ^{**}PPPD pylorus-preserving pancreatoduodenectomy, ^{††}TP total pancreatectomy

Table 2. Intraoperative characteristics and hospital stay

	Overall (n = 27)	Whipple/PPPD [†] (n = 14)	Total pancreatectomy (n = 13)
Time of surgery in minutes, median [range]	304 [183 – 480]	315 [198 – 425]	304 [183 – 480]
Blood loss in mL, median [range]	825 [100 – 3350]	500 [100 – 2000]	900 [200 – 3350]
Length of stay in days, median [range]	16 [7 – 291]	16.5 [7 – 78]	16 [10 – 291]
Re-laparotomy	3 (11%)	1 (7%)	2 (15%)
ICU [‡] admission	7 (26%)	3 (21%)	4 (31%)
Duration of ICU admission in days, median [range]	1 [1 – 46]	8 [1 – 46]	1 [1 – 6]
Readmission	10 (37%)	4 (29%)	6 (46%)
Duration of readmission in days, median [range]	12.5 [2 – 72]	18 [2 – 31]	12.5 [5 – 72]
ICU during readmission	5 (19%)	2 (14%)	3 (23%)
ICU during readmission duration in days, median [range]	4 [2 – 69]	2 [2 – 2]	10 [4 – 69]

Abbreviations: [‡]ICU intensive care unit, [†]PPPD pylorus-preserving pancreatoduodenectomy

A severe complication (Clavien-Dindo grade III or higher) occurred in 17 patients (63%), of whom 13 (76%) developed at least one more severe complication. A severe complication occurred in 6/9 (67%) patients after a Whipple/PPPD alone, 5/5 (100%) patients after a Whipple/PPPD plus distal pancreatectomy, 4/8 (50%) patients after a total pancreatectomy, and 2/5 (40%) patients after a completion pancreatectomy. All patients who underwent a Whipple/PPPD plus distal pancreatectomy developed multiple severe complications (Table 3). Median hospital stay was 19 days [range 10–291] for patients who developed a severe complication and 14.5 days [range 8–30] for patients without a severe complication.

The CCI[‡] is presented in Fig. 2 and Table 3. The overall median CCI[‡] was 47.8 [range 8.7–100] and the median CCI[‡] was higher for patients in the Whipple/PPPD group (50.6 [range 8.7–95.3]) compared with patients in the total pancreatectomy group (32.0 [range 12.2–100]). A CCI[‡] ≥ 50 was observed in seven patients (50%) after a Whipple/PPPD and five (38%) after a total pancreatectomy. The CCI[‡] of individual patients is visualized in Supplementary Fig. 1.

Occurrence of Pancreatic Surgery-Specific Complications According to the International Study Group of Pancreatic Surgery Definitions and Grading

Pancreatic surgery-specific complications according to the ISGPS definitions and grading are presented in Table 4. Half of the patients (7/14) undergoing a Whipple/PPPD developed

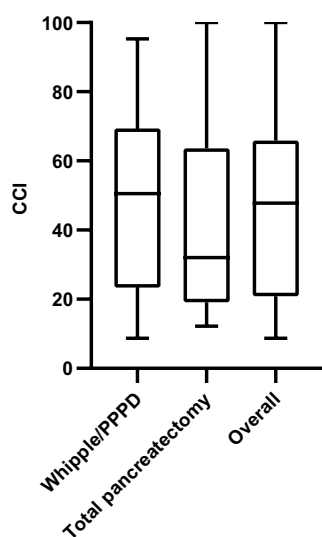


Figure 2. Boxplots of the Cumulative Complication Index (CCI) for the total cohort and stratified by procedure. Abbreviations: CCI Cumulative Complication Index, pylorus-preserving pancreaticoduodenectomy.

a POPE, of whom 6/7 (86%) were grade B. In terms of percentages, more patients in the Whipple/PPPD group had DGE grade C compared with the total pancreatectomy group (5/14 [36%] versus 1/13 [8%], respectively). Grade B/C PPH occurred in 26% (7/27) of patients. Three patients underwent re-laparotomy for PPH – one underwent relaparotomy for a splenic artery bleeding in an emergency setting (hypovolemic shock and resuscitation), one had a resection of the jejunum for severely bleeding ulcers, and one had evacuation of multiple hematomas. The source of PPH could be adequately coiled in three patients and one patient was managed conservatively. Within the patients who underwent a Whipple/PPPD plus distal pancreatectomy, POPE, DGE, bile leakage, PPH, and chyle leakage occurred in 4/5 (80%), 5/5 (100%), 1/5 (20%), 2/5 (40%) and 0/5 (0%) patients (Table 4).

Factors Associated with a Severe Complication

Regarding preoperative characteristics, a severe complication was more frequently observed in males compared with females (65% vs. 35%, $p = 0.09$) and after a Whipple/PPPD compared with a total pancreatectomy (65% vs. 35%, $p = 0.09$) (Supplementary Table 2). No meaningful differences were observed for age at surgery, tumor size, the presence of a pNET ≥ 2 cm, ASA score, center volume and period of surgery (Supplementary Table 2). Patients with a severe complication had a longer operative time (356 vs. 265.5 minutes, $p = 0.01$) [OR 1.19, 95% CI 1.05–1.41] per 10 min and intraoperative blood loss was higher in patients developing a severe complication (900 vs. 425 mL, $p = 0.02$) [OR 1.22, 95% CI 1.04–1.59] per 100mL.

Table 3. Severity of postoperative complications according to the Clavien-Dindo classification

Outcomes	Overall (n = 27)	Whipple/PPPD ^s (n = 14)			Total pancreatectomy (n = 13)
		Overall (n = 14)	Whipple/PPPD only (n = 9)	Whipple/PPPD plus distal pancreatectomy (n = 5)	
Clavien-Dindo grade I	4 (15%)	2 (14%)	2 (22%)	0 (0%)	2 (15%)
II	6 (22%)	1 (7%)	1 (11%)	0 (0%)	5 (29%)
III A	6 (22%)	5 (36%)	2 (22%)	3 (60%)	1 (8%)
III B	1 (4%)	1 (7%)	0 (0%)	1 (20%)	0 (0%)
IV A	7 (26%)	4 (29%)	3 (33%)	1 (20%)	3 (23%)
IV B	2 (7%)	1 (7%)	1 (11%)	0 (0%)	1 (8%)
V	1 (4%)	0 (0%)	0 (0%)	0 (0%)	1 (8%)
Clavien-Dindo grade III or higher complication	17 (63%)	11 (79%)	6 (67%)	5 (100%)	6 (46%)
Multiple Clavien-Dindo grade III or higher complications	13 (48%)	9 (64%)	4 (44%)	5 (100%)	4 (31%)
Clavien-Dindo grade III	7 (26%)	6 (43%)	2 (22%)	4 (80%)	1 (8%)
Clavien-Dindo grade III specified	POPF ^r (1) POPF + anastomotic leakage (1) POPF + DGE ^r (1) POPF + bile leakage (1) POPF + PPH ^r + abdominal abscess + wound infection/abscess (1) Chyle leakage (1) DGE (1)	POPF (1) POPF + anastomotic leakage (1) POPF + DGE (1) POPF + bile leakage (1) POPF + PPH + abdominal abscess + wound infection/abscess (1) DGE (1)	POPF + anastomotic leakage (1) POPF + DGE (1) POPF + bile leakage (1) POPF + PPH + abdominal abscess + wound infection/abscess (1)		DGE (1)
Clavien-Dindo grade IV	9 (33%)	5 (36%)	4 (44%)	1 (20%)	4 (31%)
Clavien-Dindo grade IV specified	PPH (4) Respiratory insufficiency (2) Cardiac tamponade (1) Esophageal perforation + PPH (1) POPF + PPH (1)	PPH (2) Respiratory insufficiency (2) POPF + PPH (1)			PPH (2) Cardiac tamponade (1) Esophageal perforation + PPH (1)

Table 3 continues on page 252

Table 3 continued from page 251

Outcomes	Overall (n = 27)	Whipple/PPPD [§] (n = 14)			Total pancreatectomy (n = 13)
		Overall (n = 14)	Whipple/PPPD only (n = 9)	Whipple/PPPD plus distal pancreatectomy (n = 5)	
Clavien-Dindo grade V	1 (4%)	0 (0%)	0 (0%)	0 (0%)	1 (8%)
Cumulative Complication Index, median [range]	47.8 (8.7 – 100)	50.6 (8.7 – 95.3)	35.7 (8.7 – 95.3)	54.8 (47.8 – 69.2)	32.0 (12.2 – 100)
Cumulative Complication Index					
0 – 19	5 (19%)	2 (14%)	2 (22%)	0 (0%)	3 (23%)
20 – 39	7 (26%)	3 (21%)	3 (33%)	0 (0%)	4 (31%)
40 – 59	7 (26%)	5 (36%)	1 (11%)	4 (80%)	2 (15%)
60 – 79	5 (19%)	3 (21%)	2 (22%)	1 (20%)	2 (15%)
≥ 80	3 (11%)	1 (7%)	1 (11%)	0 (0%)	2 (15%)

Abbreviations: ¹DGE delayed gastric emptying, ²POPF postoperative pancreatic fistula, ³PPH post-pancreatectomy hemorrhage, ⁴PPPD pylorus-preserving pancreateoduodenectomy

Table 4. Pancreatic surgery-associated complications according to the ¹ISGPS definitions and grading

Complication	Overall (n = 27)	Whipple/PPPD ^{II} (n = 14)			Total pancreatectomy (n = 13)
		Overall (n = 14)	Whipple/PPPD only (n = 9)	Whipple/PPPD plus distal pancreatectomy (n = 5)	
POPF [‡]	7 (26%)	7 (50%)	3 (33%)	4 (80%)	NA
POPF grade					
B	6 (22%)	6 (86%)	2 (67%)	4 (100%)	NA
C	1 (4%)	1 (214%)	1 (33%)	0 (0%)	
DGE [†]	19 (70%)	10 (71%)	5 (56%)	5 (100%)	9 (69%)
DGE grade					
A	9 (33%)	4 (40%)	2 (40%)	2 (40%)	5 (56%)
B	4 (15%)	1 (10%)	(0%)	1 (20%)	3 (33%)
C	6 (22%)	5 (50%)	3 (60%)	2 (40%)	1 (11%)
Bile leakage	4 (15%)	2 (14%)	1 (11%)	1 (20%)	2 (15%)
Bile leakage grade					
A	3 (11%)	1 (50%)	1 (100%)	(0%)	2 (100%)
B	1 (4%)	1 (50%)	(0%)	1 (100%)	0 (0%)
C	0 (0%)	0 (0%)	(0%)	(0%)	0 (0%)
PPH [§]	7 (26%)	4 (29%)	2 (22%)	2 (40%)	3 (23%)
PPH grade					
A	0 (0%)	0 (0%)	(0%)	(0%)	0 (0%)
B	3 (11%)	1 (25%)	(0%)	1 (50%)	2 (67%)
C	4 (15%)	3 (75%)	2 (100%)	1 (50%)	1 (33%)
Chyle leakage	4 (15%)	3 (21%)	3 (33%)	(0%)	1 (8%)
Chyle leakage grade					
A	1 (4%)	1 (33%)	1 (33%)	(0%)	0 (0%)
B	3 (11%)	2 (66%)	2 (67%)	(0%)	1 (100%)
C	0 (0%)	0 (0%)	(0%)	(0%)	0 (0%)

Abbreviations: [†]DGE delayed gastric emptying, ¹ISGPS International Study Group of Pancreatic Surgery, [‡]POPF postoperative pancreatic fistula, [§]PPH post-pancreatectomy hemorrhage, ^{II}PPPD pylorus-preserving pancreatoduodenectomy

Long-Term Pancreatic Function

The frequencies of exocrine and endocrine pancreatic insufficiency frequencies are reported in Table 5. Of the patients undergoing a Whipple/PPPD with and without concurrent distal pancreatectomy, 67% (6/9) and 60% (3/5) had pancreatic insufficiency postoperatively, respectively. After a Whipple/PPPD, 1/13 patients (8%; one patient suffered from diabetes preoperatively) developed new-onset diabetes without exocrine insufficiency whereas exocrine insufficiency after Whipple/PPPD occurred in 9/14 (64%) patients. Overall, patients were taking insulin for a median of 5.5 years [range 0–13.8] and taking pancreatic enzymes for a median of 5.1 years [range 0–13.8]. After a Whipple/PPPD, none of the patients could stop their endocrine or exocrine treatment during follow-up

Table 5. Occurrence of pancreatic exocrine and endocrine insufficiency stratified by type of resection

Resection	No insufficiency	Endocrine only	Exocrine only	Both
Whipple/PPPD* (n = 14)	5 (36%)	0 (0%)	7 (50%)	2 (14%) [‡]
Whipple/PPPD only (n = 9)	3 (33%)	0 (0%)	5 (56%)	1 (11%)
Whipple/PPPD plus distal pancreatectomy (n = 5) [‡]	2 (40%)	0 (0%)	2 (40%)	1 (20%) [‡]
Total pancreatectomy (n = 13)	0 (0%)	0 (0%)	0 (0%)	13 (100%) [§]

*Abbreviations: PPPD pylorus-preserving pancreaticoduodenectomy.

[‡]In order to be classified as Whipple/PPPD plus distal pancreatectomy, a part of the pancreatic body or tail had to be preserved in situ.

[‡]One patient already had diabetes before surgery.

[§]Two patients had diabetes before total pancreatectomy/ completion pancreatectomy.

DISCUSSION

Within this nationwide cohort of patients undergoing major duodenopancreatic surgery for MEN1-related dpNETs, rates of morbidity, severe morbidity and mortality were 100%, 63%, and 4%, respectively. These results demonstrate that, even in high-volume academic HPB centers, major duodenopancreatic surgery in patients with MEN1 is associated with a very high rate of severe complications and cumulative burden of morbidity, and underscore the importance of patient selection and adequate preoperative patient counseling. No preoperative patient characteristics were associated with complications. If patients are exposed to major duodenopancreatic surgery, longer duration of surgery and more blood loss warrant more intensified perioperative care.

Previous studies investigating complications after pancreatic surgery for MEN1-related dpNETs observed rates of complications ranging from 26 to 58%,^{11,13–17,23,34} however, complications were not systematically addressed according to accepted classification systems in the majority of studies. The only study that has overcome these issues was previously conducted by our group and observed a relatively high rate of severe complications of 33%. This high rate can be explained by the fact that complications were the primary outcome of this study and because all complications in individual patients were systematically scored.¹⁰ Few other studies have described complications after MEN1-associated major duodenopancreatic surgery. Lopez et al. included 13 patients, of whom 4 (31%) developed a complication;¹¹ Bartsch et al. observed complications in 3 out of 4 (75%) patients;¹⁷ Vezzosi et al. included 9 patients, of whom 6 (67%) developed a short-term complication;¹⁴ and Tonelli et al. investigated 14 patients, of whom 5 (36%) developed an abdominal complication.¹⁶ These low numbers of patients express the rarity of the disease. In comparison to the previous DMSG study on complications after pancreatic surgery in the setting of MEN1, the currently observed percentage of 63% of severe complications most likely

reflects the high percentage of major pancreatoduodenectomies, and indicates that this specific subgroup carries a formidable risk of developing severe complications. This is also underscored by the high median CCI* of 47.8 [range 8.7–100], compared with a median CCI* of 20.9 [range 0–33.5] in benchmark cases, defined as patients without significant comorbidities and major vascular resection, across 23 high-volume centers for pancreatic surgery.³⁵

Although current MEN1 guidelines do not routinely recommend Whipple procedures because of an increased operative mortality and long-term morbidity, these recommendations are not substantiated by underlying scientific evidence in patients with MEN1.⁶ A systematic review investigating POPF after Whipple/PPPD in non-MEN1 patients, observed POPF grade B/C in 22–26% of patients.³⁶ Patients with MEN1 have multiple risk factors, i.e. soft pancreas, pathology (neuroendocrine tumor), small pancreatic duct, for POPF and combined procedures are often performed due to the multiplicity of dpNETs.^{37,38} Three of the nine Whipple patients and four of five patients undergoing a Whipple/PPPD plus distal pancreatectomy developed a POPF grade B/C. On the contrary, approximately one in three patients (36%) in the Whipple/PPPD group did not suffer from pancreatic insufficiency, and the incidence was similar between the Whipple/PPPD only and Whipple/PPPD plus distal pancreatectomy groups. A recent study suggested that the risk of exocrine pancreatic insufficiency is related to the type of procedure and not the underlying hereditary syndrome.³⁹ Although no comparison between underlying pNET etiology was performed in the present study, the percentages of exocrine insufficiency were relatively similar between both studies – six out of nine patients in the Whipple/PPPD-only group in this study, compared with three of six patients in the cohort described by McDonald et al.³⁹ Future studies should evaluate the differences between patients with MEN1-related and sporadic pNETs, also adjusting for age and the estimated volume of the remnant pancreas.

At present, MEN1 clinical practice, European Neuroendocrine Tumor Society (ENETS), and North American Neuroendocrine Tumor Society (NANETS) guidelines recommend surgery for MEN1-related functioning pNETs.^{6,40,41} For NF-pNETs larger than 2 cm, surgery is advised by guidelines, which is also substantiated by population-based cohort studies in MEN1.^{8,9,40–43} Medical management is indicated in most patients with gastrinomas; surgical indications include failure of medical therapy, a pancreatic gastrinoma, a pNET larger than 2 cm and lymph node metastases.^{6,40,41} Due to the increased use of ⁶⁸Gallium-labeled PET/CT, more lymph node metastases might be detected, thus increasing the number of patients fulfilling the criteria for (extensive) surgery.⁴⁴ Within this series, the majority of patients had suspected lymph node metastases preoperatively, or single or multiple pNETs ≥ 2 cm, indicating that most patients had a surgical indication according to the current insights.

Besides surgical indications, the ENETS guideline recommends enucleations or limited resections whenever possible and MEN1 guidelines discourage the routine use of Whipple procedures.^{6,40} Although enucleations or limited resections provide a cure for MEN1-related pNETs, in some patients a Whipple/PPPD seems appropriate.^{15,34,45} Arguments in favor of a Whipple/PPPD instead of an enucleation of the pancreatic head include the technical feasibility of an enucleation (i.e. >2–3 mm from main pancreatic duct), multiple pNETs in the head, concurrent (duodenal) gastrinomas, as well as suspected lymph node metastases. Patients with diffuse pNETs throughout the pancreas pose a specific challenge for surgeons, especially if multiple pNETs fulfill criteria for operative resection. Instead of a total pancreatectomy or Whipple plus distal pancreatectomy, an enucleation plus distal pancreatectomy could be considered to reduce the risk of early and late complications. Based on currently accepted prognostic factors, a concomitant distal pancreatectomy can be considered based on the size, growth rate or tumor grade, in case of a functioning pNET, or if lymph nodes are suspected to originate from a pancreatic tail tumor. However, in these situations, the risk of malignancy of every individual dpNET is preferably estimated, but the major unmet need for MEN1-related NF-pNETs and gastrinomas is proper risk stratification.⁴⁶ Recent insights in the presence of alpha and beta cell subtypes – based on transcription factors ARX and PDX1 – of MEN1-related pNETs should be used for risk stratification and patient selection for major surgery.⁴⁷ Prognostic factors such as World Health Organization (WHO) grade, alternative lengthening of telomeres, DNA methylation and expression of p27^{Kip1} and p18^{Ink4c} can additionally be taken into account.^{47–50} Molecular prognostic factors for gastrinomas have been far less developed, and therefore patient selection should be guided by more traditional and readily available factors, such as gastrin levels, (aggressive) tumor growth and pNET size.^{1,9,20,25,51,52} The high risk of severe complications underscores the need for better risk stratification for MEN1-related dpNETs, therefore major surgery will be offered selectively in the future.

Although all of the procedures were performed in tertiary referral centers by experienced surgeons with a vast experience in endocrine and HPB surgery, some centers performed only one to three procedures, whereas 78% were performed in two centers. Hence, further centralization of these rare and extremely complex cases within the landscape of endocrine and HPB surgery should be encouraged.⁵³ Nationwide centralization of pancreatic surgery for adenocarcinomas has decreased the rates of postoperative complications in The Netherlands.⁵⁴ In addition, preoperative patient selection is of utmost importance to expose only those who will clinically benefit. Preoperative assessment by multidisciplinary teams with vast experience in both neuroendocrine tumors and MEN1 could aid in selecting the right patients for these high-risk procedures.⁵⁵ Distant metastases, which reduce the prospects of curative surgery, are ideally excluded preoperatively on ⁶⁸Gallium-labeled PET/CT, particularly when major surgery is considered. Considering the very high risk of severe

complications, referral to experienced endocrine surgeons to explore the technical possibilities of limited resections without compromising oncologic outcomes is of utmost importance, especially since no risk factors were observed to preoperatively identify patients with post-surgery severe complications. If patients undergo major surgery, well-known intraoperative factors (blood loss and operative time) might identify patients with a higher risk of a severe complication, and subsequently warrant close perioperative monitoring to enable early identification and timely treatment of complications.

A recent study reported lanreotide to be more effective than active surveillance for MEN1-related pNETs <2 cm;⁵⁶ however, patients were free to choose either lanreotide or active surveillance, and thus baseline characteristics differed between both groups. In addition, the rate of progression in the lanreotide group was similar to that observed in population-based cohorts from France and The Netherlands without treatment.^{8,43} Although the major unmet need is adequate risk stratification and patient selection for surgery, alternative treatment options can be considered in individual patients unwilling or unfit to undergo major surgery. Nevertheless, these therapies, such as lanreotide, should be investigated in randomized controlled trials.

The strengths of this study include the population-based cohort, the relatively high number of patients in spite of the rarity of disease, and the systematic assessment and grading of postoperative complications according to generally accepted classifications and definitions in each individual patient. In addition, this study provides insight into the cumulative burden of complications in MEN1. Furthermore, due to the nationwide collaboration, loss to follow-up is prevented, indicating that data were available even if patients were readmitted for complications to other university hospitals. Limitations include the high number of centers and subsequent number of surgeons who performed the procedures. Although 12 patients were previously described, by expanding indications to all dpNETs and extending the inclusion period up to 2018, the number of eligible patients could more than double.¹⁰ Furthermore, the relatively long time span of 15 years for patient inclusion is prone for changes in patient care. This is also reflected in the surgical indications, which showed an increase in Whipple procedures and a decrease in total pancreatectomies (excluding completion pancreatectomies) in the most recent period. Although this study aimed to investigate the incidence and severity of postoperative complications after major duodenopancreatic surgery in MEN1, a direct comparison of different pancreatic procedures, taking a broad spectrum of early complications as well as long-term outcomes, such as survival, liver metastases, and the occurrence of new dpNETs, would be interesting since this might further contribute to evidence-based surgical decision making regarding the extent of surgery.

