Recurrence and survival of pancreatic neuroendocrine tumors

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Recurrence and survival of pancreatic neuroendocrine tumors

Recidief en overleving van pancreas neuroendocriene tumoren

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General introduction and outline of the thesis

01

01

GENERAL INTRODUCTION

Neuroendocrine tumors of the pancreas (pNET) are heterogeneous tumors originating from the endocrine tissue of the pancreas and accounting for 3-7% of all pancreatic tumors[1, 2]. These neoplasms have been regarded as rare and present many clinical challenges in diagnosis, classification and treatment. They share some common clinical features, frequently have unpredictable and unusual biological behavior, and the majority of patients present late and have a delayed diagnosis. The incidence of pNET is estimated at 2/100.000, with a predicted rise faster than other malignant neoplasms[3, 4]. According to the Surveillance, Epidemiology and End Results (SEER) data, the incidence of neuroendocrine tumors (NET) in general has shown a 2.7-fold increase between 1973 and 2004[4]. The reason for this increase is often explained by improved awareness and the widespread use of advanced endoscopic and high-definition radiological imaging[3, 5]. However, several studies also suggest no improvement in outcome of patients diagnosed with pNET over a similar period[6, 7]. PNET in general are considered to be indolent, jet some subtypes can be highly malignant and resistant to therapy[8]. Clinical behavior is strikingly divers, with an overall 5-year survival varying from 97% for insulinomas to 30% for non-functioning tumors who are clinically silent[9]. These data necessitate reconsideration of the idea that pNET are slow growing, fairly benign lesions.

Nomenclature

The nomenclature of neuroendocrine neoplasms (NENs) has evolved considerably over the last two decades. Siegfried Oberndorfer is the first who described and depicted NENs in 1907. He initially considered them benign and “carcinoma-like”, calling them carcinoids, before detailing their malignant behavior in 1929. NEN of the pancreas were often called “islet cell tumor”, which denotes the presumed origination in the islets of Langerhans. The use of both terms has generally declined; however "carcinoid syndrome" is still used frequently as a generic description for a characteristic syndrome that results from hormonal overproduction that occurs in some patient with NEN[10]. In recent years, the term "pancreatic neuroendocrine tumor” has been adopted by most practitioners, as well as by the European Neuroendocrine Tumor Society (ENETS) and the World Health Organization (WHO), for well-differentiated tumors regardless of histologic grade. The term “pancreatic

neuroendocrine carcinoma" (NEC) is reserved for those cases with poorly differentiated histology and a high proliferative rate.

Clinical presentation and diagnosis

Based on the hormonal overproduction and associated clinical syndrome, pNET can be divided in functional (F-pNET) and non-functional (NF-pNET) tumors[11, 12]. Patients with functional tumors show symptoms due to hypersecretion of peptides and amines such as hypoglycemia as a result of increased insulin levels caused by an insulinoma or the Zollinger-Ellison syndrome caused by excessive gastrin production by a gastrinoma. Other, less common, functional pNET include VIPoma, glucagonom and ACTHoma. The majority of pNET (70-80%) are non-functional and do not secrete hormones that cause symptoms[4, 13, 14]. As a result, many are clinically silent until late presentation. These patients are either incidentally diagnosed through radiological imaging performed for unrelated conditions or present with symptoms of mass effects or distant (usually hepatic) metastases, or both[6, 15]. Symptoms usually consist of abdominal pain, weight loss, anorexia and nausea[11]. Several analyses have shown that approximately 65% of patients with non-functioning tumors are diagnosed with distant metastases, resulting in unfavorable prognosis[4, 7, 14, 16]. A small subgroup of patients with pNET is known with hereditary tumor syndromes such as Multiple Endocrine Neoplasia Type 1 (MEN-1) or Von Hippel Lindau disease (VHL). These patients may develop multiple well-differentiated functional or non-functional tumors in the pancreas.

Diagnosis of pNET is based on clinical presentation, hormone assays and pathology. A variety of biomarkers may be used to aid the diagnosis and post-treatment follow-up. Chromogranin A (CgA) is the most commonly secreted and measured tumor marker associated with NEN in the gastroentero-pancreatic tract. This glycoprotein is normally contained in neuroendocrine cell vesicles and is often elevated in patients with pNET. Plasma levels of CgA are thought to correlate with tumor burden, and some studies show a higher overall diagnostic sensitivity in patients with metastatic disease compared to patients with localized disease, although other studies show conflicting results[17-20]. Overall, the general accuracy of CgA is moderate as false positive increased levels of CgA often occur[15, 21, 22].

To diagnose pNET, appropriate cross-sectional imaging is recommended for all patients. Technical developments in radiological imaging have facilitated the characterization and detection of primary pancreatic NETs and liver

metastases over the last few decades. Computed tomography (CT) with intravenous contrast, magnetic resonance imaging (MRI) and endoscopic ultrasound (EUS) show the highest sensitivity and specificity, making it appropriate initial diagnosis, surgical planning and follow-up of primary tumors, distant metastases or after surgical resection[23-25]. Since somatostatin receptors are highly expressed on most neuroendocrine tumors, somatostatin-receptor scintigraphy (Octreoscan®) can be used for optimal staging and determining eligibility for nuclear treatment that targets these receptors. Position emission technology (PET) used to be recommended only for the diagnosis or staging of poorly differentiated NEC. However, the use of ^{68}Gallium as a positron emitter showed higher spatial resolution and image quality compared to Octreoscan, making it the recommended imaging modality to aid detection and staging of pNET[26, 27].

Pathology and prognosis

One of the concerns with pNET is the accurate prediction of clinical outcome. Tumor stage and grade have proven to be useful in estimating disease course and have been confirmed repeatedly in valuable studies[11, 28-32]. Staging is performed using the tumor, node, metastasis (TNM) classification, recently updated to incorporate ENETS definitions[33]. Tumor grade is an estimate of malignant potential and divides pancreatic NEN into prognostic groups based on the expression of the nuclear antigen Ki67. Well differentiated pNET are separated into G1 (low-grade) with a Ki67 expression <3% and G2 (intermediate-grade) with a Ki67 expression of 3-20%. High-grade pancreatic neoplasms expressing Ki67 >20% are classified as G3, of which the WHO recently has updated their classification dividing these tumors into G3 NET and G3 NEC[34]. G3 NET are well-differentiated and have a Ki67 in the 20-55% range, whereas poorly differentiated G3 NEC have higher Ki67 values and poorer prognosis.

Treatment

Lack of data on pNET disease poses challenges for treatment and clinical decision making, often based on experience and expert opinion rather than high-level scientific evidence. Treatment should be individualized and managed in a multidisciplinary setting based on the tumor burden and symptoms. The best therapeutic choice will depend on whether the main aim is to cure, to slow tumor growth and improve oncological outcome or improve

the quality of life by inhibition of hormonal overproduction or refrain from surgical resection with possible adverse events.

Surgery is essential in many stages of pNET management and remains the only curative treatment option[9]. Based on tumor location and size, different surgical procedures are available: pancreatoduodenectomy, total, central or distal pancreatectomy and enucleation, either performed open or laparoscopically. All except for the latter technique usually includes lymphadenectomy of locoregional lymph nodes, although clear consensus on location and total number to harvest is not available. Pancreatic surgery is often associated with complications, mainly consisting of pancreatic fistula, delayed postoperative emptying and postoperative bleeding. Because patients with pNET who undergo surgery have a relatively good survival, long term complications consisting of endocrine or exocrine pancreatic insufficiency can be developed.

The management of incidentally discovered small NF-pNET is under debate. A growing body of literature show little to no tumor progression in pNET <2cm of size and suggests safety in surveillance [35, 36]. In contrast, other studies advocate that surgical resection is the best curative option for all pNET, including the small tumors[36-38]. Although the risk of malignant potential still remains unknown for NF-pNET <2cm with respect to tumors >2cm, and the benefits due to adopting a more conservative strategy have not been described, the ENETS has updated their guidelines advocating the possibility of surveillance in small pNET. Prospective studies are necessary to provide insight on the optimal management of NF-pNET <2cm.

Follow-up and recurrence

Until recently, recurrence was generally considered to be uncommon, making is unnecessary for patients to be monitored after resection. Currently, it is assumed that early detection and treatment of pNET recurrence will lead to a favorable outcome, although supportive data is missing. Follow-up is therefore designed to monitor patients and optimize oncologic outcome[39-41]. Nevertheless, clear consensus on follow-up has not yet been reached and multiple international guidelines provide different recommendations on the frequency, modality and duration of follow-up for different stages of disease. Commonly, follow-up is the same for all patients who have undergone a curative resection of pNET. Although tumor stage and grade have proven to be useful in estimating disease course, postoperative surveillance protocols or adjuvant treatment options based on the expected recurrence rates are

not available. Reliable recurrence rates are difficult to deduct from literature because of the rarity of the disease and the inhomogeneous group of patients with resected pNET. The majority of published studies include patients with hereditary syndromes, distant metastases or locally advanced disease, who have been known to have a different probability of tumor recurrence and survival.

AIM AND OUTLINE OF THIS THESIS

This thesis aims to provide insight into the postoperative outcome of patients who have undergone a curative resection of well-differentiated pNET. Curative resection is defined as a complete resection (R0 or R1 resection[42]) of localized G1/G2 pNET, as surgery is considered the only curative treatment option available. High-grade pNET, patients with distant metastases (regardless of complete metastasecomy) and hereditary syndromes are not included as these tumors are known for their highly malignant behavior, frequent recurrences and unfavorable prognosis, making it difficult to curatively treat these patients. Some studies in this thesis include only NF-pNET to limit the heterogeneity of the patient population. Because NF-pNET do not have an overproduction of hormones and the accompanying clinical symptoms, the presentation of recurrences is often delayed compared to patients with F-pNET. For this reason, it is believed that the survival of patients with NF-pNET is worse, despite the assumption of similar pathophysiology, emphasizing the need for (improved) detection possibilities for recurrence.

In **Chapter 2** an overview of the treatment and related survival of pNET in the Netherlands from 2008-2013 is provided. **Chapter 3** presents the results of a systematic review and meta-analysis on recurrence after curative resection of well-differentiated pNET. In **Chapter 4** the Recurrence Score is introduced, an easy tool to estimate the risk of recurrence after curative resection of G1/G2 NF-pNET and identify high-risk patients. A method to alter this scoring system is described in **Chapter 5**, in which the Ki67 proliferation index is analyzed with regards to recurrence after complete resection of pNET with Ki67 of 0-20%. **Chapter 6** investigates the utility of a multianalyte blood-test, the NETest, during postoperative follow-up of pNET. The results of a consensus study with a large group of international NET experts are presented in **Chapter 7**. Eleven recommendations were formulated that may

lower the threshold for future researchers to investigate the role of adjuvant treatment for patients with a high-risk of recurrence. **Chapter 8** presents the PANDORA study, a prospective nationwide observational cohort of patients with small pNET to investigate the effect of a conservative vs. surgical treatment for non-functioning tumors<2cm.

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A Nationwide Population-Based Study on the Survival of Patients with Pancreatic Neuroendocrine Tumors in The Netherlands

02

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ABSTRACT

02

Background. Large population based studies give insight in the prognosis and treatment outcomes of patients with pancreatic neuroendocrine tumors (pNET). Therefore, we provide an overview of the treatment and related survival of pNET in the Netherlands.

Methods. Patients diagnosed with pNET between 2008-2013 from the Netherlands Cancer Registry were included. Patient, tumors and treatment characteristics were reported. Survival analyses with Log-rank testing were performed to compare survival.

Results. In total, 611 patients were included. Median follow-up was 25.7 months, and all-cause mortality was 42%. Higher tumor grade and TNM-stage were significantly associated with worse survival in both the overall and metastasized population. The effect of distant metastases on survival was more significant in lower tumor stages (T1-3 $p<0.05$, T4 $p=0.074$). Resection of the primary tumor was performed in 255 (42%) patients. Patients who underwent surgery had the highest 5-year survival (86%) compared to PRRT (33%), chemotherapy (21%), targeted therapy (NR) and somatostatin analogs (24%) (all $p<0.001$). Patients with T1M0 tumors (n=115) showed favorable survival after surgical resection (N=95) compared to no therapy (N=20, $p=0.008$). Resection also improved survival significantly in patients with metastases compared to other treatments (all $p>0.05$). Without surgery, PRRT showed the best survival curves in patients with distant metastases. Grade 3 tumors and surgical resection were independently associated with survival (HR 7.23 and 0.12, respectively).

Conclusion. Surgical resection shows favorable outcome for all pNET tumors, including indolent tumors and tumors with distant metastases. Prospective trials should be initiated to confirm these results.

INTRODUCTION

According to Surveillance, Epidemiology and End Results (SEER) data, the incidence of neuroendocrine tumors (NET) showed a 2.7-fold increase between 1973-2004 [1]. The incidence of pancreatic NET (pNET) is estimated at 2/100.000, with a predicted rise faster than other malignant neoplasms [2]. Although pNET in general are considered to be indolent, some subtypes can be highly malignant and resistant to therapy [3]. As the majority of tumors are not associated with secretion of hormones that cause clinical symptoms, patients are predominantly diagnosed with disseminated disease for whom curation is not possible [4, 5]. SEER analyses demonstrate that 64% of patients with well-differentiated (G1 and G2) pNET are diagnosed with distant metastases and have a poor 5-year survival of 27%. For these patients, different treatment options are available in order to reduce tumor load, to inhibit tumor growth or to alleviate symptoms. Treatment options include somatostatin receptor analogs (SSA), targeted therapy, chemotherapy or peptide receptor radionuclide therapy (PRRT).

Our knowledge on pNET has improved considerably in the last decade, as is evident from the fast development of staging and grading systems proposed by the World Health Organization (WHO), the European Neuroendocrine Tumor Society (ENETS) and the American Joint Committee on Cancers (AJCC). In the present study, we provide an overview of patients diagnosed with pNET in the Netherlands identified through the nationwide Netherlands Cancer Registry (NCR). The NCR covers the complete Dutch population and receives lists of newly diagnosed cancer cases from the nationwide Automated Pathology System (PALGA) on a weekly basis [6]. In addition, lists of discharged cancer patients from the national registry of hospital discharge diagnosis are obtained to capture pNET cases with only a clinical diagnosis [7]. Checks on completeness of the data shows a national coverage of about 95% [8][9]. We aim to provide more insight in the treatment related survival of patients with pNET. This knowledge will support decisions on treatment regimens and help identify priorities in research for the future. To our knowledge, this is the first comprehensive survey on pNET epidemiology in the Netherlands.

METHODS

Cases of pNET diagnosed from January 2008 to December 2013 were obtained from the nationwide, population based NCR database, managed by the Netherlands Comprehensive Cancer Organization (IKNL). Registration and coding in this registry was conducted according to the guideline of the WHO and the International Association of Cancer Registries [10]. Topography and histology were coded according to the International Classification of Diseases for Oncology, third edition (ICD-O-3) [11]. To identify patients with neuroendocrine tumors of the pancreas (ICD-O-3 codes C251, C252, C254, C258 and C259) were combined with histology codes (8000-8011, 8013, 8041-8044, 8150-8153, 8155-8157, 8240-8242, 8246-8249, 8574 and 9990) from the PALGA network. Clinical and pathological information was obtained from hospital records.

Only patients with pancreatic NET were included. NET of other origin, as well as patients diagnosed from postmortem autopsies and tumors with mixed histology, such as adenocarcinoma of the pancreas, were excluded. Tumor-Node-Metastasis (TNM) assessment was based on the TNM-classification 6th edition proposed by ENETS [12]. Missing TNM-stage was assessed using supplementary data on “extend of disease” present in the NCR database. In addition, unrecorded data on TNM-classification, tumor size and resection margins for surgically resected tumors were requested from all pathology centers and manually complemented for each patient. Data on functionality of the tumors was not present in the registry. Tumors were considered localized when the malignant tissue was confined to the pancreas, regional if there was extension into adjacent organs or metastasis to regional lymph nodes, and distant if metastasis to other organs was present. Grading was performed using the WHO grading system from the time of diagnosis, meaning that patients diagnosed before 2010 were graded according to the WHO 2004 grading system, and patients diagnosed in 2010 and later using the WHO 2010 grading system. First-line treatment of all patients was recorded. Surgery was defined as surgical resection of the primary tumor. Patients who underwent resection of distant metastases were excluded from this category, as well as patients who underwent bypass surgery or an endoscopic procedure without resection of the tumor. Targeted therapy included either treatment with a tyrosine/kinase inhibitor (-nib) or everolimus. Other treatments included peptide receptor radionuclide therapy (PRRT), chemotherapy or somatostatin analogs (SSA).

Statistical analyses were performed using SPSS Statistics for Windows version 23.0 (IBM Corp. Armonk, NY). On the basis of the distribution, data was described as median with interquartile range (IQR) for skewed distributions and as mean with standard deviation (SD) for normal distribution. For categorical data, the number and proportion (%) was displayed. Differences between patient-groups based on tumor characteristics were investigated using a chi-square statistic. Kaplan-Meier curves were plotted and Log-rank statistics computed to detect differences between survival curves for various sub-populations. Survival was defined as the time from diagnosis until death (if known) or last follow-up (last known alive date, or 31 December 2013). Median survival was defined as the length of time, from the date of diagnosis, that half of the patients are still alive. Univariable and multivariate Cox proportional hazards regression models were used to estimate hazard ratios (HR) with 95% confidence intervals (95%CI) for factors associated with survival.

RESULTS

Demographics

Patient, tumor and treatment characteristics are presented in Table 1. In total, 611 patients diagnosed with pNET were included in the analyses. The diagnosis pNET was made at an academic hospital in 36% and in a peripheral hospital in 63% of cases. Treatment was received more often in an academic hospital (46% vs. 30% peripheral hospital). Median follow-up was 25.7 months (IQR 10-45 months), all-cause mortality was 42%. Patients were diagnosed with distant metastases in 53% in 2008 and 44% in 2013 ($p=0.390$). Most patient had a grade 1 tumor (32%). Nodal metastases were seen in 23% of G1 tumors, 43% of G2 tumors and 71% of G3 tumors, respectively ($p<0.001$). Distant metastases were present in 25% of G1, 51% of G2 and 71% in G3 tumors ($p<0.001$). Nodal or distant metastases were significantly more frequent in patients with higher tumor stage (both $p<0.001$). Patients with positive lymph nodes also had distant metastases in 62%, whereas node negative patients had distant metastases in 27% ($p<0.001$).

Table 1 – Patient, tumor and treatment characteristics

	Data available N (%)	Overall	Surgery	PRRT	Chemotherapy	Targeted therapy	SSA	No therapy
N		611	255 (42%)	41 (7%)	44 (7%)	21 (3%)	62 (10%)	150 (25%)
Year of diagnosis	611 (100%)							
- 2008		64 (11%)	17/64 (27%)	10/64 (16%)	7/64 (11%)	0 (0%)	7/64 (11%)	16 (25%)
- 2009		90 (15%)	44/90 (49%)	7/90 (8%)	8/90 (9%)	2/90 (2%)	7/90 (7%)	16/90 (18%)
- 2010		97 (16%)	32/97 (33%)	9/97 (9%)	8/97 (8%)	2/97 (2%)	8/97 (8%)	36/97 (37%)
- 2011		105 (17%)	51/105 (49%)	5/105 (5%)	7/105 (7%)	3/105 (3%)	12/105 (11%)	12/105 (11%)
- 2012		135 (22%)	59/135 (44%)	4/134 (3%)	8/120 (6%)	8/135 (6%)	14/135 (10%)	35/135 (26%)
- 2013		120 (20%)	52/120 (43%)	6/120 (5%)	6/120 (5%)	6/120 (5%)	14/120 (12%)	24/120 (21%)
Median age (range)	611 (100%)	62 (53-71)	59 (19-81)	57 (38-85)	60 (38-81)	60 (63-82)	67.5 (40-87)	69 (20-90)
Gender	611 (100%)							
- Male		314 (51%)	121 (48%)	20 (49%)	26 (59%)	8 (38%)	40 (65%)	74 (49%)
- Female		297 (49%)	134 (53%)	21 (51%)	18 (41%)	13 (62%)	22 (36%)	76 (51%)
Tumor grade	348 (57%)							
- G1		197 (32%)	143 (56%)	6 (15%)	3 (7%)	2 (10%)	18 (29%)	20 (13%)
- G2		101 (17%)	56 (22%)	7 (17%)	2 (5%)	8 (38%)	16 (26%)	8 (5%)
- G3		50 (8%)	12 (5%)	1 (2%)	11 (25%)	3 (14%)	0 (0%)	19 (13%)
T-stadium	462 (76%)							
- T1		131 (22%)	99 (39%)	0 (0%)	1 (2%)	1 (5%)	1 (2%)	28 (19%)
- T2		172 (28%)	82 (32%)	12 (29%)	10 (23%)	6 (29%)	17 (27%)	38 (25%)
- T3		117 (19%)	62 (24%)	6 (15%)	8 (18%)	8 (38%)	10 (16%)	16 (11%)
- T4		42 (7%)	3 (1%)	4 (10%)	6 (14%)	2 (10%)	13 (21%)	10 (7%)
N-stadium	479 (81%)							
- N0		315 (52%)	181 (71%)	14 (34%)	16 (36%)	11 (52%)	22 (36%)	62 (41%)
- N+		182 (30%)	69 (27%)	10 (24%)	15 (34%)	6 (29%)	18 (29%)	47 (43%)
M-stadium	246 (96%)							
- M0		314 (51%)	232 (91%)	9 (22%)	3 (7%)	1 (5%)	7 (11%)	50 (33%)
- M+		290 (48%)	20 (8%)	31 (76%)	40 (91%)	20 (95%)	55 (89%)	98 (65%)
Deaths	611 (100%)	259 (42%)	35 (14%)	20 (49%)	39 (89%)	12 (57%)	33 (53%)	98 (65%)
5-year survival		53%	86%	33%	21%	Not reached	24%	30%
Median survival		25.7 months	36.2 months	43.6 months	7.6 months	16.2 months	23.1 months	9.9 months

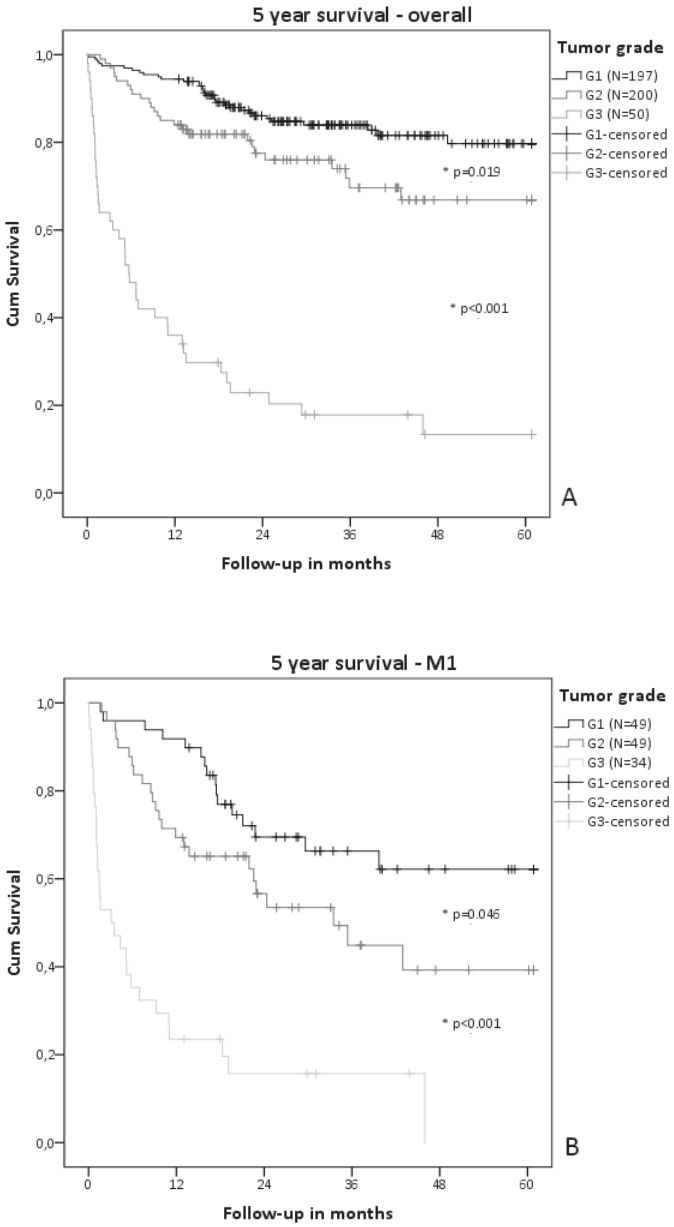

Figure 1 - Overall survival of patients with different tumor grades. **A** Overall patient population. **B** Patient with metastatic disease.

Survival

Overall, 5-year survival was 53%. There was no significant difference in overall survival for patients diagnosed in different years separately analyzed. Five-year survival was 78% without and 27% with distant metastases ($p<0.001$). In the absence of lymph node metastases 5-year survival was 72%, compared to 44% in patients with nodal metastases ($p<0.001$). In the absence of distant metastases, positive lymph nodes had a significant negative effect on survival ($p=0.003$). With distant metastases, the effect of lymph node metastases on survival was not significant, however close ($p=0.053$).

A higher tumor grade was associated with worse survival, in both localized as well as distant metastatic disease (Fig 1). Overall, 5-year survival was 80% for G1, 67% for G2 and 13% for G3 tumors. Median survival was decreased by 7.4 months for G1 ($p<0.001$), 11.3 months for grade 2 ($p<0.001$) and 12.4 months for grade 3 tumors ($p=0.022$) in the presence of distant metastases. Nodal metastases (N0 vs N1) were not associated with a survival difference of patient with different tumor grade.

Survival was worse with higher tumor stage in patients with localized disease (Fig. 2A). Five-year survival was 79% for T1, 67% for T2, 52% for T3 tumors and 30% for T4 tumors. In the presence of distant metastases, increase of tumor stage showed no worsening of survival (Fig 2B.). Median survival with and without distant metastases was 33.1 vs. 10.1 months for T1 tumors ($p<0.001$), 36.3 vs. 9.2 months for T2 tumors ($p<0.001$) and 25 vs. 16.6 months for T3 tumors ($p=0.002$), respectively. In T4 tumors, M0 and M1 patients had comparable survival curves ($P=0.074$).

Surgical and other treatment

Resection of the primary tumor was performed in 255 cases (25%). The number of patient who underwent surgery increased from 27% in 2008 to 43% in 2013 ($p=0.02$). Chemotherapy, PRRT, targeted therapy or SSA was received by 168 patients. Hundred-fifty patients received no treatment. Patient and tumor characteristics per treatments are presented in table 1. Tumor size was only known for patients who underwent surgical resection. Lymph node metastases were detected in 16% of tumors <2cm, 38% of tumors of 2-4cm and 37% of tumors >4cm of size ($p=0.002$).

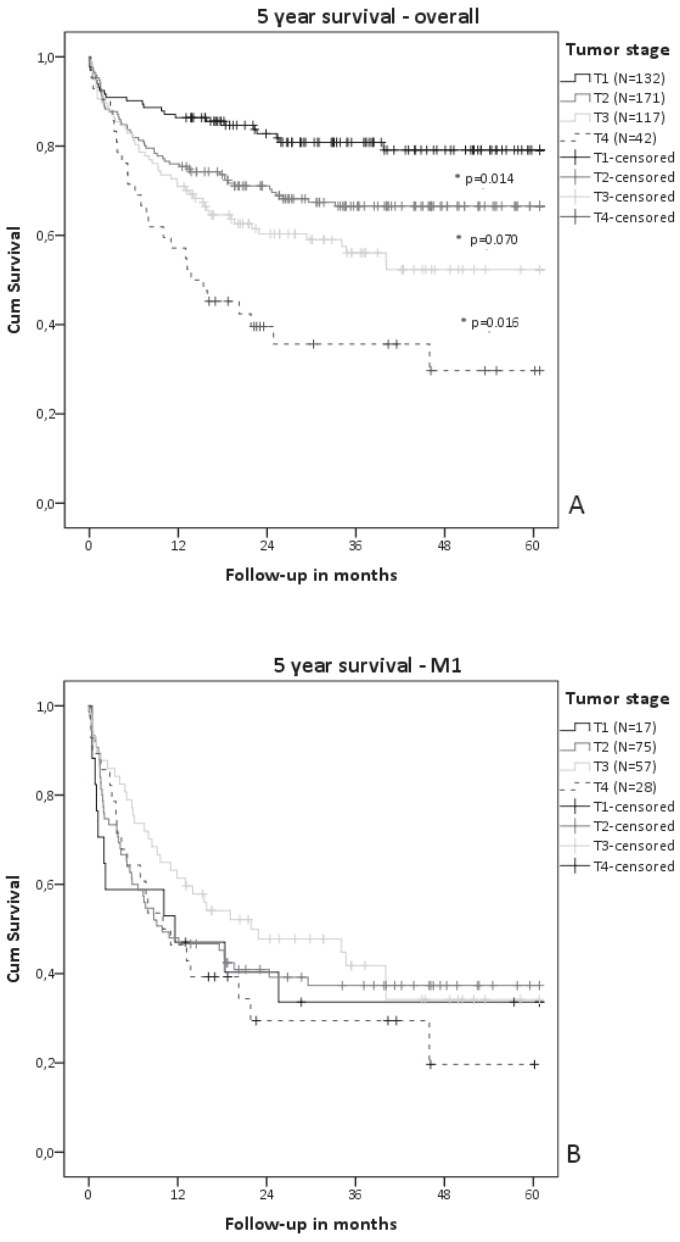

Figure 2 - Overall survival of patients with different tumor stages. **A** Overall patient population. **B** Patients with metastatic disease. No significant difference in survival was seen between each tumor stage.

Overall, survival was favorable for patients who underwent surgical resection of the primary tumor compared to PRRT, chemotherapy, targeted therapy, SSA and no therapy (all p<0.001. Fig 3A). For patients with the most indolent tumors (T1M0) a significant survival benefit was seen for surgical resection compared to no treatment (p=0.008), with a 5-year survival of 91% vs. 65% (Fig 4 – T1M0). Focusing on patients with distant metastatic disease, surgical resection of the primary tumor showed a significant better survival, with a 5-year survival of 90% compared to 50% for PRRT (p=0.016), 29% for SSA (p<0.001) and 14% for no therapy (p<0.001) (Fig 3B). Five-year survival of patients receiving chemotherapy (p<0.001) or targeted therapy (p=0.002) was not reached. When surgical resection was not performed in the presence of distant metastases, patients who received PRRT showed significant better survival compared to chemotherapy (p<0.001) or SSA (p=0.04) but not to targeted therapy (p=0.062). Tumor grade significantly differed in this population between patients who received PRRT and chemotherapy (p=0.002) and between chemotherapy and targeted therapy (p=0.017). Other patient and tumor characteristics were not significantly different between the treatment groups.