CONCLUSION

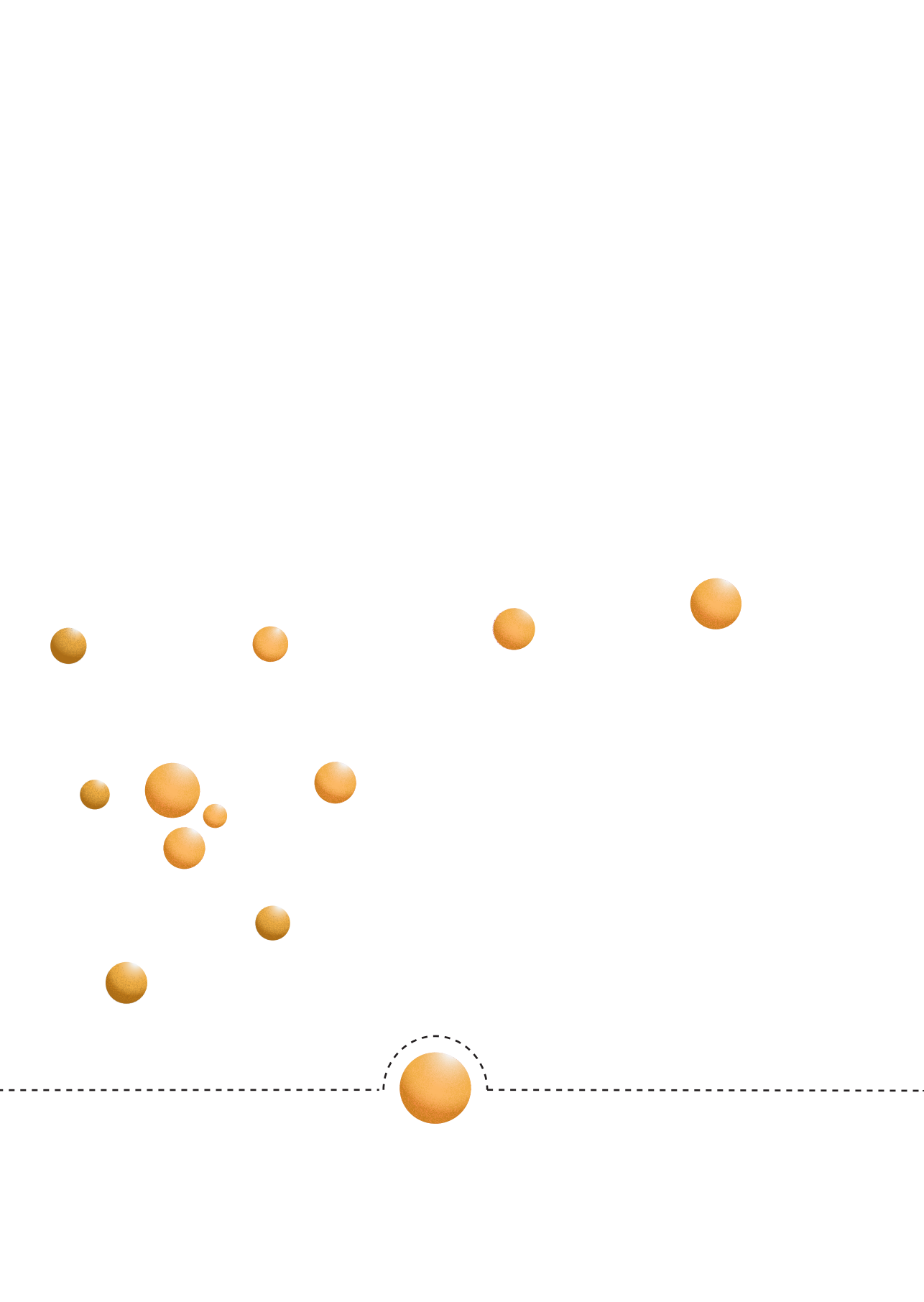
Major duodenopancreatic surgery is associated with a high rate of severe complications and cumulative morbidity and should therefore be reserved for a select subgroup of patients with MEN1-related dpNETs. These data underscore the need for adequate risk stratification for MEN1-related dpNETs. Patients should be discussed in multidisciplinary tumor boards with vast experience in MEN1-related dpNETs. In addition, individual surgical decision making should be undertaken in conjunction with the patients and their families, carefully weighing the pros and cons of major duodenopancreatic surgery.

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CHAPTER XI

ASO Author Reflections: Severe morbidity after major surgery in patients with MEN1

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D.J. van Beek, W.M.C.M. Vorselaars, I.H.M. Borel Rinkes, M.R. Vriens



PAST

Formerly, extensive procedures such as the Thompson procedure (i.e., distal pancreatectomy to the level of the superior mesenteric vein, enucleation of tumors in the pancreatic head, duodenotomy with local excision of tumors in the duodenum and peripancreatic lymph node dissection) were proposed to achieve cure for multifocal duodenopancreatic neuroendocrine tumors (dpNETs) in patients with multiple endocrine neoplasia type 1 (MEN1).¹ A shift toward more conservative surgical indications as well as better localization enabling focused resections has taken place. Unfortunately, limited resections will not suffice. If major duodenopancreatic surgery is required, the pros and cons should be carefully weighted, but data on complications after major duodenopancreatic surgery in MEN1 are limited, and no studies have assessed the cumulative burden of complications.

PRESENT

The current study observed a severe complication (Clavien-Dindo grade ≥ 3) in nearly two-thirds of patients.² Of those with severe morbidity, 76% had at least one more severe complication. Overall, the cumulative burden of complications was substantial. After a pancreatoduodenectomy patients had a higher cumulative morbidity than after total pancreatectomy. Nevertheless, in the pancreatoduodenectomy group, one-third of patients did not suffer from any form of pancreatic insufficiency. The occurrence of complications could not be predicted by preoperative characteristics, which thus hampers preoperative decision-making. These results demonstrate the significant burden associated with major duodenopancreatic surgery in MEN1.

FUTURE

To improve patient care, future perspectives include optimization of equivocal surgical indications to facilitate improved preoperative patient selection as well as a reduction of the burden of complications once major surgery is performed.

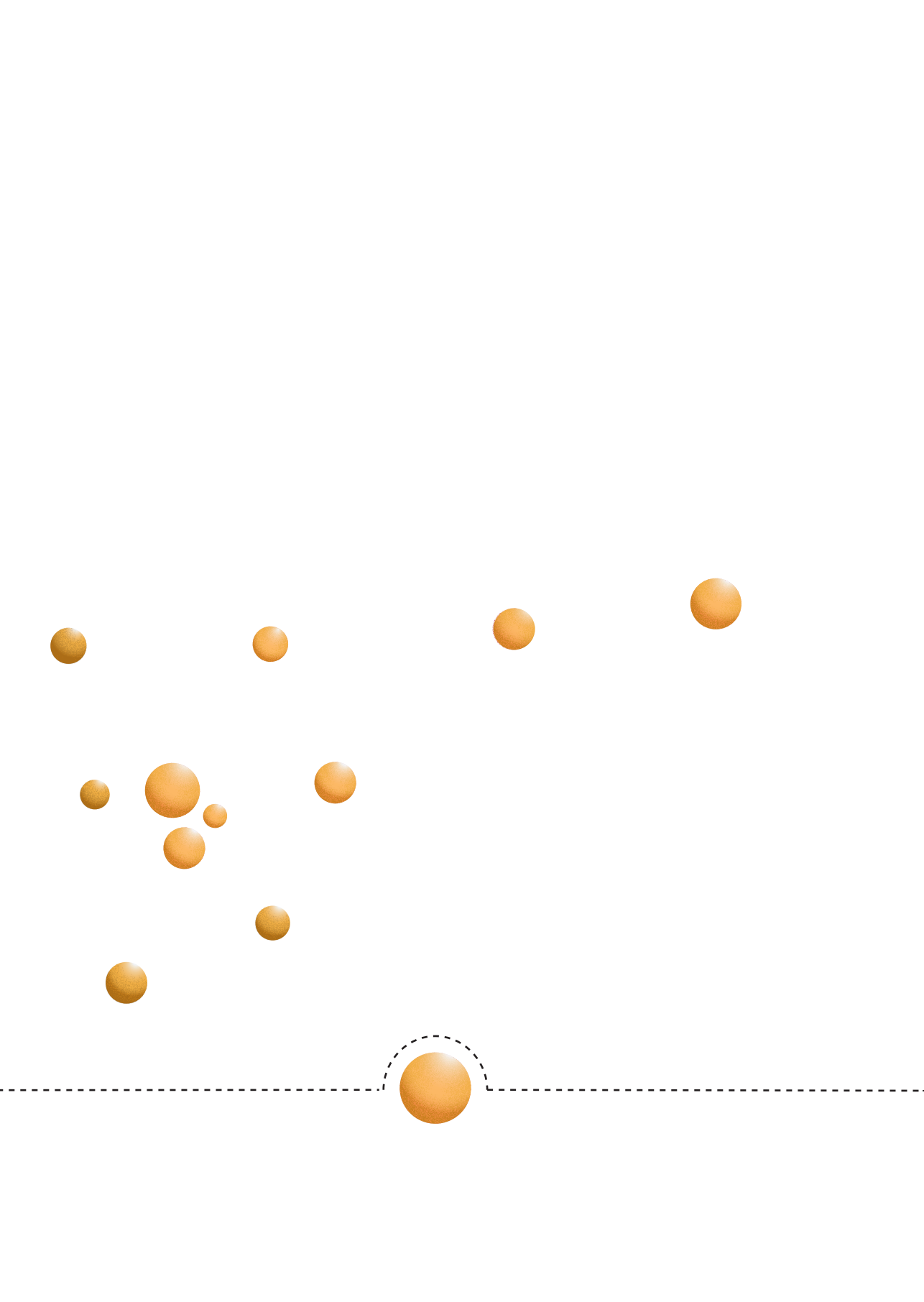
First, more specific and mainly tumor-based risk factors are needed to improve patient selection and to abandon the *'one size fits all'* treatment principle. For example, recent clinical data imply that a shift from 2 to 3 cm as surgical cut-off might yield similar oncological outcomes for pNETs, and one might therefore consider a prolonged wait-and-scan policy in some patients.³ Transcription factors are currently being investigated for the purpose of tumor-based risk stratification.⁴ However, as these transcription factors depend on pre- or

intraoperatively obtained tumor tissue, other techniques such as imaging-based risk stratification will likely be more patient-friendly and suit the radiological screening program in MEN1. Upcoming molecular imaging, particularly receptor-based imaging techniques, will hopefully enable imaging-based risk stratification. In the best-case scenario, these techniques will identify malignant dpNETs when these are still small and potentially eligible for enucleations because of size and favorable localization.⁵ In addition, further research is warranted to elucidate patterns of metastases since all patients undergoing a pancreato-duodenectomy plus distal pancreatectomy suffered from a severe complication, which stresses that oncological benefits should outweigh the complications associated with this procedure.

Second, centralization of care will likely improve patient outcomes with MEN1. Preoperative multidisciplinary and ideally multicenter team discussions will enable decision-making regarding the most complex patients with MEN1 within expert teams. When major surgery is considered, centralization of surgery within 'high volume' teams consisting of endocrine and hepatopancreatobiliary surgeons with vast experience in MEN1-related dpNETs should not be influenced by geography or travel distances and should potentially not be limited by national borders. Aims of these teams should be to reduce the incidence as well as the impact of complications. Whether patients with MEN1-related dpNETs have an increased risk of complications as compared to patients with sporadic dpNETs should be investigated in further studies to optimize referral patterns.

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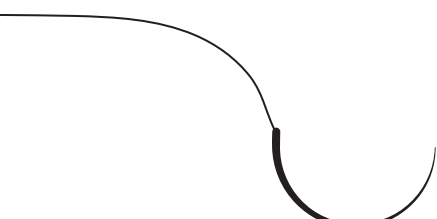
CHAPTER XII

Risk factors for complications after surgery for pancreatic neuroendocrine tumors

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D.J. van Beek*, T.J. Takkenkamp*, E.M. Wong-Lun-Hing, R.H.J. de Kleine, A.M.E. Walenkamp,
J.M. Klaase, M.W. Nijkamp, G.D. Valk, I.Q. Molenaar, J. Hagendoorn, H.C. van Santvoort,
I.H.M. Borel Rinkes, F.J.H. Hoogwater, M.R. Vriens

*Both authors contributed equally.



ABSTRACT

Background

Surgical resection is the only potentially curative treatment for pancreatic neuroendocrine tumors. The choice for the type of procedure is influenced by the expected oncological benefit and the anticipated risk of procedure-specific complications. Few studies have focused on complications in these patients. This cohort study aimed to assess complications and risk factors after resections of pancreatic neuroendocrine tumors.

Methods

Patients undergoing resection of a pancreatic neuroendocrine tumor were identified within 2 centers of excellence. Complications were assessed according to the Clavien-Dindo classification and the comprehensive complication index. Logistic regression was performed to compare surgical procedures with adjustment for potential confounders (Clavien-Dindo ≥ 3).

Results

The cohort comprised 123 patients, including 12 enucleations, 50 distal pancreatectomies, 51 pancreatoduodenectomies and 10 total/combined pancreatectomies. Mortality was 0.8%, a severe complication occurred in 41.5%, and the failure-to-rescue rate was 2.0%. The median comprehensive complication index was 22.6 (0–100); the comprehensive complication index increased after more extensive resections. After adjustment, a pancreatoduodenectomy, as compared to a distal pancreatectomy, increased the risk for a severe complication (odds ratio 3.13 (95% confidence interval 1.32–7.41)). Of the patients with multiple endocrine neoplasia type 1 or von Hippel-Lindau, 51.9% developed a severe complication versus 38.5% with sporadic disease. After major resections morbidity was significantly higher in patients with multiple endocrine neoplasia type 1/von Hippel-Lindau (comprehensive complication index 45.1 versus 28.9, $p=0.029$).

Conclusions

Surgery for pancreatic neuroendocrine tumors is associated with a high rate of complications, but low failure-to-rescue in centers of excellence. Complications are procedure specific. Major resections in patients with multiple endocrine neoplasia type 1/von Hippel-Lindau appear to increase the risk of complications.

INTRODUCTION

Pancreatic neuroendocrine tumors (pNETs) have an estimated incidence of less than 1 per 100,000 persons, but their incidence is rising.^{1–3} Less than 10% of all pancreatic operations and only 2.7–6.3% of the pancreatoduodenectomies are performed for pNETs.^{4,5} Less than 1 in 10 occurs in a familial fashion, predominantly multiple endocrine neoplasia type 1 (MEN1) and von Hippel-Lindau disease (VHL).⁶ When the peptides secreted by the pNET induce symptoms, the tumor is regarded as a functioning pNET (F-pNET); all others are considered as non-functioning (NF-pNETs).

Surgical resection is the cornerstone of curative treatment and is increasingly performed for pNETs.⁷ The European Neuroendocrine Tumor Society (ENETS) and North American Neuroendocrine Tumor Society (NANETS) guidelines recommend operative resection of F-pNETs, irrespective of their size.^{8,9} Both guidelines recommend resection of NF-pNETs larger than 2 cm but suggest that surgical resections could also be considered for NF-pNETs less than 2 cm.^{8,9} The decision to proceed to surgical resection and the choice for the type of procedure are influenced by the expected oncological benefit and the anticipated risk of procedure-specific complications.

Data on complications after pancreatic resections are abundant, but published data specifically on complication rates after pancreatic surgery for pNETs are limited. Patients with pNETs also undergo atypical pancreatic resections, have a soft pancreas and non-dilated pancreatic duct, which increase the risk of complications.^{10–13} Most studies including at least 100 patients with resected pNETs focused on postoperative pancreatic fistula (POPF), whereas patients are at risk for other pancreatic and general surgery-related complications as well. In these studies, patients have been included over almost 2 decades^{13–17}, mainly before 2010^{14–19} and specific inclusion criteria have been applied based on size or metastases^{15,19}, functionality^{16,17}, MEN1^{20,21} or sporadic nature¹⁴, thereby limiting the applicability of these studies during patient counselling in general practice. Besides, most studies lacked a detailed and comprehensive assessment and grading of complications according to accepted criteria, and risk factors are largely unknown.^{15–19,22,23} This study aimed to assess procedure-specific complications and risk factors for complications after operations for pNETs in 2 ENETS Centers of Excellence. The secondary aims were to compare postoperative complications of patients with (multifocal) MEN/VHL-related with (solitary) sporadic pNETs and those with F-pNETs versus NF-pNETs.

METHODS

Study design

Patients who underwent resection of a histopathologically proven pNET between 2008 and 2019 at the University Medical Center Utrecht (UMCU) and University Medical Center Groningen (UMCG) were identified retrospectively by extensive review of the surgical procedures database and histopathological archives. Both centers are high-volume centers for pancreatic surgery – defined as at least 20 pancreatoduodenectomies annually in the Netherlands²⁴ – and are specialized in pNETs. Both are certified as ENETS Center of Excellence. Patients were identified by an extensive search of the pathology reports and review of the surgical procedures databases. Patients undergoing pancreatic resections for a duodenal NET were not eligible. The study was approved by medical ethics committees from both centers (Research Register number 201900734 [UMCG] and 19/670 [UMCU]).

Clinical definitions

Data regarding patient demographics, work-up, operation, postoperative complications and follow-up were collected from the electronic patient records. No data were collected on race/ethnicity. Tumors without distinct clinical symptoms caused by excessive hormone excretion were regarded as NF-pNETs. Tumors were considered as F-pNET in case of excessive hormone production leading to distinct clinical symptoms and referred to as insulinomas, gastrinomas, glucagonomas and somatostatinomas, depending on the respective hormone secreted.

The presence of MEN1 or VHL disease was assessed per clinical practice guidelines or statements.^{25,26} Length of hospital stay (LOS) was calculated from the date of resection until the date of discharge. Readmission was defined as an admission after initial discharge for a complication within 30 days.

Preoperative tumor size of the largest pNET was based on the maximum observed size on conventional imaging. If conventional or functional imaging was positive for local lymph node or liver metastases, the patient was considered as having the respective metastases.

Surgery

Surgical indications and strategies were based on multidisciplinary team discussions and intraoperative findings. In both centers, surgical procedures were performed in teams of surgeons with vast competence in pancreatic surgery and experience in pNETs. An enucleation was defined as a local resection of a pNET without the resection of surrounding tissue. Pancreatoduodenectomies considered Whipple procedures and pylorus-preserving pancreatoduodenectomies (PPPD). Combined resections, eg, Whipple/PPPD and distal pancreatectomy (DP), were reserved for patients with multifocal tumors occurring in

MEN1/VHL disease. To be classified as a Whipple/PPPD plus DP, preservation of at least a part of the pancreatic body or tail was demanded.²¹ No central pancreatectomies were performed. Enucleations and DP were regarded as minor resections and Whipple/PPPDs, total and combined pancreatectomies were considered as major resections.

Outcome measures and definitions

Postoperative complications within 30 days after surgical resection or during hospitalization were graded according to the Clavien-Dindo classification.²⁷ The primary outcome of the study was the occurrence of a severe postoperative complication (Clavien-Dindo ≥ 3).²⁷ Mortality included deaths within 90 days.

The cumulative burden of complications was expressed as the comprehensive complication index (CCI), which is calculated as the sum of all complications weighted for their severity and expressed on a continuous scale ranging from 0 (no complication) to 100 (death).^{28,29} The CCI scores were used to calculate estimated costs, in Euros and United States dollars, based on the type of resection, age and CCI.³⁰ A cumulative CCI of ≥ 37.1 , which reflects the burden of at least 2 Clavien-Dindo grade 3a complications, was previously used to determine high morbidity due to operative complications.³¹

Secondary outcomes included the presence and severity of pancreatic surgery-associated complications – POPF, delayed gastric emptying (DGE), post-pancreatectomy hemorrhage (PPH), bile leak and chyle leak – which were assessed and graded according to definitions and criteria formulated by the International Study Group of Pancreatic Surgery (ISGPS).^{32–36} Grade B/C complications were considered clinically relevant. Patients with a total pancreatectomy were excluded from the POPF analysis. For the analysis of bile leak, only patients undergoing resection of the pancreatic head (Whipple/PPPD, total pancreatectomy or enucleation of the pancreatic head) were included. Failure-to-rescue was defined as death due to a severe complication.³⁷

Statistical analysis

Continuous variables were presented as mean (\pm standard deviation (SD)) or as median (range). Categorical variables were presented as count (percentage). Differences in continuous variables were assessed using Mann-Whitney U or Kruskal Wallis tests, and categorical data were compared by using the chi-square analysis or Fisher exact test. Differences in characteristics were compared between patients with MEN1/VHL versus those with sporadic disease and patients with a NF-pNET versus those with a F-pNET, respectively.

Severe complications, ISGPS grade B/C complications and CCI with estimated associated costs were compared between different surgical procedures (enucleation versus DP versus Whipple/PPPD versus total/combined pancreatectomy), patients with MEN1/VHL versus sporadic disease, and F-pNETs versus NF-pNETs. The analyses were stratified by the extent of resection.

The potential associations between preoperative characteristics and the occurrence of a severe complication or POPF were assessed using univariable logistic regression providing odds ratios (OR) with corresponding 95% confidence intervals (CI). Variables evaluated for an association with a severe complication were age at surgery in years, sex, American Society of Anesthesiologists (ASA) score, type of pancreatic resection, type of pNET, MEN1/VHL, radiological size in mm, radiological pNET ≥ 2 cm, radiological lymph node metastases, radiological liver metastases and surgical approach. The same variables were analyzed for an association with a POPF.

In multivariable analysis, the effect of surgical procedures was adjusted for age at surgery, ASA score, pNET functionality, and presence of a radiological pNET ≥ 2 cm, based on clinical experience and reasoning. Because only a limited number of covariates could be adjusted for, we performed sensitivity analyses by including different combinations of covariates.

Missing data were considered missing at random and therefore imputed using multiple imputation with the iterative Markov chain Monte Carlo method, creating 20 datasets.^{38,39} Variables listed in Table 1 as well as hospital, solitary or multiple radiological pNETs, and the period of operation (before and in 2014 or later), and the occurrence of a severe complication, were used as predictor variables for multiple imputation. For the POPF analysis, POPF was included as the outcome for imputation. Odds ratios and 95% CI were pooled using Rubin's rules.⁴⁰ Data were analyzed using SPSS version 25.0 (IBM Corp, New York, USA) and R version 3.5.1 with 'Mice' package (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Patient characteristics

In total, 123 patients were included with a mean age of 54 (± 14) years (Table 2). Sixty-four (52%) were female patients, and 27 (21.9%) patients had a genetic syndrome. In 25 patients, MEN1 or VHL was confirmed by genetic testing; in 1 patient fulfilling clinical criteria, no mutation was found by genetical testing, and in 1 patient, it was unclear if genetic testing was performed. The latter patient fulfilled clinical criteria and had a proven mutation within the family. Patients with MEN1/VHL were younger at surgery, more often had multiple pNETs on imaging, and more frequently underwent a total/combined pancreatectomy as compared to patients with sporadic disease (Table 3). The median age at surgery in patients with MEN1 and in patients with VHL was 41.5 years (6–62) and 42.0 years [33–51], respectively. Overall, the majority of patients had a NF-pNET (70.7%). Preoperatively, NF-pNETs were generally larger than F-pNETs, and more NF-pNETs were ≥ 2 cm (72.3 versus 34.3%; Table 3). Three patients had prior resection of a pNET.

Table 1. Risk factors for a severe complication.

	Severe complication [*]		Univariable analysis		Multivariable analysis	
	Yes N = 51 (41.5%)	No N = 72 (58.5%)	Crude OR	95% CI	Adjusted OR	95% CI
Age surgery in years [†]	55 [18 – 81]	56 [6 – 77]	1.00	0.97 – 1.03	1.00	0.97 – 1.03
Sex						
Female	23 (45.1%)	41 (56.9%)	1	Ref.	-	-
Male	28 (54.9%)	31 (43.1%)	1.61	0.78 – 3.34		
ASA grade						
ASA 1 or 2	39 (76.5%)	61 (84.7%)	1	Ref.	1	Ref.
ASA 3 or 4	12 (23.5%)	11 (15.3%)	1.71	0.68 – 4.29	1.51	0.56 – 4.04
Pancreatic resection						
Distal pancreatectomy	16 (31.4%)	34 (47.2%)	1	Ref.	1	Ref.
Enucleation	1 (2.0%)	11 (15.3%)	0.19	0.02 – 1.66	0.19	0.02 – 1.73
Whipple/PPPD	29 (56.9%)	22 (30.6%)	2.80	1.23 – 6.37	3.13	1.32 – 7.41
Total or other	5 (9.8%)	5 (6.9%)	2.13	0.53 – 8.52	2.14	0.50 – 9.11
Type of tumor						
NF-pNET	35 (68.6%)	52 (72.2%)	1	Ref.	1	Ref.
F-pNET	16 (31.4%)	20 (27.8%)	1.19	0.54 – 2.63	1.59	0.59 – 4.26
Hereditary syndrome						
Absent	37 (72.5%)	59 (81.9%)	1	Ref.	-	-
Present	14 (27.5%)	13 (18.1%)	1.72	0.72 – 4.09		
Size imaging in mm	23 [6 – 140]	24 [8 – 140]	0.99	0.98 – 1.01	-	-
pNET ≥ 2 cm						
Absent	20 (39.2%)	28 (38.9%)	1	Ref.	1	Ref.
Present	31 (60.8%)	44 (61.1%)	1.01	0.47 – 2.17	0.98	0.38 – 2.55
Lymph node metastases						
Absent	37 (72.5%)	56 (77.8%)	1	Ref.	-	-
Present	14 (27.5%)	16 (22.2%)	1.31	0.57 – 3.04		
Liver metastases						
Absent	40 (78.4%)	61 (84.7%)	1	Ref.	-	-
Present	11 (21.6%)	11 (15.3%)	1.50	0.59 – 3.83		
Approach						
Open	37 (72.5%)	45 (62.5%)	1	Ref.	-	-
Laparoscopic	4 (7.8%)	12 (16.7%)	0.41	0.12 – 1.38		
Robot-assisted	10 (19.6%)	15 (20.8%)	0.81	0.32 – 2.03		

Multivariable analysis includes age, ASA grade, pancreatic resection, type of tumor, pNET ≥ 2 cm.

Missing data were observed for size pNET imaging (6.5%), pNET size ≥ 2 cm (6.5%), suspected lymph node metastases on imaging (0.8%), suspected liver metastases on imaging (0.8%). For all other variables no missing data were observed.

^{*}Data given after multiple imputation.

[†]For comparison between patients with and without a severe complication median (range) is presented.

Abbreviations: ASA American Society of Anesthesiologists, CI confidence interval, F-pNET functioning pancreatic neuroendocrine tumor, NF-pNET non-functioning pancreatic neuroendocrine tumor, No. number of, OR odds ratio, Ref. reference category

Table 2. Baseline characteristics

Characteristic	Overall n = 123
Age at surgery in years, mean (SD)	54 (\pm 14)
Sex	
Male	59 (48.0%)
Female	64 (52.0%)
Surgical indication	
NF-pNET	87 (70.7%)
Insulinoma	26 (21.1%)
Gastrinoma	2 (1.6%)
Glucagonoma	1 (0.8%)
Somatostatinoma	1 (0.8%)
NF-pNET and gastrinoma	6 (4.9%)
Hereditary syndrome	
MEN1	22 (17.9%)
Von Hippel-Lindau	5 (4.1%)
ASA grade	
1	25 (20.3%)
2	75 (61.0%)
3	22 (17.9%)
4	1 (0.8%)
Preoperative imaging performed	
Magnetic resonance imaging	53 (43.1%)
Computed tomography	110 (89.4%)
Endoscopic ultrasonography	82 (66.7%)
Somatostatin receptor scintigraphy	28 (22.8%)
⁶⁸ Gallium labelled PET/CT	65 (52.8%)
⁶⁸ Ga-DOTATATE PET/CT	28 (22.8%)
⁶⁸ Ga-DOTATOC PET/CT	8 (6.5%)
⁶⁸ Ga-DOTANOC PET/CT	1 (0.8%)
¹⁸ F-DOPA PET/CT	15 (12.2%)
¹⁸ F-FDG PET/CT	1 (0.8%)
¹¹ C-5-HTP PET	12 (9.8%)
pNETs on preoperative imaging, n=122 (99.2%)	
Solitary	91 (74.6%)
Multiple	31 (25.4%)
Preoperative tumor localization	
Head	59 (48.0%)
Body	9 (7.3%)
Tail	48 (39.0%)
Multifocal	7 (5.7%)
Size largest pNET on conventional imaging in mm, median [range], n=115 (93.5%)	23 [6 - 140]
pNET \geq 2 cm on preoperative imaging, n=115 (93.5%)	71 (61.7%)
Suspected lymph node metastases on imaging, n=122 (99.2%)	30 (24.6%)
Suspected liver metastases on imaging, n=122 (99.2%)	22 (17.9%)
Procedures	
Enucleation	12 (9.8%)
Distal pancreatectomy	50 (40.7%)
PPPD/Whipple	51 (41.5%)
Total pancreatectomy	6 (4.9%)
Combined resection	4 (3.3%)

Table 2 continues on page 277

Table 2 continued from page 276

Characteristic	Overall n = 123
Approach	
Conventional/open	82 (66.7%)
Laparoscopic	16 (13.0%)
Robot-assisted	25 (20.3%)
Additional resection for suspected pNET metastases	7 (7.3%)

Abbreviations: ASA American Society of Anesthesiologists, *MEN1* multiple endocrine neoplasia type 1, *N* number of, *NF-pNET* non-functioning pancreatic neuroendocrine tumor, *PET* positron emission tomography, *pNET* pancreatic neuroendocrine tumor, *PPPD* pylorus-preserving pancreatoduodenectomy, *SD* standard deviation

Surgical procedures

The majority of patients (n=51; 41.5%) underwent a Whipple procedure (n=9) or PPPD (n=42). Fifty patients (40.7%) underwent a DP, of whom 27 (54.0%) had a spleen-preserving procedure; spleen-preserving DP were more often performed robot-assisted, and tumors were generally smaller (Supplementary Table 1). In 21 patients, splenectomy was planned due to preoperative or intraoperative suspicion of either direct tumor involvement or close relation with the spleen or splenic vessels. In the remaining 2, splenectomy was performed owing to intraoperative iatrogenic damage to the splenic vessels. Enucleation was performed in 12 patients (9.8%) – 5 tumors were located in the pancreatic head and 7 in the body or tail. In 10 of these, the distance to the main pancreatic duct was determined by intraoperative ultrasound. In 7 patients in the enucleation group, no anastomosis was constructed. A Roux-en-Y reconstruction was performed in 3 patients, of whom 1 also had a serosal patch. Six patients (4.9%) underwent a total pancreatectomy – 2 were duodenum-preserving. Four patients (3.3%) underwent a combined resection; these were exclusively performed in patients with MEN1 and multifocal disease and included a Whipple/PPPD plus DP in 3 and an enucleation plus DP in 1. Synchronous hepatic resections for pNET-related metastases were performed in 6 patients (4.9%), and 1 patient underwent synchronous resections of metastases in the stomach and kidney. Liver resections included hemihepatectomy (n=2), right hemihepatectomy plus wedge resection (n=1), wedge resection plus radiofrequency ablation (n=1), multiple wedge resections (n=1), and segmentectomies (n=1). Two patients underwent multivisceral resections due to tumor progression, and 4 other patients underwent synchronous resections due to compromise of the colonic (n=1) or omental vasculature (n=1), gastric NET (n=1) or renal cell carcinoma (n=1). Forty-one of the procedures (33.3%) were minimally invasive, of which 3 were converted.

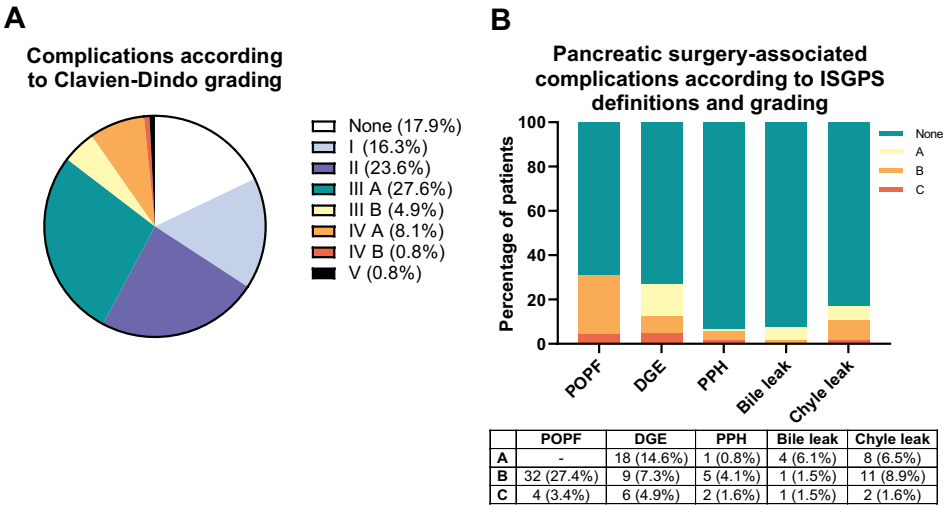


Figure 1. Postoperative complications. A) Pie-chart showing the percentage of patients with complications according to the Clavien-Dindo classification. B) Bar graphs showing the percentage of patients with pancreatic surgery-associated complications according to the ISGPS definitions and grading. For the bile leak analysis, only patients with resections of the pancreatic head were included, that is, patients after a Whipple/ pylorus-preserving pancreatoduodenectomy, total pancreatectomy, combined pancreatectomy, and enucleation of the pancreatic head. Abbreviations: *DGE* delayed gastric emptying, *ISGPS* International Study Group of Pancreatic Surgery, *POPF* postoperative pancreatic fistula, *PPH* post-pancreatectomy hemorrhage

Intraoperative and postoperative complications

The median durations of surgery and blood loss were 309 minutes (76–1,062) and 450 milliliters (0–5,000), respectively. A relaparotomy was performed in 8 patients (6.5%) – indications were POPF in 3 patients (all grade C), PPH in 2 (grade B and C), bile leak in 1 (grade C), and fascial dehiscence in 1. The remaining patient underwent 3 relaparotomies for omental necrosis, POPF grade C, and abdominal abscesses, respectively. The median LOS was 11 days (4–260).

A severe complication was observed in 51 patients (41.5%) (Figure 1); 13 of these had a grade 3 complication due to endoscopic feeding tube placement. The only death (0.8%) within 90 days occurred in a patient that suffered from a perioperative stroke with a fatal prognosis. The overall failure-to-rescue rate was 2.0% (n=1/51) and for ISGPS grade B/C complications 0% (n=0/51). Patients with a severe complication had a longer LOS (20 [6–260] versus 8 [4–30] days; $p<0.001$) than patients with no severe complication. The median CCI[†] was 22.6 (0–100), leading to associated estimated costs of €15,336 (€10,098–€46,402) and \$45,775 (\$30,140–\$138,497), respectively. Twenty-nine patients (23.6%) had a high CCI[†].

Table 3. Characteristics and outcomes of patients with a MEN1/VHL-related versus sporadic pNET and F-pNET versus NF-pNET.

Characteristic	Sporadic n = 96	MEN1/VHL n = 27	p-value	NF-pNET n = 87	F-pNET n = 36	p-value
Age at surgery in years, median [range]	57 [21 – 81]	42 [6 – 62]	<0.001	56 [19 – 79]	53 [6 – 81]	0.178
Sex						
Male	46 (47.9%)	13 (48.1%)	0.983	46 (52.9%)	13 (36.1%)	0.090
Female	50 (52.1%)	14 (51.9%)		41 (47.1%)	23 (63.9%)	
Hereditary syndrome						
MEN1	NA	NA	NA	12 (13.8%)	10 (27.8%)	0.079
Von Hippel-Lindau				5 (5.7%)	0 (0%)	
Type of pNET						
NF-pNET	70 (72.9%)	17 (63.0%)	0.315	NA	NA	NA
F-pNET	26 (27.1%)	10 (37.0%)				
ASA grade						
1	21 (21.9%)	4 (14.8%)	0.804	19 (22.8%)	6 (16.7%)	0.523
2	57 (59.4%)	18 (66.7%)		54 (62.1%)	21 (58.3%)	
3	17 (17.7%)	5 (18.5%)		13 (14.9%)	9 (25.0%)	
4	1 (1.0%)	0 (0%)		1 (1.1%)	0 (0%)	
Number of pNETs imaging, n=122 (99.2%)						
Solitary	83 (87.4%)	8 (29.6%)	<0.001	65 (74.7%)	24 (66.7%)	0.364
Multiple	12 (12.6%)	19 (70.4)		22 (25.3%)	12 (33.3%)	
Size largest pNET on conventional imaging in mm, median [range], n=115 (93.5%)	22 [6 - 140]	24 [8 – 98]	0.781	27 [8 - 120]	15 [6 – 140]	<0.001
pNET ≥ 2 cm on preoperative imaging, n=115 (93.5%)	52 (58.4%)	19 (73.1%)	0.176	60 (72.3%)	11 (34.4%)	<0.001
Suspected lymph node metastases on imaging, n=122 (99.2%)	21 (22.1%)	9 (33.3%)	0.232	23 (26.7%)	7 (19.4%)	0.393
Suspected liver metastases on imaging, n=122 (99.2%)	17 (17.9%)	5 (18.5%)	0.941	17(19.8%)	5 (13.9%)	0.441
Procedures						
Enucleation	10 (10.4%)	2 (7.4%)	<0.001	7 (8.0%)	5 (13.9%)	0.185
Distal pancreatectomy	41 (42.7%)	9 (33.3%)		32 (36.8%)	18 (50.0%)	
PPPD/Whipple	43 (44.8%)	8 (29.6%)		42 (48.3%)	9 (25.0%)	
Total pancreatectomy	2 (2.1%)	4 (14.8%)		4 (4.6%)	2 (5.6%)	
Combined resection	0 (0%)	4 (14.8%)		2 (2.3%)	2 (5.6%)	
Approach						
Open	63 (65.6%)	19 (70.4%)	0.714	60 (69.0%)	22 (61.1%)	0.654
Laparoscopic	12 (12.5%)	4 (14.8%)		10 (11.5%)	6 (16.7%)	
Robot-assisted	21 (21.9%)	4 (14.8%)		17 (19.5%)	8 (22.2%)	

Patients with both a NF-pNET and gastrinoma were analyzed as F-pNET.

Abbreviations: ASA American Society of Anesthesiologists, DGE delayed gastric emptying, F-pNET functioning pancreatic neuroendocrine tumor, MEN1 multiple endocrine neoplasia type 1, N number of, NF-pNET non-functioning pancreatic neuroendocrine tumor, pNET pancreatic neuroendocrine tumor, POPF postoperative pancreatic fistula, PPH post-pancreatectomy hemorrhage, PPPD pylorus-preserving pancreatoduodenectomy

Table 4. Procedure-specific intraoperative outcomes and postoperative complications.

	Enucleation (n = 12)	Distal pancreatectomy (n = 50)	PPPD/ Whipple (n = 51)	Total or combined pancreatectomy (n = 10)	p-value
Intraoperative outcomes					
Duration of surgery in minutes, n=122 (99.2%)	224 [76 – 485]	254 [115 – 582]	378 [166 – 810]	359 [289 – 1089]	<0.001
Blood loss in ml, n=117 (95.1%)	125 [0 – 1000]	400 [0 – 5000]	600 [150 – 4500]	775 [200 – 2000]	<0.001
Hospital stay					
Length of stay in days, n=117 (95.1%)	7 [4 – 12]	8 [4 – 64]	15 [7 – 51]	23 [7 – 260]	<0.001
Re-laparotomy	0 (0%)	3 (5.9%)	3 (5.9%)	2 (20.0%)	0.274
ICU admission	0 (0%)	2 (4.0%)	7 (13.7%)	3 (30.0%)	0.033
Postoperative complications					
Number of complications	1 [0 – 5]	1 [0 – 10]	2 [0 – 12]	4 [0 – 17]	0.001
Clavien-Dindo grade					
None	4 (33.3%)	14 (28.0%)	3 (5.9%)	1 (10.0%)	-
I	3 (25.0%)	9 (18.0%)	6 (11.8%)	2 (20.0%)	
II	4 (33.3%)	10 (20.0%)	13 (25.5%)	2 (20.0%)	
III A	1 (8.3%)	12 (24.0%)	19 (37.3%)	2 (20.0%)	
III B	0 (0%)	3 (6.0%)	3 (5.9%)	0 (0%)	
IV A	0 (0%)	2 (4.0%)	6 (11.8%)	2 (20.0%)	
IV B	0 (0%)	0 (0%)	0 (0%)	1 (10.0%)	
V	0 (0%)	0 (0%)	1 (2.0%)	0 (0%)	
Clavien-Dindo ≥ III	1 (8.3%)	16 (32.0%)	29 (56.9%)	5 (50.0%)	0.006
CCI ^a	12.25 [0 – 33.17]	20.92 [0 – 92.94]	29.58 [0 – 100]	32.12 [0 – 100]	<0.001
CCI ^a ≥ 37.1	0 (0%)	6 (12.0%)	18 (35.3%)	5 (50.0%)	0.002
Pancreatic surgery specific complications					
POPF B/C	0 (0%)	16 (32.0%)	18 (35.3%)	2 (50.0%) [*]	0.088
DGE B/C	0 (0%)	1 (2.0%)	11 (21.6%)	3 (75.0%)	0.003
PPH B/C	0 (0%)	2 (4.0%)	1 (2.0%)	4 (40.0%) [^]	<0.001
Bile leak B/C [†]	0 (0%)	NA	2 (4.0%)	0 (0%)	0.800

Table 4 continues on page 281

	Enucleation (n = 12)	Distal pancreatectomy (n = 50)	PPPD/ Whipple (n = 51)	Total or combined pancreatectomy (n = 10)	p-value
Chyle leak B/C	0 (0%)	5 (10.0%)	8 (15.7%)	0 (0%)	0.258
Any B/C complication	0 (0%)	19 (38.0%)	26 (51.0%)	6 (60.0%)	0.007
Estimated associated costs					
Euro	12444 [11400 – 17292]	14577 [10098 – 42035]	16442 [10098 – 46402]	17695 [10098 – 46402]	<0.001
USD	37143 [34025 – 51611]	43507 [30140 – 125463]	49300 [30140 – 138497]	52817 [30140 – 138497]	<0.001

Continuous variables reported as median [range]

*For the POPF analysis, patients with a total pancreatectomy were removed from the denominator.

*For the bile leak B/C analysis, only patients with surgery of the pancreatic head were included, i.e., patients after a Whipple/PPPD, total pancreatectomy and enucleation of the pancreatic head.

*Two of the six patients after a total pancreatectomy and two of the four patients after a combined resection developed a PPH.

Abbreviations: CCI comprehensive complication index, DGE delayed gastric emptying, POPF postoperative pancreatic fistula, PPH post-pancreatectomy hemorrhage, PPPD pylorus-preserving pancreatoduodenectomy

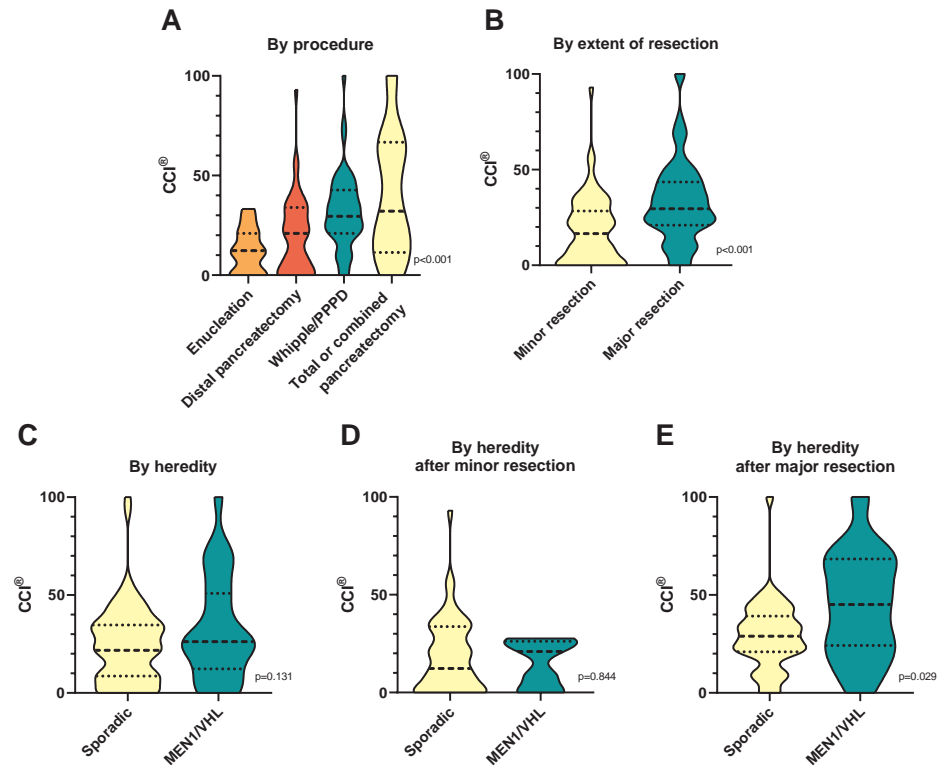


Figure 2. Violin plots showing the distribution of the CCI. A) By surgical procedure. B) By extent of resection. C) By hereditary syndrome. D) By heredity. E) By heredity after minor resection. Abbreviations: CCI comprehensive complication index, MEN1 multiple endocrine neoplasia type 1, pNET pancreatic neuroendocrine tumors, PPPD pylorus-preserving pancreatoduodenectomy, VHL von Hippel-Lindau

Procedure-related outcomes are presented in Table 4. The risk for a severe complication, a cumulative CCI ≥ 37.1 , and any grade B/C complication and the cumulative CCI as well as the number of complications were procedure related (Table 4; Figure 2). A POPF grade B/C was the most frequently occurring pancreas surgery-specific complication ($n=36$ [29.3%]). After an enucleation, none of the patients developed any ISGPS grade B/C complication. Delayed gastric emptying was significantly more often observed after a Whipple/PPPD or total/combined pancreatectomy. In addition, a grade B/C PPH occurred more often after a total/combined pancreatectomy. One of the 2 patients with a combined pancreatectomy suffered from POPF and the other from multiple abscesses and a pseudoaneurysm of the superior mesenteric artery. In the total pancreatectomy group, 1 patient suffered from an intraluminal jejunal bleeding and 1 patient from bleeding in the retroperitoneal dissection area.

Factors associated with a severe complication and grade B/C POPF

A severe complication was observed more often in male patients versus female patients, in patients with a MEN1/VHL-related pNET versus sporadic pNET, and in patients with an ASA grade of 3/4 versus 1/2, respectively. Besides procedure type, no characteristics were significantly associated with the occurrence of a severe complication in univariable analysis (Table 1). After adjusting for age at surgery, ASA grade, type of pNET, and presence of a pNET ≥ 2 cm, patients in the Whipple/PPPD group had an increased risk for a severe complication compared to those in the DP group (OR 3.13 [95% CI 1.32–7.41]; Table 1). Sensitivity analyses did not substantially influence the effect size (point estimate or 95% CI; Supplementary Table 2). No factors were associated with a POPF grade B/C (Supplementary Table 3).

MEN1/VHL versus sporadic pNET

In terms of percentage, patients with MEN1/VHL more often had a severe complication, POPF, any grade B/C complication, and a cumulative CCI ≥ 37.1 ; DGE, and PPH occurred significantly more often (Table 5). After minor resections, complication percentages were similar. In the major resection group, a severe complication and ISGPS grade B/C POPF, DGE, PPH occurred more often in patients with MEN1/VHL. The number of complications and the CCI was significantly higher in patients with MEN1/VHL as compared to sporadic disease after major resections (45.1 versus 28.9, $p=0.029$). In addition, the percentage of patients with a cumulative CCI ≥ 37.1 was higher in MEN1/VHL-related pNETs as compared to those with sporadic pNETs.

Of the patients with MEN1/VHL and a major resection, 68.8% ($n=11/16$) developed a severe complication compared to 27.3% ($n=3/11$) after a minor resection. Within the subgroups of patients with sporadic and MEN1/VHL-related pNETs, the CCI was significantly higher after major resections (sporadic 28.9 versus 12.2, $p=0.001$ and MEN1/VHL 45.1 versus 20.9, $p=0.005$, respectively). In patients with a resected sporadic pNET a severe complication occurred significantly more often after major resections (51.1% versus 27.5%, $p=0.017$).