Predictors for survival

Age at diagnosis, tumor grade, tumor stage, lymph node metastases, distant metastasis and first line treatment showed an association with survival in univariable analysis. Multivariable analysis showed that G3 tumors (HR 7.23, 95%CI 3.25-16.13) and surgical resection (HR 0.12, 95%CI 0.05-0.30) were independently associated with survival (Table 3). Excluding G3 tumors from multivariable analysis resulted in comparable results for surgical resection (HR 0.12, 95%CI 0.04 – 0.38) and only an additional significance for age without clinical relevance (HR 1.03, 95%CI 1.00 – 1.07).

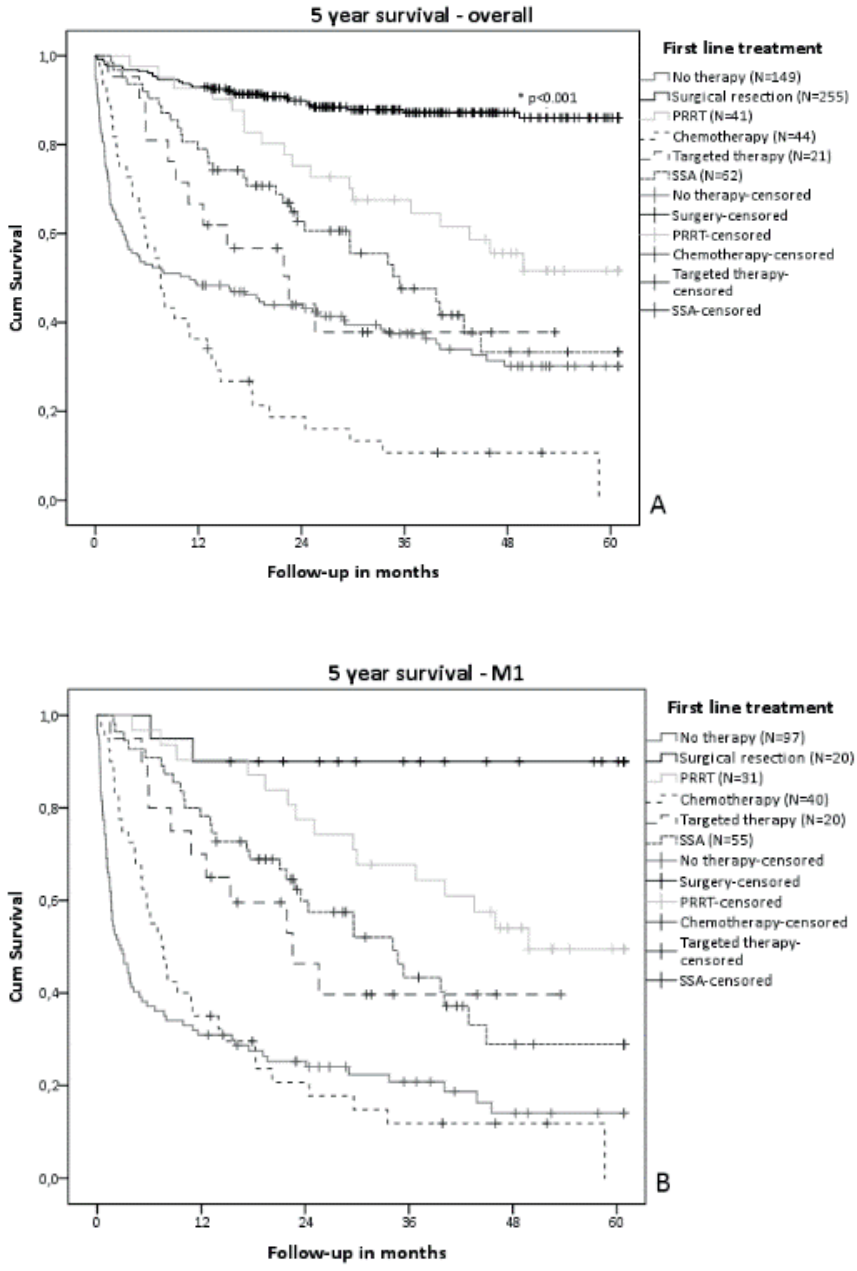

Figure 3 - Overall survival of patients based on first line treatment. **A** All patients; Surgical resection showed significantly superior survival compared to the other treatments (all p<0.001). **B** Patients with distant metastases.

Table 2 - Cox Regression Analysis

Risk factors	Univariable			Multivariable		
	HRs	*95%CI*	*p-value*	*HRs*	*95%CI*	*p-value*
Gender	1.03	0.81 – 1.31	0.817	-	-	-
Age at diagnosis	1.04	1.03 – 4.05	<0.001	1.03	1.00 – 1.05	0.076
Year of diagnosis						
- 2008	ref.	ref.	ref.	-	-	-
- 2009	0.92	0.60 – 1.43	0.73			
- 2010	0.95	0.61 – 1.48	0.81			
- 2011	0.67	0.42 – 1.07	0.09			
- 2012	0.93	0.60 – 1.44	0.74			
- 2013	0.93	0.58 – 1.50	0.77			
Tumor grade						
- G1	ref.	ref.	ref.	ref.	ref.	ref.
- G2	1.87	1.13 – 3.10	0.015	1.36	0.63 – 2.94	0.431
- G3	11.1	7.0 – 17.57	<0.001	7.23	3.25 – 16.13	<0.001
Tumor stage						
- T1	ref.	ref.	ref.	ref.	ref.	ref.
- T2	1.75	1.11 – 2.77	0.016	0.73	0.31 – 1.72	0.465
- T3	2.39	1.50 – 3.82	<0.001	1.10	0.46 – 2.63	0.826
- T4	4.25	2.49 – 7.26	<0.001	0.90	0.28 – 2.89	0.863
Nodal status	2.62	1.95 – 3.54	<0.001	1.22	0.68 – 2.16	0.507
Distant metastasis	4.79	3.60 – 6.37	<0.001	1.25	0.56 – 2.77	0.587
First line treatment						
- No therapy	ref.	ref.	ref.	ref.	ref.	ref.
- Surgical resection	0.12	0.08 – 0.18	<0.001	0.12	0.05 – 0.30	<0.001
- PRRT	0.42	0.26 – 0.68	<0.001	0.76	0.23 – 2.50	0.649
- Chemotherapy	1.63	1.12 –2.38	0.011	0.49	0.20 – 1.19	0.115
- Targeted therapy	0.75	0.41 – 1.37	0.347	0.75	0.21 – 2.67	0.656
- SSA	0.58	0.39 – 0.87	0.008	0.84	0.29 – 2.42	0.743

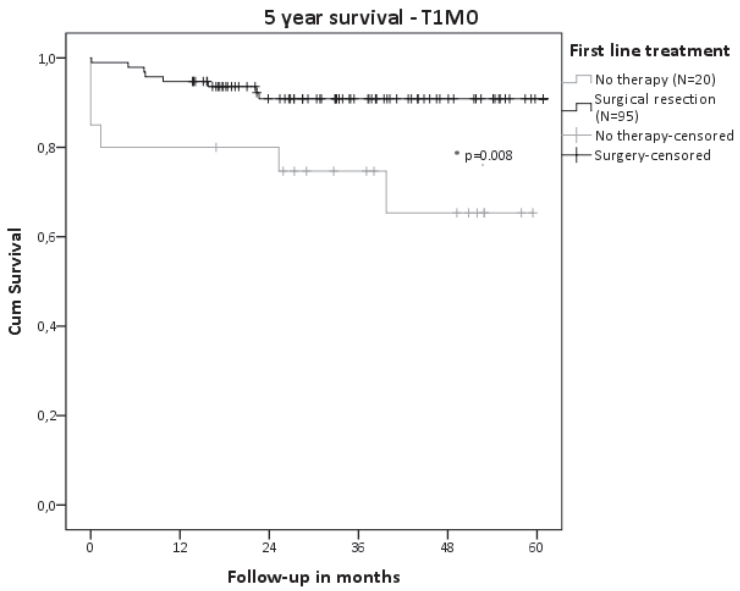

Figure 4 - Survival of patients with T1M0 tumors.

DISCUSSION

In this study, we present the treatment and survival of patients diagnosed with pNET in the Netherlands over a 6 year period. Patients undergoing surgical resection show superior outcomes in terms of survival, regardless of the presence of distant metastases. Apart from surgery and allowing for selection bias, PRRT shows the best survival curves in patients with disseminated disease.

For a long time, surgical treatment was the golden standard for patients diagnosed with localized pNET. However, there have recently been changes in the guidelines advising a conservative, rather than a surgical approach, for small non-functional tumors [13]. Data supporting this observational option are controversial as is evident from the presented results: T1M0 patients with a resection have a survival benefit compared to those without treatment. Still, issues of selection bias, small sample and missing data limit our ability to make valid conclusions. Similar studies support or contradict our findings, indicating comparable study bias and the need for prospective data [14-16]. It is imaginable that the reason to refrain from surgery might influence the

outcome in both directions. As there are no RCTs or meta-analyses that can assist the optimal management of small pNET, a prospective study to register and monitor all patients with small pNET (the PANDORA-study) is currently being conducted in the Netherlands.

A more aggressive approach has increasingly been described in the literature with regards to metastasized disease [17-20]. Similarly, our results promote surgical resection for patients with distant metastases, with a survival benefit of 40-76% in 5 years. Inclusion bias, with relative stable M1 patients, warrants that future studies clearly describe patients related treatment determinants, tumor progression and time to progression as markers. Definitions of metachronous and synchronous metastases should be established, preferably in international guidelines, for research to be univocal and comparable. Only then, the presented results can be confirmed in prospective trials that weigh the effect of resection in the presence of metastases (i.e. resection of the primary tumor with/without synchronous resection of solitary liver metastases) against the current, less invasive, systemic and nuclear options, taking into account the risks of both treatments.

The effect of PRRT has not previously been described in a population-based study. In this cohort, 41 patients received treatment with PRRT and showed the longest median survival compared to other treatments. Furthermore, survival analyses showed that PRRT had comparable outcomes to surgical resection in the overall population, and favorable outcome in patients with distant metastatic disease who did not undergo surgery. Nevertheless, there is a clear selection bias since less G3 tumors received PRRT compared to chemotherapy, implying that the favorable outcome of PRRT might be explained by the selection of patients with less aggressive disease. Significant differences for tumor grade between the treatment groups support this theory. Unfortunately, the available data was too small to provide reliable sub-analyses on tumor grade for the non-surgical treatment groups.

The results of this study must be seen in light of its limitations. Data were evaluated retrospectively and pathology reports were not standardized at the time of treatment. This may explain the considerable amount of missing data for tumor stage and grade, as other population based studies also report [1, 21]. It is worth mentioning that registration improved up to 78% for grade and 89% for tumor stage in 2013. An additional increase is anticipated in the Netherlands as national pathological guidelines for pNET have been

published the in 2013, and 4 hospitals have been named ENETS Centers of Excellence after the study period. Nevertheless, the amount of patients treated in non-academic centers show that there may be bias due to lack of centralization, as pNET requires complex knowledge and care. Furthermore, heterogeneity remains a difficult and recurring issue in pNET research. Accurate assessment of patient and tumor characteristics, along with strict selection criteria in future studies should be pursued to limit bias and draw reliable conclusions from study results.

Conclusion and future perspectives

Despite efforts, the overall survival of patients diagnosed with pNET is not improving. An effective and purposeful treatment approach is therefore necessary. Besides survival, patient related outcomes should be included in future studies. Tumor grade and TNM-stage remain the most important prognostic factors, and need to be clearly defined in each patient, to determine prognosis and treatment. Surgical treatment of small pNET and patients with M1 disease improves survival compared to all other treatments. Prospective trials must be encouraged to achieve fast and reliable results. Emphasis of future research should be on whether or not to resect pNET in patients with small lesions as well as patients with distant metastatic disease. Clear definitions for synchronous/metachronous lymph node and distant metastases, time to progression and treatment indication should be established and used in all studies concerning pNET.

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COLLABORATORS

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Recurrence after curative surgery of well-differentiated pancreatic neuroendocrine tumors: a meta-analysis

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Submitted

ABSTRACT

Background. Reliable recurrence rates after resection of pancreatic neuroendocrine tumors (pNET)are difficult to deduct from literature. Since recurrence is the most important predictor for poor outcome, this knowledge is crucial to optimize postoperative treatment of pNET patients. This meta-analysis aims to gain insight into prognosis after curative resection of well-differentiated pNET by investigating recurrence rate, time to recurrence, predictive factors and survival after recurrence.

Methods. Pubmed, Embase and the Cochrane library were searched for studies reporting recurrence rates in patients after complete resection of grade 1 or 2 pNET, without distant metastases or hereditary syndromes. Studies with <20 patients, high grade neuroendocrine carcinoma or patients who underwent (neo)adjuvant chemotherapy were excluded.

Results. Eight studies, including 734 patients, were found suitable. Meta-analysis showed an overall pooled recurrence rate of 13% (CI95% 9-18%). Weighted mean follow-up was 46.4 months. Sub-analyzes showed pooled recurrence rates of 11% for non-functional tumors ($p>0.05$), 8% for grade 1 ($p=0.003$) and 10% for R0-resections ($p=0.003$) compared to the remaining studies. Locoregional recurrence was seen in 6% versus 9% distant metastases ($p=0.011$). Weighted mean time to recurrence was 39.4 months and survival after recurrence was 38.9 months. Factors associated with recurrence were: tumor size, tumor grade, lymph node metastases, perineural invasion and R1 resection.

Conclusion. Recurrence after curative resection of grade 1 or 2 pNET is seen in 9-18%. Survival after recurrence is limited. A useful and evidence based follow-up regimen for the early detection of recurrence after curative surgery is proposed.

INTRODUCTION

Patients with pancreatic neuroendocrine tumors (pNET) are still primarily diagnosed at advanced stage, however the expanding use of high-quality radiological imaging has led to an increase in diagnosis of localized disease[1, 2]. For patients with non-functioning tumors >2cm, without distant metastasis, complete surgical resection is suggested as curative treatment[3, 4]. Postoperatively, patients are monitored to detect recurrence at an early stage, as treatment of limited disease shows favorable outcomes[5-7]. Nevertheless, knowledge on recurrence after curative surgery is still very limited, making it difficult to determine the best management. In general, it is thought that recurrence occurs sporadically after resection of localized well-differentiated (= grade 1 and 2) pNET, yet some studies report rates up to 50%[8]. Furthermore, recurrence has shown to predict a poor 10-year disease specific survival[9].

The importance of prognostic knowledge after resection of pNET seems to be generally undervalued. This becomes evident, for example, by the lack of a comprehensive follow-up program in the current international guidelines[3, 4, 10]. Knowledge on recurrence can help develop postoperative treatment strategies, based on the incidence of recurrence after surgical resection. If recurrence is indeed sporadic, follow-up after resection might perhaps be unnecessary. However, if recurrence is more common, recommendations on the frequency and optimal diagnostic modalities of postoperative hospital visits should be specified. In many cases, early detection of recurrence offers more favorable treatment options, sometimes with curative intent; such as resection of the remnant pancreas or solitary liver metastases[11]. Liver-directed, locally ablative procedures are recommended for patients with limited, non-resectable tumor burden[12-14]. When recurrence is discovered with extensive disseminated disease, systemic treatment is often the only option[15]. Despite the variety of treatment options, there is uncertainty with regard to the optimal treatment regimens. Furthermore, when identifying high-risk patients, adjuvant therapy might be beneficial to prevent the development of recurrence. At the moment, adjuvant therapy is not indicated in patients with pNET and has not yet been described in the literature. Gaining more insight in true recurrence rates and associated factors is essential to identify high-risk patients and to investigate the effect of specific treatments.

The aim of this meta-analysis is to systematically summarize existing data on recurrence after resection of non-metastasized grade 1 and 2 pNET, and to

provide more insight in the prognosis of these patients by investigating recurrence rate, time to recurrence, predictive factors and survival after recurrence.

METHODS

Search strategy

A literature search in Medline, Embase and the Cochrane Library was performed on January 2017 using the search terms “neuroendocrine”, “neuroendocrine tumor” or “NET”, combined with the terms “pancreas” and “recurrence” or “relapse” or every possible variant of these terms. Limitations used were English language and studies on humans, without limitations for period of publication. In addition, reference lists of the included studies were searched manually for additional relevant studies. All studies from the literature search were independently assessed for eligibility by three investigators (CG, END and TM) according to predetermined selection criteria. Discrepancies in appropriateness for inclusion and data-extraction in this meta-analysis were discussed.

Selection criteria

All published literature reporting recurrence rates of patients who underwent surgical resection of a grade 1 or 2 pancreatic NET, without distant metastases, were found eligible. As the functional status of the tumor is defined by the clinical syndrome and not by the immunohistochemical profile or histopathologic appearance of the tumor, studies including both functional and nonfunctional pNET (NF-pNET) were included. Studies reporting NET of other origin, including fewer than 20 patients (in total as well as in subgroups) or patients who underwent (neo)adjuvant chemotherapy were excluded. In addition, studies that included patients with metastasized disease (regardless of curative metastasectomy), gross incomplete resection (all other than R0/R1 resection as classified by the Royal College of Pathologists[16]), high grade neuroendocrine carcinoma (NEC) or patients with hereditary syndromes (Multiple Endocrine Neoplasia type-1 (MEN-1) or Von Hippel Lindau (VHL) disease) were excluded or could only be included if data of these patients was separately mentioned and could be extracted from the manuscript. Eligibility regarding grade was assessed using the World Health Organization (WHO) classification, of which studies including patients with

"poorly-differentiated" tumors according to the WHO classification before 2010, as well as patients with grade 3 NEC according to the 2010 WHO classification were excluded[17, 18]. Lymph node metastases were considered as locoregional disease and were therefore not an exclusion criterion. Corresponding authors were contacted in case patient criteria could not reliably be extracted from the manuscripts. If more than one study was reported from the same institution, research group or presented data-set, either the study with the largest sample size or the most recent study was included, provided the outcome measures were not mutually exclusive.

Data extraction and critical appraisal

After screening on title and abstract, a full text screening was performed to determine if the studies fulfilled the inclusion criteria. The following data was extracted from the included studies: first author, year of publication, year of data collection, country of origin, study type, characteristics of study population, number of patients meeting the inclusion criteria, follow-up duration and range/standard deviation (SD), recurrence rates, time to recurrence, survival period after recurrence and predictive factors for recurrence. Recurrence was defined as any recurrence according to the included studies, including local, regional, or distant disease. Overall results, as well as sub-analyzes for specific subpopulations of patients were performed in order to improve homogeneity.

The Newcastle-Ottawa Scale (NOS) is a risk of bias assessment tool for observational studies that is recommended by the Cochrane Collaboration[19,20]. We used a modified version of the NOS, since none of the studies used a comparing cohort. Each study was examined on two, instead of the regular three factors: comparability of the study groups was discarded. A score of 0-7 stars was assigned to each study according to the coding manual for cohort studies of the NOS.

Statistical analysis

Outcome data of the included studies were pooled and evaluated for all studies reporting recurrence, as well as separately for sub-group data. Pooled weighted proportions with corresponding 95% confidence intervals (95%CI) were calculated using an inverse variance method in R statistical software (2016 The R Foundation For Statistical Computing, platform i386-w64-mingw32/i386 [32-bit], cran-Rproject.org)21. A random effects model was used considering the variation between and within the studies.

Heterogeneity of the included studies was evaluated by calculating the I^2 statistic. The I^2 describes the percentage of total variation across studies as a result of heterogeneity rather than chance. The value of I2 ranges from 0% to 100%, with a value of 0% indicating no observed heterogeneity and larger values indicating increasing heterogeneity. Weighted mean follow-up duration, time to recurrence and survival after recurrence was calculated with medians by the method described by Hozo et al.[22]. Chi-square test was used to compare recurrence rates from sub-analyzes with the patient population in the remaining studies. Recurrence rates of locoregional recurrence versus distant metastases were compared with the z-statistic for proportions. Sensitivity analyzes were performed to evaluate the robustness of the results[23]. Each study was manually removed and meta-analysis was repeated to evaluate its effect on the summary estimates.

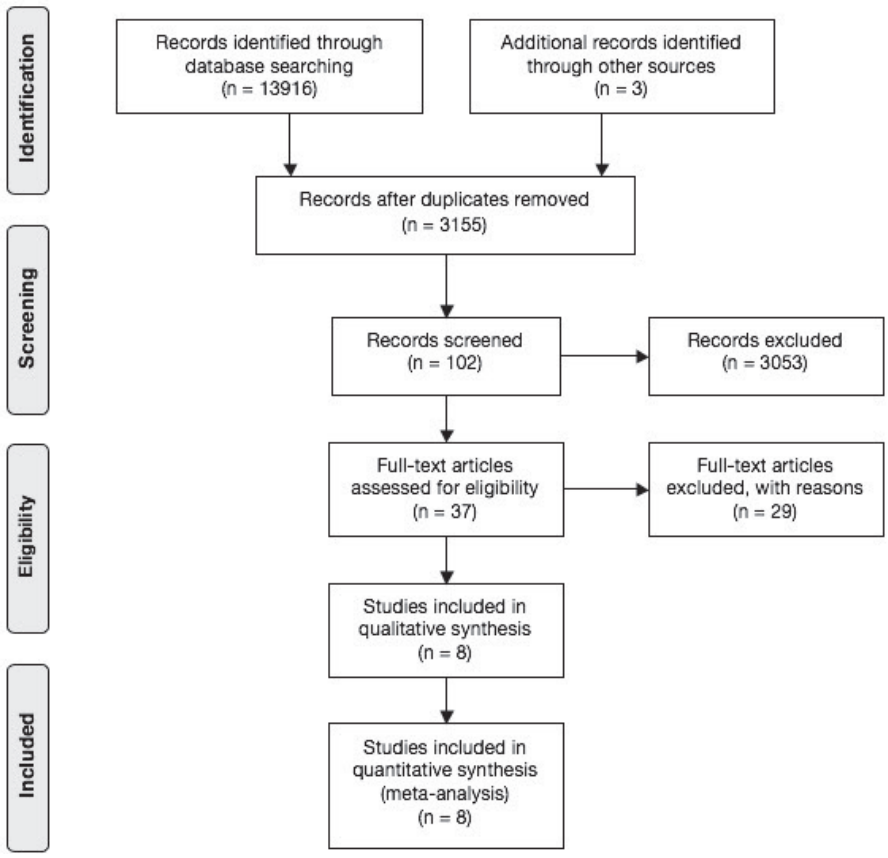

Figure 1 - PRISMA Flow-diagram of the study selection process

RESULTS

Literature search

A total of 13916 potentially eligible studies were identified in the literature search. A flow-chart of the selection process is shown in Figure 1. After excluding duplicates, 3152 studies were screened on title and abstract. Thirty-five studies were selected for full text screening, of which 5 studies met the inclusion criteria and 30 studies met (multiple) exclusion criteria. Cross-referencing of the 5 included studies led to the inclusion of 3 additional studies, eventually resulting in 8 full-text articles suitable for this meta-analysis[24-31]. The characteristics of the included studies are summarized in Table 1. All studies were published between 2007 and 2017 and described single (n=6) or multicentre (n=2) non-randomized retrospective cohorts. Four studies included only patients with non-functioning pNET (NF-pNET), of which Lee et al. only included patients with T1-2 <4cm without nodal metastases. Four studies included functional tumors, with Tsang et al. including only patients with sporadic benign insulinomas diagnosed with a prorogued fasting test[24, 25, 29, 31]. A total of 734 patients who underwent curative surgery for a grade 1 or 2 pNET between 1982 and 2013 were included for meta-analysis.

Quality assessment

Quality scores were assessed for all included studies (Table 1). The overall quality of the studies was comparable, with two studies scoring seven stars on the NOS[25, 31]. The remaining studies did not provide a sufficient follow-up period for all patients to detect recurrence (minimal of 5 years) and therefore received six stars on the NOS.

Recurrence - overall

Recurrence was radiologically diagnosed in all studies; pathological confirmation was not standard across the studies. Seven studies provided a follow-up duration for the patients in the studies[9, 24, 26-29, 31]. Four studies reported the time to recurrence[9, 25, 29, 31] and 3 studies reported a survival period after the onset of recurrence[9, 24, 25].

Table 1 – Characteristics of the included studies

Study	*Period*	*Country*	*Study type*	*NOS*	*Functional status*	*Resection margin status*	*Follow-up months (range)*	*Patients (n)*	*Recurrence (n)*	*Location recurrence*
Ferrone[5] 2007	1982-2005	USA	Retrospective single center	6*	29% functional 16% insulinoma	NA	Median 44m (1-226)	166	30	6 retroperitoneal lymph nodes 22 liver 2 bone, 1 ovary, 1 stomach
Ballian[6] 2009	1991-2007	USA	Retrospective single center	7*	NA	R0 82% R1 18%	Median 68m	43	7	3 regional (pancreas + lymph nodes) 4 distant
Kim[7] 2012	1994-2010	Korea	Retrospective single center	6*	0% functional	R0 100%	Median 31.5m (3.6-145.1)	62	2	NA
Lee[8] 2012	2000-2011	USA	Retrospective single center	6*	0% functional	R0 100%	Mean 52m (max 138)	52	0	NA
Birnbaum[9] 2014	1994-2001	France	Retrospective single center	6*	0% functional	R0 94% R1 6%	Median 42m (5-187)	108	19	5 lymph nodes 12 liver, 2 other distant organs
Tsutsumi[10] 2014	1987-2011	Japan	Retrospective multicenter	6*	47% functional 34% insulinoma	R0 100%	Median 46m (1-278)	62	6	1 local pancreas 2 lymph nodes 3 liver
Tsang[11] 2016	1998-2013	Hong Kong	Retrospective single center	7*	100% insulinoma	NA	Mean 87.9m (SD 58)	30	1	NA
Genc[12] 2017	1992-2015	Netherlands Italy	Retrospective multicenter	6*	0% functional	R0 85% R1 15%	Median 51m (IQR 29-72)	211	35	24 local pancreas 1 lymph node 5 liver
Total								**734**	**100**	

NOS: Newcastle Ottowa Scale; NA: Not Available

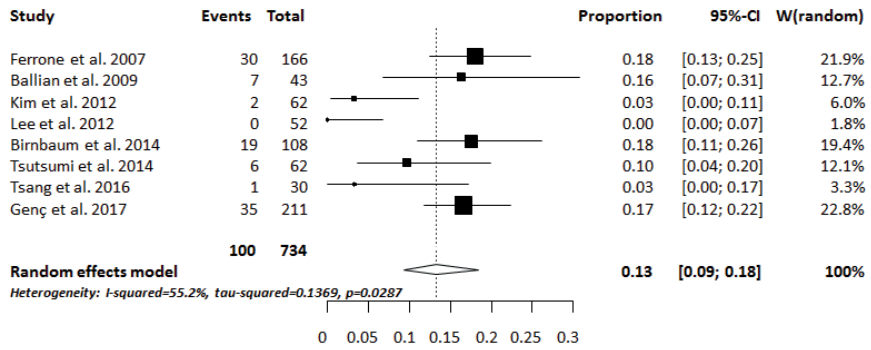

Figure 2 - Meta-analysis of recurrence rates of all included studies.

Of the 734 curatively resected pNET patients, 100 patients developed recurrence. The weighted average follow-up period for all patients was 46.4 months (SD = 38.9). Meta-analysis showed a pooled recurrence rate after curative surgery of 13% (95%CI 9-18%, I2 = 55.2%, Figure 2). Weighted mean time to develop recurrence was 39.4 months (SD = 30.0) after surgery, ranging from 23-132 months. Weighted mean survival after recurrence was 38.9 months, with a range of 32-68 months. Meta-analysis was repeated with the exclusion of the study by Tsang et al., as recurrence is known to be very uncommon for insulinomas32. In this patient population, recurrence was seen in 99 of the 704 patients, resulting into a pooled recurrence rate of 14% (95%CI 10-19%, I2 = 53,5%).

Sub-analyzes

NF-pNET

Non-functioning pNET (NF-pNET) were seen in 433 patients, reported in 4 studies[9, 26-28]. Patients with NF-pNET had a weighted mean follow-up period of 44.3 months (SD = 31.2). Recurrence was seen in 56 of 433 patients, resulting in a pooled recurrence rate of 11% (CI95% 6-21%, I2 = 71.5%, Figure 3A). Compared to the patients in the remaining studies, the recurrence rate of patients with NF-pNET showed no significant difference (p=0.297). Only one study reported recurrence rates of patient with functioning tumors[31].

Grade

In total, 231 patients with grade 1 tumors were identified in 3 studies[9, 29, 31]. Of these patients, 18 had a recurrence. Pooled recurrence rate was 8% (CI95% 5-12%, I2 = 0%, Figure 3B), with a weighted mean follow-up of 55

months (SD = 50.7). Recurrence was diagnosed after a weighted mean period of 41.1 months (SD = 32.2) following resection. As data on grade 2 tumors was insufficient the recurrence rate of grade 1 tumors was compared to the patients in the remaining studies, and showed significantly lower recurrence rates (p = 0.0027). Furthermore, the pooled recurrence rates of studies reporting grade 1 tumors were significantly lower compared to the recurrence rate of patients with grade 2 tumors reported by Genc et al. (p<0.001), as this was the only study reporting recurrence for grade 2 tumors.

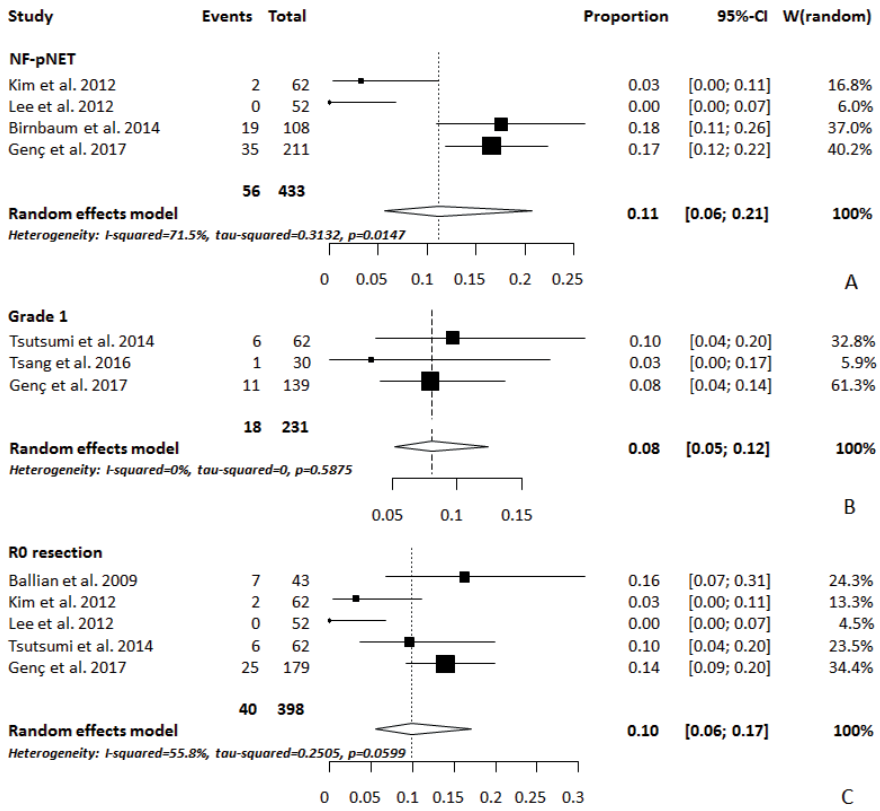

Figure 3 - Sub-analyses of recurrence rates of studies including only patients with NF-pNET (A), grade 1 tumors (B) or R0-resection (C).