F-pNET versus NF-pNET

The occurrence of complications between patients with a F-pNET as compared to those with a NF-pNET are presented in Supplementary Table 4. Overall occurrence of complications and pancreatic surgery-associated complications was similar between both groups. In the group undergoing minor resections, no differences were observed, whereas after major resections, the CCI was higher in the F-pNET group (37.1 versus 27.6, $p=0.031$). Patients with functioning pNETs more often underwent total or combined resections (30.8% versus 12.5%) and had MEN1/VHL (46.2% versus 20.8%), which likely contributed to the observed

Table 5. Complications according to extent of resections and presence of MEN1/VHL

Characteristic	Overall		Minor resection			Major resection		
	Sporadic n = 96	MEN1/VHL n = 27	p-value	Sporadic n = 51	MEN1/VHL n = 11	p-value	Sporadic n = 45	MEN1/VHL n = 16
Clavien-Dindo ≥ III	37 (38.5%)	14 (51.9%)	0.215	14 (27.5%)	3 (27.3%)	1.000	23 (51.1%)	11 (68.8%)
POPF B/C ^a	27 (28.7%)	9 (39.1%)	0.332	13 (25.5%)	3 (27.3%)	1.000	14 (32.6%)	6 (50.0%)
DGE B/C	8 (8.3%)	7 (25.9%)	0.014	1 (2.0%)	0 (0%)	1.000	7 (15.6%)	7 (50.0%)
PPH B/C	3 (3.1%)	4 (14.8%)	0.041	2 (3.9%)	0 (0%)	1.000	1 (2.2%)	4 (25.0%)
Bile leak B/C ^b	1 (2.1%)	1 (5.6%)	0.464	0 (0%)	0 (0%)	1.000	1 (2.2%)	1 (6.3%)
Chyle leak B/C	11 (11.5%)	2 (7.4%)	0.731	4 (7.8%)	1 (9.1%)	1.000	7 (15.6%)	1 (6.3%)
Any B/C	36 (37.5%)	15 (55.6%)	0.092	15 (29.4%)	4 (36.4%)	0.724	21 (46.7%)	11 (68.8%)
CCI ^c	20.92	26.22	0.131	12.25	20.92	0.844	28.94	45.12
	[0 – 100]	[0 – 100]		[0 – 92.94]	[0 – 27.61]		[0 – 100]	[0 – 100]
CCI ≥ 37.1	19 (19.8%)	10 (37.0%)	0.062	6 (11.8%)	0 (0%)	0.580	13 (28.9%)	10 (62.5%)
Number of complications	1 [0 – 10]	2 [0 – 17]	0.034	1 [0 – 10]	1 [0 – 2]	0.618	2 [0 – 6]	4.5 [0 – 17]
Euro (€)	15336	14683	0.864	14564	13534	0.160	16400	19953
	[10098 – 46402]	[10098 – 46402]		[10098 – 42035]	[10098 – 15710]		[10098 – 46402]	[10098 – 46402]
USD (\$)	45775	44362	0.924	43471	40396	0.144	49074	59556
	[30140 – 138497]	[30140 – 138497]		[30140 – 125463]	[30140 – 46890]		[30140 – 138497]	[30140 – 138497]

Continuous variables presented as median [range].

Minor resections include enucleations and distal pancreatectomies.

Major resections include Whipple/PPPDs, total pancreatectomies and combined resections.

^aFor the POPF analysis, patients with a total pancreatectomy were removed from the denominator.^bFor the bile leak B/C analysis, only patients with surgery of the pancreatic head were included, i.e., patients after a Whipple/PPPD, total pancreatectomy, combined pancreatectomy and enucleation of the pancreatic head.

Abbreviations: CCI comprehensive complication index, DGE delayed gastric emptying, N number of, POPF postoperative pancreatic fistula, PPH post-pancreatectomy hemorrhage, USD United States dollars

differences. No differences were observed in metastatic status. For both the F-pNET and NF-pNET subgroups, patients undergoing major resections had a significantly higher risk for a severe complication, DGE and cumulative CCI* ≥ 37.1 , and a higher number of complications, CCI*, and estimated costs.

No differences in complications were observed between patients with a radiological NF-pNET of <2 or ≥ 2 cm. However, in patients with a NF-pNET <2 cm, those undergoing major resections suffered significantly more often from a severe complication, any grade B/C complication, and had a higher CCI* (Supplementary Table 5).

DISCUSSION

This study investigated the incidence and severity of complications and risk factors for complications after resections of pNETs in 2 ENETS Centers of Excellence. Although mortality was low (0.8%), a severe complication occurred in 41.5% of patients. An increased risk for a severe complication, independent of age, ASA grade, tumor functionality and a radiological pNET of 2.0 cm or more, was observed in patients undergoing a Whipple/PPPD versus those undergoing a DP. A higher percentage of patients with MEN1/VHL – especially those undergoing major resections – had complications than those with sporadic disease, and the cumulative burden of complications was higher.

A meta-analysis reported mortality rates after operations for pNETs as high as 3 to 6%, depending on the procedure performed.²² In contrast, mortality was 0.8% (procedure specific range 0 to 2%) in the present study, which is similar to several more recent cohort studies within expert centers reporting mortality rates between 0 and 1.5%.^{14–19,41} This most likely reflects improved outcomes after centralization of pNET care and pancreatic surgery.²⁴ The rate of complications was substantial, but only 2% of patients with a severe complication died, indicating that the failure-to-rescue was low. Moreover, no patient died of a grade B/C pancreatic surgery-associated complication. This is in line with observations in the Netherlands that the failure-to-rescue is generally lower in high-volume than in low-volume centers.⁴² Mortality and failure-to-rescue rates were below benchmark cut-offs – established within low-risk pancreatoduodenectomy cases in 23 international high-volume centers in pancreatic surgery – whereas the CCI* and severe complications fell within the 75th percentile.⁴³ Over the years, a nationwide collaboration has been established to improve outcomes after pancreatic surgery. Although their results and experience likely have improved surgical outcomes, ongoing prospective studies – which predominantly included patients after the present study – will evaluate whether nationwide standardization of postoperative care will decrease the rates of major morbidity and POPE.

Rates of a severe complication ranged from 15.0 to 30.7% in other series, which is lower than in the present data.^{14,19,41} Complications were the primary outcome of the present study and were therefore precisely assessed and graded according to the Clavien-Dindo and ISGPS criteria and definitions. In addition, the present study included a high number of major pancreatectomies – 49.6% of the current cohort – compared to 21.3% to 31.7% in these other studies.^{14,19,41} The risk of complications was procedure-specific, as severe morbidity was observed in more than half of the Whipple/PPPD cases. Overall, endoscopic feeding tube placement for DGE contributed to the incidence of severe morbidity, as 13 patients had a single Clavien-Dindo grade 3 complication due to endoscopic feeding tube placement for DGE.

The overall rate of POPF grade B/C is considerably higher than the 12% to 13% after ‘general’ pancreatic operations.⁴⁴ Patients with a pNET generally have a soft pancreas, which induces a higher exocrine activity with more enzyme-rich pancreatic fluid, a main pancreatic duct of less than 3 mm, more side branches, and a reduced suture holding capacity, which complicate the operation.^{12,13,45} In this respect, others have shown that pancreatoduodenectomy for pNET as compared with adenocarcinoma is associated with POPF.¹³ In that study, 82% of patients with pNETs had a soft pancreas, and the median main pancreatic duct size was 3 mm.¹³ The combination of soft texture and a main pancreatic duct diameter ≤ 3 mm gives the highest risk of POPF following pancreatoduodenectomy.⁴⁶ Nevertheless, the POPF rate in the present study was 30.8%, which compares favorably to the 34.3% observed after surgical resection for pNETs in another high-volume expert center adopting the 2016 ISGPS criteria.²³ Intervention driven complication classification systems, such as the Clavien-Dindo and ISGPS definitions, lead to high percentages of complications, whereas early identification and timely treatment, such as percutaneous drainage for POPE, are aimed at reducing the incidence of multiorgan failure and mortality.

In contrast to several other studies comparing complications after resections for pNETs, which have reported a higher rate of POPF in patients undergoing a pancreas-sparing (ie, enucleation) versus a standard resection (ie, DP or pancreatoduodenectomy), none of the patients in the enucleation group suffered from a POPE.^{16,19,22,23} Potential explanations include the low number of patients undergoing an enucleation, improved patient selection – in most patients, intraoperative ultrasound was used to determine the distance to the main pancreatic duct – and expertise in surgical teams in the present series. Although a meta-analysis observed a higher rate of POPF after enucleations compared to standard resections, this risk was not increased in high-volume centers.⁴⁷ Only 1 patient developed a severe complication in the present series, indicating that enucleation may be superior regarding complications in selected patients. The feasibility of enucleation depends on tumor location, size, and distance from the main pancreatic duct.⁴⁸

In the context of complications, little is known about patients with MEN1/VHL versus

sporadic disease. Patients with MEN1 are generally affected by multifocal pNETs and even duodenal tumors, whereas patients with VHL usually have cystic pancreatic tissue, making surgery more difficult. In the present study, a severe complication, POPF, DGE and PPH, occurred more often in patients with MEN1/VHL. A previous study observed POPF more often in patients with hereditary pNETs and those with combined resections.¹⁸ The stratified analysis demonstrated that the risk of complications was similar between both groups after minor resections. In contrast, after major resections, patients with a MEN1/VHL-related syndrome had a 17.5% higher risk of a severe complication, and the percentage of PPH and DGE and the cumulative burden of complications were significantly higher. This extremely high risk – 2 out of 3 patients developed a severe complication – can be attributed to the high proportion of total and combined pancreatectomies, which were almost exclusively performed in patients with multifocal pNETs. The combined resections have only rarely been described.^{21,48,49} Along with the risks of a soft pancreas, patients undergoing a Whipple/PPPD plus DP are more prone to leakage from the pancreatic anastomosis as well as stump leakage from the cutting surface. This severe morbidity underscores the importance of adequate risk stratification and centralization of patients with MEN1/VHL-related pNETs – which are substantially younger and affected by multifocal disease – in multidisciplinary tumor boards and surgical teams with vast experience in pNETs and MEN1/VHL.

Performing surgery for pNETs is a risk-benefit balance between the oncological benefit versus the risk of complications. The complication rate and morbidity are high. Nevertheless, the latter does not mean that these operations should not be performed since surgeons have an excellent rate of rescue from complications and these tumors can be malignant. Within the present study, no disease-related factors, such as tumor size, were identified that were related to the occurrence of complications and could subsequently contribute to patient selection. The extent of surgery was the most important predictor for severe complications. These data are relevant to guide preoperative patient counselling and enable shared decision-making regarding the timing and the extent of resection.

The observed complication and failure-to-rescue rates were observed in 2 ENETS Centers of Excellence. Therefore, these results might not necessarily be applicable to lower-volume hospitals. Although no comparison was made with lower-volume hospitals, these data imply that surgery for pNETs should be reserved for centers of excellence. First, as shown, within these centers, the failure-to-rescue rate is low, likely due to timely detection of complications on the wards and adequate management of these patients by surgeons, gastroenterologists or interventional radiologists. Second, surgeons should be familiar with all (unconventional) pancreatic procedures, as these might provide excellent oncologic outcomes, and the risk of complications is procedure-specific. Third, centers of excellence often have a dedicated multidisciplinary team that enables adequate preoperative risk stratification. Fourth, patients with hereditary syndromes often undergo extensive procedures,

such as combined pancreatectomies, which are associated with a high risk of complications, therefore underscoring the importance of adequate risk stratification of each tumor in these patients.

The major strength of the present study is the comprehensive, sequential cohort of patients, including both sporadic and MEN1/VHL-related pNETs, from 2 expert centers, including results from many surgeons operating patients with pNETs over a recent period, thereby accurately reflecting current day practice. Large administrative databases are often not specific enough to provide a detailed assessment of postoperative complications. The recent study period assured electronic patient records with extensive information, including postoperative notes, discharge letters, imaging reports, laboratory values, and reinterventions, thereby ensuring complete and reliable assessment of every individual patient's postoperative course. To accurately capture and grade every complication demands substantial effort, which is a specific strength of this study. Complications were systematically assessed and graded according to the most recent accepted and validated classifications.^{29,32,50} Missing data were encountered and imputed using multiple imputation, which currently is the best statistical method to handle missing data.⁵¹ The main limitation includes the relatively small sample size, which limited covariate adjustment. In addition, pancreatic texture and pancreatic duct size, known risk factors for POPF after pancreatoduodenectomy, were not available for analysis and could not be retrieved given the retrospective study design. The number of patients in the enucleation group was limited, and, therefore, enucleations of the head and those of the body/tail were grouped. Ideally, enucleations would be the reference group for multivariable analysis, but this was impossible considering the low number of complications. Estimated costs were only estimated by methods proposed by Staiger et al.³⁰ and not directly calculated by combining all costs associated with the operation and subsequent postoperative care.

This study shows that resections for pNETs can be safely performed in ENETS Centers of Excellence. Although a considerable and procedure-dependent risk of severe morbidity was observed, mortality and failure-to-rescue were low. These data will aid preoperative patient counselling and might additionally be used for shared-decision making regarding the timing and the extent of surgical resection. Patients with MEN1/VHL-related pNETs are a challenging surgical entity and therefore warrant specialized care.

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Supplementary Table 1. Differences between patients undergoing spleen-preserving distal pancreatectomy and distal pancreatectomy with splenectomy.

Characteristic	Spleen-preserving n = 27 (54.0%)	Splenectomy n = 23 (46.0%)	p-value
Age at surgery in years	56 [6 – 81]	56 [18 – 76]	0.386
Sex			
Male	12 (44.4%)	12 (52.2%)	0.586
Female	15 (55.6%)	11 (47.8%)	
Type of pNET			
NF-pNET	14 (51.9%)	18 (78.3%)	0.077
F-pNET	13 (48.1%)	5 (21.7%)	
Size largest pNET on conventional imaging in mm, median [range], n=48 (96.0%)	19 [6 – 93]	38 [9 – 140]	<0.001
pNET ≥ 2 cm on preoperative imaging, n=48 (96.0%)	11 (44.0%)	20 (87.0%)	0.003
Indication			NA
Suspicion of spleen/splenic vessels involvement	NA	21 (91.3%)	
Intraoperative iatrogenic damage		2 (8.7%)	
Suspected lymph node metastases on imaging	2 (7.4%)	9 (39.1%)	0.014
Suspected liver metastases on imaging	1 (3.7%)	7 (30.4%)	0.017
Approach			
Open	11 (40.7%)	8 (34.8%)	0.002
Laparoscopic	2 (7.4%)	11 (47.8%)	
Robot-assisted	14 (51.9%)	4 (17.4%)	
Intraoperative outcomes			
Duration of surgery in minutes, n= 49 (98.0%)	200 [115 – 400]	288 [121 – 582]	0.016
Blood loss in ml, n=48 (96.0%)	250 [0 – 1350]	400 [100 – 5000]	0.113
Hospital stay			
Length of stay in days	7 [4 – 36]	10 [7 – 64]	0.001
Re-laparotomy	0 (0%)	3 (13.0%)	0.090
Postoperative complications			
Clavien-Dindo ≥ III	8 (29.6%)	8 (34.8%)	0.697
POPF B/C	8 (29.6%)	8 (34.8%)	0.697
DGE B/C	1 (3.7%)	0 (0%)	1.000
PPH B/C	1 (3.7%)	1 (4.3%)	1.000
Chyle leak B/C	0 (0%)	5 (21.7%)	0.016
Any B/C complication	9 (33.3%)	10 (43.5%)	0.563
CCI†	8.7 [0 – 56.5]	20.9 [0 – 92.9]	0.533
CCI ≥ 37.1	4 (14.8%)	2 (8.7%)	0.674
Number of complications	1 [0 – 5]	1 [0 – 10]	0.452
Spleen-specific complications			
Thrombosis/pulmonary embolism	0	0	-
Arterial thrombosis (TIA/CVA/MI)	0	0	-
Splenic infarction/ischemia	4 (14.8%)	0	0.115
Infections (pulmonary, urologic, catheter related infections)	4 (14.8%)	3 (13.0%)	1.000

Continuous variables are reported as median [range].

Abbreviations: CCI† comprehensive complication index, DGE delayed gastric emptying, F-pNET functioning pancreatic neuroendocrine tumor, MEN1 multiple endocrine neoplasia type 1, N number of, NF-pNET non-functioning pancreatic neuroendocrine tumor, pNET pancreatic neuroendocrine tumor, POPF postoperative pancreatic fistula, PPH post-pancreatectomy hemorrhage, PPPD pylorus-preserving pancreatoduodenectomy

Supplementary Table 2. Sensitivity analyses.

	Covariates	Adjusted OR	95% CI
Pancreatic resection			
Distal pancreatectomy	None	1 [*]	Ref.
Enucleation		0.19	0.02 – 1.66
Whipple/PPPD		2.80	1.23 – 6.37
Total or combined		2.13	0.53 – 8.52
Pancreatic resection			
Distal pancreatectomy	Age	1	Ref.
Enucleation		0.19	0.02 – 1.66
Whipple/PPPD		2.81	1.23 – 6.38
Total or combined		2.11	0.52 – 8.55
Pancreatic resection			
Distal pancreatectomy	Age	1	Ref.
Enucleation	ASA (1 or 2 versus 3 or 4)	0.20	0.02 – 1.77
Whipple/PPPD		2.84	1.24 – 6.50
Total or combined		2.10	0.51 – 8.59
Pancreatic resection			
Distal pancreatectomy	Age	1	Ref.
Enucleation	ASA (1 or 2 versus 3 or 4)	0.13	0.02 – 1.71
Whipple/PPPD	pNET functionality (NF-pNET vs. F-pNET)	3.13	1.33 – 7.36
Total or combined		2.12	0.51 – 8.79
Pancreatic resection			
Distal pancreatectomy	Age	1 [†]	Ref.
Enucleation	ASA (1 or 2 vs. 3 or 4)	0.19	0.02 – 1.73
Whipple/PPPD	pNET functionality (NF-pNET vs. F-pNET)	3.13	1.32 – 7.41
Total or combined	pNET ≥2 cm (present vs. absent)	2.14	0.50 – 9.11

^{*}Univariable model as presented in Table 4.

[†]Multivariable model as presented in Table 4.

Abbreviations: ASA American Society of Anesthesiologists, CI confidence interval, F-pNET functioning pancreatic neuroendocrine tumor, NF-pNET non-functioning pancreatic neuroendocrine tumor, OR odds ratio, Ref. reference category, vs. versus

Supplementary Table 3. Risk factors for postoperative pancreatic fistula.

	POPF [*]		Univariable analysis	
	Yes N = 36 (30.8%)	No N = 81 (69.2%)	Crude OR	95% c.i.
Age surgery in years [†]	53 [18 – 81]	57 [6 – 79]	0.98	0.96 – 1.01
Sex				
Female	17 (47.2%)	45 (55.6%)	1	Ref.
Male	19 (52.8%)	36 (44.4%)	1.40	0.63 – 3.10
ASA grade				
ASA 1 or 2	31 (86.1%)	64 (79.0%)	1	Ref.
ASA 3 or 4	5 (13.9%)	17 (21.0%)	0.61	0.20 – 1.82
Pancreatic surgery				
Distal pancreatectomy	16 (44.4%)	34 (42.0%)	1	Ref.
Enucleation	0 (0%)	12 (14.8%)	-	-
Whipple/PPPD	18 (50%)	33 (40.7%)	1.16	0.50 – 2.67
Combined resection	2 (5.6%)	2 (2.5%)	2.13	0.27 – 16.9
Type of tumor				
NF-pNET	26 (72.2%)	57 (70.4%)	1	Ref.
F-pNET	10 (27.8%)	24 (29.6%)	0.91	0.38 – 2.20
Hereditary syndrome				
Absent	27 (76.2%)	67 (82.7%)	1	Ref.
Present	9 (23.8%)	14 (17.3%)	1.60	0.61 – 4.16
Size imaging in mm	24 [6 – 120]	23 [8 – 140]	1.00	0.98 – 1.01
pNET ≥ 2 cm imaging				
Absent	12 (33.3%)	35 (43.2%)	1	Ref.
Present	24 (66.6%)	46 (56.8%)	1.49	0.62 – 3.54
Lymph node metastases				
Absent	26 (72.2%)	66 (81.5%)	1	Ref.
Present	10 (27.8%)	19 (23.5%)	1.23	0.50 – 3.03
Liver metastases				
Absent	27 (75.0%)	70 (88.4%)	1	Ref.
Present	9 (25.0%)	11 (13.6%)	2.10	0.77 – 5.70
Approach				
Open	29 (80.5%)	51 (63.0%)	1	Ref.
Laparoscopic	4 (11.1%)	12 (14.8%)	0.68	0.20 – 2.35
Robot-assisted	7 (19.4%)	18 (22.2%)	0.79	0.29 – 2.17

Missing data were observed for size pNET imaging (6.5%), pNET size ≥ 2 cm (6.5%), suspected lymph node metastases on imaging (0.8%), suspected liver metastases on imaging (0.8%). For all other variables no missing data were observed.

Patients with a total pancreatectomy were excluded from the analysis.

^{*}Data given after multiple imputation.

[†]For comparison between patients with and without a severe complication median (range) presented.

Abbreviations: ASA American Society of Anesthesiologists, CI confidence interval, F-pNET functioning pancreatic neuroendocrine tumor, NF-pNET non-functioning pancreatic neuroendocrine tumor, No. number of, OR odds ratio, POPF postoperative pancreatic fistula, Ref. reference category

Supplementary Table 4. Complications in patients with a F-pNET versus NF-pNET.

Characteristic	Overall		Minor resection			Major resection		
	F-pNET n = 36	NF-pNET n = 87	p-value	F-pNET n = 23	NF-pNET n = 39	p-value	F-pNET n = 13	NF-pNET n = 48
Clavien-Dindo ≥ III	16 (44.4%)	35 (40.2%)	0.666	6 (26.1%)	11 (28.2%)	0.857	10 (76.9%)	24 (50.0%)
POPF B/C*	10 (29.4%)	26 (31.3%)	0.839	5 (21.7%)	11 (28.2%)	0.574	5 (45.5%)	15 (34.1%)
DGE B/C	6 (16.7%)	9 (10.3%)	0.330	1 (4.3%)	0 (0%)	0.371	5 (38.5%)	9 (18.8%)
PPH B/C	4 (11.1%)	3 (3.4%)	0.193	1 (4.3%)	1 (2.6%)	1.000	3 (23.1%)	2 (4.2%)
Bile leak B/C†	0 (0%)	2 (3.9%)	1.000	0 (0%)	0 (0%)	-	0 (0%)	2 (4.2%)
Chyle leak B/C	1 (2.8%)	12 (13.8%)	0.106	0 (0%)	5 (12.8%)	0.148	7 (14.6%)	1 (7.7%)
Any B/C	16 (44.4%)	35 (40.2%)	0.666	6 (26.1%)	13 (33.3%)	0.550	10 (76.9%)	22 (45.8%)
CCI‡	23.44 [0 – 100]	22.64 [0 – 100]	0.915	12.25 [0 – 55.11]	20.90 [0 – 92.94]	0.377	37.08 [22.64 – 100]	27.58 [0 – 100]
CCI‡ ≥ 37.1	8 (22.2%)	21 (24.1%)	0.820	1 (4.3%)	5 (12.8%)	0.398	7 (53.8%)	16 (33.3%)
Number of complications	2 [0 – 17]	1 [0 – 12]	0.995	1 [0 – 4]	1 [0 – 10]	0.417	3 [1 – 17]	2 [0 – 12]
Euro	15336 [10098 – 46402]	15336 [10098 – 46402]	0.728	12917 [10098 – 24751]	14564 [10098 – 42035]	0.327	18590 [14178 – 46402]	16480 [10098 – 46402]
USD	45775 [30140 – 138497]	45775 [30140 – 138497]	0.734	38555 [30140 – 73876]	43471 [30140 – 125463]	0.286	55485 [42318 – 138497]	49187 [30140 – 138497]

Continuous variables presented as median [range].

Minor resections include enucleations and distal pancreatectomies.

Major resections include Whipple/PPPDs, total pancreatectomies and combined resections.

*For the POPF analysis, patients with a total pancreatectomy were removed from the denominator.

†For the bile leak B/C analysis, only patients with surgery of the pancreatic head were included, i.e., patients after a Whipple/PPPD, total pancreatectomy, combined pancreatectomy and enucleation of the pancreatic head.

Abbreviations: CCI‡ comprehensive complication index, DGE delayed gastric emptying, F-pNET functioning pancreatic neuroendocrine tumor, N number of, NF-pNET non-functioning pancreatic neuroendocrine tumor, POPF postoperative pancreatic fistula, PPH post-pancreatectomy hemorrhage, USD United States dollars

Supplementary Table 5. Outcomes in patients with a NF-pNET.

Characteristic	NF-pNET overall			NF-pNET <2 cm		
	<2 cm n = 23	≥2 cm n = 60	p-value	Minor n = 10	Major n = 13	p-value
Clavien-Dindo ≥ III	10 (43.5%)	25 (41.7%)	0.881	1 (10.0%)	9 (69.2%)	0.010
POPF B/C*	6 (26.1%)	19 (33.9%)	0.496	1 (10.0%)	5 (38.5%)	0.179
DGE B/C	4 (17.4%)	5 (8.3%)	0.254	0 (0%)	4 (30.8%)	0.104
PPH B/C	0 (0%)	3 (5.0%)	0.557	0 (0%)	0 (0%)	-
Bile leak B/C†	0 (0%)	2 (6.1%)	1.000	0 (0%)	0 (0%)	-
Chyle leak B/C	3 (13.0%)	9 (15.0%)	1.000	0 (0%)	3 (23.1%)	0.229
Any B/C	9 (39.1%)	25 (41.7%)	0.833	1 (10.0%)	8 (61.5%)	0.029
CCI‡	26.2 [0 – 76.0]	24.2 [0 – 100]	0.520	8.7 [0 – 34.6]	33.5 [8.7 – 76.0]	0.010
CCI ≥ 37.1	4 (17.4%)	17 (28.3%)	0.305	0 (0%)	4 (30.8%)	0.104
Number of complications	2 [0 – 12]	1 [0 – 10]	0.879	1 [0 – 5]	2 [1 – 12]	0.036

Four patients with a NF-pNET had missing preoperative radiological tumor size.

Continuous variables presented as median [range].

Minor resections include enucleations and distal pancreatectomies.

Major resections include Whipple/PPPDs, total pancreatectomies and combined resections.

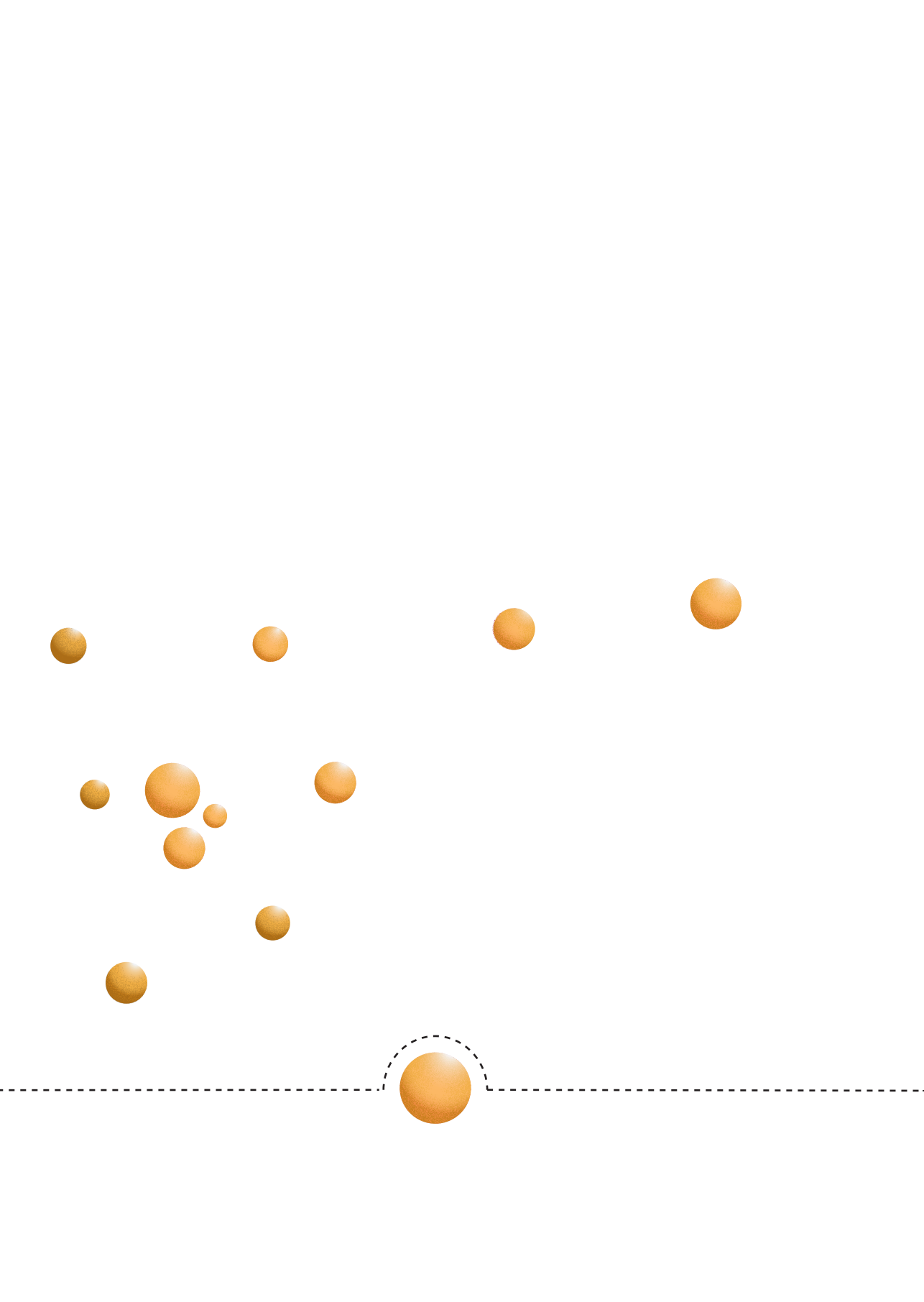
*For the POPF analysis, patients with a total pancreatectomy were removed from the denominator.

†For the bile leak B/C analysis, only patients with surgery of the pancreatic head were included, i.e., patients after a Whipple/PPPD, total pancreatectomy, combined pancreatectomy and enucleation of the pancreatic head.

Abbreviations: CCI‡ comprehensive complication index, DGE delayed gastric emptying, N number of, NF-pNET non-functioning pancreatic neuroendocrine tumor, POPF postoperative pancreatic fistula, PPH post-pancreatectomy hemorrhage

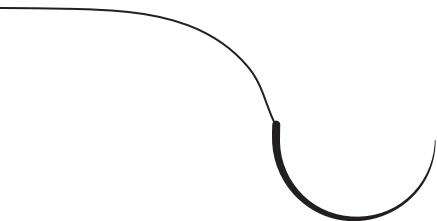
PART V

SUMMARY AND GENERAL DISCUSSION



CHAPTER XIII

Summary



Multiple endocrine neoplasia type 1 (MEN1) is a very rare autosomal disease, which hampers establishing guidelines or consensus statements based on the highest quality of evidence. Especially for MEN1-related duodenopancreatic neuroendocrine tumors (dpNETs) – which are the leading cause of death, occur in over 50% of patients and contribute to the overall psychological morbidity of intensive screening programs and the fear of malignant disease – well-grounded recommendations for diagnosis and therapy are lacking. One other important aspect is the lack of data regarding prognosis and prognostic factors, which could be used to guide clinical decisions.

This thesis aimed to improve patient care and to provide a first step toward individualized cancer care and tailored surgery for patients with MEN1-related dpNETs. Whereas a ‘*one size fits all*’ regimen is generally proposed for patients with a common disease, those with MEN1 are affected by multiple manifestations and multifocal pancreatoduodenal involvement, which demands specialized care taking multiple aspects of the disease and patient into account. To improve clinical care for the individual patient, several important aspects must be enlightened. Therefore, the studies presented in this thesis propose improvements regarding research strategies (Part I), diagnosis (Part II), prognosis (Part III) and surgical therapy (Part IV) of MEN1-related dpNETs. In this chapter the observations of the studies presented in this thesis are summarized.

PART I. RESEARCH STRATEGIES

Chapter 2. Research strategies in multiple endocrine neoplasia type 1

The foundation of the DutchMEN Study Group (DMSG) in 2008 has provided the first step in the development of the Dutch MEN1 database. The database has enabled true population-based studies which have significantly improved understanding of MEN1. In this study, the process from the initial formulation of clinical research questions, nationwide collaboration, patient enrolment, database development, data collection and handling was critically evaluated and described. It was concluded that, when randomized clinical trials or large prospective studies are unfeasible, retrospective observational population-based studies having minimized bias, improve patient care. The stepwise approach could be used by other researchers involved in MEN1 care or in rare diseases with similarities to MEN1 to implement similar databases in their centers or countries.

PART II. DIAGNOSIS

Chapter 3. Diagnosing non-functioning pancreatic neuroendocrine tumors

Over the past decades, studies have shown that non-functioning pancreatic neuroendocrine tumors (NF-pNETs) – associated with the highest risk of liver metastases development and death – are the most frequently occurring pNETs in MEN1. Therefore, guidelines recommend screening programs to enable early identification and timely treatment, watchful waiting or surgery, for MEN1-related NF-pNETs. However, no evidence-based consensus has been established on whether screening should include blood-based tumor markers, which imaging modality should be preferred and at what age screening should commence. Furthermore, if NF-pNETs are diagnosed, no recommendations on follow-up protocols exist, due to a general lack of understanding of tumor growth rates. Therefore, this study systematically reviewed the literature to answer four questions related to diagnosis and follow-up of MEN1-related NF-pNETs. Based on the results from 11 studies, diagnostic accuracy measures of tumor markers were low, which indicates that these should not be used for NF-pNET diagnosis in MEN1. In 16 studies, reported sensitivities of endoscopic ultrasound (EUS) (range between studies 75 – 100%) were superior over magnetic resonance imaging (MRI) (74 – 88%) and computed tomography (CT) (54 – 81%). A strategy combining EUS and MRI is recommended, since clinically relevant tumors are missed on EUS. ⁶⁸Gallium-Dota positron emission tomography (PET)/CT was superior over somatostatin receptor scintigraphy using ¹¹¹Indium pentetreotide. This indicates that the aforementioned should be preferred, but ⁶⁸Gallium-Dota PET/CT should not be routinely used for first line screening. The average growth rate ranged from 0.1 to 1.32 mm per year in eight studies, which suggests that the frequency of pancreatic imaging could be individualized and extended to once per 1 to 2 years. Based on the currently available literature no recommendations can be provided on the optimum timing of screening, but screening should not be extended until the age of 16.

Chapter 4. Pancreatic imaging and biopsies

Little is known on the effects of the imaging program and guidelines lack recommendations on a preferred imaging modality. In addition, when a radiologic lesion suspicious for a pancreatic neuroendocrine tumor (pNET) is observed, the question remains as to whether histopathological confirmation is demanded before clinical decisions are made. The overall diagnostic accuracy of imaging studies (MRI, CT and EUS) was investigated in 377 patients from the DMSG database. Over the study period the absolute number of imaging studies increased, and a preference for MRI was observed in the last decade. Diagnostic accuracy for all imaging studies irrespective of type was good with positive and negative predictive values of around 90%. The diagnostic accuracy of MRI was superior over CT. Most resection

specimens and fine needle aspiration (FNA) correctly showed a pNET. In four out of ten for which the initial FNA did not show a pNET, the FNA was considered as pancreatic ductal adenocarcinoma – in only two this diagnosis was correctly made, all other patients were diagnosed with a pNET. Of the entire cohort three patients, all older than 60 years, had a final diagnosis of pancreatic ductal adenocarcinoma. The accuracy for diagnosing MEN1-related pNET of MRI was higher than CT and therefore MRI should be the preferred (non-invasive) imaging modality for pNET screening/surveillance. Routine EUS with FNA is of little additional value and can be reserved for those patients considered to be at high risk for adenocarcinoma because of age and radiologic tumor characteristics.

Chapter 5. Reproducibility of imaging-based tumor size

Tumor size is regarded as the main prognostic factor for MEN1-related pNETs. Therefore, surgical indications for NF-pNETs are based on size. Recent data suggested that preoperative tumor size is frequently overestimated. Therefore, reliability and agreement of radiological and pathological tumor size were investigated in 73 patients in the Dutch population-based cohort. Overall, no systematic overestimation or underestimation was observed for imaging, nor for the subtypes of MRI, CT or EUS. For pNETs categorized as <2 and ≥ 2 cm, agreement was 81.5%, indicating that imaging and pathology classified patients differently in 18.5% of the patients. Agreement between MRI, CT, and EUS, and pathology was 88.6, 85.7, and 75.0%, respectively. These data indicate that preoperative tumor size in MEN1-related pNETs is not systematically overestimated and suggest that MRI should be the preferred imaging modality.

PART III. PROGNOSIS

Chapter 6. Survival of patients with gastrinomas and factors associated with survival

Although the majority of patients with MEN1-related gastrinomas can be adequately managed medically, surgery may be considered for a subgroup to prevent metastatic disease. Factors to guide surgical management are lacking. Therefore, overall survival and risk factors associated with survival were assessed in 63 patients with a MEN1-related gastrinoma from the Dutch population-based cohort. Ten-year overall survival was 65%, which was lower than for age and sex matched patients with MEN1. Higher initial fasting gastrin levels, a pNET ≥ 2 cm, liver metastases, suspected gastric neuroendocrine tumors and multiple concurrent NETs were associated with survival. These data show that life expectancy of patients with MEN1-related gastrinomas is reduced. Fasting serum gastrin levels and the presence of a large pNET could aid in selecting patients for operative resection.

Chapter 7. Metastatic patterns

In patients with multifocal MEN1-related dpNETs, determining of the origin of metastasis is challenging. Understanding metastatic patterns could aid clinical and surgical decision making, and improve post-resection prognostication by adequate application of tumor, node, metastasis staging systems. The relationship between dpNETs and associated metastases was studied by endocrine differentiation – transcription factors ARX and PDX1 as surrogates – and gastrin expression in 137 primary dpNETs and microadenomas, and 36 matched locoregional and distant metastases of 10 patients with MEN1. In patients with MEN1-related gastrinomas, all peri-duodenal lymph node metastases expressed gastrin, and clustered with minute duodenal gastrinomas, not with larger pNETs. In three patients, multiple primary tumors of origin were suggested. Metastases of pNETs often clustered with high grade or ALT-positive primary tumors. These results express a duodenal origin of lymph node metastases. The anticipated extent of pancreatoduodenal disease will contribute to shared decision-making regarding the timing and extent of surgery in MEN1.

Chapter 8. Prognosis of patients with a resected pancreatic neuroendocrine tumor

Metastasized pNETs are the leading cause of death, but risk factors are largely unknown. It is unknown if prognosis is affected by tumor type. Therefore, this study assessed whether patients with a resected MEN1-related NF-pNET have a different prognosis as compared to patients with a resected MEN1-related insulinoma. Furthermore, tumor type specific risk factors were studied. A total of 153 patients, 61 with a NF-pNET and 92 with an insulinoma were included from an international multicenter cohort. Estimated 10-year liver metastases-free survival was significantly reduced for patients with a resected NF-pNET than those with a resected insulinoma. Liver metastases-free survival was associated with tumor type, tumor size and World Health Organization tumor grade. Liver metastases-free survival was similar for patients with resected tumors <2 cm and 2-3 cm, but worse for those tumors ≥3 cm. These data imply that subtypes of pNETs have different malignant behavior. Therefore, postoperative counseling and follow-up regimens should be tumor type specific and at least consider size and World Health Organization grade.

PART IV. SURGICAL THERAPY

Chapter 9. Outcomes after surgery for insulinomas

Surgical resection of insulinomas is aimed at removing the insulin-producing tumor as well as to minimize the change of future hypoglycemia recurrence and to prevent pancreatic insufficiency. The optimal extent of surgery, i.e., limited or formal pancreatic resections, is unknown. Ninety-six patients with a resected MEN1-related insulinoma were identified

in 46 centers from Europe and North America. Persistent or recurrent hypoglycemia occurred in 7% and the estimated 10-year hypoglycemia-free survival rate was 91%. Of the patients with localized disease, persistent disease occurred in 1 out of 46 patients after a pancreatic resection and recurrent disease occurred in 1 out of 17 after an enucleation. These data show that surgery for MEN1-related insulinoma is more successful than previously thought. Enucleation appears to be the favorable surgical strategy for treatment of localized MEN1-related insulinoma, owing to the absence of pancreatic insufficiency and high rate of symptom resolution.

Chapter 10. Complications after major surgery for duodenopancreatic neuroendocrine tumors

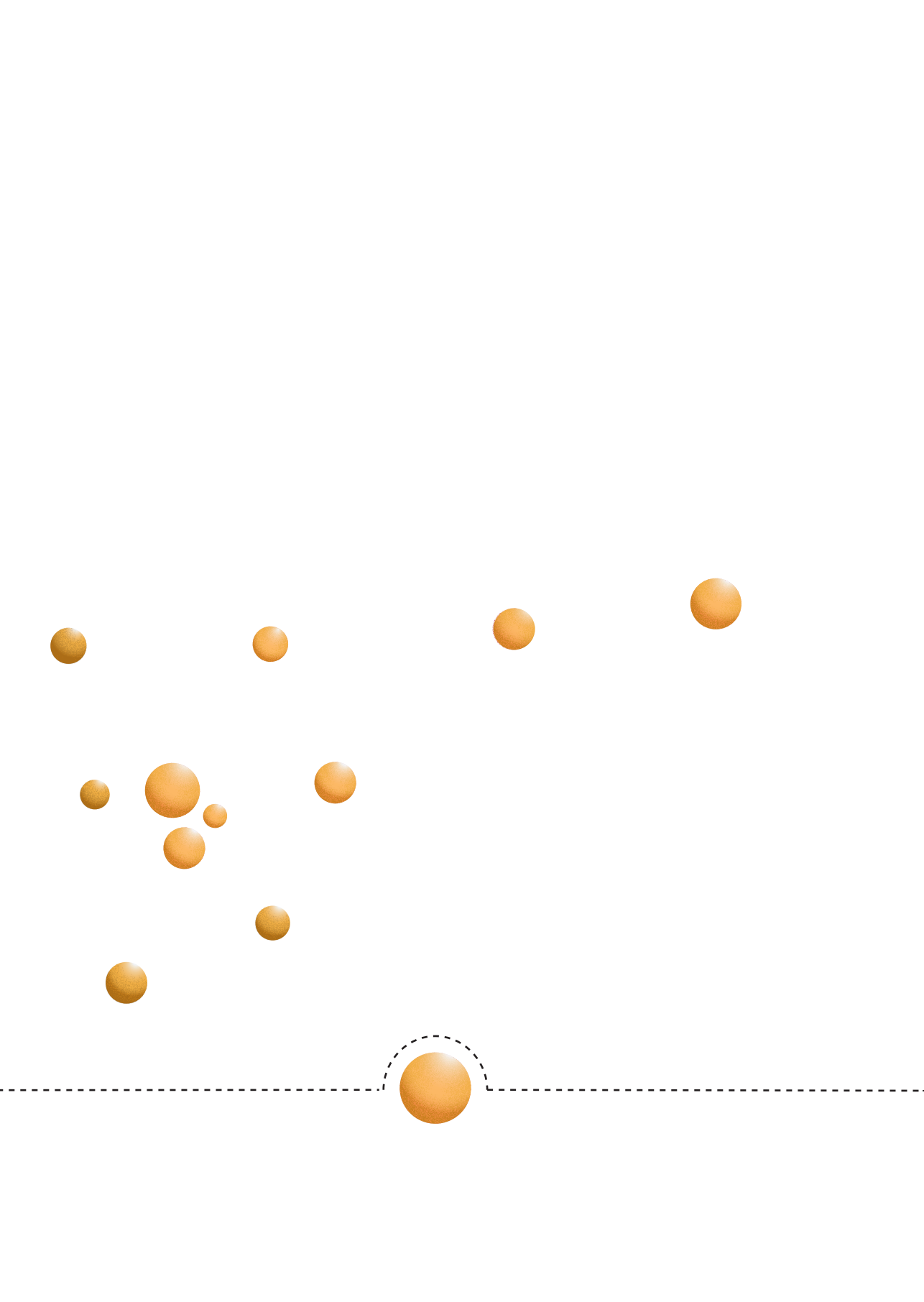
Pancreatic surgery for MEN1-related NF-pNETs is associated with a severe complication in every one out of three patients. For duodenal gastrinomas and pancreatic head tumors ineligible for enucleations, major resections i.e., Whipple procedures or total pancreatectomies, provide the only viable strategy for potential cure. Complication data in these specific patients are absent. Twenty-seven patients undergoing a major duodenopancreatic resection for MEN1-related dpNETs were identified in the Netherlands. Patients undergoing these procedures were young. A severe complication occurred in approximately two out of three patients and the cumulative burden of complications was tremendous. No preoperative factors were associated with a severe complication. These results underscore the importance of patient selection and adequate preoperative patient counseling and implicate that major duodenopancreatic surgery in MEN1 should be reserved for a selected subgroup.

Chapter 11. Perspectives major surgery in MEN1

Major surgery for MEN1-related duodenopancreatic neuroendocrine tumors is associated with a severe risk of complications and cumulative burden of complications. Future perspectives include the optimization of equivocal surgical indications to facilitate improved preoperative patient selection as well as a reduction of the burden of complications once major surgery is performed. Imaging-based risk stratification will enable early identification of malignant tumors when these are still small and potentially eligible for minimally invasive enucleations. Insight in metastatic patterns will contribute to tailoring of the extent of surgery. Indications for major surgery should be centralized to specialized multidisciplinary teams, and once major surgery is considered, centralization of these procedures to teams consisting of endocrine and hepato-pancreato-biliary surgeons with vast experience in MEN1-related dpNETs to reduce the incidence and impact of complications.

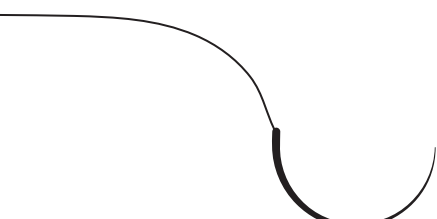
Chapter 12. Risk factors for complications after pancreatic surgery for neuroendocrine tumors

Large studies investigating complications after surgery for pNETs according to accepted classifications and definitions are lacking. Furthermore, little is known about risk factors for complications in these specific patients. Therefore, procedure-specific outcomes and risk factors for complications were assessed in 123 patients undergoing surgery for pNETs from 2008 – 2018 in the University Medical Center Utrecht and the University Medical Center Groningen. Mortality was 0.8%, a severe complication occurred in 41.5%, and the failure-to-rescue rate was 2.0%. A pancreatoduodenectomy, as compared to distal pancreatectomy increased the risk for a severe complication. After major surgery, the cumulative burden of complications was higher in patients with MEN1/VHL. The data express that surgery for pNETs is associated with a high rate of procedure-specific complications, but low failure-to-rescue in expert centers. In addition, the risk of complications is increased after major surgery for MEN1/VHL-related pNETs.



CHAPTER XIV

General discussion



The work included in this thesis provides insight in the diagnosis, follow-up, prognostication, and surgical outcomes of patients with MEN1-related dpNETs. These studies will contribute to the management of patients with MEN1-related dpNETs and subsequently provide a step towards personalized care in MEN1.