R0 resection

Overall, five studies reported separate recurrence rates of patients with R0 resected tumors[9, 25-27, 29]. Recurrence was seen in 40 of the 398 patients with initially no evidence of microscopic disease on histopathology after resection. Within a weighted mean follow-up period of 44.6 months (SD 38.1), pooled recurrence rate was 10% (CI95% 6-17%, I2 55.8%, Figure 3C). Recurrence was diagnosed after a weighted mean time of 36.1 months (SD = 30.0) after surgery and was seen significantly less in patients reported to have R0 resection margins compared to the patients from the remaining studies including both R1 and R0 resections in their analyzes (p = 0.0030) (Table 1).

Location

Five studies reported the anatomical location of the recurrence in 590 patients[9, 24, 25, 28, 29]. These were summarized as either locoregional recurrence, in the remnant pancreas and/or regional lymph nodes, or as distant metastases. Recurrence was seen in 97 of 590 patients. Weighted mean follow-up for all patients was 46.5 months (SD = 40.6) and recurrence was diagnosed after a weighted mean period of 37.5 months (SD = 30.0) after resection. Forty-one patients developed recurrence in the remnant pancreas or regional lymph nodes, resulting in a pooled locoregional recurrence rate of 6% (CI95% 4-11%, I2 59.1%). In the same population, distant metastases were reported in 52 patients, resulting in a pooled recurrence rate of 9% (CI95% 6-15%, I2 69.9%). Local recurrence was seen significantly less often compared to distant metastases (p = 0.0109).

Predictors for recurrence

Three studies presented variables associated with the development of recurrence[9, 25, 28]. These included cox regression analyzes on disease specific and disease free survival, as well as specific predictors for recurrence. Factors associated with recurrence from univariate analysis were size, grade, microscopic incomplete resection (R1), perineural and vascular invasion and lymph node metastases. Size, R1 resection, lymph node metastases and perineural invasion were mentioned in both studies presenting results on univariate analysis[9, 25]. From multivariate analysis size, grade, lymph node metastases and perineural invasion were presented as independent predictors for recurrence or disease free survival. The first two factors were associated in both studies that present data on multivariate Cox regression analysis[9, 28].

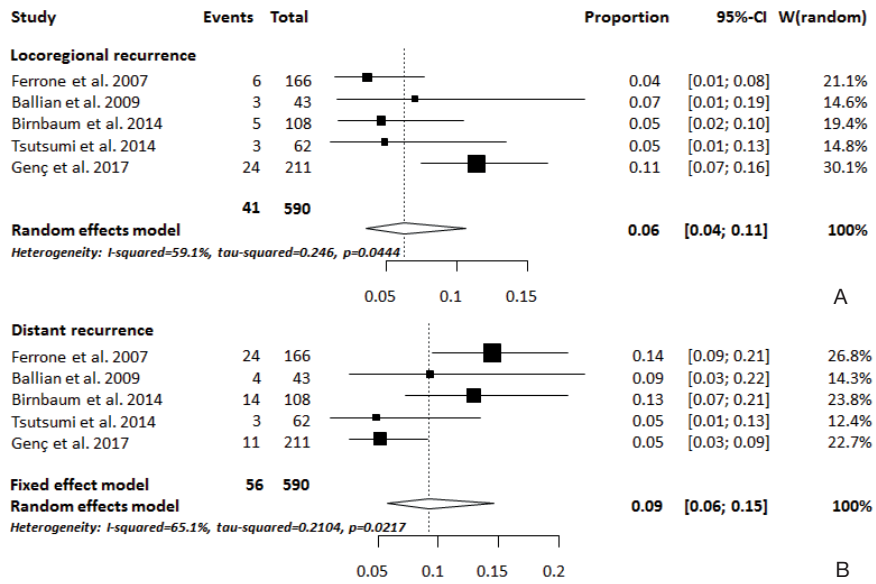

Figure 4 - Sub-analyses of recurrence rates stratified for locoregional (A) and distant (B) recurrence.

Sensitivity analysis

Sensitivity analysis showed no change in the overall results for meta-analyzes of all patients, as well as for sub-analyzes of patients with NF-pNET, R0 resections or grade 1 tumors separately. Therefore, it is assumed that no single study significantly contributed to the overall results and the conclusions of this meta-analysis.

DISCUSSION

This meta-analysis is the first to systematically establish reliable recurrence rates in patients treated curatively for a grade 1 or 2 pNET. Pooled rates of 9-18% show that recurrence after curative resection is not rare and typically occurs within the first years after surgery. These results may be seen as a guide, rather than exact values, to emphasize the importance of better knowledge and understanding of recurrent pNET. In a recent meeting of the NET Task Force of the National Cancer Institute GI Steering Committee multiple NET expert highlighted the importance of this knowledge[33]. The lack

of data regarding recurrence have presented a major obstacle to design studies on the role of adjuvant therapy, the benefit of early detection of recurrence and the effect of recurrence treatment. Hereby, we provide the necessary evidence, based on all published literature, to facilitate the development of future studies and aid therapeutic decisions after resection of pNET.

At the moment, international guidelines do not provide clear recommendations on the content and frequency of hospital visits for patients who underwent a resection of a pNET[3, 4]. Regularly, all patients are monitored the same, for it is assumed that surgery will provide a favorable outcome. Nevertheless, the presented results show that a uniform follow-up regimen is warranted in order to detect recurrence in a controlled manner and at an early stage. Various predictive factors are known to identify patients with a high risk of recurrence or poor prognosis, some of which are confirmed by sub-analyses in this study. Based on the study results, we therefore recommend a standard follow-up protocol for at least 10 years is encouraged, as clear examples of late recurrence are known[9, 28]. In addition, we suggest that the frequency of hospital visits is dependent on the time after resection and the risk profile of the patient. The first few years after resection intensive monitoring must be considered, taking into account that the time to recurrence is approximately 3 years. After this period the frequency of observations can be either maintained or reduced, based on the presence/absence of unfavorable tumor characteristics.

There is no international consensus on the definition of recurrence in pNET, especially with regards to the localization. This is evident from the wide range of interpretations of recurrence in the different studies. From our point of view, recurrence is defined as the new localization of pNET tissue, either locally in the remnant pancreas, in locoregional lymph nodes or metastases in distant organs or lymph nodes. A discrimination between these three locations should be made when recurrence is assessed, to prevent underestimation of recurrence and to gain insight in recurrence patterns that can aid detection during follow-up. Unfortunately, only 3 out of the 8 included studies shared this view[9, 25, 29]. Two studies did not provide a definition of recurrence[27, 31]. Some of the excluded studies only reported distant metastases as recurrence. Without clear agreement on these important matters, it becomes impossible to establish optimal treatment protocols for patients with pNET. Local recurrence might require different treatment and have a different prognosis than distant metastases, and the same might be

true for single versus diffuse metastatic disease. Therefore, we propose that the abovementioned definition of recurrence will be implemented in the International Study Group of Pancreatic Surgery (ISGPS) criteria to ensure uniformity in both management as well as research on pNET in the future (Table 2). During the study selection process, it became also evident that the majority of available pNET research is retrospective and mainly focused on patients with advanced disease. Multiple studies report recurrence rates but often include patients with hereditary syndromes, metastatic disease or gross incomplete resections. As these patients are, by definition, known to have a different prognosis, these results cannot be translated to curatively treated patients[3, 34-36]. Although we used strict selection criteria and performed sub-analyzes to reduce the effect of heterogeneity, it remains questionable if the available literature provides sufficient data to come to a conclusion. With the current available literature, it is unrealistic to reach lower values of heterogeneity while providing reliable results on recurrence rates after curative surgery for pNET. Nevertheless, these findings are important to share, as for a long time recurrence after curative surgery for pNET was an elusive concept, allowing the management to be based solely on personal experience. Improved techniques, such as Gallium based PET-CT, will lead to better detection of resectable pNET and recurrence and an increase in patients in need for advanced treatment is expected. Although the quality of the included studies may not provide exact expectations on the risk for recurrence, the presented results show that a specific focus on postoperative treatment strategies is urgently needed.

Table 2 – Proposed pNET recurrence criteria

Definition	New localization of tumour tissue
Localization	- Local in remnant pancreas - In regional lymph nodes of the pancreatic area - Distant in other organs or distant lymph nodes

In conclusion, recurrence after curative surgery for grade 1 and 2 non-metastasized pNET is not rare with 18% and mean survival of 38.9 after diagnosis. We propose an adequate follow-up strategy to detect recurrence at an early stage. Through proper international agreements on follow-up programs, patient selection and definition of recurrence, we believe that optimal postoperative management and reliable research to predict, and possibly even prevent, recurrence in the future can be achieved.

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A new scoring system to predict recurrent disease in grade 1 and 2 non-functional pancreatic neuroendocrine tumors

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ABSTRACT

Objective. To predict recurrence in patients with grade 1 or 2 non-functioning pancreatic neuroendocrine tumors (NF-pNET) after curative resection.

Background. Surgical resection is the preferred treatment for NF-pNET, however recurrence still occurs frequently after curative surgery, worsening prognosis of patients.

Methods. Retrospectively patients with NF-pNET of three institutions were included. Patients with distant metastases, hereditary syndromes or grade 3 tumors were excluded. Local or distant tumor recurrence was scored. Independent predictors for survival and recurrence were identified using Cox-regression analysis. The recurrence-score was developed to predict recurrence within 5-years after curative resection of grade 1-2 NF-pNET.

Results. With a median follow-up of 51 months, 211 patients with grade 1-2 NF-pNET were included. Thirty-five patients (17%) developed recurrence. The 5- and 10-year disease specific/overall survival was 98%/91% and 84%/68%. Predictors for recurrence were tumor grade 2, lymph node metastasis and perineural invasion. Based on these predictors the recurrence-score was made. Discrimination (c-statistic 0.81, CI95% 0.75-0.87) and calibration (Hosmer Lemeshow chi-square 11.25, p=0.258) indicated that the ability of the recurrence-score to identify patients at risk for recurrence is good.

Conclusion. This new scoring system could predict recurrence after curative resection of grade 1 and 2 NF-pNET. With the use of the recurrence-score less extensive follow-up could be proposed for patients with low recurrence-risk. For high-risk patients, clinical trials should be initiated to investigate whether adjuvant therapy might be beneficial. External validation is ongoing due to limited availability of adequate cohorts.

INTRODUCTION

In patients with curative resected non-functional pancreatic neuroendocrine tumor (NF-pNET), the overall prognosis is usually favorable. Currently The main focus during follow-up is to detect recurrence at an early stage[1-3]. However, follow-up regimens after resection of pNET are generally the same and no distinction is made between patients based on the presence or absence of specific tumor characteristics. In addition, Reliable recurrence rates are difficult to deduct from literature, because of the rarity of the disease and the inhomogeneous group of patients with resected pNETs. Most studies include patients with hereditary syndromes, hormonal overproduction, incidentally detected pNET and patients with metastases or locally advanced disease[4-8]. All these patients have a different probability of tumor recurrence and survival.

In the general practice, knowledge about the prognosis of a patient provides support when determining the frequency of the follow-up visits. Better estimation of long-term results prognosis of curable patients is therefore desirable. With this knowledge, postoperative management can be customized based on the expected risk of recurrence, as is common in some other malignancies[9]. This approach can have advantages for the patient as well as the hospital and the health care system. Despite international guidelines[10,11], there is still much uncertainty about the frequency of follow-up visits and radiological imaging. Current European Neuroendocrine Tumor Society (ENETS) and North American Neuroendocrine Tumor Society (NANETS) guidelines provide recommendations on the management of pNET but do not include statements on postoperative follow-up regimens. Moreover, the latest guidelines propose a conservative approach in the surgical management of small tumors <2cm. This opinion is based on retrospective analyses and the indolent nature of these tumors. This strategy could be adopted for a select group of patients after surgical resection of tumors without unfavorable characteristics.

In comparison with other types of cancer, including pancreatic cancer, adjuvant treatment after surgical resection is not recommended for patients with NF-pNET[4]. In metastatic patients, different treatment options are available in order to reduce tumor load, to inhibit tumor growth or to alleviate the symptoms[12]. These include chemotherapy[13,14], long acting somatostatin analogues[15,16], mTOR or tyrosine kinase inhibitors (everolimus, sunitinib)[17,18] and peptide receptor radionuclide therapy (PRRT)[19]. Theoretically, one or

more of these treatment options could serve as adjuvant therapy in patients with a risk of recurrent disease after curative resection. Clinical trials are needed to evaluate this benefit. However, it is difficult to identify high-risk patients, most likely explaining why this has never been investigated before.

Until now, it is unclear which combination of risk factors for recurrence matter most in patients with grade 1 or 2 NF-pNETs in daily practice. A recent study by Birnbaum et al reported tumor size and tumor grade to be independent predictors for recurrence in patients with sporadic NF-pNET without distant metastases[20]. However, studies on predictive factors are scarce and frequently include patients with either distant metastasis present during surgical resection, hereditary syndromes or high-grade carcinoma. The aim of this study was to analyze the long-term outcome in a very selective group of patients with low- to intermediate-grade NF-pNET without hereditary syndromes, grade 3 tumors or distant metastasis at time of diagnosis. Recurrence rates and significant predictors for recurrence were analyzed. With these predictors, the recurrence-score was developed to calculate the risk of recurrence of the individual patient and to identify high-risk patients after curative resection.

METHODS

Retrospectively, all NF-pNET with a curative resection from 1992 to 2015 of three academic institutions were included: the Erasmus Medical Center in Rotterdam and the Academic Medical Center in Amsterdam, both in the Netherlands, and the Ospedale San Raffaele in Milano, Italy. All institutions are high volume centers for pancreatic surgery and specialized in the treatment of neuroendocrine tumors. The pathology reports of all pancreas resections in the selected period were reviewed for the diagnosis of pNET. Patients were included if a histopathology proven pNET was present. Inclusion criteria for this study were adults with a curative resected grade 1 or 2 NF-pNET without distant metastases at the time of diagnosis. Patients with ampullary or duodenal NETs and all patients with (unresectable) locally advanced disease or distant metastases, successfully treated or not, were excluded. Patients with hereditary syndromes, such as Multiple Endocrine Neoplasia type 1 (MEN-1) or Von Hippel-Lindau syndrome (VHL) or with grade 3 NF-pNET, even if diagnosed after resection of the pNET, were also excluded.

NF-pNET was defined as a pNET without clinical syndrome based on symptoms associated with hormone overproduction. The medical records, radiological imaging reports and operation reports were reviewed for the demographics and clinical data including, age of surgery, sex, tumor size (based on preoperative radiological imaging), tumor location and type of surgery. Radiological imaging consisted of abdominal CT scan and in some patients of endoscopic ultrasonography and/or octreotide scintigraphy (Octreoscan®/^{68}Ga-DOTATATE PET-CT).

Depending on tumor location, pancreatoduodenectomy, distal or total pancreatectomy was performed. Central pancreatectomy or tumor enucleation was performed in patients with small pNET far enough from the pancreatic duct. Lymphadenectomy was not routinely performed in patients with tumor enucleation. All included NF-pNET were reassessed with emphasis on for tumor grade, lymph node involvement, vascular or perineural invasion by three experienced pathologists (FJ van K, S van E and JV). Mitotic count and histological grade were based on the World Health Organization (WHO) classification of 2010 in grade 1 to 3[21]. Resection margins were classified according the Royal College of Pathologists[22]. Completely excised tumors were classified as R0, tumors with microscopic margin involvement <1mm were classified as R1. Pathology was performed according to the local protocols. Major complications after surgery were defined as pancreatic fistula grade B/C, delayed gastric emptying grade B/C or post-operative bleeding grade B/C, scored according to the ISGPF classifications[23-25].

Since small pNET may show a more indolent recurrence pattern, separate analyses were performed concerning patient with NF-pNET <2cm.

Besides routine control of physical symptoms, the follow-up program consisted of physical examination, laboratory tests and radiological imaging. The first year after surgery, patients were seen every 6 months. Thereafter, follow-up was annually or in case of elevated chromogranin A or dubious radiology results continued every 6 months. Follow-up was indicated for 10 years after surgery. Recurrence was defined as local recurrence in the pancreas, new localization in lymph nodes or the development of distant metastases. Recurrence free survival was defined as the percentage of patients without recurrence after resection. Disease specific survival was the percentage of patients who have not died due to pNET.

Statistical analysis

Statistical analyses were performed using IBM SPSS statistics 20 and R for windows version 3.0.2 (cran-Rproject.org). Based on the distribution, the data was described with mean and standard deviation (SD) or median and interquartile range (IQR). For categorical data, the number and proportion (%) were displayed. Kaplan-Meier curves were used to determine the median time for recurrence and survival. To identify predictors for survival within 10 years after curative surgery a Cox proportional hazard regression was performed. This was repeated for predictors for recurrence within 5-years. The assumption of proportional hazard regression was tested by visually inspecting the log minus log plots. No violations were detected for any of the variables included in the model. The results were presented with the Hazard Ratio (HR) and the 95% confidence interval (CI95%). To determine predictors for recurrence a backwards selection with a P-value of <0.05 was used to select the variables one by one from the multivariable Cox regression analysis. Based on the hazard ratios of the significant predictors of the multivariable Cox regression, a scoring system was made. The hazard ratio was translated into a score (the recurrence-score) for each predictor and were multiplied by 10 to prevent loss of information due to rounding. The overall recurrence-score corresponds to the risk for recurrence within 5 years after a curative resection. ROC analysis was performed to determine the most suitable cut-off of the recurrence-score. Both the Youden's index as well as the Log Rank method was used to determine the recurrence-score with the most appropriate sensitivity and specificity. Model performance was assessed by measurements of discrimination and calibration. Discrimination is the ability to separate the persons who will have recurrence from the persons who will not have recurrence. Calibration is the ability to correctly quantify the observed absolute risk. The discriminative ability of the model was examined by calculating Harrel's c-statistic26 with 95% CI and the calibration of the model was assessed by calculating the goodness of fit Hosmer–Lemeshow Chi-square test. Moreover, we examined the discrimination of the WHO grade model and compared the c-statistics of the two models using a z-test. The c-statistic may vary from 0.5 to 1.0. A discriminative value of 0.5 was considered as good as chance and a value above 0.9 was excellent. Calibration was not significant; the prediction of the model was comparable with the actual outcome. A 2-sided p-value < 0.05 was considered significant. The Medical Ethics Review Committee has approved the study.

RESULTS

With a mean age of 60 years (range 19 – 83) at diagnosis, 211 patients were included in the analysis. Patient and tumor characteristics are listed in Table 1. In total, 139 patients had a G1 tumor. Median tumor size was 25mm (IQR 15 – 44) and most frequently located in the pancreatic head (40%). Pancreatoduodenectomy was performed in 64 (30%), left pancreatectomy in 101 (48%), tumor enucleation in 29 (14%), central pancreatectomy in 11 (5%) and total pancreatectomy in 5 (2%) patients. Postoperatively, major complications were seen in 58 patients (27%) and consisted of pancreatic fistula grade B/C in 46 patients (22%), delayed gastric emptying grade B/C in 7 patients (3%) and postoperative bleeding grade B/C in 2 patients (1%). One patient experienced a pancreatic fistula and postoperative bleeding. Complete resection (R0) was performed in 179 patients (85%), whereas the remaining 32 (15%) showed either microscopic tumor cells at the resection margin or within 1 mm (R1). R1 resections were found in 12 (18.8%) patients who underwent pancreatoduodenectomy, 8 (7.9%) patients who underwent left pancreatectomy, 6 (20.7%) patients who underwent enucleation, 4 (36.4%) patients with central pancreatectomy and 2 (40%) with a total pancreatectomy.

Long-term follow-up

Median follow-up time was 51 months (IQR 29-72). Recurrence was seen in 35 patients (17%): 16 (46%) after pancreatoduodenectomy, 14 (40%) after left pancreatectomy, 3 (9%) after enucleation, one after central pancreatectomy and one after total pancreatectomy. In 24 patients (69%) the recurrence was located in the pancreatic remnant, whereas 5 patients (14%) developed recurrence as distant metastases and one had lymph node metastasis. Mean tumor size of patients with recurrence was 36.8mm versus 32.9mm for patients without recurrence ($p>0.05$). Grade 1 was seen in 11 (31.4%) patients and grade 2 in 24 (68.6%) patients. Ten (28.6%) patients with recurrence had R1 resection and 20 (57.1%) had lymph node metastases in the resected specimen of the initial surgery. Median time to recurrence was 43 months (IQR 23 – 62). Mean survival of patients without recurrence was 163 months, compared to 139 months for patients with recurrence ($p=0.011$), Figure 1. Overall, 19 patients (9%) deceased, including 9 patients due to tumor progression. The 5- and 10-year disease specific survival was 98% and 84%, respectively. Overall survival was 91% within 5

years and 68% within 10 years. Recurrence free survival of all patients is presented in Figure 1.

Table 1 - Patient and tumor characteristics (n=211), n (%)

Age, median	60 (IQR 50 – 66)
Male	103 (48.8%)
Tumor location,	
Head	80 (37.9%)
Body	59 (28%)
Tail	72 (34.1%)
Tumor grade	
G1	139 (65.9%)
G2	72 (34.1%)
Tumor size, median	25 mm (IQR 15 – 44)
Major complications	58 (26.5%)
Resection margin	
R0	179 (84.8%)
R1	32 (15.2%)
Positive lymph nodes	51 (24.2%)
Perineural invasion	28 (13.3%)
Vascular invasion	50 (23.7)
Mortality	19 (9%)
Disease related deaths	9 (4.3%)
Tumors <2cm	84 (39.8%)
Size, median	14 (IQR 11-17)
G2	14 (16.7%)
R1 resection margin	11 (13.1%)
Positive lymph nodes	10 (11.9%)
Recurrence	4 (4.8%)
Mortality	7 (8.3%)
Recurrence	35 (16.6%)
G2	24 (68.6%)
R1 resection margin	10 (28.6%)
Positive lymph nodes	20 (57.1%)
Local recurrence	24 (68.6%)
< 5 years after surgery	32 (91.4%)

IQR: interquartile range

Tumor size <2cm

Based on the latest ENETS guidelines, a sub-analysis for tumors <2cm was performed. In this cohort, 84 of the 211 patients had a tumor smaller than 2 cm. Thirty-seven patients were male (44%) and 47 females (56%), with a median tumor size of 14mm. Tumor location was equally distributed between the head, corpus and tail of the pancreas (28.6%, 36.9% and 34.5% respectively). Enucleations were performed in 23 cases (27.4%), the remaining 51 patients underwent pancreatic resection. Eleven patients had a R1 resection (13.1%) and 14 patients had a grade 2 tumor (16.7%). Lymph node metastases were present in 10 patients (11.9%) and perineural invasion was seen in 8 patients (9.5%). Recurrence was seen in 4/84 patients (4.8%).

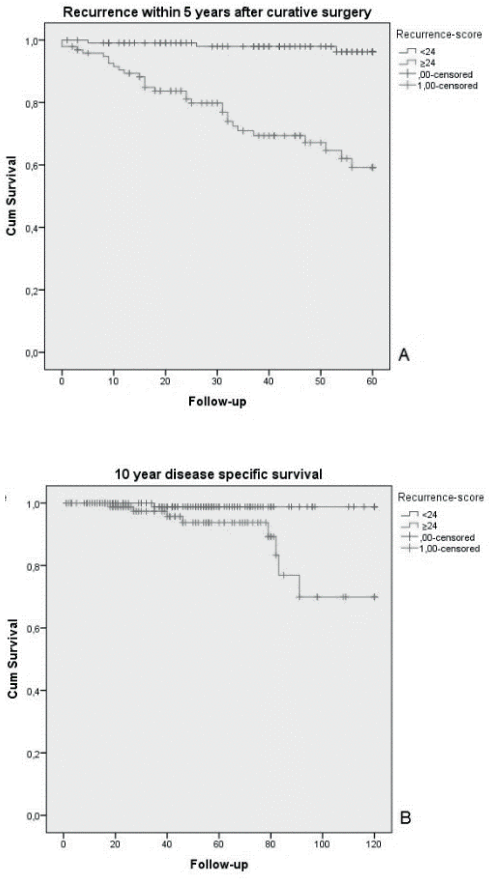

Figure 1 - Kaplan-Meier analysis of patients with a grade 1 or 2 NF-pNET. A, Ten-year overall survival of patients with and without recurrent disease. B, Ten-year recurrence-free survival of all patients.

Seven patients died, of which 2 were related to pNET. From univariable analysis, tumor grade (HR 18.5, CI95% 1.91-179.13, p=0.012), positive lymph nodes in the resected specimen (HR 7.8, CI95% 1.09-55.16, p=0.041), perineural invasion (HR 30.7, CI95% 3.19-295.74, p=0.003) and vascular invasion (HR 6.9, CI95% 0.97-49.03, p=0.05) were predictors of recurrence within 5 year after curative surgery. Multivariable analysis was not performed due to patient numbers. Disease specific survival was 97% in 5 years and the same for 10 years. The 5- and 10-year overall-survival was 91% and 79% respectively.

Predictors for survival and recurrence

A Cox regression analysis was performed to identify risk factors for mortality within 10 years after surgery (Table 2). pNET related death was associated with perineural invasion (HR 3.8 CI95% 1.51-9.63) and recurrence (HR 2.7, CI95% 1.4-6.56). Cox regression analysis was repeated for predictors for recurrence within 5 years after surgery (Table 3). Univariable analysis was significant for tumor size, R1 resection, tumor grade, positive lymph nodes in the resected specimen and perineural invasion. With a backwards selection, tumor grade (HR 4.07, CI95% 1.87-8.84), positive lymph nodes in the resected specimen (HR 2.44, CI95% 1.17-5.09) and perineural invasion (HR2.38, CI95% 1.11-5.10) were significant to predict recurrence in the multivariable analysis. Recurrence within five years after curative resection was seen in 25% of patients with only tumor grade 2, in 30% of patients with only positive lymph nodes and in 14% of patients with perineural invasion. In the presence of two predictive factors recurrence was seen in 38% of patients with a grade 2 tumor and positive lymph nodes, 40% of patients with positive lymph nodes and perineural invasion, and in 33% of patients with a grade 2 tumors and perineural invasion. When all predictive factors were present 60% of the patients showed recurrence. Of the 107 patients with none of these factors present, only 2 developed recurrent disease.

The recurrence-score

A scoring system was made based on the independent predictors from the multivariable Cox regression analysis (Figure 2). The recurrence-score predicts the probability to develop recurrence within 5 years after curative resection in patients with a grade 1 or 2 NF-pNET. For each patient, a total recurrence-score was calculated based on the presence or absence of these factors. Patients with recurrent disease showed significantly higher

recurrence-scores (49.3) compared to the recurrence-scores of patients without recurrence (17.7, p<0.001).

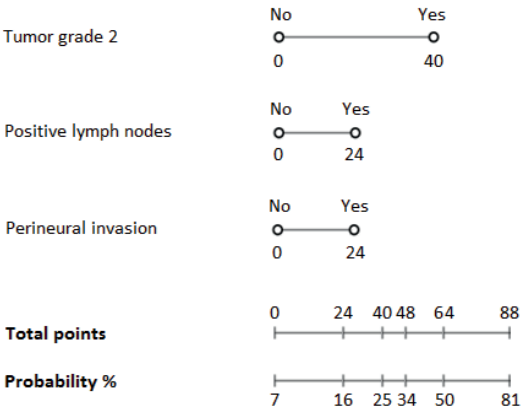

Figure 2 - The recurrence score to predict recurrent disease within 5 years after curative resection. Patients score points for the presence or absence of each of the tumor characteristics. The total points can be translated into the probability of recurrent disease within 5 years after curative surgery.

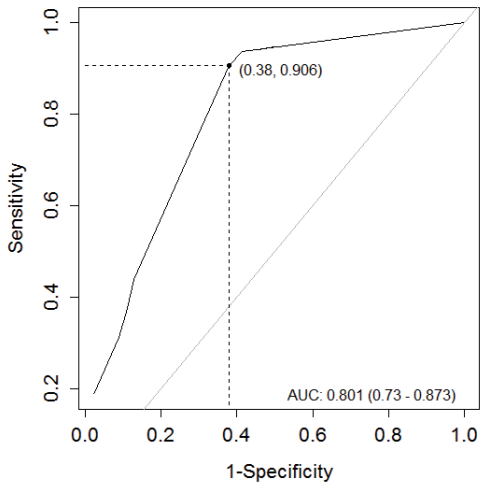

Figure 3 - ROC analysis of recurrence scores to determine the most appropriate cut-off to identify high-risk patients for recurrence within 5 years after curative surgery.

The recurrence-score was internally validated. The discriminative ability of the recurrence-score was good, with a Harrel's c-statistic of 0.81 (CI95% 0.75-0.87) and a Hosmer Lemeshow chi-square test of 11.25 (p=0.258). In practice, the WHO grading is used to predict recurrence15. The discrimination of the WHO grading was lower compared to the recurrence-score with a c-statistic of 0.72 (CI95% 0.64-0.79). However close, this was not significant (p-value=0.059). Calibration of this model was not examined because it only consists of two variables, grade 1 and grade 2.

To determine the appropriate cut-off to identify high-risk patients for recurrence within 5 years after surgery, an ROC analysis of the recurrence-score was performed, Figure 3. This resulted in an optimal recurrence-score cut-off of 24, with a sensitivity of 91% and specificity of 62%. Kaplan-Meier analysis showed a significant difference in recurrence within 5 years after surgery for patients with a recurrence-score below 24 with a mean time to recurrence of 59 months, compared to patients with a recurrence-score of 24 and higher and mean time to recurrence of 46.9 months ($p<0.001$). Mean 10-year disease specific and overall survival was 181.3 months (CI95% 178.0 – 184.6) and 110.3 months (CI95% 103.4 – 117.3) respectively for patients with a recurrence-score below 24, compared to 167.0 months (CI95% 140 – 193.6) and 99.4 months (CI95% 90.3 – 108.6) respectively for patients with a recurrence-score of 24 and higher (DSS p=0.008, OS p=0.038).