RESEARCH STRATEGIES

The population-based DutchMEN Study Group (DMSG) MEN1 database, with retrospective data collection covering 1990 up to 2014, provided tremendous new insights in MEN1 (**Chapter 2**). The initiation of such a study group is hard, constructing a longitudinal database covering multiple manifestations even harder, and continuously updating and improving the database according to new standards likely is the hardest. The latter steps must be preceded by a solid foundation. Internal improvements of the DMSG database as well as external advancements are needed to advance to the next generation research in MEN1. In addition, acknowledgement and adequate allocation of funding sources for rare diseases are demanded to continue this process.¹

Internal improvements

Internal improvements include prospective data collection and flexible updating of variables, such as incorporating new imaging or surgical techniques. Ideally, the database will be structured to automatically capture standardized values, such as laboratory data, from electronic patient records. Besides data collection, another step forward is the incorporation of Biobanks, enabling tissue and blood sample storage. Crosslinking clinical data and patient tissue characteristics will facilitate translational research. The DMSG including the eight University Medical Centers each maintaining its own Biobank, will enable these studies within a population-based cohort.

Several of these processes have yet commenced. Linking transcription factor analyses of individual tumors to long-term clinical data, facilitates the identification of tumor-based risk factors. The correlation between tissue micro arrays from MEN1-related pNETs and clinical data provided novel insights in transcription factors associated with prognosis.² In addition, organoids of pNETs are being developed based on resected tumor tissue.³ Organoids – stem cell-derived three-dimensional cultures replicating organs or specific cell types – can be used to study cell biology, genomic analyses, interactions with their environment, and evaluate the effectiveness of medical therapies.⁴ Besides the aforementioned internal improvements, additional cross-border hurdles need to be taken to improve global knowledge in MEN1.

External improvement – Teamwork makes the dream work

The next step is expanding existing networks and databases, such as the DMSG and the French Endocrine Tumor Study Group (Groupe d'étude des Tumeurs Endocrines (GTE)) databases to other regions of the world. Although understanding has increased through population-based and multicenter studies from these study groups, absolute patient numbers are still limited. Particularly for manifestations which have a low(er) incidence, such as insulinomas which occur in 10 – 15% of patients, extensions to these databases are needed. An example is the International Insulinoma Study Group, which involves 46 centers from Europe and North America (**Chapter 9**). Such international collaborations are particularly suitable for answering specific questions for which the database is built (**Chapter 9**) and subsequent questions (**Chapter 8**). Most databases exclusively include surgically treated patients, focus on only one single MEN1 manifestation and generally lack a standardized longitudinal design with long-term follow-up. Their use and continuity in the long-term are therefore limited for rare and complex diseases such as MEN1.

The combined efforts of international collaborations and databases like the DMSG will enable high-quality multinational population-based studies, preferably including tissue collection, in the near future. The major challenge of these large collaborations will be to generate unselected populations.

Randomized controlled trials in MEN1 – One step closer?

International collaborative research groups will provide a step toward randomized controlled trials in MEN1. Up to today, only one randomized controlled trial has been performed for MEN1-related primary hyperparathyroidism which occurs in almost all patients. However, it took 18 years to include 32 patients from two centers.⁵ Several topics for which observational studies have provided management advice still suffer from residual confounding, which can only be prevented by performing adequately powered randomized controlled trials with long-term follow-up.

International consortiums with up-to-date prospective databases will enable cross-sectional identification of patients eligible for inclusion in trials. Besides, surrogate markers for long-term outcomes are much needed, since these will accelerate prospective cohort studies and trials.

DIAGNOSIS & IMAGING

Diagnosing NF-pNETs in MEN1

The MEN1 life is a repetitive process of diagnosis and follow-up, which starts with genetic testing at birth at birth and subsequently leads to a life-long burden of biochemical testing

and imaging studies. These screening programs induce the continuous fear of having a tumor as well as developing their metastasis. Although most of these are irreversible, tailoring of the diagnostic and follow-up regimen could reduce their burden. The primary goal of screening is to identify clinically relevant non-functioning pNETs (NF-pNETs), which lead to morbidity and mortality. On the contrary, the overdiagnosis of small and indolent NF-pNETs, which induce anxiety and demand intensive follow-up, should be minimized. Imaging protocols should follow these aims.

In **Chapter 4** it was observed that almost two-thirds of the patients had a pNET on imaging during their follow-up. The number of imaging studies, as well as the positive number of imaging studies, increased gradually over the past decades regardless of a simultaneous increase in age. In the debate on the optimal imaging modality, the burden of these imaging techniques should be considered. In **Chapters 3 & 4**, it was shown that MRI is valuable for detecting clinically relevant (NF-)pNETs. Furthermore, it is non-invasive and particularly diffusion-weighted imaging (DWI) Magnetic resonance imaging (MRI) is useful for diagnosing liver metastases.⁶ One potential disadvantage of MRI is the use of gadolinium, which deposits in the brain. However, at present there is no evidence that these gadolinium deposits lead to clinical disease.⁷ Furthermore, a recent study reported that the diagnosis of MEN1-related pNET can be well established by an abbreviated MRI protocol using DWI indicating that contrast medium administration is unnecessary.⁸ Alike MRI, computed tomography (CT) is a systemic imaging modality. However, due to its undesirable radiation exposure unsuitable for long-term screening in MEN1. After eight years of CT or nuclear imaging scanning, the estimated effective radiation dose was above the threshold associated with developing secondary solid tumors or leukemia.⁹ The effective radiation dose was especially higher in patients with dpNETs and those with metastatic disease.⁹ In **Chapter 4** it was shown that CT decreased, and MRI increased gradually over the past decades. Moreover, in a recent MEN1 cohort the sensitivity of MRI was higher as compared with CT.

Endoscopic ultrasound (EUS) has proven to be the most sensitive, however, it is invasive, detects many small and clinically irrelevant pNETs and, on the contrary, misses clinically relevant pNETs particularly in the pancreatic tail (**Chapter 3**). Furthermore, MRI instead of EUS could lead to an estimated cost reduction of 0 – 67%.¹⁰

Follow-up of NF-pNETs

There is a lack of clarity regarding the optimal follow-up of NF-pNETs. Once NF-pNETs are diagnosed, patients continue the ‘*one size fits all*’ regimen of annual pancreatic imaging. However, as shown in **Chapter 3**, the growth rate of NF-pNETs was generally low. In addition, within the Dutch population-based cohort liver metastases rarely developed in patients with NF-pNETs smaller than 2 cm after a median follow-up of 3 years.¹¹ Thirty

percent of these tumors showed growth during follow-up, but no factors were identified that were associated with stable versus growing tumors.¹¹ If tumors were growing, patients with germline missense mutations versus nonsense and frameshift mutations had faster growing tumors.¹¹ The rate ratio for new pNETs was 1.04.¹¹ Based on the estimated growth rate, the minimal risk of adverse clinical events and the low rate of new pNETs, annual pancreatic imaging for all patients is deemed unnecessary. Nevertheless, personalized screening protocols are still nonexistent and at present reducing surveillance intensity is only possible in consultation with the patient. Further research including longer follow-up of these tumors is demanded to test whether these observations persist after long-term follow-up and whether germline mutations or other prognostic factors could guide surveillance intensity.

Imaging-based risk stratification

Clinical practice guidelines or consensus statements lack evidence-based recommendations on EUS-guided fine needle aspirations (FNA).¹²⁻¹⁵ In **Chapter 4** it was shown that the added value of FNA over a radiological diagnosis of pNETs in MEN1 is limited and should therefore not be routinely performed. However, an advantage of EUS is the opportunity to take EUS-guided FNA to determine World Health Organization (WHO) tumor grade.¹⁶ Tumor grade is associated with prognosis (**Chapter 8**) and could serve as criterion for resection.^{13,15,17} Considering the radiological screening program in MEN1, imaging-based risk stratification will be more patient friendly. ⁶⁸Gallium (⁶⁸Ga) labeled somatostatin analogues positron emission tomography (PET)/CT provides more accurate staging, particularly regarding metastases, which subsequently altered management decisions in 31% of patients.¹⁸ Proper studies evaluating the diagnosis of dpNET-related lymph node metastases in MEN1 are lacking. ⁶⁸Ga labelled PET/CT mainly focusses on diagnosing or ruling out metastatic lesions, whereas clinical care would particularly benefit from improved staging and possible indications for operative resection of pNETs in the absence of metastases. In a study of 49 patients with MEN1 undergoing ¹⁸F-fluorodeoxyglucose (¹⁸F-FDG) labelled PET/CT, FDG avidity was associated with a Ki-67 $\geq 5\%$ of pNETs or distant metastasis leading to a sensitivity of 85.7% (95% confidence interval (CI) 48.7 – 99.3%) and specificity of 95.2% (95% CI 84.2% – 99.2%), respectively.¹⁹ Despite their potential, additional studies are needed before ¹⁸F-FDG PET/CT should be implemented in MEN1 protocols.²⁰

Optimizing current imaging protocols

Apart from pNET diagnosis, imaging is used for tumor, node, metastasis (TNM) staging by determining tumor size, the presence of locoregional lymph node metastases and liver metastases. Estimation of tumor size and categorization of pNETs as smaller or larger than 2 cm based on MRI and CT is accurate (**Chapter 5**). Imaging-based size measurement

could be further improved by identifying the optimal measurement methodology, for example by obtaining radiological acquisitions suitable for 3D measurements to develop a protocol for size measurements of pNETs.

Besides tumor size as a prognostic factor for metastases, conventional imaging studies provide more data for imaging-based risk stratification. MRI provides morphological information – such as signal intensity, tumor heterogeneity, tumor extension, vascular behavior and enhancement patterns – and functional data. Functional features include apparent diffusion coefficients and true diffusion coefficients, which were inversely correlated to histopathological Ki67 labelling in sporadic pNETs.²¹ More likely, the future will embrace radiomics, a novel method for the quantitative description of medical imaging which extracts disease-related information within images which is not perceptible with the human eye. For sporadic pNETs, CT-based radiomic features are associated with tumor grade and survival.^{22–24} The MRI-based radiomics field is gradually expanding, which could hopefully lead to imaging-based risk stratification using conventional imaging for MEN1-related pNETs.²²

Localization

Conventional imaging is the key to NF-pNET diagnosis and ⁶⁸Ga labelled PET/CT plays the central role in determining the extent of pancreatoduodenal involvement (**Chapter 3**). Surgical resection of insulinomas under current preoperative conventional imaging studies is mostly successful in reversing hyperinsulinemic hypoglycemia (**Chapter 9**). However, as shown in **Chapter 9**, the preferred strategy for MEN1-related insulinomas is an enucleation if technically feasible. In this respect, the major motive to opt for an enucleation is the adequate and precise localization of the insulin-producing pNET in the background of diffuse NF-pNETs, which demands specific imaging strategies. While NF-pNETs and gastrinomas express somatostatin receptors type 2, insulinomas express the glucagon-like peptide-1 receptor which can be specifically targeted using ⁶⁸Ga-DOTA-exendin-4 ([Nle¹⁴, Lys⁴⁰](Ahx-DOTA-⁶⁸Ga)NH₂) exendin-4. For sporadic insulinomas, ⁶⁸Ga-DOTA-exendin-4 PET/CT is superior over Single Photon Emission Computed Tomography/CT or CT or MRI.²⁸ For MEN1-related insulinomas, ⁶⁸Ga-DOTA-exendin-4 PET/CT results are promising. Ten out of 11 insulinomas in six patients were adequately localized by ⁶⁸Ga-DOTA-exendin-4 PET/CT.²⁹ This promising technique should be evaluated in more patients with MEN1, especially as an add-on to MRI and ⁶⁸Ga-DOTA-labelled PET/CT, since these might enable focused pancreatic resections. Besides, in complex patients with insulinomas and NF-pNETs or gastrinomas, the combined use of functional imaging modalities will truly have added value by determining the lymph node and liver involvement of insulinomas versus NF-pNETs and gastrinomas. For functioning tumors functioning prospects include accurate localization techniques and for NF-pNETs specific imaging techniques to identify and localize tumors based on aggressiveness and thus prognosis.

PROGNOSIS

'Biology is King, Selection is Queen, and the technical details of surgical procedures are the Princes and Princesses of the realm who frequently try to overthrow the powerful forces of the King or Queen, usually to no long-term avail, although with some temporary apparent victories' were regarded as the main principles of surgical oncology by Blake Cady in 1997.³⁰ These fundamental concepts in the respective order will form the basis of future improvements in prognosis and therapy of patients with MEN1-related dpNETs.

Biology is King

Population-based risk factors

Studies have assessed risk factors for death in MEN1.³¹ However, these generally used static models. Individual risk prediction based on a single factor is often limited. Drawing accurate prognosis is a mix of multiple factors ideally combined in a prognostic model. Considering the longitudinal disease pattern in MEN1, population-based time-to-event studies including time-dependent variables, such as the presence of manifestations, tumor size and treatment at specific timepoints are needed. Taking the low absolute number of deaths or liver metastases into account, these models should apply techniques to reduce overfitting in case of few events.³²

In addition to these well-known prognostic factors, upcoming studies should focus on new biological factors that can predict long-term outcomes of patients with MEN1. Potential factors include genotype, tumor-based epigenetic factors, or blood-based biopsies. A recent study observed genetic anticipation within successive generation, hypothesizing potential molecular mechanisms leading to this phenomenon.³³

Unraveling the prognosis of MEN1-related gastrinomas

Besides population-based risk factors for survival, prognostic factors for dpNETs are what we need to move forward from here. The GTE analyzed risk factors within their patients with dpNETs.³⁴ Gastrinomas, pNETs of 2 cm or larger, and age were associated with metastases, but only age and pNETs of 2 cm were associated with death once metastasis occurred.³⁴ By contrast, within the Dutch MEN1 population, patients with gastrinomas had a reduced overall survival as compared to age and sex matched controls (**Chapter 6**). Several factors contribute to the knowledge gap and varying survival rates among studies. The reference group in **Chapter 6** consisted of age and sex matched controls regardless of the presence of a pNET. Those without a pNET likely have a better survival as compared to those with a pNET. The diagnosis of gastrinomas is particularly challenging, and although previous guidelines demanded secretin testing as reference standard, a more recent consensus statement acknowledges that secretin tests are not widely available.¹⁵ A combination of

fasting hypergastrinemia of at least 10 times the upper limit of the reference value and a gastric pH below 2 confirms the presence of a gastrinoma.¹⁵ However, in most patients gastrin levels will be less than 10 times elevated, thereby complicating gastrinoma diagnosis. This could also reflect the relatively high age at diagnosis in the Dutch cohort. Additional cross-sectional diagnostic studies, including biochemical and radiological studies, such as ⁶⁸Ga-DOTA-labelled PET/CT, are demanded to provide uniform gastrinoma diagnosis. These will subsequently enable large cohort studies with standardized work-up on prognostic factors to identify indications for medical or surgical management.

Tumor-based risk stratification

Unraveling the biological behavior of gastrinomas and NF-pNETs will be the major aspect in optimizing oncological outcomes. Population-based studies enable the identification of basic factors associated with long-term outcomes. From a surgical point of view, individual tumor risk stratification is demanded to improve surgical indications. Specifically, for multifocal dpNETs, resolving metastatic patterns increases understanding of these tumors. As shown in **Chapter 7**, in patients with lymph node metastases and hypergastrinemia, lymph node metastases located around the pancreatic head originated from duodenal gastrinomas. The limitation of the present study was the relatively low number of tumors and metastases. These analyses are ideally replicated in larger cohorts including more metastasis. In addition, further research on this topic should aim to identify the exact tumor which caused the metastasis. Determining a correlation between fasting serum gastrin levels and radiological or pathological tumor load should be explored, since this could improve staging and clinical decision making.

Translational research

Malignant dpNETs should be identified timely to reduce the number of patients with metastases and associated mortality. The biologic behavior of pNETs is largely unknown. In **Chapter 8** it was observed that patients with resected NF-pNETs had a worse liver metastases-free survival as compared to patients with resected insulinomas, adjusted for age, size and WHO grade. This hypothesizes differences in underlying biological mechanism which drive tumor progression. Insights in genetic and epigenetic factors driving the development and progression of pNETs have contributed to the understanding.³⁵ The majority of sporadic pNETs harbors a somatic mutation in the *MEN1* or death domain-associated protein (*DAXX*) and alpha-thalassemia/mental retardation X-linked (*ATRX*) genes, which are associated with alternative lengthening of telomeres (ALT) which subsequently leads to decreased survival.^{36–41} Nevertheless, *DAXX*, *ATRX* and ALT are not routinely used in clinical practice. Transcription factors ARX and PDX1 – which are surrogate markers of endocrine differentiation – determine distinct alpha and beta subtypes

of pNETs.^{2,42,43} In a large cohort including 67 sporadic and 77 MEN1-related pNETs, ARX subtype tumors were associated with prognosis.² Other prognostic factors which have been reported for MEN1-related pNETs include WHO grade, DNA methylation and expression of p27^{Kip1} and p18^{Ink4c}.^{17,44,45} Before widespread implementation, these factors should be replicated and validated in large independent cohorts.

The aim of future research projects should be to enable personalized prognostication through risk scores and the identification of molecular pathways associated with tumor development and tumor progression. Recent insights into polyamines have identified a polyamine signature which is associated with progression of MEN1-related dpNETs.⁴⁶ In a corresponding mouse model, the signature was elevated early during disease progression.⁴⁶ Besides the genetic mutations previously mentioned, epigenetic changes likely contribute to differences in aggressiveness of pNETs.^{35,47} Epigenetics contribute to the regulation of gene expression through processes such as DNA methylation and histone modification. Specific DNA methylation patterns have been identified which are associated with tumorigenesis and could subsequently be used to stratify prognosis of sporadic pNETs.⁴⁸ These processes must be further explored for patients with germline MEN1 mutations.

Selection is Queen

At present, patient selection for surgical resection is primarily guided by size, WHO grade, functionality and presence of lymph node metastases.^{13–15,49} In **Chapter 8**, size categorized to 2 cm and on a continuous scale, functionality and WHO were associated with liver metastases-free survival.

Whom to operate?

A population-based cohort study from the Netherlands investigated surgical resection versus watchful waiting for MEN1-related NF-pNETs; confounding by indication was reduced by propensity score adjustment.⁵⁰ Operative resection was not superior over watchful waiting for tumors smaller than 2 cm.⁵⁰ Subgroups including tumors 2 – 3 cm and larger than 3 cm were too small for statistical comparisons, but within the 2 – 3 cm group, resection did not reduce liver metastases-free survival.⁵⁰ In the French cohort synchronous or metachronous metastasis were observed in 11% (7/65), 27% (3/11) and 11/21 (52%) of NF-pNETs smaller than 2 cm, 2 – 3 cm, and larger than 3 cm, respectively.⁵¹ Similar figures were observed after resection in **Chapter 8**, where patients with resected pNETs smaller than 2 cm and between 2 and 3 cm had similar survival probability estimates 10 years post-resection. However, for patients with pNETs of 3 cm or larger survival was significantly reduced. These data hypothesize that one could consider postponing resection beyond 2 cm. Further research is needed to test this hypothesis. Particularly for the 2 – 3 cm group, more information is needed to determine when surgical resection is indicated. For von

Hippel-Lindau-related pNETs, a specific cut-off of 2.8 cm is used for surgical resection.⁵² Future studies should evaluate cut-offs, and likely specific cut-offs based on transcription factors or WHO grade, for MEN1-related pNETs.

When to operate?

The decision to proceed to surgery is a risk-benefit balance analysis between the oncological benefits and the risks of early and late complications. The aim of dpNET treatment is to maintain a good quality of life.⁴⁹ From this perspective, identifying those who will benefit from treatment in terms of quality of life is of added value. Insights in how they weigh the burden of repetitive screening with a watchful waiting strategy against the burden of invasive procedures with associated complications and how these affect their quality of life and time to functional recovery are needed. Further research should elaborate on baseline differences in quality of life between patients with non-metastasized and metastasized dpNETs. The degree of change in quality of life pre- and post-surgery and before and after the development of distant metastases could contribute to this debate.

Postoperative follow-up regimen

Post-resection, patients will re-enter the regular MEN1 screening, since no specific protocols exists for postoperative follow-up. For sporadic NF-pNETs, prediction models have been developed to predict recurrence in order to tailor follow-up.⁵³ Prediction models for clinically relevant outcomes are demanded in MEN1 to tailor follow-up intensity and aid choice of imaging study. Further research is needed to develop postoperative risk prediction models, which should at least include tumor type, tumor size and WHO grade (**Chapter 8**). For sporadic pNETs, a multigene liquid biopsy blood biomarker (NETest) has been reported to accurately identify patients with residual disease after curative resection, thereby enabling personalized follow-up.^{54,55} Considering that sporadically occurring pNETs are generally solitary, future studies should assess whether blood based biomarkers could guide the postoperative follow-up of MEN1-related pNETs.

SURGICAL THERAPY

Technical maneuvers are the Prince and Princess

Minimally invasive surgery

Technical advances should aim to improve the impact of interventions and reduce the incidence and burden of complications. In general, minimally invasive surgery as compared to traditional open surgery reduces postoperative pain, complications and hospital stay. For pancreatic surgery, laparoscopic distal pancreatectomy is superior over open distal

pancreatectomy in terms of functional outcomes, length of stay, quality of life and occurrence of delayed gastric emptying, but overall complications were similar.⁵⁶ In contrast, for pancreatoduodenectomies a multicenter randomized controlled trial was prematurely terminated because of an increased 90-day complication-related mortality in the laparoscopic group.⁵⁷ Minimally invasive surgery considers both laparoscopic and robot-assisted surgery; robot-assisted surgery improves dexterity, high-resolution visualization and a three-dimensional view. A propensity-score matched cohort study, including 253 patients with a pNET, observed similar rates of severe complications, postoperative pancreatic fistula (POPF) grade B/C and mortality between the robot-assisted and laparoscopic distal pancreatectomy groups before and after matching.⁵⁸ However, the robot-assisted group had a lower conversion rate, higher spleen preservation rate, and lower re-admission rate at the cost of longer operative time and hospital stay.⁵⁸

Observational studies in MEN1 without adjustment for confounders, reported similar advantages in patients undergoing minimally invasive distal pancreatectomies and enucleations. Those undergoing a minimally invasive resection versus those with an open resection, more often had spleen preservation, lower blood loss and operative time, shorter hospital stay and less lymph nodes removed.⁵⁹ Robot-assisted compared with laparoscopic procedures led to less conversions and splenectomies and no differences were observed regarding morbidity and mortality.⁶⁰ The major disadvantage and potential drawback to widely implement minimally invasive surgery for multifocal MEN1-related dpNETs is the lack of intraoperative palpation of the pancreas. In one series, in two out of 21 patients (10%) undergoing minimally invasive distal pancreatectomy the primary tumor was not resected.⁶⁰ Fluorescence-guided surgery combining tumor-specific fluorescent dye and a near-infrared imaging system could aid in intraoperative tumor localization. In 10 patients with sporadic pNETs, intraoperative fluorescence identified all lesions during laparoscopy.⁶¹ So far, only one patient with multifocal MEN1-related pNETs has been reported.⁶² Multiple fluorescent lesions were observed which corresponded to pNETs and microadenomas. In the sporadic cohort, two patients had fluorescent lymph nodes – albeit with lower intensity as compared to the pNETs – which were normal at pathology.⁶¹ Ideally, in the setting of MEN1 tumor-specific tracers should be developed with could be used for intraoperative imaging techniques as well as regular nuclear imaging. Furthermore, intraoperative surgical decision making would benefit from the identification of lymph node metastases using fluorescence imaging. Perceptions on lymph node dissections vary. A minimum of 13 and 12 resected lymph nodes after pancreatoduodenectomy and distal pancreatectomy have been suggested for accurate staging.^{63,64} Particularly during enucleations systematic lymph node dissections are not regularly performed. In this respect, intraoperative blood-based biomarkers could be of value. Considering the heterogenous behavior of tumors, intraoperative localization could therefore aid in tailoring the extent of surgical resections and lymph node dissections for MEN1-related dpNETs.