Table 2 - Predictors for mortality within 10 years

Risk factors	Univariable Cox Regression			Multivariable Cox Regression		
	HR	*CI95%*	*p-value*	*HR*	*CI95%*	*p-value*
Male gender	1.218	0.515 – 2.877	0.654			
Age						
<40	ref	ref	ref			
40-50	0.307	0.019 – 4.919	0.404			
51-60	1.276	0.153 – 10.610	0.822			
61-70	1.949	0.247 – 15.412	0.527			
>70	2.300	0.256 – 20.624	0.457			
Tumor location						
Head	ref	ref	ref			
Body	0.684	0.248 – 1.884	0.463			
Tail	0.629	0.214 – 1.846	0.399			
Tumor size						
<2cm	ref	ref	ref			
2-4cm	1.249	0.464 – 3.361	0.659			
>4cm	1.088	0.345 – 3.431	0.885			
Major complication	0.660	0.244 – 1.787	0.414			
R1 resection	2.439	0.980 – 6.072	0.055			
Tumor grade 2	1.816	0.768 – 4.291	0.174			
Positive lymph nodes	2.105	0.872 – 5.085	0.098			
Perineural invasion	4.130	1.644 – 10.375	0.003	3.813	1.510 – 9.627	0.005
Vascular invasion	1.755	0.723 – 4.263	0.214			
Recurrence	2.977	1.237 – 7.169	0.015	2.730	1.137 – 6.554	0.025

Table 3 – Predictors for recurrence within 5 years

Risk factors	Univariable Cox Regression			Multivariable Cox Regression		
	HR	*CI95%*	*p-value*	*HR*	*CI95%*	*p-value*
Male gender	1.149	0.573 – 2.3	0.696			
Age						
<40	ref	ref	ref			
40-50	0.249	0.05 – 1.236	0.089			
51-60	0.775	0.213 – 2.818	0.699			
61-70	0.830	0.238 – 2.891	0.769			
>70	0.294	0.049 – 1.763	0.181			
Tumor location						
Head	ref	ref	ref			
Body	1.969	0.842 – 4.605	0.118			
Tail	1.099	0.412 – 2.929	0.851			
Tumor size						
<2cm	ref	ref	ref	-	-	-
2-4cm	3.957	1.302 – 12.028	0.015			
>4cm	5.920	1.946 – 18.008	0.002			
Major complication	0.932	0.531 – 1.636	0.806			
R1 resection	2.722	1.286 – 5.763	0.009	-	-	-
Tumor grade 2	5.625	2.653 – 11.927	<0.001	4.066	1.871 – 8.835	<0.001
Positive lymph nodes	4.039	2.014 – 8.102	<0.001	2.439	1.170 – 5.085	0.017
Perineural invasion	4.088	1.970 – 8.485	<0.001	2.380	1.111 – 5.097	0.026

HR: hazard rate, CI: confidence interval, ref: reference

DISCUSSION

Patients with pancreatic neuroendocrine tumors generally have a favorable prognosis. However, in case of recurrence, these patients have a poor survival. Assessment of risk factors for recurrence could therefore be of importance. In this study the recurrence-score is presented that can identify patients at risk to develop recurrence within 5 years after curative surgery of a grade 1 or grade 2 NF-pNET. For these patients adjuvant therapy after curative resection might improve prognostic outcomes. For these patients, postoperative follow-up regimens can be customized based on their risk profile. Further research is warranted to investigate if adjuvant therapy after curative resection might improve prognostic outcomes.

The recurrence-score can be calculated from the presented scoring-system based on the presence or absence of predictors for recurrence. The predictors presented in this study correspond to the ones reported in the literature and can be translated into a probability to develop recurrence[20,27-29]. With the recurrence-score, a selection of patients who have a high or low risk for recurrence after curative resection can be made. For example, patients with a recurrence-score of 0 have a 7% risk of recurrence within 5 years. Cost-effectiveness of follow-up with imaging should be evaluated for this group of patients. On the other hand, patients with a recurrence-score of 40 or higher have a 25% or more risk of recurrence, which will be a clear indication for follow-up with imaging techniques and possibly even adjuvant treatment to reduce this recurrence risk. To our knowledge, no literature exists that describes the role and effects of adjuvant therapy for patients after curative surgery of pNET. Based on the treatment of patients with advanced pNET, different treatment options are available that can serve as adjuvant treatment[30]. The presented recurrence-score sets a basis for future trials to select patients to investigate the role of adjuvant therapy based on risk stratification for recurrent pNET.

According to the new ENETS guidelines, patients with NF-pNET smaller than 2cm of size no longer have to undergo surgery to achieve optimal oncologic outcomes. Evidence for these changes in the management of this disease is based on retrospective analyses only[31-33]. Prospective cohorts are necessary to confirm this assumption. Theoretically, the same strategy could be translated to low-risk patients without unfavorable characteristics after surgical resection of pNET.

In this cohort, the recurrence-score was a better predictor of recurrence within 5 years compared to tumor grade of the WHO classification, with an almost statistically significant lower c-statistic of 0.72 (p=0.058). This effect may be explained by the comparison of a model with two extra independent predictors in comparison with one in the model of the WHO. However, in the recurrence-score grading is the strongest independent predictor with a hazard ratio of 4.01.

Most studies on risk factors for recurrence after resection of pNET include patients with distant metastases present at resection, functional and non-functional tumors combined or patients with familial syndromes[28,34-36]. By including all these patients, the results are difficult to interpret and sometimes misleading since the risk of recurrence and survival is different for these patients. In this study, a very selective group of NF-pNET was included and analyzed on risk factors for recurrent disease. These strict criteria limit the amount of patients suitable for inclusion considerably. To overcome this problem, cohorts from experienced international academic centers with close relations to the European Neuroendocrine Tumor Society (ENETS) were combined to increase the sample size and therefore reliability of the recurrence-score. However, the same limitations were experienced in finding an adequate validation cohort. External validation is needed in order to investigate whether the recurrence-score is useful in another population. Because the relevance of the recurrence-score can be of clinical value, we have decided to publish these data while external validation is ongoing.

The majority of the patients in this study showed recurrence located in the pancreatic remnants (69%) as opposed to distant metastases. In the literature, there is inconsistency on the definition of recurrence. Some studies only score recurrence when it is diagnosed as distant metastases[20,37,38], whereas new localization of tumor tissue in the remnant pancreas or regional lymph nodes should also be considered as recurrence. In addition, it is not yet known if recurrence occurs more frequently locally or as distant metastases. Therefore, it is unclear whether these results are influenced by selection bias.

There are some limitations in this study. First is the extended inclusion period. In the beginning of the study, the follow-up program was not standardized for every patient. For example, in patients with elevated chromogranin A, radiological imaging was more frequently performed. On the other hand, in patients with a grade 1 tumor without positive lymph nodes, a less strict

follow-up program was followed. This may bias the time to detect recurrence. However, until now there is no exact follow up program in the guidelines[4,10,39]. Furthermore, it has been a challenge to obtain a cohort of this size. An unrealistic large cohort is needed to meet up to the standard recommendations for Cox regression analysis. Since this study investigated a rare disease with a recurrence rate that corresponds to the literature, Cox regression analysis has been performed nevertheless and three predictors have been included in the recurrence-score[1-3].

In this cohort of 211 NF-pNET patients, microscopic positive resection margins were seen in 15% after pancreatic resection. Similar results have been reported in studies with comparable patient populations[38,40]. However, it is noteworthy that in this cohort incomplete resections were seen in 17.4% of the patients that underwent a surgical resection before 2012, whereas this was 8.9% from 2012-2015. The proportion of patients with an incomplete resection might therefore be explained by the period in which they underwent surgery. In previous years it was generally assumed that oncologic outcome was not affected by positive resection margins, due to the indolent nature of pNET. Even in the present-day, the role of resection margins remains unclear. Without this knowledge, surgeons balance the risk of postoperative complications against the prognostic value of an extensive resection.

Our future goal in the treatment of grade 1 or 2 NF-pNET is adjuvant treatment for high-risk patients with NF-pNET based on the recurrence-score. External validation with a different cohort is needed in order to investigate whether this scoring system is valid for worldwide use. Furthermore, clinical trials are needed to investigate if these high-risk patients may benefit from adjuvant treatment after curative resection. It is beyond the topic of this study to discuss the most optimal design for future research.

In conclusion

Tumor grade, positive lymph nodes and perineural invasion are independent predictors for tumor recurrence. Based on these risk factors, the recurrence-score is presented to predict recurrence after surgical resection of grade 1 and 2 NF-pNETs. External validation is required to investigate whether this scoring system can be used in the clinical practice. Patients with a recurrence-score ≥ 24 are considered to be high-risk and may benefit from adjuvant therapy.

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Recurrence of Pancreatic Neuroendocrine Tumors and Survival Predicted by Ki67

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ABSTRACT

Background. Despite evidence of different malignant potentials, postoperative follow-up assessment is similar for G1 and G2 pancreatic neuroendocrine tumors (pNET) and adjuvant treatment currently is not indicated. This study investigated the role of Ki67 with regard to recurrence and survival after curative resection of pNET.

Methods. Patients with resected non-functioning pNET diagnosed between 1992 and 2016 from three institutions were retrospectively analyzed. Patients who had G1 or G2 tumor without distant metastases or hereditary syndromes were included in the study. The patients were re-categorized into Ki67 0–5% and Ki67 6–20%. Cox regression analysis with log-rank testing for recurrence and survival was performed.

Results. The study enrolled 241 patients (86%) with Ki67 0–5% and 39 patients (14%) with Ki67 6–20%. Recurrence was seen in 34 patients (14%) with Ki67 0–5% after a median period of 34 months and in 16 patients (41%) with Ki67 6–20% after a median period of 16 months ($p<0.001$). The 5-year recurrence-free and 10-year disease-specific survival periods were respectively 90 and 91% for Ki67 0–5% and respectively 55 and 26% for Ki67 6–20% ($p<0.001$). The overall survival period after recurrence was 44.9 months, which was comparable between the two groups ($p = 0.283$). In addition to a Ki67 rate higher than 5%, tumor larger than 4 cm and lymph node metastases were independently associated with recurrence.

Conclusion. Patients at high risk for recurrence after curative resection of G1 or G2 pNET can be identified by a Ki67 rate higher than 5%. These patients should be more closely monitored postoperatively to detect recurrence early and might benefit from adjuvant treatment. A clear postoperative follow-up regimen is proposed.

INTRODUCTION

One of the concerns for patients with pancreatic neuroendocrine tumors (pNET) is the accurate prediction of clinical outcome. Tumor stage and grade have proved to be useful in estimating disease course and have been confirmed repeatedly in valuable studies[1–6]. Despite this, follow-up assessment is the same for all patients who have undergone curative resection of pNET. Neither surveillance protocols nor adjuvant treatment options based on expected recurrence rates are available, although the recurrence rate is reported to be 17% after resection of well-differentiated pNET, with considerable consequences for survival[7].

The 2010 tumor grade classification of the World Health Organization (WHO) divides pNET into three prognostic groups based on the proliferation index assessed through the expression of the nuclear antigen Ki67, with Ki67<3% classified as low-grade pNET (G1), Ki67 3–20% classified as intermediate-grade pNET (G2), and Ki67>20% classified as high-grade neuroendocrine carcinoma (NEC) (G3)[8–11].

Multiple studies have shown a good correlation between the Ki67 index and tumor size, angioinvasion, and biologic behavior of neuroendocrine tumors[12–14]. However, heterogeneity of pNET is increasingly described, and the wide range of the Ki67 distribution in the grading systems is under debate[15–17]. Therefore, WHO proposed an updated classification system for pNET this year, in which high-grade tumors with Ki67>20% are subdivided into well-differentiated G3 NET and poorly differentiated G3 NEC[18]. Although clear upper or lower limits for G3 NET and G3 NEC are not provided, differences in genetic basis and the course of disease are suggested[19–21]. Similar assumptions also are apparent for tumors with Ki67<20%. A Ki67 cutoff of 10% is used to select patients suitable for liver transplantation according to the Milan criteria, comparable with the inclusion criteria of the Clarinet study and of many oncologists generally when choosing a systemic treatment[22,23].

For non-metastasized patients, the latest European Neuroendocrine Tumor Society (ENETS) guidelines also discriminate between ''low-G2'' and ''high-G2'' pNET without providing cutoff values, suggesting different treatment responses within this patient population[1]. Furthermore, several studies describe a higher discriminating capacity when G1 and G2 pNET are divided by a Ki67 cutoff of 5% instead of 3% to predict disease progression[5,24,25].

After curative surgery of pNET, follow-up assessment is focused on early detection of recurrence. The use of the Ki67 proliferation index to guide postoperative management has not been described previously[7,26,27]. Based on the capacity of Ki67 to predict disease outcome in general, it is likely that the proliferation index of surgically treated pNET could also be predictive in estimating the risk for the development of recurrence. Therefore, we hypothesized that pNET with Ki67<20% indicates a heterogeneous group of tumors with a different postoperative disease course and aimed to investigate the role of Ki67 in predicting recurrence and survival after curative resection.

METHODS

The study enrolled patients who underwent a curative resection of a non-functioning pNET with Ki67<20% between 1992 and 2016 from the following three academic centers: The Academic Medical Center Amsterdam and The Erasmus University Rotterdam in the Netherlands (both ENETS Centers of Excellence) and the Ospedale San Raffaele in Milan, Italy. The data for 211 patients (75%) also have been presented in a previous study of this group[7].

All the patients were free of distant metastatic disease at diagnosis and not associated with a genetic predisposition for the development of pNET. Pathology reports were reviewed for the diagnosis of pNET, and patients were included in the study if pNET was histologically proven. All patients with (unresectable) locally advanced or distant metastatic disease, successfully treated or not, were excluded from the study.

The functional status of the tumors was based on the clinical presentation of symptoms associated with hormonal overproduction. The Ki67 proliferation index was retrieved from pathology reports. Tumor tissue of patients with a diagnosis before 2010 or with pathology reports containing insufficient information on the Ki67 index (n = 24) were reassessed with an emphasis on Ki67 by experienced pathologists.

For all the patients, visual assessment (‘‘eyeballing’’) was used to assess Ki67, and histologic grade was based on the WHO classification of 2010[28]. Classification according to the Royal College of Pathologists was used to assess resection margins[29]. Depending on the tumor location, pancreaticoduodenectomy, distal pancreatectomy, or total pancreatectomy was performed. Central pancreatectomy or enucleation was performed for

patients with a small pNET far enough from the pancreatic duct. Lymphadenectomy was not routinely performed with enucleation.

The patients were categorized into groups based on the Ki67 proliferation index of the tumor. Because pathologists frequently did not report an exact number to indicate Ki67, but rather provided a range for the proliferation rate, groups were initially defined by the most commonly used cutoffs provided in the pathology reports as follows: G1 (Ki67 0–2%), low G2 (Ki67 3–5%), mid-G2 (Ki67 6–10%), and high G2 (Ki67 11–20%). Because early Kaplan–Meier analysis (Figure 1a) showed similar results for patients with G1 and low G2, as well as for patients with mid-G2 and high G2, and because the cutoff of 5% also was supported by Cox proportional hazard regression (Table 1), the patients were re-categorized into two groups: Ki67 0–5 and Ki67 6–20% (Figure 1b).

Follow-up assessment after resection consisted of physical exams, laboratory tests, and radiologic imaging. The frequency of hospital visits was at least every 6 months for the first 2 years and yearly thereafter. Follow-up time was defined as the time to the last known date the patient was alive or the time until death. Recurrence was defined as local recurrence in the pancreas, a new location in lymph nodes, or the development of distant metastases. All recurrences were identified through radiologic imaging.

Statistical analyses were performed using IBM SPSS Statistics 23 (IBM Corp., Armonk, NY). On the basis of the distribution, the data were described using mean and standard deviation (SD) or using median and interquartile range (IQR). For categorical data, the number and proportion (%) were displayed. Differences between patient and tumor characteristics were investigated using a Chi-square statistic for categorical values and a Mann–Whitney U test for numeric values.

Kaplan–Meier survival analyses with log-rank testing were performed to investigate recurrence-free and disease-specific survival. To identify variables associated with recurrence within 5 years after surgery, Cox proportional hazard regression analyses were performed. Receiver-operating-characteristic (ROC) analysis with area-under-curve (AUC) determination was performed to investigate the diagnostic ability with regard to recurrence and disease-specific survival. The results were presented with the hazard ratio (HR) and the 95% confidence interval (CI). The discriminative ability of the model was examined by calculating the Harrel c-statistic with

95%.30 Moreover, we examined the discrimination of the WHO grade model and compared the c-statistics of the two models using a z test.

The net reclassification improvement (NRI) analysis was used to quantify how well our new proposed model reclassified subjects compared with the current WHO grading classification[31,32].

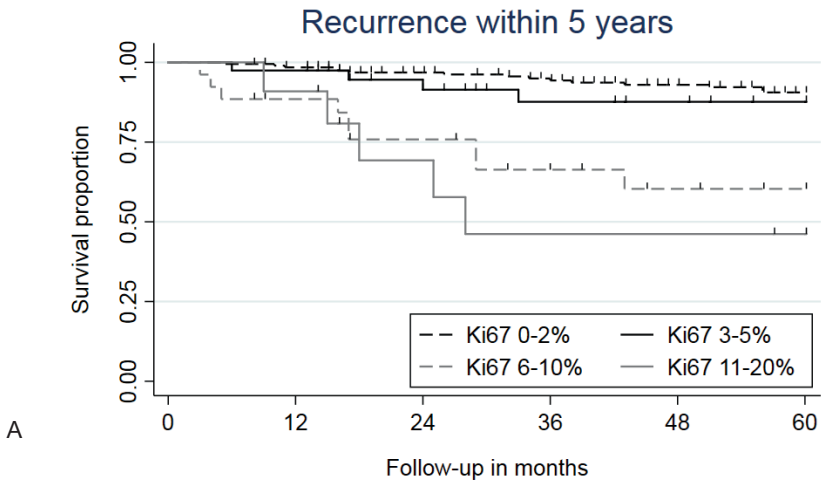
RESULTS

This study analyzed 280 patients. Patient and tumor characteristics are presented in Table 2. Left pancreatectomy was performed for 136 patients (49%), pancreaticoduodenectomy for 80 patients (29%), enucleation for 45 patients (16%), central pancreatectomy for 13 patients (5%) and total pancreatectomy for 5 patients (2%). Tumors with Ki67 0–5% were seen in 241 patients, whereas 39 patients had a pNET with Ki67 6–20%. The patients with Ki67 6–20% more frequently had lymph node metastases (53 vs 22%; p = 0.0002), perineural invasion (28 vs 11%; p = 0.0129), vascular invasion (51 vs 20%; p < 0.0001), and R1 resection (36 vs 12%; p = 0.0438) than the patients with Ki67 0–5%.

Recurrence and Survival

Recurrence was experienced by 49 patients (18%), and the majority (53%) of these recurrences were located in distant organs. The patients with recurrence more often had tumors in the pancreatic head (45 vs 36%; p = 0.0174), tumors larger than 2 cm (86 vs 54%; p < 0.0001), WHO 2010 grade 2 tumors (47 vs 25% G1; p = 0.0033), R1 resection (26 vs 11%; p=0.0126), lymph node metastases (60 vs 19%; p<0.0001), perineural invasion (30 vs 10%; p = 0.0004), and vascular invasion (49 vs 19%; p = 0.0342) than the patients without recurrence.

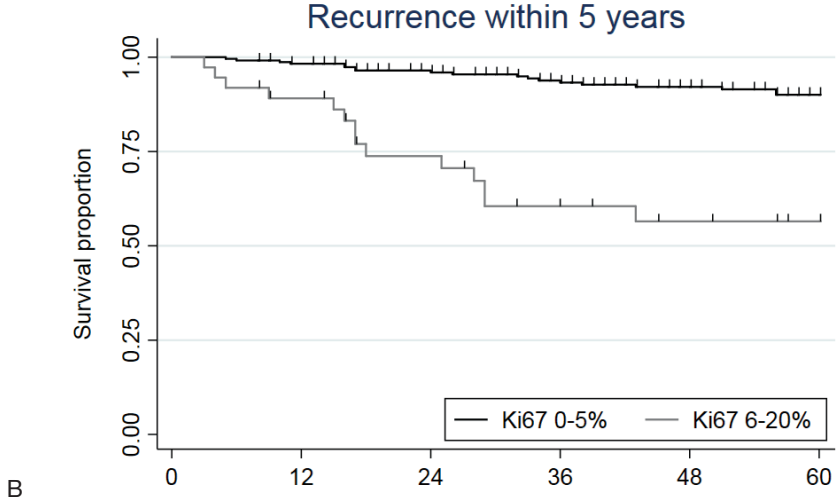
Of the 241 patients with Ki67 0–5%, 34 (14%) had a recurrence. Local recurrence in the pancreas of 12 patients was observed and recurrence in the regional lymph nodes of 2 patients. Distant metastases developed in 18 patients. Of the 39 patients with Ki67 6–20%, 16 (41%) had a recurrence, with 1 found locally in the pancreas, 2 found in regional lymph nodes, and 8



A

Number at risk

Ki67 0-2%	193	187	164	147	125	100
Ki67 3-5%	39	38	30	23	19	16
Ki67 6-10%	26	21	17	13	9	7
Ki67 11-20%	11	10	6	4	4	3



B

Number at risk

Ki67 0-5%	232	225	194	170	144	116
Ki67 6-20%	37	31	23	17	13	10

Figure 1 - Recurrence within 5 years after curative resection. **A** Patients categorized into four groups based on Ki67. **B** Patients categorized in two groups based on Ki67.

Table 1 - Predictors for recurrence within 5 years (N=280)

Risk factors	Univariate Cox regression			Multivariable Cox regression		
	HR	95%CI	P value	HR	95%CI	P value
Male sex	0.99	0.49 – 2.00	0.976			
Age						
<40	ref	ref	ref			
41-50	0.72	0.18 – 2.88	0.639			
51-60	0.86	0.23 – 3.26	0.826			
61-70	1.06	0.30 – 3.74	0.923			
>70	0.20	0.02 – 1.95	0.167			
Tumor location						
Head	Ref	Ref	Ref			
Body	0.86	0.36 – 2.05	0.738			
Tail	0.85	0.37 – 1.97	0.704			
Tumor size (mm)						
<20	ref	ref	ref			
21-40	2.45	0.84 – 7.16	0.102			
>41	6.13	2.24 – 16.75	<0.001	2.27	1.10 – 4.72	0.027
R1 resection	1.72	0.71 – 4.19	0.233			
WHO Tumor grade	0.24	0.12 – 0.47	<0.001	-	-	-
Ki67 (%)						
0-2	ref	ref	ref			
3-5	1.99	0.72 – 5.52	0.188			
6-10	5.88	2.46 – 14.05	<0.001			
11-20	7.68	2.52 – 23.42	<0.001			
Ki67 >5%	5.54	2.68 – 11.43	<0.001	5.21	1.47 – 18.4	0.010
Positive lymph nodes	4.95	2.32 – 10.58	<0.001	3.36	1.48 – 7.61	0.004
Perineural invasion	3.17	1.41 – 7.17	0.005	-	-	-
Vascular invasion	3.09	1.50 – 6.37	0.002	-	-	-

found as distant metastases. Kaplan–Meier analysis showed significantly less recurrence within 5 years after surgery for the patients with Ki67 0–5% than for the patients with Ki67 6–20% ($p < 0.001$; Figure 1b). The 5-year recurrence-free survival rate was 90% for the patients with Ki67 0–5 and 55% for the patients with Ki67 6–20%. Overall, the median time to recurrence (TTR) was 31.7 months (IQR 10.5–47 months): 34 months (IQR 16–59 months) for the patients with Ki67 0–5% and 16 months (IQR 4.25–23.25 months) for the patients with Ki67 6–20% ($p = 0.005$).

The median survival time was 63 months for the patients with Ki67 0–5% tumors and 45 months for the patients with Ki67 6–20% tumors ($p = 0.017$). The 10-year disease-specific survival was 91% for the patients with Ki67 0–5% tumors and 26% for the patients with Ki67 6–20% tumors ($p < 0.001$, Figure 2). The median survival time after recurrence was 44.9 months (IQR 16–68.3 months), which was statistically comparable between the two groups ($p = 0.283$).

Table 2 - Tumor and patient characteristics (N=280)

Male : Female	136 : 144
Age	Median 59 years (IQR 48.8 – 66)
Follow-up	Median 62 months (IQR 36– 84)
Tumor location	
Head	105 (38%)
Body	81 (29%)
Tail	94 (34%)
Ki67 (%)	Mean 2.8 (SD = 3.7)
0-2	199 (71%)
3-5	42 (15%)
6-10	28 (10%)
11-20	11 (4%)
Tumor size	Median 25mm (IQR 15 – 40)
<20mm	113 (40%)
21-40mm	100 (36%)
>40mm	67 (24%)
R0 : R1	240 : 39
Lymph node metastases	65 (23%)
	Missing: 12%
Perineural invasion	34 (13%)
	Missing: 9%
Vascular invasion	65 (25%)
	Missing: 5%
Recurrence	49 (18%)
Local	12 (25%)
Regional	4 (8%)
Distant	26 (53%)
Unknown location	7 (14%)
Size	Median 40mm (IQR 25-59)
Ki67	Mean 4.8% (SD = 5.4)
G2*	23/49 (47%)
R1 resection	13/49 (27%)
Lymph node metastases	27/49 (55%)
Perineural invasion	13/49 (27%)
Vascular invasion	23/49 (47%)
Time to recurrence	Median 31.7 months (IQR 10.5 – 47)
Survival after recurrence	Median 44.9 months (IQR 16 – 68.3)
>30-day mortality	25 (9%)
Disease related deaths	14 (5%)

* According to the 2010 WHO classification (Klimstra et al. 2010)

The ROC analysis for Ki67 showed an AUC of 0.683 for the prediction of recurrence within 5 years. The highest sensitivity and specificity were reached at a Ki67 cutoff value of 5%, with a sensitivity of 37% and a specificity of 87%. An AUC of 0.737 was found for 10-year disease-specific survival.

The discriminative ability of this Ki67 model showed a Harrel c-statistic of 0.672 (95% CI 0.591–0.753). The discrimination of the WHO grading with

regard to predicting recurrence was comparable, with a c-statistic of 0.681 (95% CI 0.602–0.760). This was not statistically significant (p = 0.781).

Net Reclassification Improvement Analysis

Table 3 presents the results of the NRI analysis. The additive NRI of the proposed Ki67 cutoff value was 0.866, indicating that the new cutoff value had good additive value for the WHO grading classification. The absolute NRI was 10%, indicating that 10% of patients were correctly reclassified in our proposed model based on their risk for the development of recurrence within 5 years. This effect can best be attributed to the reclassification of patients with a low risk for the development of recurrence.

Cox Proportional Hazard Analysis

The factors related to recurrence within 5 years after surgery from the univariable Cox regression analyses were tumor size greater than 4 cm, WHO tumor grade, Ki67>5%, lymph node metastases, and perineural and vascular invasion. The independent predictors for recurrence were tumor size greater than 4 cm (HR 2.5; 95% CI 1.14–5.40), Ki67>5% (HR 3.0; 95% CI 1.34–6.81), and lymph node metastases (HR 3.3; 95% CI 1.40–7.70) (Table 1). Tumors larger than 4 cm were seen in 67 patients, 21 (31%) of whom experienced a recurrence. The absolute NRI of Ki67 compared with size was 6.5%. Lymph node metastases were present in 65 patients, 27 of whom experienced recurrence (42%). The absolute NRI of Ki67 compared with lymph node metastases was 5%.

Table 3 – Reclassification among patients with and without recurrence

WHO grading model	New proposed Ki67 cut-off		
	Ki67 0-5%	Ki67 6-20%	*Total*
Patients with recurrence (N=49, 17,5%)			
Grade 1	26	0	26
Grade 2	7	16	23
Total	33	16	49
Patients without recurrence (N=231, 82,5%)			
Grade 1	173	0	173
Grade 2	35	23	58
Total	208	23	231

Net reclassification of patients with recurrence: 0-7 = -7. Net reclassification of patients without recurrence: 35-0=35. Additive NRI: ((-7/49) x 100) + ((35/231) x 100) = 0.866. Absolute NRI: ((-7 + 35)/280) x 100) = 10%.

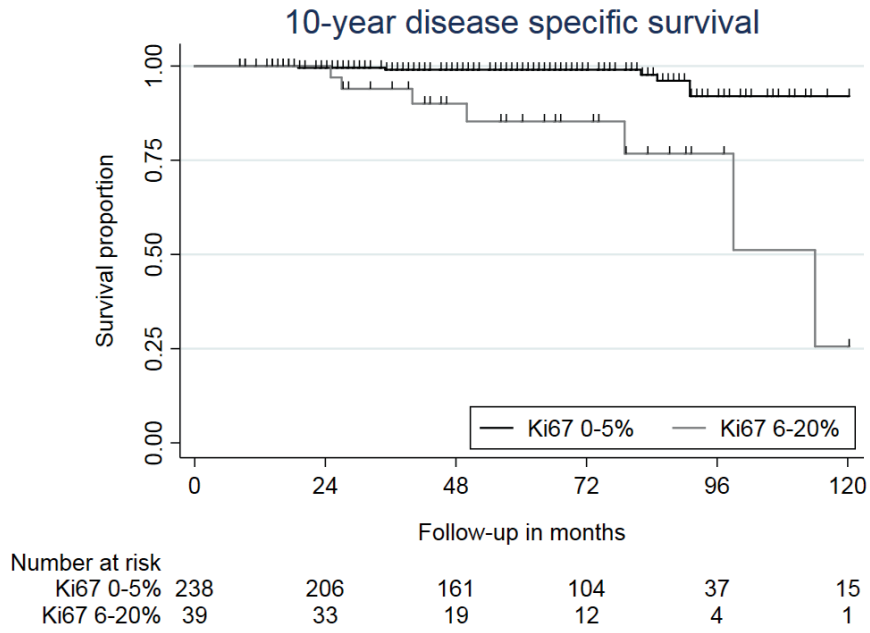


Figure 2 - The 10-year disease-specific survival times for patients with Ki67 0–5 and Ki67 6–20%.

The 10-year disease-specific survival was associated with Ki67 >5% and perineural invasion in the univariate analysis, but only Ki67 >5% was independently associated with 10-year disease specific survival in the multivariable Cox regression analysis (HR 6.5; 95% CI 1.93–21.79; p = 0.003).

DISCUSSION

We propose a novel categorization of low- and intermediate-grade pNET based on the Ki67 index to predict recurrence after curative resection. Tumors with Ki67 6–20% have a threefold higher risk for the development of recurrence within 5 years and show significantly shorter survival than tumors with Ki67 ≤5%. With this cutoff value, a reliable method for stratifying patients into groups of high and low risk for recurrence after surgery is presented.

In a previous study, we presented a scoring system to identify high-risk patients through three predictors for recurrence[7]. The current study contributes to strengthening of this scoring system. When the criteria for

grade 2 tumors are modified for tumors with Ki67>5%, it will be possible to identify high-risk patients more accurately. The recurrence score showed a sensitivity of 91% and a specificity of 62% and is expected to increase with this revision. Furthermore, patients with Ki67 3–5% (15% of our cohort) will be downgraded by this modification, limiting unnecessary treatment or monitoring. External validation of the scoring system currently is being performed and will include this new Ki67 distribution as well.

Postoperative follow-up assessment of patients with pNET typically consists of hospital visits combined with laboratory tests and/or radiologic or nuclear imaging. A clear guideline for postoperative management such as the frequency of hospital visits, the method for diagnostic testing, or the duration of follow-up assessment has not been recommended to date. Combining the presented results with preexistent literature, we propose a postoperative surveillance protocol based on the risk of recurrence for patients who have non-metastasized pNET with K67<20% (Table 4). This scheme comprises yearly consultations with imaging for all patients and additional half-yearly consultations with clinical assessments and laboratory tests (chromogranin A) for high risk-patients. Based on clinical findings and laboratory results, additional imaging may be obtained.

Ideally, imaging is alternated between radiologic and somatostatin receptor imaging to achieve the highest accuracy. Findings have shown that gallium-based nuclear imaging has the highest sensitivity and specificity for the detection of pNET and is therefore the preferred nuclear imaging method[33–36]. Radiologic imaging with either contrast-enhanced computed tomography

Table 4 – Surveillance protocol after curative resection of pNET with Ki76 <20%

	Yearly follow-up	Additional follow-up	Frequency	Duration
Low-risk patients*	Clinical assessment Imaging**	--	Yearly	At least 5 years
High-risk patients*	Clinical assessment Imaging**	Clinical assessment Laboratory tests***	Every 6 months	10 years

* Risk stratification either through the newly proposed Ki67 distribution, or more accurately through to the modified version of the recurrence score by Genç et al. (Genc et al. 2017a)
** Alternating between anatomical and nuclear modalities
*** Chromogranine A

(CT) or (diffusion weighted) magnetic resonance imaging (MRI) is advised[37–39]. Based on the median time to recurrence, a follow-up period of 10 years is encouraged because late recurrences have been described[7]. The interval between assessments can be increased if the disease is stable after 5 years, especially for low-risk patients.

Due to the retrospective nature of this study, it was not possible to assess exact Ki67 rates for each patient. It is questionable, however, whether exact rates for each tumor will be more meaningful in determining postoperative prognosis. At this writing, exact Ki67 values have limited clinical relevance because the choice for treatment is often determined by tumor grade or smaller ranges of Ki67. Furthermore, the proliferation index of a tumor may have different prognostic significance in different stages of disease or treatment. This is already evident, for example, in determination of systemic treatment options for patients with disseminated disease. A Ki67 cutoff of 10% often is used by oncologists, confirming heterogeneity in malignant potential within one WHO grading group. The treatment of localized nonfunctioning tumors smaller than 2 cm might also be influenced by different Ki67 cutoffs, in which the choice for surgical versus conservative treatment may change for G2 tumors with higher or lower Ki67 values. In addition, assessing the exact amount of Ki67-positive cells, either manually on printed images or determined through computer software, also can create a false sense of accuracy because each method for counting positive cells is associated with an error margin. Likewise, differences in practice can lead to intra- and inter-observer variability. Therefore, it might be both more reliable and more feasible to agree on smaller ranges of Ki67 (e.g., <5, 5–10, 15–20%) rather than exact values, with stratification of patients into their risk for the development of recurrence.

The current results must be seen in light of their limitations. Data were evaluated retrospectively, and pathology reports were not standardized at the time of treatment. Furthermore, the treatment of recurrence was not taken into account when survival was analyzed. Because survival after recurrence was comparable between Ki67 0–5 and Ki67 6–20% tumors, we expect the treatment of these patients to be similar. Nevertheless, results might be biased, and survival after recurrence might show treatment results rather than the effect of recurrence itself. In addition, these results could be interpreted with the assumption that early detection and treatment of recurrence will result in survival benefit. However, no studies support this theory, and prospective clinical trials are necessary to confirm these hypotheses.

At this writing, the clinical relevance of this study may be limited except for de-escalation of follow-up regimens for Ki67 0–5% patients and intensification of follow-up regimens for patients with Ki67 6–20%. Adjuvant therapy to prevent recurrence in the future could be a possibility. However, the vicious circle of nonexistent data, together with the difficulty of obtaining prospective studies for this purpose, forms an obstacle to the development of such treatments. To overcome these issues, a consensus study has been initiated among European pNET experts to discuss possibilities for investigating the role of adjuvant treatment for high-risk patients. The results of this consensus will be published shortly. The current study might bring us one step closer to achieving this necessary research by clarifying the selection of patients who should be eligible for adjuvant treatment.

In conclusion, this study is the largest study to describe the use of the Ki67 proliferation index to estimate postoperative recurrence. These results contribute to the assumption of tumor heterogeneity among patients with a Ki67<20%. Future studies should focus on determining Ki67 rates, preferably in prospective trials, to propose a further alteration of the grading system for well-differentiated pNET.

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Measurement of circulating transcript levels (NETest) to detect disease recurrence and improve follow- up after curative surgical resection of well- differentiated pancreatic neuroendocrine tumors

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ABSTRACT

Background. Recurrence of pancreatic neuroendocrine tumors (pNET) after surgery is common. Strategies to detect recurrence have limitations. We investigated the role of clinical criteria and the multigene polymerase chain reaction–based NETest during post-operative follow-up of pNET.

Methods. We studied 3 groups of resections: R0 with no recurrence (n = 11), R0 with recurrence (n = 12), and R1 with no recurrence (n = 12). NETest levels (>40%) were compared with chromogranin A (CgA) and clinicopathological criteria (CC; grade, lymph node metastases, size). Nonparametric, receiver operating characteristics, logistic regression, and predictive feature importance analyses were performed.

Results. NETest was higher in R0 with recurrence (56 ± 8%) compared with R1 with no recurrence (39 ± 6%) and R0 with no recurrence (28 ± 6%, $P < .005$). NETest positively correlated with recurrence (area under the curve: 0.82), CgA was not (area under the curve: 0.51 ± 0.09). Multiple regression analysis defined factor impact as highest for NETest ($P < .005$) versus CC ($P < .03$) and CgA ($P = .23$). NETest gave false positive or negative recurrence in 18% using a 40% cutoff. Logistic regression modeling of CC was 83% accurate; it was 91% when the NETest was included. Combining CC and NETest was approximately 2× more effective than individual CC alone (increase in R2 value from 43% to 80%).

Conclusion. A multigene blood test facilitates effective identification of pNET recurrence, prediction of disease relapse, and outperforms CgA.

INTRODUCTION

Although the majority of patients with pancreatic neuroendocrine tumors (pNET) are diagnosed at an advanced stage, improvements in imaging modalities, awareness of the disease, and pathological recognition have contributed to the improvement in detection of localized disease[1-3]. For patients with nonfunctioning pNET ≥20mm in size without distant metastasis, complete surgical resection is recommended as the primary curative strategy[4,5]. Thereafter, effective follow-up programs are designed to detect recurrence at an early stage, given that treatment of limited disease has the most favorable outcome[6-8]. However, data on post-curative surgical recurrence remains limited, making it challenging to determine the best follow-up strategy. In general, recurrence is thought to occur sporadically, yet some studies report rates up to 48%.9 Furthermore, recurrence is known to be an independent predictor for a poor 10-year disease-specific survival[10].

A key unmet need in improving outcome is the early detection of recurrent disease and the timely initiation of treatment after pNET resection. In many cases, early detection of recurrence offers more favorable treatment options, sometimes with curative intent, such as resection of the remnant pancreas or solitary liver metastases[11,12]. Liver-directed, locally ablative procedures are recommended for patients with limited, nonresectable tumor burden[13-15]. When recurrence is discovered with an extensive disseminated disease, systemic treatment is often the only option[16]. Despite the variety of treatment options, there is uncertainty with regard to the optimal treatment regimen. Newly introduced molecular-based markers, along with clinical trials comparing the efficacy of treatment modalities, offer a chance to move the treatment of neuroendocrine tumor disease toward personalized patient care. Given the multiple treatment options available for NET disease, the early detection of recurrence and the judicious introduction of therapy should be considered to optimize pNET outcome.

Current guidelines to evaluate tumor recurrence recommend radiological examinations and biomarker evaluation during follow-up[4,5]. Chromogranin A (CgA) used to be considered as the most useful biomarker for detection of metastases after curative resection of pNET[17]. However, its low sensitivity of 67% and specificity of 68%, as well as controversy regarding technical criteria of the assay, have led to a significant diminution in enthusiasm for its clinical utility[17,18]. Overall, the general accuracy of CgA is moderate, given its poor metrics as a biomarker and the high false positives noted[19-21]. Although

some reports describe an increased diagnostic accuracy with a high tumor load, this is of limited value in an early detection and treatment strategy[17,22]. The limited clinical utility of CgA and the overall lack of efficacy of monoanalyte peptide or amine secretory biomarkers has led to considerable interest in developing novel and effective tools for the surveillance of neoplasia. In this respect, considerable attention has focused on the evaluation of the molecular characteristics of cancer and the development of sensitive techniques to define the molecular biology of the tumor as opposed to measuring its secretory products. The term liquid biopsy has been coined to describe the technique of detecting a tumor in blood and has been effectively used in other cancers including breast and colon[23,24]. Recently, a liquid biopsy strategy for neuroendocrine tumors has been described[25]. It comprises a multianalyte polymerase chain reaction–based blood test specific for neuroendocrine tumors (the NETest) with a sensitivity and specificity of >93% for diagnosis. This multianalyte biomarker tool has been successfully used to demonstrate residual disease and the early detection of recurrent disease after surgical resections in small bowel and lung NETs[26,27].

This prospective surgical cohort study aimed to determine the prognostic accuracy of neuroendocrine transcript expression in blood, compared with CgA and other known clinical criteria associated with pNET recurrence, to determine its usefulness as a biomarker for assessment of surgical efficacy and detection of recurrence after curative resection.

METHODS

Patient selection

All patients that were operated on for pNET in the Academic Medical Center Amsterdam between 2006 and 2015 were screened for inclusion (Figure 1). The pathology reports of all pancreatic resections in the selected period were reviewed for the diagnosis of pNET. Only patients with histologically confirmed diagnosis of pNET were eligible for enrollment to the study. Included were 35 patients of 18 years or older and surgically treated for G1 or G2 localized pNET (per pNET Ki-67 cutoff classification[28]) without distant metastases or hereditary syndromes at initial diagnosis. The study group demographics and clinicopathological characteristics are included in Table 1.

Patients were divided into 3 groups based on the pathological assessment of the pancreatic resection margins and the clinical disease status at the time of the blood draw. Resection margins were classified according to the Royal College of Pathologists. Completely excised tumors were classified as R0, whereas tumors with microscopic margin involvement <1 mm were classified as R1. Assessment of completeness of surgical resection and disease staging at the time of initial diagnosis or during follow-up was based upon anatomical imaging (computed tomography [CT]/magnetic resonance imaging [MRI]). Tumor recurrence was defined as local recurrence in the remnant pancreas, new localization in lymph nodes (LNs), or the development of distant metastases after initially being rendered free of disease, and was diagnosed in accordance with RECIST 1.0 criteria.

The 3 groups comprised of: (1) R0 resection and no signs of recurrence during follow-up (R0NR); (2) R0 resection and evidence of recurrent disease on imaging during follow-up (R0R); (3) R1 resection and therefore residual tumor in situ, without evidence of recurrence on imaging (R1NR).

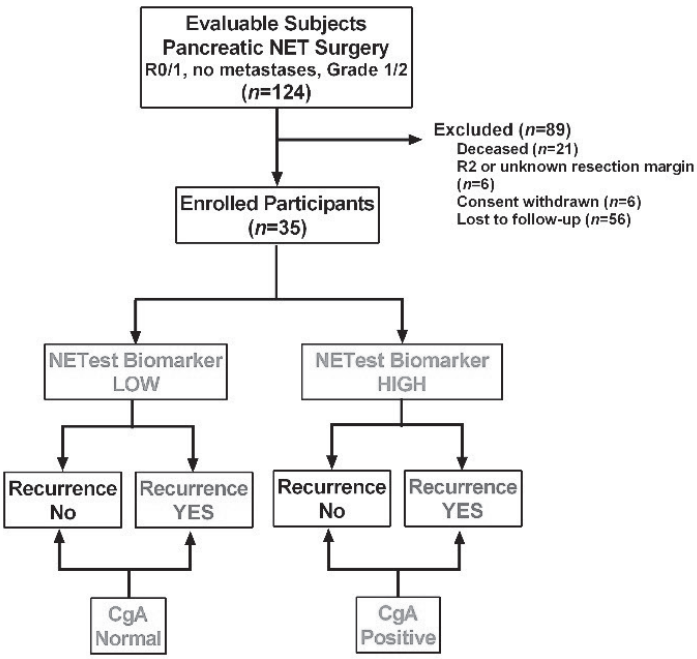

Figure 1 - STARD diagram outlining the study. NETest Low (scores <40%) and NETest High (scores >40%). CgA normal: values <108 ng/mL; CgA positive: values >108 ng/mL. CgA, chromogranin A; STARD: Standards for Reporting Diagnostic Accuracy

The medical records, pathology reports, radiological imaging reports, and operation reports were reviewed for the demographics and clinicopathological data, including patient's age at the time of surgery, sex, tumor functionality, tumor location within the pancreas, type of surgery, tumor size (based on post-operative pathology), grade, LN involvement, and perineural and vascular invasion. Radiological imaging consisted of abdominal CT or MRI scans and in some patients, endoscopic ultrasonography of the pancreas and/or functional imaging (Octreoscan or 68Ga-DOTATATE PET/CT) were performed. Use of proton pump inhibitors (PPIs) was determined. A control group of healthy volunteers was included. The local Medical Ethics Committee approved the study (protocol number: NL50925.018.15).

Sample collection

Two blood samples per patient were collected according to the local protocol of the laboratory in the outpatient clinic of the Academic Medical Center Amsterdam. Following informed consent, each patient donated two 5mL whole blood samples collected into ethylenediaminetetraacetic acid tubes. The blood draw was combined with other regular blood tests performed during the follow-up. After sampling, 1 blood specimen was immediately stored at –20°C, whereas the second ethylenediaminetetraacetic acid tube was spun at 800 rpm (10 minutes) to separate plasma dedicated for CgA measurement by enzyme-linked immunosorbent assay, as previously described.29,30 Thereafter, both samples were stored at –80°C within 2 hours from blood collection.

NETest blood measurement

Details of PCR methodology, mathematical analysis, and validation have been previously published in detail[31-34]. In brief, this comprises a 2-step protocol (RNA isolation, complementary DNA production, and PCR) from ethylenediaminetetraacetic acid–collected whole blood[29,31,32]. Target transcript levels are normalized and quantified versus a population control[31]. Thereafter, multianalyte algorithm analyses are undertaken. Final gene expression results are expressed as an activity index score from 0% to 100% based on the integration of the majority vote and summated expression of 5 gene clusters that include the proliferome, epigenome, growth factor signalome, and genes involved in pluripotency[33].

CgA enzyme-linked immunsorbent assay

CgA was measured using the NEOLISATM CgA kit (EuroDiagnostica, Malmo, Sweden)[35]. CgA enzyme-linked immunosorbent assay normal values were ≤108 ng/mL[34].

Data analysis

The primary outcome was obtaining the NETest score of the patients in the 3 various pNET groups as specified above and the control group. A NETest score between 0% and 100% was obtained and a value of >20% was considered as a positive test[25]. Scores ranging between 0% and 20% were considered as negative. Previous studies have identified that a cutoff of 40% differentiates low activity (stable) disease from active (progressive) disease[34,36]. Therefore, scores ranging between 41% and 100% were evaluated as predictive of disease recurrence. Scores for each patient and each group were collated and assessed. In addition, CgA levels were compared with the NETest score. Data are presented as mean ± standard error of mean (median: [interquartile ranges]).

Statistical analysis

Statistical analysis was performed using SPSS Statistics for Windows version 23.0 (IBM Corp., Armink, NY), Prism 6.0 for Windows (GraphPad Software, La Jolla, CA; www.graphpad.com), and MedCalc Statistical Software version 12.7.7 (MedCalc Software bvba, Ostend, Belgium; www.medcalc.org; 2013). Sensitivity comparisons using Fisher's exact test, nonparametric tests, and receiver operating characteristics analysis were made between the NETest and CgA and/or other selected tumor clinicopathological characteristics known as predictive for recurrence. The accuracy of each of the variables separately was compared with the NETest, as well as in various combinations, using receiver operating characteristics curve analyses and the sensitivity, specificity and area under the curve (AUC) were calculated. Area under the curves were compared and the Z-statistic (values >1.96 are significant) derived and the Youden J index (performance of a diagnostic) was calculated. Multiple regression and logistic regression analyses were undertaken to identify which parameters were associated with recurrence. The odds ratio (OR), χ^2 value, and Nagelkerke R2 coefficient (coefficient of determination) were derived to assess the strength of the association or "relatedness" of each factor or combination of factors to recurrence[37]. Predictive feature importance analysis (FIA) was undertaken to define the

"importance value" for each factor (biomarker or clinical criterion) alone or in combination. Importance values were derived using a random forest approach that evaluates the output from decision tree algorithms used to define the relationship of a variable for example, a biomarker, to an output for example, recurrence. A random forest model is generated with 10-fold cross-validation and examined to determine mean decreases in the Gini coefficient.[38] The Gini coefficient provides a measure of how each variable contributes to the structure of a random forest plot. Variables that result in nodes with higher purity (ie, more accurately model disease recurrence) have a higher decrease in Gini coefficient[39]. As such, the greater the decrease in the Gini coefficient, the greater the "Importance" value and better the relation to predicting recurrence. Biomarkers (CgA, NETest) and 3 clinical variables criteria (tumor grade, size, and LN involvement) were each evaluated to determine which factor (or combination of factors) had the highest "Importance" value score.

RESULTS

Demographics and follow-up

Patients and tumor characteristics are presented in Table 1. In total, 35 patients were included: 11 patients with R0NR, 12 patients with R0R, and 12 patients with R1NR. Duration of follow-up from surgery to the last hospital visit was significantly shorter for patients in the R0NR group compared with R0R and R1NR ($p<0.002$; Figure 2). Three patients died during follow-up, of which 1 death was pNET related. The healthy control group consisted of 6 male and 5 female volunteers, with a median age of 42 years (interquartile range, 32.5-53.5).

Nineteen (54%) of the 35 patients were using PPIs at the time of blood collection; 7/11 (64%) R0NR, 7/12 (58%) R0R, and 5/12 (42%) R1NR. Ten of the 12 (83%) with recurrence received therapy to treat the disease relapse. This included somatostatin analogs (n=5), chemotherapy (n=2), embolization (n=1), metastasectomy (n=1) and peptide receptor radionuclide therapy (PRRT) (n=1). Collection of blood samples was either after or during these treatments. Two patients did not receive therapy for their recurrence. All patients without recurrence did not receive any systemic therapy between resection and collection of the blood samples.

Table 1 - Patient and tumour characteristics

	R0 with no Recurrence (R0NR) (n=11)	*R0 with recurrence (R0R) (n=12)*	*R1 resection with no recurrence (R1NR) (n=12)*
Male: Female	4:7	6:6	6:6
Age, median (IQR)	63 years (59-65)	62,5 years (61-64)	57 years (50-59.5)
F:NF	0:11	1:11	5:7
Tumour location			
- Head	6	4	7
- Body	0	3	4
- Tail	5	5	1
Surgical resection (*n*)			
- Pancreaticoduodenectomy	6	3	1
- Central pancreatectomy	0	2	3
- Left pancreatectomy	5	6	2
- Total pancreatectomy	0	0	0
- Enucleation	0	1	6
Grade 1	73% (8/11)	42% (5/12)	100% (12/12)
Grade 2	27% (3/11)	58% (7/12)	0% (0/12)
Tumour size, median (IQR)	20mm (17-53)	45mm (27-63.8)	15mm (7.8-27.5)
Lymph node metastases	27% (3/11)	75% (9/12)	8% (1/12)
Perineural invasion	50% (3/6)	67% (4/6)	0% (0/3)
Vascular invasion	50% (4/8)	91% (10/11)	0% (0/5)
Follow-up, median (IQR)	31 mo (24-47)	105 mo (54.8-125.3)	92.5 mo (61.8-115.8)
NETest score (%), median (SD)	27 (6.4)	50 (26)	27 (22)
CgA level (ng/ml), median (SD)	67 (439)	62 (268)	81 (315)
Time from surgery to blood collection, median (IQR)	18 mo (1-33)	104 mo (44.3-125.3)	91.5 mo (60-105.8)
Recurrence	--		--
- Local		25% (3/12)	
- Regional		17%% (2/12)	
- Distant		58% (7/12)	
Time to recurrence, median (IQR)	--	37.5 mo (26-58.3)	--
Follow-up after recurrence, median (IQR)	--	50 mo (20.8-94.8)	--
Time from recurrence to blood collection, median (IQR)	--	49.5 mo (4.5-93.8)	--
Time from blood collection to last follow-up, median (IQR)	18 mo (6-22)	5.5 mo (0-15)	2.5 mo (0.3-9)

CgA, chromogranin A; IQR, interquartile range; SD, standard deviation; mo, months.

Biomarker evaluation in controls and pNET cohorts

The NETest scores were significantly elevated (56 ± 8%: [50%:28.8-85.25]) in the R0R cohort compared with R0NR (28 ± 2%: [27%:20-40], p=0.004) (Figure 3A). Levels were not significantly different in R0R compared with R1NR (39 ± 6%: [27%:27-40], p=0.08). All pNET cohorts, irrespective of recurrence, had higher levels ($P < 0.05$) than controls (19 ± 5%: [20%:13-20]). CgA levels were not significantly different ($P = .062$-0.94) between any of the 3 pNET cohorts (mean: 205-244 ng/mL; median 62-18 ng/mL; Figure 3B). Levels were higher in the nonrecurrence (NR) cohorts ($p<0.05$) than in

controls (44 ± 5 [40:32-56]); however, CgA was not significantly different (p=0.14) between R0R and controls.

Correlation between biomarkers and recurrence

The NETest cutoff of 40%36 determined that the area under receiver operating characteristic curve (AUROC) for differentiating recurrence from NR was 0.82 ± 0.08, Z-statistic: 4.19; p<0.0001 (Figure 4). The Youden index (J) was 0.64. Using the upper limit of normal (108 ng/mL) as the cutoff for CgA, the AUROC was 0.51 ± 0.09. The Z-statistic was 0.14 and the Youden index (J) of 0.02 are not significant (p=0.88). A comparison of the NETest and CgA identified the AUC was significantly better for the former. The difference between areas was 0.31 ± 0.14; Z-statistic: 2.01; p=0.044.

Evaluation of PPIs, tumor burden at recurrence, and biomarker

NETest levels were not different in those using PPIs (44 ± 6%: [33%:27-73]) compared with nonusers (38 ± 6%: [27%:27-41]). CgA levels were higher in those using PPIs (278 ± 86 ng/mL: [142:55-451]) compared with nonusers (153 ± 69 ng/mL: [55:37-93]). This did not reach statistical significance (p=0.059).

In the R0NR group, CgA was elevated in 3 of 7 (43%) on PPI and in 1 of 4 (25%) not using a PPI. CgA was therefore elevated by PPI in 43% of surgically "cured" patients when it was used.

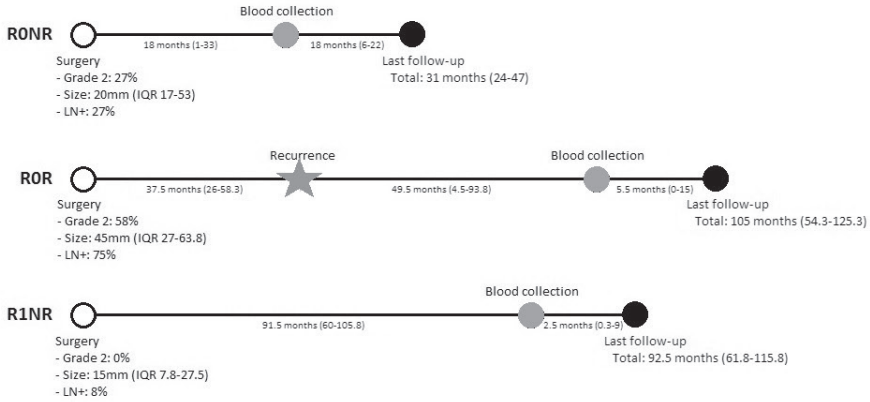

Figure 2 - Diagram defining the median times of blood collection, last follow-up, and recurrence. LN, lymph node; R0NR, R0 with no recurrence; R0R, R0 with recurrence; R1NR, R1 resection with no recurrence

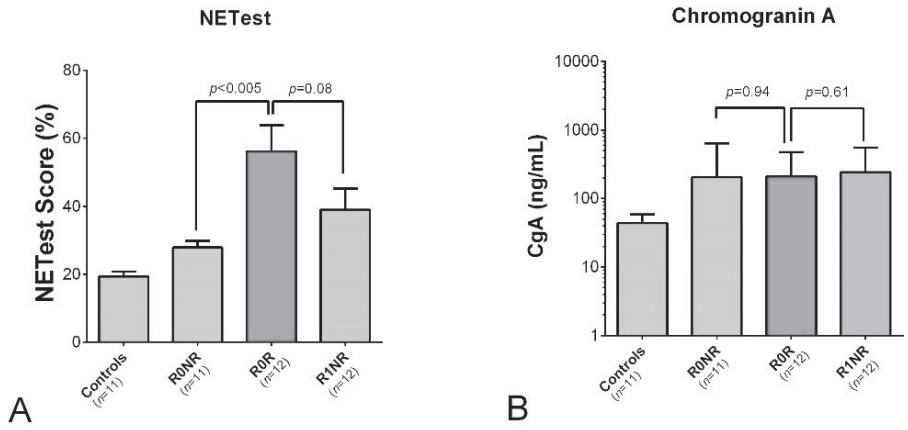

Figure 3 - NETest expression and CgA levels in controls and cohorts. **A** NETest scores were significantly elevated in the R0 resection cohort with recurrence (R0R) compared with the resection cohort that did not recur (R0NR). Levels were similar between R0NR and R1 resection with no recurrence (R1NR: p=0.08). **B** CgA levels were not significantly different between any of the pNET cohorts irrespective of the presence of recurrence or no recurrence. Mean and standard error of mean are indicated. CgA, chromogranin A; pNET, pancreatic neuroendocrine tumors.

In the R1NR group, CgA was elevated in 3 of 5 (60%) on PPI and in 2 of 7 (29%, no PPI). This indicates that approximately 17% of all R1NR have a "true" elevated CgA level, that is, CgA related to neuroendocrine tumor disease. All patients with residual disease had single LN-positive disease. This indicates that an elevated CgA is associated with <20% of lymph node–positive disease. The NETest was low (but positive >20%) in all R1NR patients consistent with accurate disease detection.

In the ROR group, CgA was elevated in 5 of the 7 (71%) on PPI. All who were not on PPI (5/5) had normal CgA levels. An evaluation of tumor burden identified that 2 patients exhibited a single LN recurrence. One had normal CgA, and the other had elevated CgA—both were using PPIs. One had an elevated NETest and the other had a low NETest. In 1 patient in which a local recurrence was identified, CgA was normal (no PPI); the NETest was elevated. In 9 patients who developed a distant metastatic disease, 4 had elevated CgA levels. All 4 were on PPIs. The NETest was elevated in 6 of the 9. Low NETest levels were ascribed to effective SSA use (n=2) and streptozotocin/5-fluorouracil (FU) treatments (n=1) at the time of the blood draw. CgA elevation in ROR was therefore related to PPI use.

Evaluation of biomarkers and clinical factors as predictors of recurrence

Multiple regression analysis identified that the following biomarkers and the tumor clinicopathological characteristics were associated with recurrence: NETest score >40% ($p<0.001$), tumor grade ($p<0.03$), positive LNs ($p<0.03$), and tumor size >20mm ($p<0.02$; Table 2). The R2 coefficient was 0.65, the F-ratio was 13.9, and $p<0.0001$. CgA levels alone had no association with recurrence ($p=0.23$).

Examination of the individual factors identified the NETest was overall 83% accurate for disease status (Figure 5A). For clinical criteria, this ranged between 69% and 80%. Most factors were strongly associated with no recurrence (83%-91%) except for the tumor size, which was poorly associated (57%; χ^2: $p<0.05$ vs. LN positivity and NETest). Normal CgA levels were identified in 61% of patients with no recurrence and in 59% of those with recurrence ($p=1.0$). CgA levels were therefore unhelpful in the detection of recurrence ($p<0.005$ vs. all other factors: Fisher's exact test; 2-tailed).

The OR for each of the individual factors and recurrence in the regression models was CgA: 1.11 (p=NS), grade: 9.3 ($p=0.005$), LN: 14.3 ($p= 0.002$), and size: 14.3 ($p=0.003$). The OR for the NETest was 21 ($p<0.0001$).

Individual χ^2 values were CgA: 0.02, grade: 7.8, LN: 11.4, and size: 8.7. The χ^2 value for NETest was 13.

Table 2 - Multiple Regression analysis of NETest, CgA and clinicopathological parameters associated with recurrence

Independent factors	*Coefficient*	*Std. Error*	*r_*	*t*	*P*	*VIF*
(Constant)	-0.1639					
NETest score	0.4550	0.1208	0.5940	3.765	**0.0009**	1.168
Tumour size >20mm	0.3903	0.1459	0.4647	2.676	**0.0127**	2.001
Positive lymph nodes	0.3106	0.1292	0.4263	2.403	**0.0237**	1.528
Grade (G1 vs. G2)	0.3119	0.1329	0.4181	2.347	**0.0268**	1.413
Site of tumour (head vs. corpus/tail)	-0.1392	0.1099	-0.2410	-1.266	0.2166	1.182
CgA	0.1318	0.1064	0.2361	1.239	0.2264	1.064
Tumour size >40mm	-0.1934	0.1536	-0.2397	-1.259	0.2192	2.219
Non-functional status	0.1769	0.1537	0.2203	1.151	0.2601	1.314
LNR	-0.7323	0.4464	-0.3795	-1.641	0.1204	2.569

LNR = Lymph Node Ratio = number of positive lymph nodes/all dissected lymph nodes

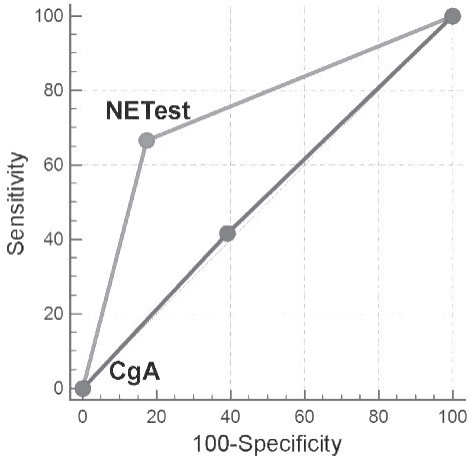

Figure 4 - Receiver operator curve analysis for the identification of recurrence. NETest: The AUROC for differentiating recurrence from no recurrence using NETest was 0.82 ± 0.08; 95%CI, 0.65-0.93; p<0.0001. CgA: The AUROC for differentiating recurrence using CgA (normal versus elevated) was 0.51 ± 0.09; 95%CI, 0.34-0.69; p=0.88. NETest (red line); chromogranin A (CgA; green line). An AUC of 0.5 is indicated by the thin diagonal line behind the CgA AUC line (green). AUC, area under the curve; AUROC, area under the receiver operator characteristic

Nagelkerke R2 (relatedness—coefficient of determination) were CgA: 0.0008, grade: 0.27, size: 0.31, and LN: 0.39 (Figure 5B). For the NETest, R2 was 0.43.