Choosing the optimal surgical strategy

In contrast to extensive and even near-total pancreatectomies such as the Thompson procedure, focused resections shift the essence to estimating the malignant potential of each individual tumor and technical feasibility of resections. For patients with solitary insulinomas, enucleations do not seem to increase the risk for long-term hypoglycemia (**Chapter 9**). Major surgery in MEN1 is associated with a tremendous risk of severe complications and cumulative burden of complications even in experienced teams of endocrine and pancreatic surgeons (**Chapter 10**). Moreover, major surgery in patients with MEN1 as compared to those with sporadic disease increases morbidity (**Chapter 12**). Could the early identification of malignant tumors deviate from a passive watch-and-wait strategy to early enucleations and thereby prevent major resections later?

Will we be able to adequately decide on the preferred procedure for the individual patient? The benefits of surgery should be weighed against early complications, late morbidity, functional recovery, quality of life, and long-term outcomes such as survival, distant metastases and growth of dpNETs left undisturbed and the occurrence of clinically relevant new dpNETs. Currently, insufficient data are published on most of these topics. A systematic review including 533 patients, concluded that formal pancreatic resections increased the chance of pancreatic insufficiency, but reduced the chance of recurrence compared with enucleations.⁶⁵ Nevertheless, the rate of reoperations was similar across both groups. Potentially relevant studies were excluded, most studies did not report all outcomes, did not report procedure specific outcomes, follow-up time differed, and recurrence was defined as any new dpNET.⁶⁵ Ideally a prospective cohort study with long-term follow-up and longitudinal data collection regarding patient related outcomes, such as quality of life and time to functional recovery, and disease-related outcomes should be realized.

Identification of complications – Intraoperative factors

As shown in **Chapter 10**, for major resections in MEN1, no preoperative factors could identify patients with a severe complication. Nevertheless, increased operative duration and estimated blood loss were associated with such a complication. This is in accordance with other studies observing increased mortality, morbidity and length of stay after intraoperative bowel or vascular accidents.⁶⁶ Moreover, the severity of intraoperative adverse events is directly related to the severity of postoperative complications, which could therefore be used to intensify postoperative monitoring.⁶⁷

Strategies to reduce the occurrence of complications

Postoperative pancreatic fistula (POPF) is the major contributor to morbidity and mortality following pancreatic surgery. The Fistula Risk Score (FRS) appoints a risk score based on pancreatic gland texture, pancreatic duct size, baseline pathology and estimated blood loss,

at the time of pancreatic anastomosis reconstruction during pancreatoduodenectomy.⁶⁸ Patients with pNETs generally fulfill most of these criteria, thereby being exposed to a high baseline risk of POPF, which indicates that those with MEN1 confront the surgeon with multiple challenges. Especially for those with the highest FRS, the risk for POPF increases up to 29.1% as compared to 0.7% for the lowest risk group.⁶⁹ Within **Chapter 12** it was shown that pancreatoduodenectomies for pNETs were associated with a POPF rate of 35%. Moreover, in those with MEN1 undergoing a pancreatoduodenectomy plus distal pancreatectomy this rate was 80% (**Chapter 10**). In both studies POPF was a major contributor to morbidity. These data indicate that strategies to reduce the occurrence of POPF would particularly benefit patients with MEN1 and dpNETs.

Fistula mitigation strategies

Up to today, multiple and varying risk mitigation strategies have been performed, but no general recommendations exist regarding the optimal anastomotic techniques for pancreatoduodenectomy. Several strategies include technical variations (e.g., pancreaticogastrostomy reconstruction, dunking/invaginating anastomosis, and autologous tissue patches) and/or utilization of intraperitoneal drains, anastomotic stents (internal or external), prophylactic somatostatin analogues, and tissue sealants. In an observational study, pancreaticojejunostomy plus external stent placement and omission of octreotide provided the lowest POPF rate in high risk patients.⁶⁹ In a randomized controlled trial evaluating pancreaticojejunostomy versus pancreaticogastrostomy reconstruction including externalized stents and octreotide omission within the high risk patients, observed no differences in POPF rates.⁷⁰ Nevertheless, severe complications occurred more often after pancreaticogastrostomy and if POPF occurred, the morbidity was higher after pancreaticogastrostomy.⁷⁰ Also for distal pancreatectomies, pNETs are an independent POPF risk factor.⁷¹ Within the general population the POPF rate is 15.1% as compared to 32.0% in two ENETS centers of excellence (**Chapter 12**). No mitigation strategies, i.e., transection method, octreotide, fibrin glue/tissue sealants, autologous tissue patches have been identified which independently predict the occurrence of POPE, albeit that drain placement may reduce its impact.⁷¹

The role of the individual surgeon – surgeon's proficiency

For pancreatic resections, three of the factors of the FRS are endogenous – the only modifiable factor is intraoperative blood loss. The effect of blood loss on POPF is most pronounced in the high-risk fistula group where a potential reduction of 31% could be achieved by reducing blood loss.⁷² Surgeons who had the least blood loss had a significantly lower POPF-rate as compared to those with most blood loss.⁷² Blood loss was independently associated with surgeon experience.⁷² Besides blood loss, surgeon experience – defined as

total number of pancreatoduodenectomies and/or years of experience – was associated the risk of POPF.^{69,72,73} In addition, more experienced surgeons frequently use ‘better’ mitigation strategies.⁷³

Nevertheless, experience does not explain everything. For laparoscopic bariatric surgery, greater surgeons’ skill led to less postoperative complications.⁷⁴ In this respect, improved surgical skill during robot-assisted pancreaticojejunostomy was independently protective for POPF.⁷⁵ These data imply that the surgeon’s proficiency plays a pivotal role in reducing the occurrence of complications. Improving strategies for high-risk patients, such as those with MEN1-related or sporadic pNETs, will hopefully reduce the number of complications.

FINAL REMARKS

Centralization within current centralization

The Netherlands is leading in Europe regarding centralization of care for rare cancers.⁷⁶ At present, patients with MEN1 in the Netherlands are treated in the eight University Medical Centers. Four of these are acknowledged as European Neuroendocrine Tumor Society Center of Excellence for the treatment of neuroendocrine tumors. Having an acceptable doctor-to-patient ratio is challenging, considering that the Netherlands has approximately 370 living patients with MEN1, distributed across eight hospitals. Of the living patients with MEN1 in the Netherlands, the median number of patients per center is 36 [range 12 – 124]. Only three centers offer care to more than 50 patients of which only one includes more than 100 patients. Differences exist between these centers regarding the number of specialists involved in the care of these patients; some perform follow-up of 120 patients by three endocrinologists, whereas in others 60 patients could potentially be managed by six. Those numbers also pertain to radiologists and surgeons. Thus, at present, the anticipated quality of care is determined by being at the right place at the right time.

Centralization of complex cancer care in the United States of America led to increased traveling distances.⁷⁷ Although the inter-hospital distances are relatively short in the Netherlands, methods for centralization without increasing the burden for patients should be considered. Virtual multidisciplinary team discussions could enable patient discussion in expert teams, which is of added value where local expertise is unavailable or insufficient.⁷⁸ A Virtual MEN1 Center in the Netherlands would minimize travelling distances and offer maximal access to experts in all fields. Moreover, centralized reading of imaging by experienced radiologists and nuclear radiologists will enhance staging and improve imaging-based tumor localization and risk stratification. Virtual microscopy could improve adequate tumor interpretation and staging. By discussing the most difficult cases within these expert teams, nationwide knowledge and education will improve. Research will be intensified,

since patient inclusion for prospective cohort studies or randomized controlled trials will enhance. At last, expensive and expert diagnostics or therapies, such as GLP1 receptor PET/CT or Peptide Receptor Radionuclide Therapy, could be implemented selectively in one or a few centers thereby reducing overall costs. Before major surgical procedures are considered, patients can be discussed in expert teams, so the risks and benefits associated with these procedures can be discussed with the patients and their relatives. Although preoperative risk stratification and surgical indications could be established in virtual multidisciplinary team discussions, these procedures cannot be performed online.

The center and surgeon volume outcome relation in surgery is well-established.^{79,80} For pancreatic surgery, mortality and short-term complications are related to both hospital and surgeon volume and have been reduced by centralization.^{81–87} Pancreatic neuroendocrine tumors comprise less than 10% of the pancreatic resections annually in the Netherlands. Considering the high rate of complications associated with pancreatic surgery for pNETs (**Chapter 12**) and specifically major surgery for MEN1-related dpNETs (**Chapter 10**) further centralization within current centralization could increase center and surgeon volumes for patients with MEN1-related and sporadic pNETs. The major aim should be to reduce the incidence and impact of postoperative complications. In **Chapter 12** it was shown that the morbidity is substantial, but that the failure-to-rescue is low in two ENETS centers of excellence. Further centralization can be guided by geography, organization or volume.⁸⁴ For pancreatic surgery, optimal location-allocation based on mortality did not lead to substantially longer traveling times for patients.⁸⁸ However, the most important is that centralization for these rare and complex tumors should focus on the ability to treat a disease by multidisciplinary teams and not on the number of procedures.⁷⁹

Stage migration in MEN1?

The Will Rodgers Phenomenon, dates from the 1930's and follows the quote: *“When the Okies left Oklahoma and moved to California, they raised the average intelligence level in both states”*. The concept implies that by moving people from one group to another, the average values in both groups increase. In medicine it is referred to as ‘stage migration’.⁸⁹ Whether prognostic grading systems perform similar across different cohorts with more recent diagnostic measures, for example due to the early detection of metastases by ⁶⁸Ga-DOTA-labelled PET/CT, is questionable. In patients with lung cancer, classification of disease changed after the introduction of new diagnostic modalities.⁸⁹ Patients who migrated to a worse stage group, positively affected the survival rates of the worst stage groups. Are we facing similar patterns in MEN1? Are the today's patients systematically being ‘upstaged’ due to the use of ⁶⁸Ga-DOTA-labelled PET/CT? Does this imply that studies with long-term follow-up might have missed (micro)metastases and that observed survival estimates cannot be reliably used in daily clinical practice? The future will tell.

In the Netherlands, survival probabilities for patients with dpNET-related liver metastases were 50%.⁹⁰ Whether these observations for populations in the ⁶⁸Ga-DOTA-labelled PET/CT remains speculation.

FUTURE PERSPECTIVES

- Centralization
 - ◆ Clinical care
 - ◆ Supranational research initiatives
- Individual patient risk prediction
 - ◆ Increase understanding of tumor development and progression
 - ▶ Unraveling epigenetic factors and molecular pathways
 - ▶ Develop organoids to evaluate new therapies
 - ◆ Developing blood-based risk factors
 - ◆ Imaging-based risk stratification
 - ◆ Personalized postoperative follow-up intervals and imaging regimen
- Surgical
 - ◆ Minimally invasive surgery
 - ◆ Intraoperative imaging
 - ◆ Intraoperative or early postoperative measurements to ascertain radical resection
 - ◆ Reduce the impact of complications

CONCLUSIONS PER CHAPTER

PART I RESEARCH STRATEGIES

Chapter 2

For rare diseases, observational studies within patient cohorts, although prone to bias, seem the most feasible study design regarding the disease prevalence. Preferably, data collection is performed prospectively, however, under certain conditions, data storage in a longitudinal retrospective database with a disease-specific framework is suitable.

PART II DIAGNOSIS

Chapter 3

Biomarkers should no longer play a role in the diagnostic process for NF-pNETs, as accuracies are too low. For the detection of NF-pNETs, EUS has the highest sensitivity. A combined strategy of EUS and MRI seems to be the most useful. Gallium 68 octreotate-DOTA positron emission tomography-CT could be added if NF-pNETs are diagnosed to identify metastasis. Surveillance programs should focus on and be adapted to the presence of substantial growth in NF-pNETs. The optimal age to start screening must yet be determined, as insufficient evidence for an evidence-based recommendation was available.

Chapter 4

The accuracy for diagnosing MEN1-related PanNET of MRI was higher than CT and therefore MRI should be the preferred (non-invasive) imaging modality for PanNET screening/surveillance. Routine EUS with FNA is of little additional value and can be reserved for those patients considered to be at high risk for adenocarcinoma because of age and radiologic tumor characteristics.

Chapter 5

Within a population-based cohort, MEN1-related pNET size was not systematically over- or under estimated on preoperative imaging. Based on agreement and reliability measures, MRI is the preferred imaging modality.

PART III PROGNOSIS

Chapter 6

Life expectancy of patients with MEN1 gastrinoma is reduced. FSG levels and pancreatic NETs ≥ 2 cm are prognostic factors. FSG levels might guide surveillance intensity, step-up to additional diagnostics, or provide arguments in selecting patients who might benefit from surgery.

Chapter 7

For patients with MEN1-related hypergastrinemia and pNETs, a duodenal origin of peri-duodenopancreatic lymph node metastasis should be considered, even when current conventional and functional imaging studies do not reveal duodenal tumors preoperatively. Chapter 8 Patients with resected MEN1-related nonfunctioning pancreatic neuroendocrine tumors had a significantly lower liver metastases-free survival than patients with insulinomas. Postoperative counseling and follow-up regimens should be tumor type specific and at least consider size and World Health Organization grade.

PART IV SURGICAL THERAPY

Chapter 9

Surgery for MEN1-related insulinoma is more successful than previously thought.

Chapter 10

Major duodenopancreatic surgery in MEN1 is associated with a very high risk of severe complications and cumulative burden of complications and should therefore be reserved for a select subgroup of patients with MEN1-related dpNETs.

Chapter 11

To improve patient care, future perspectives include optimization of equivocal surgical indications to facilitate improved preoperative patient selection as well as a reduction of the burden of complications once major surgery is performed.

Chapter 12

Surgery for pNETs is associated with a high rate of complications, but low failure-to-rescue in expert centers. Complications are procedure specific. Major surgery in patients with MEN1/VHL appears to increase the risk of complications.

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ADDENDA

SUMMARY IN DUTCH / NEDERLANDSE SAMENVATTING

REVIEW COMMITTEE

AUTHORS & AFFILIATIONS

LIST OF PUBLICATIONS

ACKNOWLEDGEMENTS / DANKWOORD

CURRICULUM VITAE

SUMMARY IN DUTCH – NEDERLANDSE SAMENVATTING

Achtergrond

Het multipale endocriene neoplasie type 1 (MEN1) syndroom is een zeer zeldzame ziekte die voorkomt bij ongeveer 1 tot 10 per 100,000 personen. Naar schatting zijn er ongeveer 400 mensen in Nederland met het MEN1 syndroom. Het is een erfelijk syndroom dat een autosomaal dominant overervingspatroon kent – dit betekent dat de kinderen van een patiënt met MEN1 een kans van 50% hebben op het krijgen van MEN1. Het syndroom wordt veroorzaakt door een mutatie in het *MEN1* gen. Dit geeft een verhoogde kans op tumoren in verschillende organen, waarvan de meest voorkomende adenomen van de bijnierschors (prevalentie 77–93%), neuro-endocriene tumoren (NETs) van het duodenum (twaalfvingerige darm) en pancreas (alvleesklier) (41–57%), en hypofyse adenomen (30–48%) zijn. Daarnaast hebben patiënten een verhoogd risico op NETs van de maag, longen en thymus, bijnieradenomen, huidtumoren, spiertumoren en borstkanker. Deze manifestaties ontstaan vaak al op jonge leeftijd. De tumormanifestaties binnen het MEN1 syndroom leiden ertoe dat de levensverwachting van een patiënt met MEN1 gemiddeld 10 jaar lager is dan die van de algemene bevolking in Nederland. Voor patiënten die zijn aangedaan met tumoren van het duodenum en pancreas (dpNETs) is de gemiddelde levensverwachting 55–60 jaar, vergeleken met 73 jaar voor de MEN1 populatie als geheel. Om de levensverwachting en kwaliteit van leven van patiënten met MEN1 te verbeteren zijn betere behandelmethoden nodig voor dpNETs. De focus van dit proefschrift ligt op de MEN1-gerelateerde dpNETs.

Vanwege de onderliggende genetische mutatie zijn patiënten vaak aangedaan door multipale dpNETs. De tumoren zijn heterogeen ten aanzien van hormoonproductie leidend tot klinische symptomen en agressiviteit. Insulinomen en gastrinomen zijn de hormonaal actieve tumoren die respectievelijk insuline of gastrine in excessieve hoeveelheden produceren. De grootste groep dpNETs zijn echter niet hormonaal actief en worden derhalve aangeduid als niet-functionerende neuro-endocrienetumoren (NF-pNETs). Het voornaamste behandeldoel is om patiënten zo lang mogelijk ziekte- en symptoomvrij te houden om op die manier een goede kwaliteit van leven te waarborgen. Het huidige screeningsprogramma is gericht op vroegdiagnostiek van de tumoren om zo een tijdige interventie mogelijk te maken. De enige curatieve interventie is chirurgische resectie van de tumor(en). Gezien de jonge leeftijd waarop deze tumoren optreden, het hoge aantal tumoren, en het diverse spectrum aan tumoren, is het continu een balans vinden tussen enerzijds de voordelen van een operatie en anderzijds het risico op vroegtijdige postoperatieve alsmede lange termijn complicaties. Tot op heden is het niet mogelijk om voor iedere patiënt de juiste inschatting te maken. Het doel van dit proefschrift is het verkrijgen van inzicht in onderzoeksstrategieën

naar MEN1 (**Deel I**), het verbeteren van de diagnostiek (**Deel II**), prognose (**Deel III**) en chirurgische behandeling (**Deel IV**) van dpNETs bij patiënten met MEN1.

DEEL I. ONDERZOEKSSTRATEGIEËN

Hoofdstuk 2. Onderzoeksstrategieën voor multipele endocriene neoplasie type 1

In hoofdstuk 2 worden de onderzoeksstrategieën van de DutchMEN Study Group (DMSG) beschreven en geëvalueerd. De DMSG werd opgericht in 2008 als samenwerking tussen de acht Universitaire Medische Centra (UMC) in Nederland. De studiegroep bestaat uit endocrinologen uit ieder UMC, een endocrien chirurg en een afvaardiging van de patiëntenvereniging. Vanuit deze landelijke samenwerking werd een MEN1 database opgezet op basis van vooraf geformuleerde onderzoeksvragen gebaseerd op klinische vraagstukken. Meer dan 90% van de patiënten met MEN1 in Nederland is geïncludeerd in de database, waardoor er een representatieve groep samengesteld is en het mogelijk is geworden om 'population-based' studies te verrichten. De patiëntenvereniging heeft een essentiële rol gespeeld zowel bij het includeren van patiënten en als bij het behouden van de patiënten participatie. Vanwege de complexiteit van het syndroom, waarbij patiënten levenslang vervolgd worden en er gelijktijdig meerdere manifestaties van het MEN1 syndroom aanwezig kunnen zijn, werden ieder kwartaal ongeveer 700 variabelen verzameld. Het ontwikkelen van een dergelijke database is een uitdaging. Cruciale fases om de kwaliteit te waarborgen zijn de dataverzameling en databewerking. Het verzamelen van ruwe en niet-geïnterpreteerde data, een gecentraliseerd protocol om gestandaardiseerde dataverzameling mogelijk te maken, en het minimaliseren van het aantal dataverzamelaars zijn essentiële stappen. Deze studie laat zien dat retrospectieve 'population-based' cohortstudies met hoogwaardige methoden om bias en 'confounding' te reduceren, betrouwbare antwoorden kunnen geven op klinische vragen en daardoor de patiëntenzorg kunnen verbeteren. Deze stapsgewijze aanpak en onderzoeksmethoden kunnen gebruikt worden voor MEN1 onderzoek in andere centra en landen.

DEEL II. DIAGNOSTIEK

Hoofdstuk 3. Diagnostiek van niet-functionerende neuro-endocriene pancreastumoren

Niet-functionerende neuro-endocriene pancreastumoren (NF-pNET) zijn zowel de meest voorkomende als meest dodelijke dpNETs bij patiënten met MEN1. Zodoende is er een intensief screeningsprogramma om tumoren vroegtijdig te identificeren en om tijdig te besluiten tot een actief afwachtend beleid of chirurgische resectie. Echter, er is geen evidence-based consensus ten aanzien van het gebruik van tumor markers in het bloed, welk type beeldvorming het beste gebruikt kan worden en op welke leeftijd het screeningsprogramma

moet aanvangen. Daarnaast bestaan er geen follow-up protocollen, omdat er weinig gegevens zijn over de groeisnelheid van tumoren. Er werd een systematische literatuurstudie verricht met als doel om antwoorden te genereren op deze vier vragen ten aanzien van de diagnostiek en follow-up van MEN1-gerelateerde NF-pNETs. Op basis van 11 studies werd geconcludeerd dat de diagnostische accuratesse van tumormarkers laag is, en dat deze tumormarkers niet gebruikt moeten worden om MEN1-gerelateerde NF-pNETs te diagnosticeren. In zestien studies was de gerapporteerde sensitiviteit van endo-echografie (spreiding tussen individuele studies 75 – 100%) hoger dan die van magnetic resonance imaging (MRI) (74 – 88%) en computed tomography (CT) (54 – 81%). Een gecombineerde strategie van EUS en MRI wordt aanbevolen, aangezien klinisch relevante tumoren gemist worden op alleen de EUS. Functionele beeldvorming, die gebruik maakt van de tumoractiviteit of receptorexpressie van tumoren, is een groeiende manier om deze tumoren of metastasen van tumoren te diagnosticeren. Van deze typen functionele beeldvorming was ⁶⁸Gallium-Dota positron emission tomography (PET)/CT beter dan somatostatine receptor scintigrafie. Eerstgenoemde moet de voorkeur krijgen, echter, niet als eerste stap tijdens de screening. In acht studies bedroeg de gemiddelde groeisnelheid 0.1 tot 1.32 mm per jaar. Dit impliceert dat de frequentie van pancreasbeeldvorming geïndividualiseerd kan worden en verlengd tot één keer per 1 tot 2 jaar, in plaats van jaarlijks zoals de richtlijn adviseert. Op basis van de huidige literatuur kon er geen aanbeveling gedaan worden ten aanzien van de aanvang de screening, maar uitstellen tot het 16^e levensjaar lijkt niet verstandig.

Hoofdstuk 4. Pancreasbeeldvorming en biopten

Er is weinig bekend over de effecten van het radiologische screeningsprogramma en de huidige richtlijnen geven geen aanbeveling ten aanzien van de optimale beeldvormingsmodaliteit. Daarnaast is het onbekend of het noodzakelijk is om een pathologische bevestiging te verkrijgen van een geobserveerde tumor voordat patiënten verwezen worden voor een operatie of een ‘watchful waiting’ beleid wordt gevoerd. De diagnostische accuratesse van beeldvorming (MRI, CT en EUS) werd onderzocht in 377 patiënten uit de Nederlandse MEN1 database. Over de studieperiode nam het aantal onderzoeken toe, en in de laatste decade werd een stijging in het percentage MRI gezien. De diagnostische accuratesse van de beeldvorming was goed, met positief en negatief voorspellende waarden van ongeveer 90%. In de directe vergelijking tussen MRI en CT, was MRI beter ten opzichte van CT. De meeste chirurgisch resectiepreparaten en biopten toonden terecht een neuro-endocriene pancreastumor (pNET). Bij vier van de tien bij wie het initiële biopt geen pNET toonde, betrof het een diagnose adenocarcinoom – bij slecht twee patiënten was deze diagnose terecht, alle andere patiënten hadden een pNET. Van het hele cohort kregen drie patiënten, allen ouder dan 60 jaar, terecht een diagnose pancreasadenocarcinoom. De diagnostische accuratesse voor het diagnosticeren van

MEN1-gerelateerde pNET is hoger voor MRI vergeleken met CT en zodoende moet MRI de voorkeur krijgen als niet-invasieve modaliteit voor het screenen en vervolgen van pNETs. Het routinematig verkrijgen van weefselbevestiging draagt nauwelijks bij en moet achter de hand gehouden worden voor patiënten met een hoog risico op een adenocarcinoom, zoals hogere leeftijd en radiologische karakteristieken.