Combining all 3 clinical criteria resulted in a χ^2 value of 21.7 with a relatedness value of 0.64. Different combinations of the NETest and individual clinical criteria exhibited χ^2: 15.5-24.2 and R2: 0.49-0.69. The combination of the 3 clinical criteria and the NETest in a logistic regression model provided the best fit with a χ^2 of 30.31 ($p<0.0001$), a relatedness value of 0.80, and an AUC of 0.96 ± 0.04. The model accuracy was 91.4%. Twenty-two of 23 (96%) NR patients were correctly classified and 10 of 12 (83%) recurrences were correctly identified.

FIA (see Section 2) was then undertaken to further examine the importance of individual biomarkers and clinical criteria in respect of disease recurrence. The NETest (1.8) was 4.5× more important than CgA (0.4) and identified as the predominant variable related to recurrence (Figure 6). A combination of all 3 clinicopathological characteristics yielded an importance value of 2.3. FIA assessment of the value of inclusion of the NETest to the clinicopathological characteristics increased the importance numerator to 4.3. The measurement of tumor transcript levels in blood (NETest) significantly improved the utility of grade, tumor size, and LN metastases in the accurate prediction of recurrence by almost 2-fold.

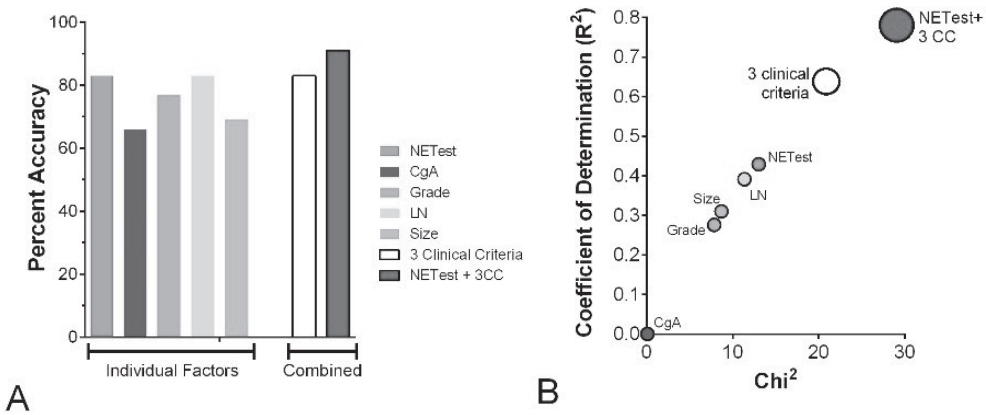

Figure 5 - Association between biomarkers, clinical criteria, and disease recurrence. **A** Accuracy of individual factors for the assessment of disease recurrence. CgA accuracy was 66%, tumor size >20 mm: 69%, grading: 77%, lymph node (LN) involvement 80%, and NETest 83%. NETest and LN positivity were significantly more accurate than both size and CgA ($p<0.05$). Combining the 3 clinicopathological characteristics had the same accuracy as the NETest alone (83%). The inclusion of the NETest to the 3 clinical criteria increased the accuracy to 91%. **B** Logistic modeling for strength of association (χ^2) versus the coefficient of determination (Nagelkerke R2) to disease recurrence. CgA levels exhibited almost no relationship (χ^2=0.02, R2=0.0008) to recurrence compared with other clinical criteria. The individual clinical criteria exhibited χ^2 and R2 values of: grading 7.8/0.28, size 8.7/0.31, and lymph nodes 11.5/0.39. The NETest χ^2 value was 13 and the R2 0.43 (43%). The combination of all 3 clinical criteria increased the χ^2 from an average of 9.3 to 21.7 and R2-value from an average of 33% to 64%. The addition of the NET blood transcript information to the 3 clinical criteria increased the χ^2 value from 13 to 30 and the R2 value from 43% to 80%. The combination of 3 clinical criteria and the NETest exhibited the greatest association with disease recurrence. CC, clinicopathological characteristics; CgA, chromogranin A; LN, lymph node involvement; NET, neuroendocrine tumors. The coefficient of determination is a measure of "relatedness" (Nagelkerke R2).

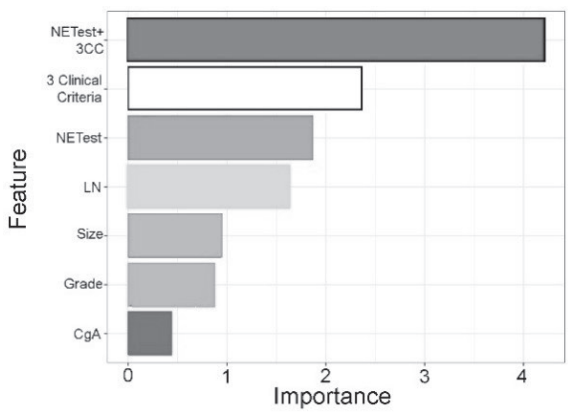

Figure 6 - Feature performance analysis defining the relative importance of biomarkers and clinicopathological characteristics for disease recurrence. CgA was the least important feature (0.4) for identifying disease recurrence. Individual clinical criteria grade (0.8), tumor size (0.92), and LN involvement (1.6) were 2-4× more important than CgA. The NETest (Importance: 1.8) was the single most important individual feature (4.5× > CgA). The combination of the 3 clinicopathological characteristics had an importance value of 2.3. The inclusion of the pNET blood transcriptome increased the importance to 4.3. A combination of 3 clinicopathological characteristics and the NETest exhibited the greatest importance value.

NETest and recurrence/NR

Six patients (17%) could overall be considered incorrectly classified by the NETest (Table 3 and Figure 7). In the NR group (n=23), the NETest incorrectly identified 2 patients. Patient 1, 66-year-old female, nonfunctional, 6mm G1 tumor, underwent enucleation. Six years after surgery had an increased NETest and CgA—93% and 497 ng/mL— respectively. An MRI 18 weeks thereafter identified no recurrent disease. Patient 2, a 67-year-old female, with a nonfunctional 10mm G1 tumor underwent a central pancreatectomy. Biomarkers were measured 8.9 years after surgery and the NETest was elevated—73%. CgA was normal. A ^{68}Ga-DOTATATE-PET-CT was undertaken 31 weeks later, and no tumor was detected. Both patients were designated R1 re sections and neither had post-operative therapy.

In the recurrence group (n=12), 4 individuals were incorrectly identified by the NETest. All 4 patients had nonfunctional tumors and all underwent R0 resections. Patient 3 was a 47-year-old male with a 115mm G2 pNET and positive LN (1/6), who underwent leftpancreatectomy. He had completed a phosphoinositide 3-kinase inhibitor clinical study (NPV-BEZ235) and also undergone radiofrequency ablation/embolization for liver metastases. NETest and CgA were measured 5.1 years after surgery before initiation of a third line therapy, streptozotocin/5-FU treatment. The NETest was 27% (positive) and CgA was 31 ng/mL (normal). Abdominal MRI, 68 weeks after biomarker measurement, identified persistent recurrent disease. Patient 4, a 74-year-old male with a 17mm G2 tumor, positive LN (1/3) and perineural invasion, underwent a pancreatic corpus resection. Biomarkers were measured 8 years after surgery. The NETest was 33% (elevated) and CgA was 210 ng/mL (elevated). CT and an Octreoscan, 1 week after biomarker evaluation, identified a small LN deposit, which was confirmed at excisional biopsy. Patient 5, a 64-year-old female, with a 55mm G2, 2/5 LN pNET underwent a pancreaticoduodenectomy. Biomarkers were evaluated 1.4 years after surgery during somatostatin analog therapy. The NETest was 27% (elevated) and CgA was 451 ng/mL (elevated). CT identified liver disease, which was then treated by streptozotocin/5-FU. Follow-up imaging 64 weeks later (MRI) was interpreted as stable disease. Patient 6, 71-year-old male, underwent a left pancreatectomy for a 25mm G1 pNET with perineural invasion and LN (0/6). Biomarker evaluation 9.8 years after surgery was NETest: 27% (elevated) and CgA: 188 ng/mL (elevated). CT identified local recurrence, and he was treated with somatostatin analogs. Follow-up MRI 68 weeks later identified stable disease.

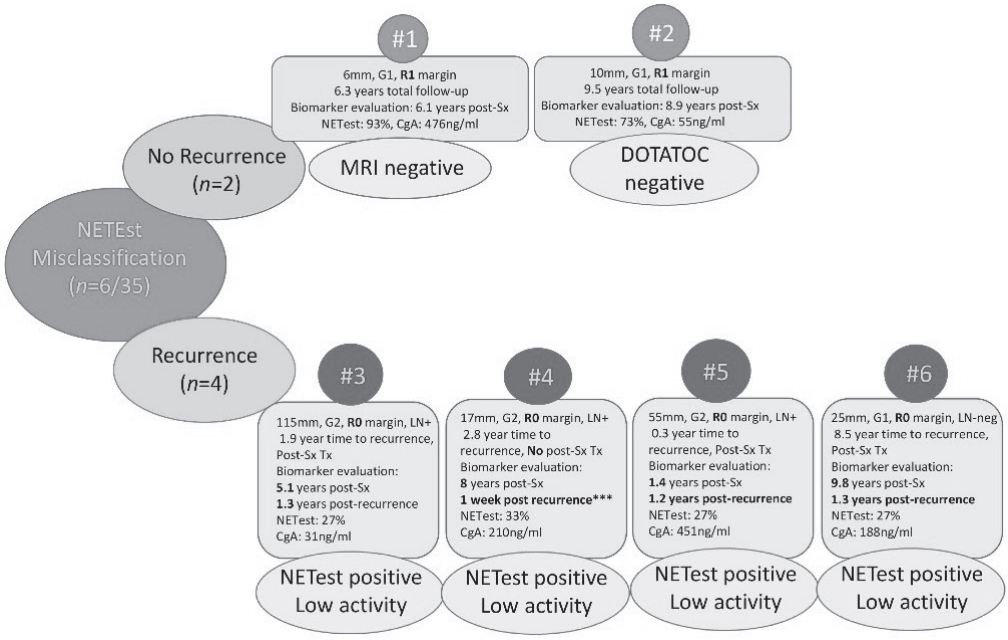

Figure 7 - Relationship between NETest and false results for recurrence. Recurrence false positive: Two patients (1 and 2) were identified with elevated NETest (scores: 93% and 73%) in the absence of image detectable disease. Both had R1 margins and had been followed for 6.3 and 8.9 years, respectively. In case 1, NETest (93%) and CgA (476 ng/mL) were elevated. The time from biomarker measurement to imaging (MRI only) was 18 weeks. In case 2 NETest was 73% and CgA normal. A ^{68}Ga-DOTATATE PET/CT was undertaken 31 weeks after biomarker measurement was negative. The inability to detect disease despite molecular evidence of a NET may reflect the limitations of image sensitivity. Recurrence false negative: Four patients (3-6) were identified with positive NETest scores (>20%) but values that are categorized as low activity (ie, <40%) range. All had R0 margins and tumors had recurred 0.3-2.8 years after surgery (in the 3 G2 cases) and within 8.5 years in the G1 case. Three of the 4 (3, 5, and 6) had in the interim undergone a variety of therapies to treat recurrence. Biomarker evaluation was undertaken 1.2-1.3 years after recurrence in these 3 cases. Tumors were all stable at imaging, which is consistent with the low NETest activity. The NETest was measured in case (4), 1 week after surgery (lymph node excision) for recurrent disease. Presumably, the low value in this instance reflects the residual circulating transcript levels from residual low burden disease. Sx, surgery (pancreatic resection); Tx, treatment. chromogranin A; MRI, magnetic resonance imaging; NET, neuroendocrine tumors.

Table 3 - Clinical characteristics of misclassified patients (*n*=6)

No.	*Cohort*	*Gender*	*Age*	*F/NF*	*Surgery*	*Resection Margin*	*Grade*	*LNR*	*Size (mm)*	*Post-Surgery Treatment*	*NETest score (%)*	*CgA (ng/ml)*	*Total FU (years)*	*TTR*	*TTRB*
1	NR	F	66	NF	Enucleation	R1	1	0/0	6	Nil	93	497	6.3	0	0
2	NR	F	67	NF	Central pancreatectomy	R1	1	0/0	10	Nil	73	55	9.5	0	0
3	R	M	47	NF	Left pancreatectomy	R0	2	6/1	115	RFA/embolization, NVP-BEZ235, S/5FU	27	31	8.2	1.9	5.1
4	R	M	74	NF	Corpus resection	R0	2	3/1	17	Prostate cancer (Chemotherapy)	33	210	10.7	2.8	7.9
5	R	F	64	NF	Pancreatico-duodenectomy	R0	2	5/2	55	SSA, S/5FU	27	451	2.6	0.3	1.1
6	R	M	71	NF	Left pancreatectomy	R0	1	6/0	25	SSA	27	188	9.9	8.5	0.2

F/NF = functional or non-functional; LNR = Lymph Node Ratio = number of positive lymph nodes/all dissected lymph nodes; FUP = follow-up (years); NR = non-recurrence; R = recurrence; RFA = radiofrequency ablation; S/5FU = streptozotocin/5-fluorouracil; SSA = somatostatin analog; TTR = time to recurrence (years); TTRB = time from recurrence to blood draw (biomarker evaluation) (years).

DISCUSSION

To improve the outcomes of patients who have been surgically treated for a localized G1/G2 pNET, post-operative management needs to be optimized to detect recurrences at an early stage. Such an identification or effective prediction of disease recurrence would facilitate stratification of the patients into those at higher risk, who would benefit from adjuvant treatments. Current clinical biomarkers in use are limited. In the study, we have demonstrated that the NETest effectively detects disease recurrence after curative surgery of pNET, and hence can be used to improve quality of the post-operative follow-up. The NETest has proven to be robust in the discrimination of pNET from healthy controls, therefore confirming its utility as a diagnostic for pancreatic NET disease[25]. As it has been already noted in other surgical series of the lung and GI tract NETs[26,27] higher NETest scores were evident in patients with recurrence compared to those without. Furthermore, NETest scores >40% were significantly correlated with recurrence, while CgA was not. In fact, the utility of CgA was significantly limited by a number of factors. First, an elevated CgA was only identified in 10 of 24 (42%) patients with residual or recurrent disease. Of these 10, 8 were taking PPIs. Therefore, a total of 2 cases (out of 24 patients) or 8% of individuals with elevated CgA levels could be unequivocally ascribed to pNET disease. Second, PPIs were used in 19 patients (54%). This use elevated CgA in 11 (58%) of them. Third, CgA was not related to disease burden in the recurrent group. Indeed, it was elevated in only 4 of 9 with distant metastases, and all 4 were receiving PPIs. The inconsistent elevation in CgA (presumably due to intermittent use) coupled to the high number of patients in whom it is prescribed, as well as its unreliable relationship to disease burden results in it being of poor clinical value.

Personalization of post-operative care for pNET is under debate by NET experts, but this has not yet led to consensus recommendations on follow-up strategies. One explanation is the limited number of monitoring strategies available to accurately detect recurrence. Imaging, either anatomical or functional, is currently the gold standard for recurrence detection. The blood-based tests currently advised by various guidelines (ie CgA) do not demonstrate reliable accuracy (sensitivity/specificity metrics) to give support to or provide an alternative to imaging. Indeed, the level of evidence for the use of CgA is classified by the National Comprehensive Cancer Network as

Type 3 “Based upon any level of evidence, there is major NCCN disagreement that the intervention is appropriate”[40].

In agreement with previously published studies, including one from Genc et al[10], predictors for recurrence have been identified and include tumor size, grade, and LN metastases. In addition, we identified the NETest, but not CgA, as the biomarker that could accurately identify recurrence. Receiver operator curve analysis, logistic regression modeling, and predictive FIA demonstrated that CgA had no value for detecting or predicting recurrence. Indeed, the OR for CgA was 1.11 (1.0 is no association). Similarly, the χ^2 and the Nagelkerke R2 were both lower than 0.1, indicating no relationship to disease status (ie, pNET disease recurrence) after surgery.

As a component of the clinical criteria for predicting recurrence CgA was identified to have the least importance. Indeed, normal CgA levels were identified in the same proportion of patients in each cohort (59% and 61%, respectively). Furthermore, while some studies describe a correlation between CgA levels and tumor load[41-43], our results do not support this hypothesis. In contrast, patients with evidence of recurrence on radiological imaging, and therefore tumor tissue in situ (R0R group), showed comparable CgA levels to patients without evidence of tumor tissue on radiological imaging. Indeed, only 4 (of 9) with distant disease exhibited elevated CgA; all 4 were being treated with PPIs. Elevated CgAs were noted in 5 of 12 with known residual (LN positive) disease (R1NR group). Three were taking PPIs. Thus, an elevated CgA may be of relevance in <20% of cases. CgA clearly is not a useful marker for either pNET disease or for recurrence. In contrast, elevated NETest levels were strongly associated with the development of a pNET recurrence. The OR and χ^2 were 21 and 13, respectively, with a relatedness value of 0.43 (43%). Levels were unaffected by PPI use and were significantly elevated irrespective of disease burden. The NETest AUC for recurrence was significantly better (0.82 vs. 0.51, $p<0.05$) than CgA. Similarly, predictive FIA demonstrated that the molecular biomarker was almost 5-fold more important than CgA (FIA).

We specifically evaluated a cutoff of 40% for the NETest in this surgical series. This level has been previously demonstrated to differentiate those with “low” risk of disease from those with a moderate or high risk of disease activity.34,36 NETest scores >40% have been identified to be prognostic (in 100% of cases) for disease progression[36]. Conversely, scores <40% in those with stable disease were 100% consistent with image-confirmation of

disease stability.36 In the current surgical study, the NETest was 83% accurate for pNET recurrence.

Two individuals (9%) were incorrectly classified in the NR group (n=23). Both had undergone R1 resections for small (<20mm) Grade I tumors. Neither exhibited LN metastases nor were being treated. The NETest score for patient 1 was 93% with an elevated CgA level (497 ng/mL). Patient 2 had a NETest level of 73% and CgA of 55 ng/mL. In patient 1 the time between biomarker measurement and imaging was 18 weeks. In patient 2 the time was 31 weeks. It has previously been noted that NETest scores >70% are associated with a median progression-free survival (PFS) of 0.7 years and that up to 25% may not have image demonstrable disease for up to 3 years[36]. Based on previous experience with NETest sensitivity compared with imaging, we suspect that both patients will exhibit image detectable disease in the future. The image positivity criteria disparity probably reflects the difference in sensitivity of detection between imaging tools (CT and MRI) compared with transcriptomic analysis. It has been noted that functional imaging detects 10% to 30% lesions not identified on an anatomic imaging[44,45]. It is possible that the use of functional (^{68}Ga-DOTATATE PET-CT) would have detected recurrent pNET disease in these patients[44].

In a group of patients (n=4) who recurred, the NETest was positive by definition (ie, >20%) but was less than the 40% that had been preselected as the cutoff point to predict disease recurrence. Blood samples were collected 1, 64, 68, and 68 weeks after detection of recurrence. Three of these patients were being treated with somatostatin analog and streptozotocin/5-FU therapy and were identified as exhibiting stable disease. Thus, the low but positive NETest levels likely reflect low activity disease presence (stable). In patient 6, the blood sample was collected 1 week after resection of the LN with microscopic disease; thus, it was predictable that the NETest score would be low (27%). The period of time between biomarker evaluation, the effective post-surgical treatments are consistent with a NETest scores (20%-40%) that fall into the low disease activity range. In essence, they confirm the presence of disease that is stable as might be predicted after effective therapy.

The individual clinical criteria identified by regression analysis exhibited 69% to 80% accuracy for predicting recurrence. While a tumor size >20mm was strongly associated with recurrence (92% of all recurrences were associated with large tumors), only 52% of tumors >20mm recurred. Grading itself was

not always indicative of recurrence. While 70% of Grade II tumors did recur, only 58% of all recurrences were G2I tumors. While LN status was associated with recurrence, the LN ratio did not appear useful as an effective marker [28]. Only 6 of 12 (50%) R0 that recurred exhibited LNR >0.2. It can thus be considered that single clinical criteria alone cannot effectively predict recurrence in pNETs. This supports the increasing enthusiasm for generating multiplex scoring systems or nomograms to predict disease recurrence[10].

Combining the 3 clinical criteria (size, grade, and LN metastases) resulted in a model with an overall accuracy of 83%. Recurrence was predicted in 75% of cases (9 of 12). The inclusion of the NETest further increased the accuracy to 91%. Ninety-six percent of those who did not recur were accurately predicted, whereas 10 of 12 recurrences were identified. This model had the highest coefficient of variation (0.8) identifying that it most accurately captured information related to disease recurrence. Furthermore, evaluation of this model using predictive feature analysis identified it to be >1.8 times more important than individual clinical criteria alone for determining recurrence. This observation would suggest a role for the measurement of blood molecular biomarker in pNET recurrence prediction modeling. More accurate stratification of pNET disease using multiple criteria would also provide a better basis for defining different clinical treatment groups in the evaluation of treatment efficacy.

The NETest+3 clinical criteria model allowed for consideration of a post-operative stratification into 3 risk categories to guide followup. Tumors with no unfavorable characteristics (eg, <20 mm, low grade, and no LN metastasis), with a low NETest score (≤40%), could be considered less likely to recur, and hence less intensive resource dependent post-operative monitoring might be possible. In the presence of a single unfavorable clinical criterion, or a NETest score >40%, more aggressive follow-up protocol with yearly consultations and radiological imaging could be advised. Those with 2 or more unfavorable clinical criteria, and a NETest score >40%, could be considered as high risk and the follow-up frequency intensified. This might involve more frequent imaging particularly utilizing more sensitive nuclear medicine strategies[44] to ascertain disease not identifiable by anatomic imaging.

Currently, patients with small pNETs also undergo intensive follow-up because surgical resection is no longer directly indicated according to current European Neuroendocrine Tumor Society (ENETS) guidelines[4]. During the

follow-up of these patients, there is no other possibility to monitor tumor progression besides imaging. Under these circumstances, the NETest might provide an opportunity to better assess the future malignant potential of the disease, particularly as its metrics are significantly better than any other biomarker, including pancreatic polypeptide and pancreastatin[38]. In this respect, an elevated NETest (>80%) is strongly associated with disease progression[34] and "omic analysis" of transcript measurement in the blood has been reported to be of value in increasing the accuracy of the prediction of tumor progression[26,33]. Thus, the gene cluster (omic analysis) of NET transcript expression in individuals with small pNETs (<20 mm) might be useful to identify those with a high risk of disease progression. Such patients might benefit from pre-emptive surgical resection despite small tumor size. Moreover, elevated levels after surgery are effective prognostic markers and can be used to identify those who would benefit from early intervention because of risk of recurrence[26,27]. Ultimately, we envisage that the NETest could be evaluated at least annually after surgery and included in the algorithm to provide an updatable real-time patient risk status. The frequency of testing would depend on the risk of progression. Cost-effectiveness would be determined through changes (decreases) in imaging as has been recently noted[46].

The current study confirms that CgA has no role in predicting pNET disease recurrence. It has little accuracy, was the poorest feature for prediction and the OR (1.1) was no different to using no biomarker at all. If the patient is receiving PPIs, the value of CgA is even further obfuscated. It seems likely that use of a molecular blood test as an accurate marker of disease recurrence or progression might be of clinical utility.

The study has some limitations. As with many investigations in the NET field sample sizes for each group are small and patients identified retrospectively. Patients were older than controls but this has not been identified to be relevant to the NETest; no correlation has been noted between age and transcript levels[29]. The follow-up duration of patients in the R0NR group is shorter compared to the other patient groups (31 months vs. 105 and 92.5 months, respectively). As recurrence is typically seen within the first 5 years after surgery, a follow up of 31 months may not be adequate to identify all of those who will recur.

CONCLUSION

Identification of, or prediction of pNET recurrence at an early time point is a critical medical necessity to facilitate further treatment and improve survival. Current clinically used criteria are effective, but inclusion of a blood biomarker that will improve accuracy would be of added value. In this respect the NETest has performance metrics that conform to NIH standards and detects and predicts recurrence after curative resection of G1/G2 pNET. Our study indicates that blood transcript analysis of pNETs added biomolecular value to support the current clinical and radiological parameters used in post-operative follow-up. Larger prospective studies are warranted to more fully explore the utility of the NETest in the identification of postoperative residual pancreatic NET disease or recurrence and to help better stratify patients for post-surgical treatment.

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Adjuvant therapy for pancreatic neuroendocrine tumors: expert consensus and recommendations for future research

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Submitted

ABSTRACT

With the exponential increase of incidentally diagnosed pancreatic neuroendocrine tumors (pNET), more patients are treated with curative intent. Although several patients will have a recurrence after surgery, the role of postoperative adjuvant therapy has not been studied and is therefore not recommended in guidelines. Multiple clinicopathological characteristics have been described in the literature to identify patients at high-risk of recurrence who might benefit from adjuvant treatment. However, currently no data is available on the indication or benefit of adjuvant treatment after potentially-curative resection. This causes a major obstacle to designing studies on the role of adjuvant therapy in these patients. The PNET Consensus Study Group was created to overcome some of these obstacles and aimed to lower the threshold for future researchers by providing expert opinions on issues essential for the design of clinical trials. Through online surveys and a subsequent meeting during the 2017 ENETS Conference, international experts on pancreatic NET treatment formulated priorities for future studies on adjuvant treatment after curative resection of well-differentiated pNET. Emphasis was placed on the development of well-designed clinical trials with clearly defined efficacy criteria. Key recommendations include the selection of high risk patients, choice of adjuvant treatment regimen and study design.

INTRODUCTION

Despite their rare nature, increasing incidence of pancreatic neuroendocrine neoplasms (pNEN) has been reported across multiple published studies[4, 7, 13, 43, 44]. PNEN account for approximately 10% (0.55 per 100.000 in 2009) of all NEN, with the incidence rate increasing six-fold within the last years according to a recent study by Klimstra et al.[45]. With this, diagnosis at an early stage is becoming more common, resulting in more patients undergoing treatment with curative intent.

Curative treatment typically consist of surgical resection of localized disease, including additional regional lymphadenectomy[46]. Post-operative adjuvant medical therapy is not recommended in recent guidelines from ENETS and NANETS [46, 47] due to the lack of robust data demonstrating benefit. Multiple studies have shown significant decrease of survival for patients with disease relapse[9, 10]. Clear guidelines for postoperative follow-up have not yet been described, presumably because it is generally assumed that recurrence is uncommon. However, a recent meta-analysis of the literature by our study-group showed an overall reported recurrence rate of 13% after curative resection of localized grade 1 or 2 pancreatic neuroendocrine tumors (pNET), without hereditary syndromes[48]. Several predictors for recurrence have been found in multiple studies, making it possible to identify high-risk patients[13, 49-51]. This provides a possibility to stratify postoperative treatment of patients based on the risk to develop recurrence. Patients with a low recurrence-risk could possibly benefit from less frequent follow-up, while high-risk patients might need intensified monitoring to detect recurrence early. Although, adjuvant therapy is currently not recommended, several pNEN experts suggest that it could be beneficial to prevent recurrence in high-risk patients. Therapeutic options to prevent recurrence after surgical resection have never been described. Multiple systemic treatment options are known for patients with advanced pNET that could potentially serve as adjuvant treatment after curative surgery. As there is no published data on the effect of these therapies on patients who underwent surgery with curative intent, implementation as adjuvant therapy is impeded.

A number of factors pose challenges for diagnosis, treatment and clinical decision making in the adjuvant setting for patients with pNET. These include the rarity of the disease in individual centers, limited cross-center collaboration to increase patient numbers and non-standardized follow-up protocols. In rare diseases, treatment is frequently based on experience and

expert opinion, presenting a major obstacle to design and complete studies of appropriate power and duration. The PNET Consensus Study Group was created to overcome some of these obstacles by formulating priorities for future studies on adjuvant treatment after curative resection of pNET, as currently there is no available literature. Meeting objectives included the development of recommendations for appropriate study end points and standardization of clinical trial inclusion criteria. Through online communications, individual surveys and an expert meeting during the 2017 ENETS Conference, scientific knowledge was brought together with existing expertise to formulate and discuss key unmet needs on this topic. The aim of this consensus-study was to encourage clinical research on adjuvant therapy in pNET, and to facilitate development and coordination of relevant clinical trials in this disease with support from experts in the field.

METHODS

A panel of clinicians consisting of international experts on pancreatic NEN treatment (including endocrinologists, oncologists, surgeons, experts in diagnostic imaging, pathologists and researchers) were asked to participate in the PNET Consensus Study Group. Experts were identified from a systematic search of published literature and peer recommendations.

This consensus study was structured by including an overview of the existing literature summarizing recent developments in the field, through a comprehensive search of the medical literature in December 2016. This overview was sent prior to and along with an online survey, which collected personal opinions and expertise from the experts. A total of 17 questions were presented in two rounds of online voting (see supplementary data). Answers were anonymously collected without presenting the results of other participants or previous rounds. Feedback was conducted and resulting statements, recommendations and amendments were processed by the executive team members. Statements achieving an agreement of 80% or more were considered as having reached consensus, whereas statements achieving less than 80% were considered as non-agreed and were planned to be a key subject of discussion for the subsequent PNET Consensus Study Group meeting. The meeting of the Study Group was held on 9th of March 2017 during the 14th Annual European Neuroendocrine Tumor Society (ENETS) Conference (Barcelona, Spain) where non-agreed statements and

clinical trial priorities to investigate adjuvant therapy in pNET were discussed. Recommendations from agreed statements of the surveys, together with newly-reached statements, ideas and concepts for future research were summarized at the end of the meeting for consensus. After the meeting, a draft of the consensus and recommendations was further developed and refined by the executive members and online communicated with all invited experts of the Study Group for final approval. This report was structured to address key issues for clinical trials to investigate the role of adjuvant therapy in curatively-treated pNETs by stating key recommendations supported by a summary of the deliberations leading to the consensus. All Study Group Members agreed the following consensus statements.

RESULTS

Twenty-one international experts on pNET treatment were invited to participate in the Study Group. In total, 18 experts (86%) participated in the online surveys and/or consensus meeting.

A total of 17 questions were posed in the online surveys. In 6 of the 17 questions (35%) consensus was reached before the meeting in March 2017. During the meeting, consensus was reached in an additional 9 questions, resulting in agreement on 15 of the 17 questions (88%). After review and discussion, consensus and recommendations were finalized and 11 comprehensive recommendations were formulated.

Is there a need for research on adjuvant therapy after curative resection of well-differentiated pNET?

RECOMMENDATION 1
Due to the high risk of recurrence and its associated disease burden the study group agrees that there is a need for research on adjuvant therapy after curative resection of pNET.

The incidence of pNET is increasing and recurrence after surgery is more frequent than generally thought. The published literature describes recurrence in 9-18% after curative resection, having considerable adverse

impact on survival[48]. Furthermore, multiple studies provide clear evidence that tumors with specific unfavorable characteristics have a higher risk to develop recurrence[13, 49-51]. Tools to identify high risk patient have been provided but have had no effect on the postoperative management of these patients. Lack of data regarding this disease, and recurrence in particular, is thought to be responsible for disregarding research on the prevention of recurrence[52]. Nevertheless, considering the recent data, it is impossible to ignore the need for studies on postoperative prognosis, the effect of early diagnosis or treatment and prevention of recurrence. Currently there are no published studies on the effect of adjuvant treatment after curative resection of pNET. Considering the different options for systemic therapy shown to be effective in the advanced disease setting, adjuvant treatment in a select group of patients could potentially be beneficial in preventing recurrence. Therefore, all participants agreed that there is an unmet need for research on the role of adjuvant treatment after curative resection of pNET. This additional therapy after primary tumor resection would aim at reducing the risk of recurrence and death by eliminating residual micro-metastatic disease.

Which patients should be included in the study?

RECOMMENDATION 2
The role of adjuvant treatment should be investigated in patients with an increased risk of recurrence after curative resection of incidental grade 1 or 2 pNET.

RECOMMENDATION 3
High-risk patients should be identified using an updated pNET recurrence score[51].

RECOMMENDATION 4
Given the good tolerability of systemic therapy and the expectation of improvement in quality of life as well as life expectancy, all patients from the age of 18 years should be eligible to be included in future studies.

Logically, adjuvant therapy must be reserved for patients with a high risk of recurrence. Uniform methods to identify these patients have not yet been described. Predictive factors for recurrence after resection of pNET have

been presented in multiple studies[50, 51, 53]. However, a majority of these studies include patients with known unfavorable characteristics such as high grade neuroendocrine carcinoma, distant metastatic disease or hereditary syndromes, affecting the reliability and clinical utility of these results. Clinicopathological characteristics associated with recurrence in patients who have undergone curative resection of grade 1 or 2 pNET without distant metastases or hereditary syndromes have only been described in a few studies[49-51, 54, 55]. The most frequently associated factors include tumor size, tumor grade, lymph node metastases, presence of perineural invasion and involved surgical margin (R1) resection. To date, these studies have not led to recommendations or methods to classify patients into high and low risk for the purpose of customizing postoperative follow-up.

A practical method to estimate the risk of recurrence has recently been published[51]. This recurrence-score is a tool for clinicians to estimate the individual risk of recurrence by scoring the absence or presence of three different tumor characteristics. The corresponding points are awarded and the total score correlates to an estimated risk to develop recurrence within 5 years after curative surgery. Given the paucity of sufficiently executed studies using homogeneous patient groups and adequate follow-up in this field, the study group agrees that the recurrence-score is a reliable method to identify high-risk patients who might benefit from adjuvant treatment. In addition to this score, and on the basis of recent developments within the grading system for pNET, the study group requested extra analyses investigating heterogeneity within grade 1 and 2 pNET based on the Ki67 proliferation index, with regards to recurrence and survival. These analyses confirmed that tumors with Ki67>5% have a significantly higher risk in developing recurrence and have a decreased 10-year disease specific survival compared to tumors with Ki67$\leq$5%[32, 56]. The study group has therefore reached the final conclusion that patients with a high risk of recurrence can be selected using a modified version of the recurrence score, in which grade 2 tumors will be defined as tumors with a Ki67>5%. Patients with a modified recurrence-score above 64 have >50% chance to develop recurrence and are therefore considered as high-risk, taking into account that patients with unfavorable tumors currently receive non-standardized monitoring and no preventive measures. To investigate the role of adjuvant treatment, these patients will be eligible for inclusion in future trials. Research on predictors for recurrence or methods to identify high-risk patients is highly encouraged by the study

group and new results should be included in the case of future research on adjuvant therapy.

Age criteria for inclusion was considered unnecessary, given the relatively good tolerability of systemic treatments in patients with advanced pNET disease general. Furthermore, adjuvant therapy is expected to expand life expectancy and improve quality of life due to the absence of recurrence and its treatment. Therefore, all adult patients (above the age of 18 years) at high risk of recurrence after radical resection can be considered suitable for inclusion in a potential study on adjuvant therapy after curative resection of pNET.

Which patients should be excluded from the study?

RECOMMENDATION 5
Patients with hereditary syndromes, high grade (Ki67>20%) pNEN and patients with distant metastases at time of resection should be excluded from the study. Prior to inclusion, the presence of distant metastases should be assessed, preferably with ^{68}GA-DOTATATE PET-CT.

RECOMMENDATION 6
Neo-adjuvant treatment, for any reason, should be a contraindication for inclusion.

RECOMMENDATION 7
General exclusion criteria should be applied; i.e. other/secondary malignancies, limited life-expectancy, poor performance status to undergo systemic treatment or pregnancy. Patients with a sufficient postoperative recovery after considered fit for surgery are believed to have an adequate performance status to receive adjuvant treatment.

All patients diagnosed with resectable pNET, without distant metastases or hereditary syndromes, should be considered for inclusion in this study. Distant metastases should be evaluated with preoperative ^{68}GA-DOTATATE PET-CT, generally advised by the study group for every pNET patient prior to surgery. The final decision for inclusion will take place after resection, as criteria for high-risk can only be established from pathology of the resected

specimen. Only patients who meet the inclusion criteria, who are sufficiently recovered from surgery and for those who general exclusion criteria do not apply will be found eligible for inclusion. All patients who received neo-adjuvant treatment (somatostatin receptor analogs, chemotherapy, PRRT or targeted therapy), for any reason, will be excluded from this study as the effects of either pre- or post-operative treatment cannot be analyzed separately.

Which adjuvant treatment regimen is preferred?

RECOMMENDATION 8
Chemotherapy with a regimen of capecitabine and temozolomide (CapTem) was considered to be the best treatment to serve as adjuvant therapy.

Systemic treatment options for pNET are typically reserved for patients with symptoms due to hormonal hypersecretion or patients with non-functioning disseminated disease. These include somatostatin receptor analogs (SSA), chemotherapy, targeted therapies (everolimus, sunitinib) or peptide receptor radiotherapy (PRRT). Data on the effects of adjuvant systemic treatment options for patients after curative resection of pNET do not exist. The lack of evidence and the rarity of this disease challenge the development of clinical trials to investigate the role of adjuvant treatment in high-risk patients. In the absence of published research, clinical results of systemic treatments indicated for patients with disseminated disease can be translated to potential effects in surgically treated patients with possible microscopic disease. This approach can help to decide which therapy is most suitable to serve as adjuvant treatment in future research.

Through a systematic review of all published studies up to November 2016, the overall response rate (ORR; all radiological evidence of regression) and clinical benefit (CB; radiological disease regression + stable disease) of the different systemic treatment options were summarized. Pooled results per treatment group and specific sub-treatments provided a clear overview of treatment results and possible adverse events to compare. As it still remains unknown what the potential benefits of adjuvant therapy will be, possible adverse events were weighed against the risk of disease recurrence and effects of (late) treatment next to expected cytoreductive effects.

Furthermore, practical aspects, such as treatment duration and costs were considered.

From the online survey, targeted therapy and SSA were found less suitable due to the generally poor cytoreductive results in clinical trials, together with unclear duration of treatment. Long-term use is expected, associated with higher costs and risks of adverse events [57-59]. Chemotherapy and PRRT were both considered eligible for adjuvant treatment based on the presented results in the systematic review on the effects of these treatments in advanced disease. General availability, costs, safety, therapy's characteristics, mechanisms of action and published evidence of both treatment modalities were extensively discussed. Ultimately, the study group favored treatment with a regimen of CapTem to serve as adjuvant therapy given the generally good tolerability, limited serious adverse events, limited costs and broad availability. Although PRRT studies show impressive results with regard to response rate and quality of life after treatment, technical challenges involving the potential unavailability of functional expression of the somatostatin receptor have led to the conclusion that this treatment is less suitable to serve as adjuvant treatment in the short term. Considering recent developments regarding FDA and EMA approval of PRRT as a systemic treatment option for pNET, a study design involving both PRRT and CapTem as treatment modalities in the adjuvant setting may be considered.

Study design

RECOMMENDATION 9
The role of adjuvant treatment in high-risk patient with pNET should be investigated in a randomized controlled trial (RCT).

RECOMMENDATION 10
The patients in the control-arm of this RCT should be high-risk patients who receive standard care without adjuvant treatment.

RECOMMENDATION 11
The primary outcome of the study should be recurrence-free survival. Secondary outcomes should include recurrence rate and disease specific survival.

Studies on rare disease are mainly challenging because results have to be drawn from small patient population and recommendations are regularly based on low evidence from retrospective studies. Therefore, despite the limited number of patients who will be eligible for a study on adjuvant treatment after pNET resection, there was a wide agreement among the participants at the meeting that the optimal study design for this study should be a randomized controlled trial (RCT). This study design was found necessary to achieve immediate reliable and clear results, regardless of the known obstacles, that can be implemented directly into the clinical setting. The control group of this RCT must consist of high-risk patient who do not receive adjuvant therapy after curative resection. Both study arms must follow the same standardized and intensive follow-up regimen. Blinding was not considered feasible. Recurrence-free survival is advised as primary outcome due to the lack of a visible target to monitor treatment response. Power calculations were made based on the expected ORR and CB from the pooled results of the systematic review and resulting estimated recurrence rates after adjuvant treatment. Using ORR results, 304 patients (152 in each arm) are required to have a 90% chance of detecting, as significant at the 5% level, an increase in recurrence-free survival from 50% in the control group to 68% in the experimental group. For results on CB, a total of 74 patients (37 in each arm) are required to detect an increase of recurrence-free survival to 83.5% in the experimental group. The study group recommends a follow-up duration of at least 5 years for all participants. The frequency of hospital visits should be at least every 6 months for all participants of the study (both experimental and control arm).

DISCUSSION

The group of international experts considered the risk of recurrence after potentially-curative resection of well-differentiated pNET of considerable concern, particularly in high-risk patients. Despite this risk of recurrence after initial curative treatment there is no international consensus on adjuvant treatment regimens, which is mainly due to a lack of evidence. Therefore, it is imperative that the potential effect of adjuvant treatment after curative resection of well-differentiated pNET is investigated further. At present, it is difficult to acquire robust and reliable results from prospective trials because of the limited number of patients that most individual centers see annually.

An international multicenter approach seems therefore necessary. Given the large number of obstacles and effort needed to set up prospective randomized trials for this purpose, the pNET Consensus Study Group aimed to lower the threshold for future researchers by providing expert opinions on issues essential for the design of such a trial. Future studies are not only important to investigate the effect of adjuvant treatment after pNET resection but may also provide insights in early detection and treatment of recurrence in the control group, given the proposed intensive follow-up protocol. In addition to the 11 recommendations on which consensus was reached, further standardization of protocols among participating centers should be agreed on. These include domains as surgical techniques, with emphasis on the extend and standardization of lymph adenectomy during primary resection. The same applies to pathological assessment of pNET specimens: pathology report should be internationally standardized to promote optimal and analogous registration across registries for future research. Analysis of such data could also help identify other predictors for recurrence to further improve accuracy of the current method to identify high-risk patients.

In conclusion, the pNET Consensus Study Group proposes to investigate the potential beneficial effect of adjuvant chemotherapy on recurrence free survival for high-risk patients with well-differentiated pNET, who underwent curative resection in an open-label randomized controlled trial.

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PANDORA - Prospective nationwide observational cohort of patients with pancreatic neuroendocrine tumors <2cm

08

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ABSTRACT

Rationale. pancreatic neuroendocrine tumors (pNET) are more often diagnosed incidentally due to the use of better imaging techniques. Surgical resection is the only curative treatment and long term follow-up indicates a survival benefit for patients who underwent primary resection. However, pancreatic resections are associated with serious postoperative morbidity. In addition, recent literature shows that incidentally found pNET have a significant smaller size and are more commonly associated with lower tumor stages. Progression or tumor growth in small incidentally found non-functioning pNET seems minimal. Therefore, the European Neuroendocrine Tumor Society (ENETS) has updated their guidelines; surveillance is now recommended for patients with non-functional pNET <2cm. Although this approach seems safe, long term follow-up data are needed to guarantee the safety of this policy.

Objective. To monitor long term effects of a non-operative management of small pNETs.

Study design. A prospective, multicentre, cohort in collaboration with all Dutch Pancreatic Cancer Group (DPCG) affiliated centers that treat patients with pNET.

Study population. patients diagnosed with a pNET <2cm.

Endpoints. Tumor progression and survival will be the primary outcomes. In addition, patients who do undergo a resection despite the guideline will be observed. The reasons to deviate from the initial therapy will be investigated. A secondary outcome will be the quality of life of all patients that are diagnosed with a pNET <2cm, regardless of received therapy.

INTRODUCTION

Neuroendocrine tumors of the pancreas (pNET) comprise 1-2% of all pancreatic malignancies and have an incidence of 1-2/million/year[1]. Improved diagnostics and pathological recognition have led to an increased detection of pNET. pNETs are mostly identified on abdominal imaging performed for other indications[2]. Since pNET are relatively rare, large prospective studies, let alone randomized controlled trials, on diagnostics and treatment are very difficult to perform.

PNET are divided in functioning and non-functioning tumors. Functioning tumors are defined as tumors with hormonal overproduction causing a clinical syndrome. Functioning pNET, regardless of size, form an indication for resection. Other pNET are diagnosed during surveillance programs for patients with genetic syndromes such as MEN1 or Von Hippel Lindau disease. The latter genetically based pNET are not the focus of this protocol, since specific guidelines for these patients are available. Non-functioning pNET are often diagnosed because of pain of abdominal complaints but also incidentally during abdominal imaging for other indications. Larger non-functioning pNET (>2cm) form an indication for resection but consensus is lacking on the treatment of small non-functioning pNETs.

Patients with pNET have a much better prognosis then patients with pancreatic adenocarcinoma, with 10-year survival rates after resection exceeding 80%. Especially patients with small pNET seem to have an excellent prognosis and the latest guidelines suggest a wait-and-see protocol for patients with non-functioning pNET <2cm[1,2]. Most of these patients in surveillance protocols do not show tumor progression. However, some studies report that eventually resection during follow-up was performed in 14-25% patients[3-5]. Because these series are retrospective, indications for a wait- and-see strategy and late surgery are difficult to assess.

Therefore, in this nationwide prospective PANDORA cohort, all patients with small non-functioning pNETs (<2cm) will be registered and prospectively followed. Indications for initial and later treatment strategies and policy changes will be recorded.

Objectives

The aim of this prospective cohort is to analyze the long-term effects of the non-operative management of pNET <2cm. Tumor progression will be

monitored and the amount of patients that will undergo a resection regardless of the indication will be assessed. In addition, the reasons to undergo a resection will be analyzed. Patients that will receive a non-operative treatment after initial diagnosis will be compared to the patients that undergo a resection despite indication for surveillance. Furthermore, a comparison with a large retrospective database of a similar patient population will also be made.

STUDY DESIGN

All patients that are diagnosed with a NF-pNET <2cm at the participating centers will be registered in the PANDORA database. Data collection will be performed in an anonymous database, not linked to private details of patients used for sending Quality of Life (QoL) questionnaire or requiring hospital data. Every hospital including patients will need a local study-representative. Registration will be performed by either the clinician or the PANDORA-representative through the study website (www.amc.nl/pandora). The study coordinator (SC) will collect data on treatment and diagnostics and will directly contact patients by mail/email for QoL assessments. The time of follow-up will be least 10 years, or till date of death.

STUDY POPULATION

Inclusion criteria

- Patients > 18 years
- Able to read and write Dutch/English and fill in QoL-questionnaires
- Radiology (or pathology) proven NF-pNET <2cm
- Imaging with CT/MRI showing no metastases or suspected lymph nodes.

Exclusion criteria

- Patients with MEN1 or another genetic predisposition for pNET
- Functioning pNET (gastrinoma, insulinoma, etc)
- pNET grade 3

DIAGNOSIS

The flowchart is presented in Figure 1. All patients are diagnosed as stated below. Patients with a pNET diagnosed after resection will be followed as stated in the PANDORA postoperative follow-up protocol.

Hormones

•	Chromogranin A	Only initially
•	Only peptic ulcers/abdominal pain / diarrhoea	Gastrin
•	Only in hypoglycemia	Insulin and C-peptide
•	Complaints unclear	Endocrine evaluation

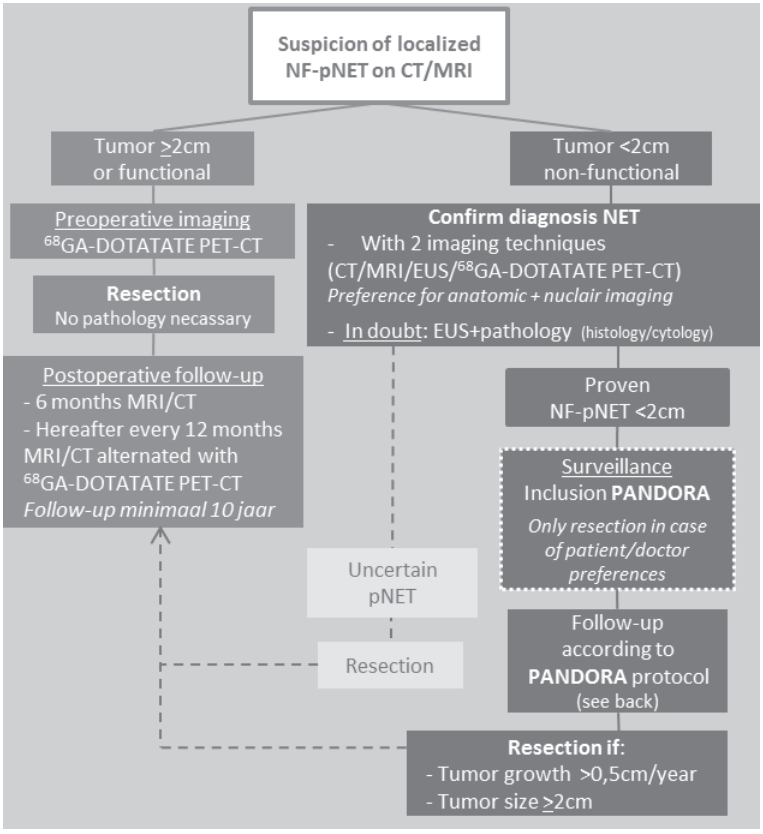

Figure 1 – PANDORA flowchart.

METHODS

Imaging

The diagnosis of pNET must be made with high suspicion on at least 2 radiological techniques, preferably by combining anatomical and nuclear imaging. Pathology through FNA is not directly recommended since the risk of pancreatitis and sampling error. Only in case of doubt cytology/histology is indicated.

- Contrast enhanced CT scan or MRI
- ^{68}Ga-DOTATATE PET-CT
- 18FDG-PET in NEC / Grade 3 pNET

^{68}Ga-DOTATATE PET-CT is advised as preoperative assessment of dissemination in all G1/G2 pNET.

Genetics

Optional: PTH /calcium, only at initial visit (MEN 1 screening)

Pathology

Endosonography (EUS) with Fine Needle Aspiration (FNA) or Fine Needle Biopsy (FNB) with Ki67 index (or grading) is only advised when there is doubt of diagnosis pNET through imaging, and not for those who will undergo resection.

FOLLOW-UP

Wait-and-see protocol for patients with non-resected pNET

Outpatient visits combined with imaging as in the following schedule (MRI favors CT scans to reduce radiation-dose):

Year 1	3 months	EUS
	6 months	MRI/CT
	9 months	Outpatient /telephone consultation
	12 months	EUS
Year 2	18 months	MRI /CT
	24 months	MRI/CT
Year 3	30 months	Outpatient visit / telephone consultation
	36 months	MRI/ CT
Year 3-10	MRI/ CT every 12 months	

If during follow-up the tumor size exceeds the >2cm, or if tumor growth >0.5cm/year is visualized, resection should be advised. All other tumor characteristics, such as suspected lymph nodes or suspected metastases should be proven with cytology and/or be considered in the treatment strategy. Preoperative imaging though ^{68}Ga-DOTATATE PET-CT to assess dissemination of disease is advised.

Follow-up protocol after resection

6 months postoperative	MRI/CT
Thereafter every 12 months	MRI/CT or ^{68}Ga-DOTATATE PET-CT

Follow-up at least until 10 years postoperatively.

ADVISORY BOARD

Two NET specialists from each of the 4 Dutch ENETS-centers are asked to be part of the PANDORA Advisory Board. This advisory board can be counselled for medical expertise, management and/or other questions associated with PANDORA.

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General discussion and future perspectives

09

GENERAL CONCLUSION AND FUTURE PERSPECTIVES

As shown by multiple population based studies, the incidence of pancreatic neuroendocrine tumors (pNET) is rising and is expected to rise further due to increasing knowledge and the use of advanced radiological and nuclear imaging techniques[1,2]. Patients with pNET generally have a favorable prognosis but despite efforts the overall survival is not improving over time[3-5]. Typically, patients experience long delays before a diagnosis (5–7 years), and most lack access to the multidisciplinary care necessary for optimal management of these complex tumors. Furthermore, the majority of patients are still diagnosed with metastatic disease. Disappointingly, the increase in the number of patients diagnosed with pNET seems to have surpassed our knowledge of this disease. In view of the slow progress made for this condition, reliable and well executed studies are ungently warranted.

Survival primarily depends on the tumor grade and tumor-node-metastasis (TNM) stage, showing superior outcomes after surgical resection for almost all patients (**Chapter 2**). Surgical resection is considered the only curative treatment option and represents the treatment of choice for any localized pancreatic neoplasm[6-8]. A more aggressive treatment approach has increasingly been described in the literature for selected patients with distant metastases, as multiple studies show survival benefit after either resection of (solitary) liver metastases and/or after resection of the primary tumor in the presence of distant metastases[9-12]. On the contrary, small pNET seem to have a more indolent behavior and the need for surgical resection of these tumors is under debate.

Recurrence

The growing incidence of pNET has led to an increase of patients diagnosed with localized, and thus resectable disease. Between 2008 and 2013 the number of patients who underwent a pancreatic resection for pNET has shown a 1.6-fold increase within the Netherlands (**Chapter 2**). To improve the outcome of patient who have been surgically treated, postoperative management needs to be optimized. Multiple studies have shown significant decrease of survival in patients with recurrence after curative treatment of pNET, suggesting that postoperative follow-up should be designed to detect recurrence (**Chapter 3 and 4**). However, clear guidelines for postoperative follow-up have not yet been described[13, 14]. Lack of data regarding recurrence rates and median time to recurrence presents a major obstacle to designing

studies of appropriate power and duration to overcome this nescience. A meta-analysis of published literature on patients with well-differentiated pNET who underwent a curative resection shows that recurrence is seen in 9-18% within 5 years after surgery (**Chapter 3**). This indicates that recurrence of well-differentiated pNET is not uncommon and should not remain untreated. Several predictors for recurrence have been presented in the literature, making it possible to identify high risk patients[3,13]. In a large database of patient with non-functioning pNET (NF-pNET) predictive factors for the development of recurrent disease were analyzed. Tumor grade, the presence of lymph node metastases and perineural invasion were independently associated with recurrence within 5 years after surgery, leading to the development of a scoring system to estimate the risk of recurrence and identify high-risk patients (**Chapter 4, Figure 1**). The predictors presented in this scoring system correspond to the literature and, when combined, showed to be a better predictor of recurrence than the grading classification of the World Health Organization (WHO). The clinical relevance of tumor grade to determine the postoperative treatment strategy of pNET has been under debate because of the wide range of the Ki67 proliferation index, in particular for grade 2 and 3 tumors[15-17]. Heterogeneity of pNET is increasingly described leading to a recent update of the WHO grading system, subdividing high-grade tumors into well-differentiated G3 neuroendocrine tumors (NET) with lower Ki67 and a better prognosis and poorly differentiated G3 neuroendocrine carcinoma (NEC) with higher Ki67 values and unfavorable prognosis[18]. Although clear upper or lower limits to differentiate between these high-grade tumors are not provided, differences in disease behavior and response to treatment are suggested[19-21]. Some NET experts promote a similar development for G2 tumors, for which the Ki67 ranges from 3-20%. Already a cutoff of 10% has being advised to select patients suitable for liver transplantation or inclusion in the Clarinet study, supporting heterogeneity for well-differentiated pNET aswell[22,23]. Furthermore, several studies describe a higher discriminative capacity when G1 and G2 pNET are divided by a Ki67 cutoff of 5% instead of 3%[24-26]. The same is seen when the proliferation index is analyzed for the prediction of recurrence after curative resection of pNET (**Chapter 5**). In a large retrospective study of 241 patients with well-differentiated pNET, patients with G1 pNET (Ki67 0-2%) showed a similar risk of recurrence within 5 years after surgery compared to pNET with Ki67 of 3-5%. Moreover, pNET with a Ki67 of 6-20% had a threefold higher risk to develop recurrence and showed significantly shorter survival than tumors with Ki67 $\leq$5%. These results

contribute to strengthen the Recurrence Score by updating the criteria for G2 tumors to pNET with Ki67 6-20%. Discrimination of high-risk and low-risk patients will be more accurate, providing the possibility to customize postoperative treatment of patients based on the risk to develop recurrence. High-risk patients might benefit from intensified postoperative monitoring or adjuvant treatment while unnecessary follow-up or treatment for low-risk patient, especially for pNET with a Ki67 of 3-5%, may be limited.

Biomarkers

The introduction of personalized postoperative treatment strategies is under debate by NET experts, resulting in the lack of uniform follow-up recommendations. A possible explanation is the limited number of monitoring strategies available to detect recurrence accurately. Radiological imaging with Computed Tomography (CT) or Magnetic Resonance Imaging (MRI), and nuclear imaging with ^{68}GA-DOTATATE PET-CT is currently the gold standard to detect recurrence[6]. A fundamental issue in the management of gastrointestinal NEN is the absence of reliable biomarkers that can provide a measure of the underlying mechanism of tumor development and growth. Chromogranin A (CgA) used to be considered as the most useful biomarker for the detection of metastases after curative resection of pNET[27]. However, CgA does not achieve the desired metrics for biomarker sensitivity and specificity. The sensitivity of CgA varies from 24-88% in the literature, showing increased accuracy in patients with high tumor load[6, 28, 29]. However, measurements show low specificity as CgA is also elevated in other neoplasia (pancreatic, prostate, small cell lung neoplasia) as well as in a variety of cardiac and inflammatory diseases, renal failure and the use of acid suppression medication, making it ineffective as first-line diagnostic for pNET[30-35].

The term liquid biopsy has been used to describe the capability of detecting tumor cells in blood and has been effectively used in other cancers[36,37]. Expanding knowledge in the genomic landscape of pNET and the relatively limited complexity of the mutational signatures that characterize their pathogenesis make these tumors ideally suited for this technique[38]. Recently a multianalyte blood-test based on circulating RNA, the NETest, has been described by Modlin *et al.* showing a sensitivity and specificity >93% for the diagnosis of NET[39]. As a biomarker, the NETest also confirmed to be effective in the detection of pNET recurrence during postoperative follow-up (**Chapter 6**). In a prospective study among 35 patients, the NETest showed an 83%

accuracy in detecting recurrence after curative resection of well-differentiated pNET, compared to 59% for CgA. NETest scores were significantly higher in patients with recurrence compared to patients without recurrence or with R1 resections, and showed the strongest association to recurrence with an odds ratio (OR) of 21 (*vs.* 1.11 for CgA). Other clinicopathological characteristics associated with recurrence included tumor grade (OR 9.3), lymph node metastasis (OR 14.3) and tumor size (OR 14.3). Combining the NETest with clinicopathological characteristics showed to be ~2x effective in detecting recurrence, reaching an accuracy of 91%. These results suggest that there is no role for CgA in predicting disease recurrence and that the NETest can improve the quality of postoperative follow-up. Together with clinicopathological criteria, the NETest is useful in accurately identifying high-risk patients and aid postoperative treatment strategies. Corresponding clinicopathological characteristics with the Recurrence Score confirm the prognostic accuracy of these variables and their clinical relevance in predicting recurrence. Furthermore, the results suggest a broader applicability of the NETest in the treatment of pNET. The NETest has proven to be robust in the discrimination of pNET from healthy controls, confirming its utility as diagnostic for pNET disease. Furthermore, other studies have shown strong association of NETest scores and tumor progression, providing the possibility to monitor treatment effect[40-42]. The NETest might be useful in patients with small pNET, discriminating between those with a high risk of disease progression who might benefit from pre-emptive surgical resection and tumors with less malignant behavior for whom the risks of an operation do not outweigh the risk of disease progression. Moreover, the NETest might also be useful in the follow-up of small pNET who undergo a conservative approach to assess tumor growth or changes in behavior. Ultimately, we envisage that the NETest is used regularly in all stages of disease to provide an updatable real-time patient risk status.

Small pNET

The improvement of cross-sectional imaging techniques significantly increases the detection of small and often asymptomatic pNET. It remains questionable if all these lesions should be routinely resected. Until recently, surgical resection was the gold standard for all localized pNET lesions. The latest update of the European Neuroendocrine Tumor Society (ENETS) guidelines for the management of well-differentiated non-functional pNET (NF-pNET) advocate the possibility of a conservative approach, rather than surgical resection, for small tumor $\leq$2cm[6]. The objective is that these small

lesions are likely benign or intermediate risk and will show limited to no growth or metastasis. Moreover, the guidelines state that only 6% of NF-pNET ≤2cm would be malignant when incidentally discovered[43]. Data supporting surveillance, however, are controversial as it is based on a small number of retrospective cohort studies[44,45]. Correspondingly, a nationwide population based study in the Netherlands shows survival benefit for patients who underwent surgical resection for T1N0 pNET (**Chapter 2**).

For many clinicians, it remains unclear whether it is safe to adopt this rigorous change of treatment at the moment, as the same 2016 ENETS guidelines clearly state that additional data are needed to guarantee the safety of this policy. Besides the lack of reliable prospective data on small pNET, a number of important variables remain underexposed when opting for conservative approach. For example, it is well demonstrated that tumor grade and lymph node metastases could play a role in the prognosis of many pNET. Both variables are known to have a strong correlation with pNET recurrence after curative resection (**Chapter 4**), suggesting increased malignant behavior for G2 pNET and those with lymph node metastases. At the moment, it is not possible to determine the grade of a pNEN other than through pathological assessment. However, fine-needle aspiration (FNA), which is undesirable in small lesions because of the high risk of post-FNA pancreatitis, is not accurate in differentiating the grade of pNEN and frequently shows sampling error[46,47]. Other methods to estimate the malignant potential of pNET have not yet been described. As described above, the NETest might be able to offer a solution in estimating the behavior of pNET in order to determine the need for surgery.