Hoofdstuk 5. Reproduceerbaarheid van radiologische tumorgrootte

Tumorgrootte is de belangrijkste prognostische factor voor MEN1-gerelateerde pNETs. Zodoende zijn de huidige chirurgische indicaties voor NF-pNETs gebaseerd op de grootte. Een recente studie suggereerde dat de tumorgrootte preoperatief vaak wordt overschat. In hoofdstuk 5 werd de reproduceerbaarheid van de radiologische en pathologische tumorgrootte onderzocht in 73 patiënten uit de Nederlandse MEN1 database. De tumorgrootte werd niet systematisch overschat of onderschat. Dit gold ook voor de subgroep analyses van MRI, CT en EUS. De tumorgrootte werd gecategoriseerd in <2 en ≥ 2 cm, de huidige afkapwaarde om patiënten voor een operatie te verwijzen. Binnen de subgroep die geïndiceerd werd als <2 of ≥ 2 cm was de overeenkomst tussen de beeldvorming en de pathologie 81.5%, dus voor de overige 18.5% van de patiënten was er geen overeenstemming tussen de beeldvorming en pathologie. De overeenkomsten tussen MRI, CT, EUS, en de pathologie bedroegen respectievelijk 88.6, 85.7, en 75.0%. De data uit deze studie laten zien dat de preoperatieve tumorgrootte van MEN1-gerelateerde pNETs niet systematisch overschat wordt en de data suggereren dat MRI de beste modaliteit is om de grootte te bepalen.

DEEL III. PROGNOSE

Hoofdstuk 6. Prognose en risicofactoren gastrinomen

De meeste patiënten met MEN1-gerelateerde gastrine producerende tumoren – gastrinomen – kunnen medicamenteus behandeld worden met maagzuurremmers. Desalniettemin kan verwijdering van de tumoren overwogen worden om gemetastaseerde ziekte te voorkomen. Er zijn weinig factoren bekend die gebruikt kunnen worden om patiënten die baat hebben bij een operatie te identificeren. De overleving en risicofactoren voor overleving werden bekeken in 63 patiënten met een MEN1-gerelateerd gastrinoom in het Nederlandse ‘population-based’ cohort. De 10-jaars overleving bedroeg 65%, welke significant lager was dan voor leeftijd en geslacht gemaakte MEN1 patiënten. Hogere initiële gastrine waarden, een pNET ≥ 2 cm, levermetastasen, een verdenking op NETs in de maag, en multiple NETs waren geassocieerd met een slechtere overleving. Deze data laten zien dat de levensverwachting van MEN1 patiënten met gastrinomen afgenomen is. Gastrine waarden en de aanwezigheid van een grote pNET kunnen bijdragen aan het selecteren voor patiënten voor een operatie.

Hoofdstuk 7. Metastaseringspatronen

Voor patiënten met multifocale MEN1-gerelateerd dpNETs met metastasen is het bepalen van de origine van de metastasen een uitdaging. Inzicht in metastaseringspatronen kan bijdragen aan de chirurgische besluitvorming ten aanzien van de uitgebreidheid van de resectie. Daarnaast wordt het mogelijk om een betere inschatting te maken van de prognose door de TNM-classificatie beter te kunnen toepassen in het geval van meerdere tumoren. De relatie tussen de dpNETs en metastasen werd onderzocht door te kijken naar de endocriene differentiatie – als maat werden hiervoor de transcriptiefactoren ARX en PDX1 gebruikt – en gastrine expressie in 137 primaire dpNETs en microadenomen, en 36 locoregionale en afstandsmetastasen in 10 patiënten met MEN1. Bij patiënten met MEN1-gerelateerde gastrinomen vertoonden alle lymfekliermetastasen rondom het duodenum gastrine expressie. Deze lymfekliermetastasen konden gelinkt worden aan duodenum gastrinomen en niet aan grote(re) pNETs. Bij drie patiënten waren er aanwijzingen voor meerdere gemetastaseerde tumoren. Metastasen van pNETs clusterden aan pNETs met een hoge World Health Organization (WHO) tumor graad (c.q. hogere delingssnelheid) en Alternative Lengthening of Telomeres (ALT)-positieve tumoren. De resultaten van deze studie laten een overheersende duodenale origine van lymfekliermetastasen zien. Een adequate inschatting van de uitgebreidheid van dpNETs zal bijdragen aan besluitvorming ten aanzien van de uitgebreidheid van de resectie in MEN1.

Hoofdstuk 8. Prognose van patiënten met een gereseceerde neuro-endocriene pancreastumor

Gemetastaseerde pNETs zijn de voornaamste doodsoorzaak van patiënten met MEN1. Er zijn nagenoeg geen risicofactoren bekend; zo is het ook niet bekend of het tumor type de prognose beïnvloedt. In deze studie werd bekeken of patiënten met een gereseceerde NF-pNET een andere prognose hebben dan patiënten met een gereseceerd insulinoom in een groot internationaal multicenter cohort. Daarnaast werden tumor specifieke risicofactoren voor levermetastasen of overlijden geanalyseerd. In totaal werden 153 patiënten, waarvan 61 met een NF-pNET en 92 met een insulinoom geïncludeerd. De geschatte 10-jaars levermetastasenvrije overleving was significant korter voor patiënten met een gereseceerde NF-pNET dan voor patiënten met een gereseceerd insulinoom. Ook na het corrigeren voor leeftijd, tumorgrootte en WHO graad was de levermetastasenvrije overleving voor patiënten met een NF-pNET significant slechter. De multivariabele analyse liet tevens zien dat de levermetastasenvrije overleving was geassocieerd met tumor type, tumor grootte, en WHO graad. De levermetastasenvrije overleving was vergelijkbaar voor patiënten met een tumor <2 cm en 2-3cm, maar slechter voor patiënten met een tumor ≥ 3 cm. Deze data impliceren dat de subtypen pNETs verschillen in agressiviteit. Zodoende dienen de postoperatieve voorlichting en follow-up tumor type specifiek te zijn. Naast tumor type, bepalen grootte en WHO-graad de prognose.

DEEL IV. CHIRURGISCHE BEHANDELING

Hoofdstuk 9. Uitkomsten na chirurgie voor insulinomen

Insulinomen zijn insuline producerende pNETs die kunnen leiden tot levensbedreigende hypoglykemieën. Naar schatting komen ze voor bij 10% van de patiënten met MEN1. De enige curatieve behandelmethode is een operatieve verwijdering. Het doel van de operatie is om de insuline producerende tumor te verwijderen, de recidiefkans te reduceren en de pancreasfunctie te behouden. Dit vormt een uitdaging bij MEN1 patiënten met multiple pNETs waarvan het vaak niet duidelijk is welke insuline produceert. Vanwege de zeldzaamheid van insulinomen werd een internationale samenwerking, the International MEN1 Insulinoma Study Group, opgericht, bestaande uit 46 centra uit Europa en Noord-Amerika. Er werden 96 patiënten geïnccludeerd. Persisterende of recidief hypoglykemie trad op in 7% van de patiënten en de geschatte 10-jaars hypoglykemievrije overleving bedroeg 91%. Van de patiënten met een gelokaliseerd insulinoom was sprake van persisterende ziekte bij 1 van de 46 na een formele pancreasresectie en recidief ziekte trad op bij 1 van de 17 patiënten na een enucleatie. Deze data laten zien dat operaties voor MEN1-gerelateerde insulinomen succesvoller zijn dan vooraf werd gedacht. Een enucleatie dient de voorkeur te krijgen voor gelokaliseerde insulinomen vanwege de hoge genezingskans en het voorkomen van pancreasinsufficiëntie.

Hoofdstuk 10. Complicaties na grote resecties voor duodenopancreatische neuro-endocriene tumoren

Pancreaschirurgie voor MEN1-gerelateerde NF-pNETs gaat gepaard met een ernstige complicatie (c.q. een complicatie die een radiologische, gastro-enterologische of chirurgische re-interventie behoeft) bij één op de drie patiënten. Voor duodenum gastrinomen en tumoren in de pancreaskop die niet geschikt zijn voor een enucleatie, bieden grote resecties, zoals een Whipple procedure of een totale pancreatectomie, de enige mogelijkheid om een patiënt te genezen. Er zijn geen gegevens over complicaties in deze specifieke subgroep. In Nederland ondergingen 27 patiënten met MEN1 zo'n procedure over een periode van 15 jaar. De patiënten waren over het algemeen jong. Een ernstige complicatie trad op in bijna twee op de drie patiënten. De cumulatieve last van alle complicaties was hoog. Er werden geen factoren geïdentificeerd die geassocieerd waren met het optreden van een ernstige complicatie. Deze studie laat de hoge kans op complicaties zien na uitgebreide resecties voor MEN1-gerelateerde dpNETs en onderstreept het belang van patiënt selectie en adequate preoperatieve voorlichting. De procedures moeten met terughoudendheid worden uitgevoerd in hooguit een selecte groep.

Hoofdstuk 11. Perspectieven van grote chirurgie binnen MEN1

Grote resecties voor MEN1-gerelateerde dpNETs gaan gepaard met een hoog risico op ernstige complicaties en een hoge cumulatieve complicatielast. Perspectieven voor de toekomst zijn het optimaliseren van chirurgische indicaties om te zorgen dat alleen patiënten die er echt baat bij hebben zo'n procedure ondergaan. Daarnaast is het belangrijk om de ernst van de complicaties te verminderen. In de toekomst kan beeldvorming gestuurde risicostratificatie binnen de MEN1 screening het hopelijk mogelijk maken om vroegtijdig kwaadaardige tumoren te identificeren die op dat moment nog middels minimaal invasieve chirurgie geënuceerd kunnen worden. Inzicht in metastaseringspatronen kan de uitgebreidheid van de resectie bepalen. De indicatiestelling voor grote resecties wordt idealiter gecentraliseerd in gespecialiseerde multidisciplinaire teams. Daarnaast moeten deze procedures bij voorkeur gecentraliseerd worden in teams bestaande uit endocrien en hepato-pancreato-biliair chirurgen getraind in MEN1-gerelateerde dpNETs om het optreden en de impact van complicaties te reduceren.

Hoofdstuk 12. Risicofactoren voor complicaties na pancreasresecties voor neuro-endocriene tumoren

Er zijn weinig gedetailleerde data beschikbaar over procedure-specifieke complicaties en risicofactoren voor complicaties na operaties voor pNETs. Zodoende bekeken wij complicaties in 123 patiënten die tussen 2008 en 2018 werden geopereerd in twee expertisecentra voor de behandeling van NETs, het UMC Utrecht en het UMC Groningen. De mortaliteit bedroeg 0.8%, een ernstige complicatie trad op in 41.5%, en de 'failure-to-rescue' (het overlijden van een patiënt na het optreden van een ernstige complicatie) was 2.0%. In de multivariabele analyse met correctie voor patiënt en tumor factoren verhoogde een pancreatoduodenectomie vergeleken met een distale pancreatectomie het risico op een ernstige complicatie. Na uitgebreide resecties was de cumulatieve last van complicaties hoger bij patiënten met MEN1 of von Hippel-Lindau (VHL). Deze data laten zien dat operaties voor pNETs gepaard gaan met een hoog procedure-specifiek risico op complicaties, maar lage 'failure-to-rescue' in expertisecentra. Daarnaast lijkt het risico op complicaties hoger na grote resecties voor MEN1 of VHL-gerelateerde pNETs.

REVIEW COMMITTEE

Prof. dr. I.H.M. Borel Rinkes

Professor of Surgical Oncology, University Medical Center Utrecht

Prof. dr. P.J. van Diest (committee chair)

Professor of Pathology, University Medical Center Utrecht

Prof. dr. C.H.J. van Eijck

Professor of Surgery, in particular pancreatic surgery, Erasmus Medical Center Rotterdam

Prof. dr. O.W. Kranenburg

Professor of Surgical and Translational Tumor Biology, University Medical Center Utrecht

Prof. dr. M.G.E.H. Lam

Professor of Nuclear Medicine, University Medical Center Utrecht

AUTHORS AND AFFILIATIONS

Detlef K. Bartsch, MD, PhD

Department of Visceral, Thoracic and
Vascular Surgery
Philipps University Marburg
Marburg, Germany

Peter H. Bisschop, MD, PhD

Department of Endocrinology and
Metabolism
Amsterdam UMC location Academic
Medical Center
Amsterdam, The Netherlands

Bert A. Bonsing, MD, PhD

Department of Surgery
Leiden University Medical Center
Leiden, The Netherlands

Inne H.M. Borel Rinkes, MD, PhD

Department of Endocrine Surgical
Oncology
University Medical Center Utrecht
Utrecht, The Netherlands

Maria Luisa Brandi, MD, PhD

Department of Surgery and Translational
Medicine
University of Florence
Florence, Italy

Lodewijk A.A. Brosens, MD, PhD

Department of Pathology
University Medical Center Utrecht
Utrecht, The Netherlands

Laurent Brunaud, MD, PhD

Department of Digestive, Hepatobiliary
and Endocrine Surgery
Université de Lorraine, Hôpital Brabois
Adultes,
CHU Nancy, France

Ioannis Christakis, MD, PhD

Department of Surgical Oncology, Section
of Surgical Endocrinology
University of Texas MD Anderson Cancer
Center
Houston, United States of America

Elfi B. Conemans, MD, PhD

Department of Endocrinology
Amsterdam University Medical Center
location VUmc University Medical Center
Amsterdam, The Netherlands
Department of Pathology
University Medical Center Utrecht
Utrecht, The Netherlands

Cornelis H.C. Dejong, MD, PhD

Department of Surgery
Maastricht University Medical Center,
NUTRIM School for Nutrition and
Translational Research in Metabolism
Maastricht, The Netherlands
Department of Surgery
Universitätsklinikum Aachen
Aachen, Germany

Olaf M. Dekkers, MD, PhD

Departments of Endocrinology and
Metabolism and Clinical Epidemiology
Leiden University Medical Center
Leiden, The Netherlands

Koen M.A. Dreijerink, MD, PhD

Department of Endocrinology and
Internal Medicine
Amsterdam University Medical Centers
Amsterdam, The Netherlands

Madeleine L. Drent, MD, PhD

Department of Internal Medicine, Section
of Endocrinology
Amsterdam UMC location VU University
Medical Center
Amsterdam, The Netherlands

Diane Donegan, MD

Division of Endocrinology, Diabetes,
Metabolism, and Nutrition
Mayo Clinic
Rochester, United States of America

Casper H.J. van Eijck, MD, PhD

Department of Surgery
Erasmus Medical Center
Rotterdam, The Netherlands

Madelon van Emst, BSc

Department of Pathology
University Medical Center Utrecht
Utrecht, The Netherlands

Shena Giri, BA

Department of Surgery
Northwestern University
Chicago, United States of America

Francesca Giusti, MD, PhD

Department of Surgery and Translational
Medicine
University of Florence
Florence, Italy

Harry van Goor, MD, PhD

Department of Surgery
Radboud University Medical Center
Nijmegen, The Netherlands

Pierre Goudet, MD, PhD

On behalf of the Groupe d'Etude des
Tumeurs Endocrines (GTE)
Department of Digestive and Endocrine
Surgery
Dijon-Bourgogne University Hospital
Dijon, France

Wenzel M. Hackeng, MD

Department of Pathology
University Medical Center Utrecht
Utrecht, The Netherlands

Jeroen Hagendoorn, MD, PhD

Department of Surgery
Regional Academic Cancer Center Utrecht
University Medical Center Utrecht & St.
Antonius Hospital
Utrecht, The Netherlands

Bas Havekes, MD, PhD

Department of Internal Medicine,
Division of Endocrinology
Maastricht University Medical Center
Maastricht, The Netherlands

Wouter W. de Herder, MD, PhD

Department of Internal Medicine
Erasmus Medical Center
Rotterdam, The Netherlands

Frederik J.H. Hoogwater, MD, PhD

Department of Hepato-Pancreato-Biliary
Surgery and Liver Transplantation
University of Groningen, University
Medical Center Groningen
Groningen, The Netherlands

**Anouk N. van der Horst-Schrivers, MD,
PhD**

Department of Endocrinology
University Medical Center Groningen,
University of Groningen
Groningen, The Netherlands

Ralph Hsiao, BSc

Department of Surgery
University Health Network
Toronto, Canada

Geert Kazemier, MD, PhD

Department of Surgery
Cancer Center Amsterdam, Amsterdam
University Medical Center location VUmc
University Medical Center
Amsterdam, The Netherlands

Electron Kebebew, MD

Endocrine Oncology Branch
National Cancer Institute, National
Institutes of Health
Bethesda, United States of America

Joost M. Klaase, MD, PhD

Department of Hepato-Pancreato-Biliary
Surgery and Liver Transplantation
University of Groningen, University
Medical Center Groningen
Groningen, The Netherlands

Ruben H.J. de Kleine, MD

Department of Hepato-Pancreato-Biliary
Surgery and Liver Transplantation
University of Groningen, University
Medical Center Groningen
Groningen, The Netherlands

Wouter P. Kluijfhout, MD, PhD

Department of Surgery
University Health Network
Toronto, Canada

Aranxa Kok, BSc

Department of Pathology
University Medical Center Utrecht
Utrecht, The Netherlands

Rachel S. van Leeuwen, MD, PhD

Department of Endocrine Oncology
University Medical Center Utrecht
Utrecht, The Netherlands

Jerena Manoharan, MD

Department of Visceral, Thoracic and
Vascular Surgery
Philipps University Marburg
Marburg, Germany

Naris Nilubol, MD

Endocrine Oncology Branch
National Cancer Institute, National
Institutes of Health
Bethesda, United States of America

I. Quintus Molenaar, MD, PhD

Department of Surgery
Regional Academic Cancer Center Utrecht
University Medical Center Utrecht & St.
Antonius Hospital
Utrecht, The Netherlands

Pavel Nockel, MD

Endocrine Oncology Branch
National Cancer Institute, National
Institutes of Health
Bethesda, United States of America

Folkert H.M. Morsink, BSc

Department of Pathology
University Medical Center Utrecht
Utrecht, The Netherlands

G. Johan A. Offerhaus, MD, PhD

Department of Pathology
University Medical Center Utrecht
Utrecht, The Netherlands

Sjoerd Nell, MD, PhD

Department of Endocrine Surgical
Oncology
University Medical Center Utrecht
Utrecht, The Netherlands

Jesse D. Pasternak, MD

Department of Surgery
University Health Network
Toronto, Canada

Elisabeth J.M. Nieveen van Dijkum, MD, PhD

Department of Surgery
Cancer Center Amsterdam, Amsterdam
University Medical Center location
Academic Medical Center
Amsterdam, The Netherlands

Nancy D. Perrier, MD

Department of Surgical Oncology, Section
of Surgical Endocrinology
University of Texas MD Anderson Cancer
Center
Houston, United States of America

Maarten W. Nijkamp, MD, PhD

Department of Hepato-Pancreato-Biliary
Surgery and Liver Transplantation
University of Groningen, University
Medical Center Groningen
Groningen, The Netherlands

Carolina R.C. Pieterman, MD, PhD

Department of Endocrine Oncology
University Medical Center Utrecht
Utrecht, The Netherlands

Emily L. Postma, MD, PhD

Department of Surgery
New York Presbyterian Hospital Weill
Cornell Medical Centre

New York, United States of America
Department of Endocrine Surgical
Oncology
University Medical Center Utrecht
Utrecht, The Netherlands

Nicolas Santucci, MD
Department of Digestive and Endocrine
Surgery
Dijon-Bourgogne University Hospital
Dijon, France

Hjalmar C. van Santvoort, MD, PhD
Department of Surgery
Regional Academic Cancer Center Utrecht
University Medical Center Utrecht & St.
Antonius Hospital
Utrecht, The Netherlands

Cord Sturgeon, MD
Department of Surgery
Northwestern University
Chicago, United States of America

Tim J. Takkenkamp, BSc
Department of Hepato-Pancreato-Biliary
Surgery and Liver Transplantation
University of Groningen, University
Medical Center Groningen
Groningen, The Netherlands

Mark J.C. van Treijen, MD
Department of Endocrine Oncology
University Medical Center Utrecht
Utrecht, The Netherlands

Gerlof D. Valk, MD, PhD
Department of Endocrine Oncology
University Medical Center Utrecht
Utrecht, The Netherlands

Adrian Vella, MD, PhD
Division of Endocrinology, Diabetes,
Metabolism, and Nutrition
Mayo Clinic
Rochester, United States of America

Annenienke C. van de Ven, MD, PhD
Department of Endocrinology
Radboud University Medical Center
Nijmegen, The Netherlands

Helena M. Verkooijen, MD, PhD
Imaging Division
University Medical Center Utrecht
Utrecht, The Netherlands

Wessel M.C.M. Vorselaars, MD, PhD
Department of Endocrine Surgical
Oncology
University Medical Center Utrecht
Utrecht, The Netherlands

Menno R. Vriens, MD, PhD
Department of Endocrine Surgical
Oncology
University Medical Center Utrecht
Utrecht, The Netherlands

Annemiek M.E. Walenkamp, MD, PhD
Department of Oncology
University of Groningen, University
Medical Center Groningen
Groningen, The Netherlands

Frank J. Wessels, MD

Department of Radiology
University Medical Center Utrecht
Utrecht, The Netherlands

Edgar Wong-Lun-Hing, MD, PhD

Department of Endocrine Surgical
Oncology
University Medical Center Utrecht
Utrecht, The Netherlands

Jonathan Zagzag, MD

Department of Surgical Oncology, Section
of Surgical Endocrinology
University of Texas MD Anderson Cancer
Center
Houston United States of America

Wouter T. Zandee, MD, PhD

Department of Endocrinology
University Medical Center Groningen,
University of Groningen
Groningen, The Netherlands

Rasa Zarnegar, MD

Department of Surgery
New York Presbyterian Hospital Weill
Cornell Medical Centre
New York, United States of America

LIST OF PUBLICATIONS

van Beek DJ, Pieterman CRC, Wessels FJ, van de Ven AC, de Herder WW, Dekkers OM, Zandee WT, Drent ML, Bisschop PH, Havekes B, Borel Rinkes IHM, Vriens MR, Valk GD. **Diagnosing Pancreatic Neuroendocrine Tumors in Patients with Multiple Endocrine Neoplasia Type 1 in Daily Practice.**
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CURRICULUM VITAE

Dirk-Jan van Beek was born on the 10th of August 1992 in Rotterdam, The Netherlands, as the first son of Ton and Yvonne van Beek. He has one younger brother, Maurits van Beek. After graduating from the bilingual Wolfert van Borselen Scholengroep, Rotterdam, he started medical school at the Utrecht University in 2010. During his study he developed a specific interest in surgery and therefore he subsequently performed his final internship and research project at the Department of Surgical Oncology of the University Medical Center Utrecht.

After obtaining his medical degree in 2017, Dirk-Jan started as a PhD candidate at the Department of Endocrine Surgical Oncology of the University Medical Center Utrecht under the supervision of prof. dr. M.R. Vriens and prof. dr. G.D. Valk. His PhD project concentrated on the treatment of multiple endocrine neoplasia type 1-related duodenopancreatic neuroendocrine tumors. Simultaneously, he obtained a Master of Science degree in Epidemiology, with specific focus on Clinical Epidemiology, at the Utrecht University.

In 2019 he was appointed as the sixth Sten Tibblin Fellow for the Sten Tibblin Research Fellowship in Endocrine Tumors at the Skånes University Hospital Lund, Sweden, under the supervision of dr. M. Almquist and dr. E. Nordenström. He coordinated multiple national and international studies of which several were presented at national and international meetings.

In 2020 he started his clinical career as a resident not in training (ANIOS) at the Department of Surgery of the Meander Medical Center, Amersfoort, The Netherlands. Since 2021 he is working as a surgical resident in training (AIOS) at the Diaconessenhuis, Utrecht.

During his youth and research time he enjoyed playing field hockey. Dirk-Jan currently lives together with Sanne Roohé in Utrecht.