The PANDORA study is a nationwide prospective cohort in which all patients with NF-pNET ≤2cm diagnosed in the Netherlands are registered and prospectively followed (**Chapter 8**). Indications for initial and later (changed) treatment strategies, tumor progression, quality of life and survival will be assessed. With this prospectively maintained database, in collaboration with the international ASPEN study of professor Falconi (ClinicalTrials.gov Identifier NCT03084770), we hope to answer the question if active surveillance in small, sporadic pNET may be a good alternative to surgical management and whether it is necessary to expose this select patient population to a high surgical risk. In contrast to the guidelines advising a conservative approach for small pNET, the PANDORA protocol presents a clear follow-up regimen for both patients who receive surveillance as well as patients who undergo a pancreatic resection. To prevent post-FNA

complications, diagnosis must be based on 2 imaging modalities, ideally a combination of anatomical imaging (i.e. CT or MRI) and nuclear imaging (preferably ^{68}GA-DOTATATE PET-CT), especially since the latter shows very high sensitivity in the detection of pNET in different studies[48,49]. Database analysis of patients who underwent surgical resection of a pancreatic mass in the Academic Medical Center Amsterdam showed that this method is safe as no adenocarcinoma was missed when the radiologist suspected pNET on imaging. Therefore, tumor tissue analysis for the diagnosis of pNET is only advised in those patients where there is doubt about the diagnosis on imaging. In addition to imaging, the NETest may have a role in the diagnosis of pNET and estimating the malignant potential of small tumors in the future.

Future perspectives

For some time, lack of knowledge has resulted in the delayed development of better treatment strategies for patients with localized pNET disease. Increasingly, it becomes clear how to assess the behavior of pNET and initiate therapy accordingly. To improve the postoperative outcomes of patients with well-differentiated pNET, a number of issues will have to be prioritized.

Firstly, there is a strong need for clear and uniform follow-up regimes, assuming survival benefit for the early detection and treatment of tumor recurrence. In Table 1 we propose such a protocol based on the expected risk profile of the patient, which can be determined by the updated Recurrence Score (**Chapter 4 and 5**) and the NETest (**Chapter 6**). Patients with a Recurrence Score of 24 or higher (meaning that one of the following

Table 1 - Proposed follow-up protocol after curative resection of well-differentiated pNET.

	Yearly follow-up	*Half-yearly follow-up*	*Minimum duration*
Low-risk patients - Ki67 0-5% - No lymph node metastases - No perineural invasion - NETest <40%	Clinical assessment NETest Imaging, alternating - CT/MRI - Nuclear imaging	↑NETest scores = follow-up as high-risk patients, until 3x stable NETest <40%	≥5 years
High risk patients - Ki67 ≥6% - Lymph node metastases - Perineural invasion - NETest ≥40%	Clinical assessment NETest Imaging, alternating - CT/MRI - Nuclear imaging	Clinical assessment NETest (additional imaging)	10 years

tumor characteristics must be present: pNET with Ki67> 6%, lymph node metastasis or perineural invasion) or with a NETest score> 40% will be considered as high-risk, while those with a Recurrence Score below 24 and a NETest <40% will be considered low-risk patients. For all patients, yearly consultations with clinical assessments, laboratory tests (NETest) and imaging is advised for the first 5 years. Ideally, imaging is alternated between radiologic and somatostatin receptor imaging to achieve the highest accuracy. Half-yearly hospital visits with laboratory tests are advised for high-risk patients and low-risk patients with increasing NETest scores. Based on the clinical findings and laboratory results, additional imaging may be obtained. For low-risk patients a minimum follow-up duration of 5 years is advised, while a follow-up of at least 10 years is encouraged for high-risk patients as late recurrences have been described[14]. Based on the median time to recurrence, the interval between hospital-visits can be increased if there are no signs of pNET recurrence after 5 years. By implementing this protocol, patients will be monitored in a more structured way, resulting in early detection and treatment of recurrence. Conceivably, this will lead to improved survival of curatively treated patient and at the same time provide the data necessary to demonstrate the beneficial effect of early detection of recurrence.

The restricted availability and limited data due to the rare nature of this disease is another reason for the slow development of optimal treatment strategies. Proper international agreements on disease stage, grade and treatment, patient selection and study design should therefore be another priority to obtain fast and reliable data from well-executed studies. During the writing of this thesis it became apparent that many studies use different definitions, for example to define the appropriate patient selection. Heterogeneity is a known problem for NEN in general, making a strict patient selection all the more important to be able to draw useful conclusions from studies. The same was seen for the definition of recurrence, resulting in data not being comparable and availability of already limited data being even further restricted. Initiation of prospective studies on pNET disease is challenging, given the low patient numbers and scattered presentation in different centers. However, well-designed and executed prospective studies will ultimately result in the availability of more reliable data in the shorter term. Although such studies are often time consuming, less reliable studies, often carried out retrospectively with poorly comparable data, lead to unreliable outcomes, discussion and noise. Therefore, we want to advocate for clear

agreements, not only to optimize the treatment of patient with pNET but also to optimize scientific research and obtain useful results.

Lastly, with the current knowledge and results presented in this thesis, it is no longer possible to deny that recurrence occurs regularly and that patients with a high-risk can be identified. In addition to intensified follow-up, these patients might also benefit from adjuvant treatment. Several systemic and local ablative treatment options are known for NEN, however data on the effect of these treatments only exists for patient with disseminated disease. The use of these treatments on patients without detectable tumor load is unknown, making it difficult to set up studies to investigate the role of adjuvant therapy. To overcome some of these obstacles, a collaboration with a large group of international experts in pNET treatment has been initiated. This consensus study resulted in 11 comprehensive recommendations to encourage clinical research on adjuvant therapy and facilitate the development and coordination of relevant clinical trials with support from experts in the field (**Chapter 7**). A randomized controlled trial investigating the recurrence preventive effect of chemotherapy with a regimen of capecitabine and temozolomide is advised. When the availability or Peptide Receptor Radionuclide Therapy (PRRT) improves, the advice is to reconsider this technique as adjuvant treatment. With these recommendations, it will be possible to initiate a study on the short term and it is expected that the first results on the effects of adjuvant therapy can become evident in a few years.

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Appendices

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SUMMARY

Pancreatic neuroendocrine tumors (pNET) are rare neoplasms with an increasing incidence due to the widespread use of cross-sectional radiological imaging and expanding knowledge. Reliable and well executed studies with an adequate patient selection are scarce, mostly because to the limited availability of data. As a result, the survival of patients diagnosed with pNET does not seem to increase. To improve the postoperative outcome of pNET it is important to understand the survival of patient who have undergone a surgical resection and identify those patients who have a high risk to develop recurrence. Clear and uniform follow-up recommendations are warranted in order to detect and treat recurrence early. This thesis provides insight into the pre- and postoperative management of patients with well-differentiated pNET (low-grade G1 and moderate-grade G2 tumors) without distant metastases or hereditary syndromes.

Because studies on rare diseases often contain small patient numbers, we have performed a large population-based study on the treatment and related survival of pNET. In **Chapter 2**, patients diagnosed with pNET in the Netherlands between 2008 and 2013 were identified from the Netherlands Cancer Registry database. The overall survival did not change during this period. Metastatic disease was seen in approximately half of the patients. Higher Tumor-Node-Metastasis (TNM) stage and tumor grade were significantly associated with worse survival. The majority of patients received surgical treatment and the number of patients who underwent a pancreatic resection increased significantly in time. Surgical resection showed favorable outcome in almost all subpopulations. For patients with the most indolent tumors (T1M0, meaning pNET <2cm without distant metastases) a significant survival benefit was seen after surgical resection compared to patients who did not receive treatment. In addition, patients with distant metastases showed significant better survival after resection of the primary tumor. At the moment, international guidelines advise surgical resection for localized pNET >2cm of size. These results suggest that patients with small tumors and those with distant metastases might also benefit from surgical resection, despite current recommendations.

Recurrence after curative resection of well-differentiated pNET has been assumed to be uncommon, because of the indolent nature of neuroendocrine tumors in general. Different recurrence rates have been reported, whereby

the importance of disease relapse remains unclear and is not always recognized. **Chapter 3** presents the results of a systematic review and meta-analysis of the existing literature to provide reliable recurrence rates for this patient population. Strict inclusion criteria were used to minimize heterogeneity, a known and common problem in pNET research. Eight studies, comprising 734 patients, met the inclusion criteria. Recurrence after curative resection of G1 or 2 pNET was seen in 9-18% after a mean time of 39.4 months. Subgroup analyses showed significant lower recurrence rates for patients with G1 tumors (8%) and R0-resentions (10%). Distant metastases, predominantly in the liver, were seen more often than local recurrence and regional lymph nodes metastases. Survival after recurrence was poor, with a mean of 38.9 months. Factors reported to be associated with recurrence include tumor size, grade, lymph node metastases, perineural invasion and R1 resection. From these results, we conclude that recurrence after curative resection of well-differentiated pNET is not rare and has serious consequences for the survival.

Identification of patients who have a high risk of recurrence may aid postoperative treatment and the development of both follow-up regimens and studies on adjuvant therapy. In **Chapter 4** we describe the development of an easy tool to estimate the risk of recurrence in patients who underwent a curative resection of well-differentiated non-functioning pNET (NF-pNET). In a large cohort of 211 patients, recurrence was seen in 17% after a median time of 43 months. Patients who developed recurrence showed significant lower survival rates compared to patients without recurrence. Recurrence-free survival curves showed that recurrence was predominantly seen in the first 5 years after resection. Predictors for recurrence were G2, lymph node metastasis and perineural invasion, corresponding with the literature. The recurrence-score was developed on the basis of these predictors. In this scoring system, patients are awarded points based on the presence or absence of each of the predictive factors. The total points correspond to the probability to develop recurrent disease within 5 years after surgery. Internal validation showed good discriminative ability of the recurrence-score that was higher compared to the grading classification of the World Health Organization (WHO). A cut-off of 24 points was determined as the appropriate score to classify patients as high or low risk of recurrence, with a sensitivity of 91% and specificity of 62%. Patients who were considered low-risk (recurrence-score <24) showed a significantly better ten-year disease specific survival compared to patients who had a high-risk in developing

recurrence (recurrence-score ≥24). In the future, the recurrence-score can be used to customize follow-up regimens on the basis of the risk profile of patients who underwent a curative resection of G1 or G2 NF-pNET.

Prognosis of pNET is highly dependent on tumor grade, which is determined by the Ki67 proliferation index. Despite this knowledge, postoperative follow-up is the same for G1 and G2 tumors and adjuvant therapy is currently not indicated. There has been debate about the wide range of Ki67 within the different grades, resulting in a recent update of the grading system for high-grade tumors. In **Chapter 5** we hypothesized that pNET with a Ki67 <20% (low and moderate grade pNET) are a heterogeneous group of tumors with different postoperative disease course as well, and investigated the role of Ki67 in predicting recurrence and survival after curative resection. In a large cohort was seen that tumors with Ki67 6-20% recurred significantly more often compared to tumors with Ki67 0-5%. Furthermore, tumors with Ki67 6-20% significantly more often showed unfavorable tumor characteristics compared to tumors with Ki67 0-5%. The Ki67 cut-off of 5% also showed adequate discriminative abilities with the highest sensitivity and specificity for predicting recurrence. Although the discriminative ability of this proposed Ki67 cut-off was comparable to that of the WHO grading classification, an additive value was seen as 10% of patients were correctly re-classified based on their risk of recurrence with this new method. In addition to Ki67 >5%, tumor larger than 4 cm and lymph node metastases were independently associated with recurrence. These results can be used to strengthen the recurrence-score presented in **Chapter 4**; by updating the criteria of 'G2 tumors' to 'tumors with Ki67>5%' it will be possible to identify high-risk patients more accurately. Moreover, patients with Ki67 3–5% (approximately 15% of our cohort) will be downgraded by this modification, limiting unnecessary treatment or monitoring for these patients.

Current guidelines recommend Chromogranin A (CgA) as the biomarker to evaluate tumor recurrence during follow-up. However, the clinical utility of CgA is questionable, given its moderate accuracy and regular false-positive values. Recently the NETest was presented, a liquid biopsy strategy showing a sensitivity and specificity of >93% for the detection of neuroendocrine tumors in the gastrointestinal tract. In **Chapter 6** we investigated the prognostic accuracy of the NETest, compared to CgA and other known clinical criteria associated with pNET recurrence, to determine its usefulness during postoperative follow-up after curative resection of pNET. In this

prospective surgical cohort study, the NETest was significantly higher in patients with recurrence compared to patients without recurrence or R1 resections. CgA did not show different values between these groups and showed no positive correlation to recurrence in several analyses. The NETest was an independent predictor for recurrence, together with grade, lymph node metastases and tumor size. Combining these three clinicopathological characteristics with the NETest showed an accuracy of 91% in predicting recurrence, and was almost 2x more effective than clinicopathological characteristics alone. In conclusion, the NETest outperforms CgA as a biomarker to guide postoperative follow-up and facilitates the effective identification of high-risk patients, especially when combined with the recurrence-score.

Accurate identification of high-risk patients provides the possibility to customize postoperative treatment. Adjuvant therapy is currently not indicated but might be beneficial to prevent recurrence after curative treatment. Lack of data regarding this disease, in particular following curative resection, presents a major obstacle to designing studies on the role of adjuvant therapy in high-risk patient. **Chapter 7** presents the results of a consensus study, a collaboration between multiple international experts in the treatment of pNET. The aim was to overcome some of these obstacles and provide recommendations to encourage clinical research on adjuvant therapy in pNET. All participating experts considered the risk of recurrence after curative resection of concern and agreed that there is a need for research on this topic. A randomized-controlled trial, both arms including high-risk patients identified with the modified version of the recurrence-score, is advised as the optimal study design. The board availability and general good tolerability have led to the decision to advise chemotherapy with a regimen of capecitabin and temozolomide (CapTem) to serve as adjuvant therapy. However, the study group notes that Peptide Receptor Radionuclide Therapy (PRRT) shows promising results and will have to be reconsidered when availability has increased. Power calculations based on the reported response rates of CapTem in patients with advanced disease show that a multicenter approach seems inevitable to acquire the number of patients necessary to achieve decent and reliable results.

For long, surgical resection was the gold standard for pNET without distant metastases. Nevertheless, recent studies report little to no progression of small pNET, suggesting excellent prognosis and low morbidity when

refrained from surgery. Based on these results the European Neuroendocrine Tumors Society (ENETS) recently updated their guidelines, recommending a wait-and-see approach for small non-functioning tumors. Because these developments are supported by a small number of retrospective studies, the PANDORA study has been introduced: a prospective cohort of all patients diagnosed with non-functioning pNET <2cm in the Netherlands. The study protocol is presented in **Chapter 8**. The aim is to investigate the long-term effects of non-operative management of small pNET. Tumor progression, reasons to refrain from the guidelines and quality of life will be analyzed. Patient and tumor characteristics, as well as long-term outcomes of patients who receive conservative treatment will be compared against those who undergo (initial/delayed) surgery. With this study, we hope to assess the need for surgical resection with regards to optimal treatment outcome within considerable time.

SAMENVATTING

Neuroendocriene tumoren van het pancreas (pNET) zijn zeldzame neoplasmata met een groeiende incidentie, met name door het toenemende gebruik van geavanceerde radiologische beeldvorming en onze stijgende kennis over dit ziektebeeld. Grote, betrouwbare studies met adequate patiënten selecties zijn schaars, veelal door de lage incidentie. Hierdoor bestaat het beeld bestaat dat de overleving van patiënten met een pNET niet lijkt toe te nemen. Om de postoperatieve uitkomsten te verbeteren is het van groot belang inzicht te krijgen in de overleving van patiënten met een pNET die een chirurgische resectie hebben ondergaan en vervolgens die patiënten te identificeren die een grote kans hebben op het krijgen van een recidief. Duidelijke en uniforme follow-up adviezen zijn noodzakelijk om recidieven vroeg te ontdekken en te behandelen. Dit proefschrift beoogt inzicht te geven in de pre- en postoperatieve zorg van patiënten met goed-gedifferentieerde pNET (laag tot middelmatig gegradeerde G1 en G2 tumoren) zonder afstandsmetastasen of erfelijke syndromen.

Omdat studies met zeldzame ziektebeelden vaak kleine patiënten aantallen bevatten, hebben we een grote populatie-studie verricht naar de behandeling gerelateerde overleving van patiënten met een pNET. Met behulp van de database van de Nederlandse Kanker Registratie (NKR) werden alle patiënten, gediagnosticeerd met pNET tussen 2008 en 2013 in Nederland, geïdentificeerd. In **Hoofdstuk 2** wordt het beloop van deze patiëntgroep beschreven. Het blijkt dat gedurende deze periode de algehele overleving van de populatie niet toenam. Ongeveer de helft van de patiënten werd gediagnosticeerd met afstandsmetastasen. Een hogere Tumor-Klier-Metastase (TNM) classificatie en tumor gradering bleken significant geassocieerd met een slechtere overleving. De meerderheid van de patiënten onderging chirurgische resectie, waarbij het aantal geopereerde patiënten significant toenam in de tijd. Chirurgische resectie liet betere uitkomsten zien in bijna alle subpopulaties. Bij patiënten met de een tumor van de laagste classificatie, een pNET <2cm zonder afstandsmetastasen (T1M0), werd er een overlevingswinst gezien wanneer zij een resectie hadden ondergaan in vergelijking met patiënten die geen behandeling ontvingen. Ook patiënten met afstandsmetastasen lieten een betere overleving zien na resectie van de primaire tumor. Op dit moment wordt chirurgische resectie alleen geadviseerd voor niet-gemetastaseerde pNET >2cm. Onze resultaten stellen dat niet alleen bij patiënten met kleine tumoren maar ook patiënten met

afstandsmetastasen ten tijde van de diagnose een operatie moet worden overwogen, ondanks de huidige richtlijn.

Over het algemeen wordt verondersteld dat recidieven van goed-gedifferentieerde pNET niet veel voorkomen, gezien het indolente karakter van deze tumoren. Omdat er verschillende recidief percentages worden beschreven hebben wij in **Hoofdstuk 3** een systematische review en meta-analyse van de bestaande literatuur uitgevoerd om duidelijkheid te scheppen over de ware frequentie van recidieven in deze patiëntenpopulatie. Strikte inclusiecriteria werden gehanteerd om heterogeniteit te minimaliseren, een bekend probleem in pNET onderzoek. Acht studies, bestaande uit 734 patiënten, voldeden aan de inclusiecriteria. Recidief na curatieve resectie van G1 of G2 pNET werd gezien in 9-18%, na een gemiddelde periode van 39,4 maanden. Subgroep analyse liet zien dat patiënten met G1 tumoren (8%) en R0-resecties (microscopisch radicaal) (10%) significant minder vaak een recidief ontwikkelden. Afstandsmetastasen, met name in de lever, werden vaker gezien dan lokale recidieven of metastasen in regionale lymfeklieren. De overleving na een recidief bleek beperkt, met een gemiddelde overlevingsduur van 38,9 maanden. Onafhankelijke factoren die werden genoemd als voorspellers voor het ontwikkelen van een recidief waren tumor grootte, gradering, lymfeklier metastasen, perineurale invasie en irradicale resecties. Uit deze studie concluderen wij dat recidieven na curatieve resectie van goed-gedifferentieerde pNET niet zeldzaam zijn en derhalve niet moeten worden onderschat.

Het identificeren van patiënten met een hoog risico op een recidief kan het postoperatieve behandelproces sturen en de ontwikkeling van zowel follow-up richtlijnen als adjuvante studies ondersteunen. In **Hoofdstuk 4** beschrijven wij de ontwikkeling van een handig hulpmiddel om het risico op een recidief in te schatten voor patiënten die een curatieve resectie hebben ondergaan van een niet-functionerende pNET (NF-pNET). In een groot cohort van 211 patiënten werden recidieven in 17% gezien, na een mediane periode van 43 maanden. Patiënten die een recidief ontwikkelden, hadden een significant slechtere overleving ten opzichte van patiënten zonder recidief. Recidief-vrije overlevingscurves lieten zien dat het overgrote deel van de recidieven zich in de eerste 5 jaar na operatie openbaarden. Onafhankelijke voorspellers voor een recidief waren G2 tumoren, lymfeklier metastasen en perineurale invasie, zoals ook in de literatuur wordt beschreven. Met deze voorspellers werd de ‘Recurrence Score’ ontwikkeld. In dit scoringssysteem krijgen patiënten

punten voor de aan- of afwezigheid van deze tumor karakteristieken, waarbij de totale score correspondeert met de geschatte kans op het ontwikkelen van een recidief binnen 5 jaar na operatie. Interne validatie liet een goed onderscheidend vermogen van de Recurrence Score zien, welke hoger was dan die van het graderingssysteem van de Wereld Gezondheids Organisatie (WHO). Een afkapwaarde van 24 punten bleek het meeste geschikt om hoog- en laag-risicopatiënten te onderscheiden, met een sensitiviteit van 91% en specificiteit van 62%. Patiënten met een laag risico op recidief (Recurrence Score <24) hadden een significant betere overleving dan patiënten met een hoog risico op recidief (Recurrence Score $\geq$24). In de toekomst kan de Recurrence Score gebruikt worden om de postoperatieve follow-up na curatieve resectie van een G1 of G2 NF-pNET aan te passen op basis van het risico om een recidief te ontwikkelen. Hoog-risicopatiënten zouden baat kunnen hebben bij intensieve follow-up of adjuvante therapie om recidieven te voorkomen.

Verschillende studies laten zien dat de prognose van pNET sterk afhankelijk is van de tumor gradering. Deze wordt bepaald op basis van de Ki67 proliferatie-index, waarbij G1 tumoren de laagste proliferatie-index hebben en G3 de hoogste. Ondanks deze kennis is de postoperatieve follow-up hetzelfde voor patiënten met G1 tumoren en patiënten met G2 tumoren, onafhankelijk van de Ki67 proliferatie-index, en is adjuvante therapie (nog) niet geïndiceerd. Er is veel discussie over de spreiding van Ki67 binnen de verschillende graderingen, resulterend in een recente update van het graderingssysteem voor hooggradige tumoren. In **Hoofdstuk 5** is de hypothese dat pNET met Ki67 <20% (niet hooggradige pNET) ook heterogeen zijn en een verschillend postoperatief ziektebeloop hebben. In dit hoofdstuk hebben wij de rol van de Ki67 index onderzocht in het voorspellen van recidieven en de overleving na curatieve resectie van G1/2 pNET. In een groot cohort werd gezien dat tumoren met Ki67 6-20% significant vaker een recidief ontwikkelde dan tumoren met Ki67 0-5%. Bovendien hadden pNET met Ki67 6-20% significant vaker ongunstige tumor karakteristieken. De Ki67 afkapwaarde van 5% had een redelijk onderscheidend vermogen, met de hoogste sensitiviteit en specificiteit voor het voorspellen van een recidief. Hoewel het onderscheidende vermogen van dit model vergelijkbaar was met die van het bestaande WHO-graderingssysteem, werd een toegevoegde waarde gezien met re-classificatie van 10% van de patiënten op basis van het risico op het ontwikkelen van een recidief. Naast een Ki67 >5% bleek ook tumor grootte >4cm en lymfeklier metastasen onafhankelijke voorspellers

voor een recidief. Deze resultaten dragen bij aan het versterken van de Recurrence Score, zoals beschreven in **Hoofdstuk 4**. Door het updaten van de criteria voor 'G2 tumoren' naar 'tumoren met Ki67 >5%' is het mogelijk geworden om accurater hoog-risicopatiënten te identificeren. Bovendien worden patiënten met Ki67 3-5% (ongeveer 15% in ons cohort) gedegradeerd naar laag risico, waardoor overbehandeling kan worden beperkt.

De huidige richtlijnen adviseren Chromogranine A (CgA) als de biomarker voor het beoordelen van pNET recidieven tijdens de postoperatieve follow-up. Echter, de klinische toepasbaarheid is twijfelachtig gezien de beperkte accuraatheid en frequent vals-positieve resultaten. Recentelijk is de NETest geïntroduceerd, een zogeheten 'liquid biopsy' (vloeibaar biopsie) techniek, die een sensitiviteit en specificiteit van >93% laat zien voor het detecteren van neuroendocriene tumoren in het gastrointestinale systeem. In **Hoofdstuk 6** onderzochten wij de prognostische waarde van de NETest, in vergelijking met CgA en andere bekende tumor karakteristieken die geassocieerd zijn met recidief, om de mogelijke bruikbaarheid in de postoperatieve follow-up van patiënten met een pNET te bepalen. In deze prospectieve studie was de NETest significant verhoogd in patiënten met een recidief in vergelijking met patiënten zonder recidief of irradicale resecties. CgA liet geen verschillen zien binnen deze groepen en toonde in verschillende analyses geen positieve correlatie met recidief. De NETest bleek een onafhankelijke voorspeller voor recidief, samen met tumor gradering, lymfeklier metastasen en tumor grootte. Wanneer deze tumor karakteristieken werden gecombineerd met de NETest werd er een 91% accuraatheid bereikt voor het voorspellen van een recidief, bijna 2 keer hoger dan de tumor karakteristieken alleen. Concluderend is de NETest te verkiezen boven CgA als tumormarker en ondersteunt de NETest een effectieve identificatie van hoog-risicopatiënten, in het bijzonder wanneer het wordt gecombineerd met de Recurrence Score.

Identificatie van hoog-risicopatiënten biedt de mogelijkheid om de postoperatieve follow-up aan te passen. Adjuvante behandeling is momenteel niet geïndiceerd, maar zou kunnen bijdragen in het voorkomen van een recidief na curatieve resectie. Het gebrek aan data over dit ziektebeeld, in het bijzonder na curatieve resectie, vormt een grote belemmering voor het ontwikkelen van studies die de rol van adjuvante behandeling in hoog-risicopatiënten onderzoeken. **Hoofdstuk 7** beschrijft de resultaten van een consensus studie, een samenwerking tussen

verschillende internationale experts in de behandeling van pNET. Het doel was om een deel van de belemmeringen weg te nemen en aanbevelingen te geven om klinisch onderzoek met adjuvante therapie in patiënten met een pNET aan te moedigen. Alle deelnemende experts hebben het risico op het ontwikkelen van een recidief na curatieve resectie onderkend en waren het erover eens dat er behoefte is aan onderzoek op dit gebied. Een gerandomiseerde studie werd geadviseerd, met in beide armen hoog-risicopatiënten geïdentificeerd met de gemodificeerde versie van de Recurrence Score. De brede beschikbaarheid en goede tolerantie heeft geleid tot de beslissing om chemotherapie met capecitabine en temozolomide (CapTem) te adviseren als adjuvante behandeling. Echter, de studiegroep benadrukt dat ook Peptide Receptor Radionuclide Therapie (PRRT) veelbelovende resultaten laat zien en adviseert deze te heroverwegen wanneer de beschikbaarheid van deze behandeling is toegenomen. Power berekeningen op basis van gepubliceerde resultaten van CapTem in patiënten met een vergevorderde ziekte laten zien dat een multicenter benadering noodzakelijk is om adequate patiënten aantallen te verzamelen en betrouwbare resultaten te bereiken.

Lange tijd was chirurgische resectie de gouden standaard voor pNET zonder afstandsmetastasen. Recentelijke studies beschrijven echter nauwelijks tot geen progressie met een excellente prognose en lage morbiditeit voor kleine pNET, wanneer van opereren wordt afgezien. Op basis van dergelijke resultaten heeft de European Neuroendocrine Tumor Society (ENETS) recentelijk zijn richtlijnen aangepast, waarbij een afwachtend beleid voor kleine niet-functionerende tumoren wordt geadviseerd. Omdat dit advies op een kleine aantal retrospectieve studies is gebaseerd, is de PANDORA-studie opgezet: een prospectief cohort van alle patiënten met een niet-functionerende pNET <2cm in Nederland. Het studieprotocol wordt gepresenteerd in **Hoofdstuk 8**. Het doel is om de lange-termijn effecten van deze niet-operatieve behandeling van kleine pNET te onderzoeken. Tumor progressie, redenen om af te zien van de huidige richtlijnen en kwaliteit van leven zullen worden geanalyseerd. Patiënt- en tumor karakteristieken van patiënten die een conservatieve behandeling ondergaan zullen worden vergeleken met patiënten die een primaire resectie ondergaan. Met deze studie hopen wij binnen afzienbare tijd de noodzaak voor chirurgische resectie in relatie tot een optimaal behandelresultaat te kunnen bepalen.

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Dr. A.M.E. Walenkamp – Department of Medical Oncology, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands.

LIST OF PUBLICATIONS

Measurement of circulating transcript Levels (NETest) to detect disease recurrence and improve follow-up after curative surgical resection of well-differentiated pancreatic neuroendocrine tumors.
C.G. Genç, A.P.J. Jilesen, I. Drozdov, A. Malcsewska, H.J. Klümpen, C.H.J. van Eijck, E.J.M. Nieveen van Dijkum, M. Kidd, I. Modlin.
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Recurrence of pancreatic neuroendocrine tumors and survival predicted by Ki67.
C.G. Genç, M. Falconi, S. Partelli, F. Muffatti, S. van Eeden, C. Doglioni, H.J. Klümpen, C.H.J. van Eijck, E.J.M. Nieveen van Dijkum.
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A nationwide population-based study on the management and survival of pancreatic neuroendocrine tumors in the Netherlands.
C.G. Genç, H.J. Klümpen, M. van Oijen, C.H.J. van Eijck, E.J.M. Nieveen van Dijkum.
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C.G. Genç, A.P.J. Jilesen, S. Partelli, M. Falconi, F. Muffatti, F.J. van Kemenade, S. van Eeden, J. Verheij, S. van Dieren, C.H.J. van Eijck, E.J.M. Nieveen van Dijkum.
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B. Edwin, M. A. Sahakyan, M.A. Hilal, M.G. Besselink, M. Braga, J.M. Fabre, L. Fernández-Cruz, B. Gaye, S.C. Kim, I.E. Khatkov and EAES Consensus Conference Study Group.
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Mol. Psych. 2014. PMID: 23999526

PhD PORTFOLIO

Promotor:	Prof. dr. C.H.J. van Eijck
Copromotor:	Dr. E.J.M. Nieveen van Dijkum
PhD period:	2015 - 2017

1. PhD training

	Year	*Workload (ECTS)*
Courses		
The AMC World of Science	2016	0.7
Practical Biostatistics	2016	1.1
Scientific Writing in English for Publication	2016	1.5
Clinical Data Management	2016	0.2
Scientific Writing in English for Publication	2016	1.5
Evaluation of Medical Tests	2016	0.6
Observational Epidemiology	2017	0.9
Systematic Review Writing	2017	0.7
Seminars, workshops and master classes		
5th IPSEN Masterclass Neuroendocrine Tumors	2015	1.0
Post-graduate course European Neuroendocrine Tumor Society (ENETS)	2016	0.5
Weekly Department Seminars	2015-2017	3.5
Journal Club	2015-2017	3.0
Oral presentations		
Management and survival of pancreatic neuroendocrine tumors in the Netherlands.		
World Congress of Surgery, Basel, Switserland.	2017	0.5
European-African HPB Association, Mainz, Germany.	2017	0.5
Nomogram to predict recurrent disease in grade 1 and 2 non-functional pancreatic neuroendocrine tumors.		
Chirurgendagen, Veldhoven, the Netherlands.	2016	0.5
European Society for Surgical Research, Prague, Czech Rp.	2016	0.5
Poster presentations		
The clinical value of circulating transcript analysis (NETest) during follow-up of resected well-differentiated pancreatic neuroendocrine tumors.		
Annual Conference of the European Neuroendocrine Tumors Society, Barcelona, Spain.	2018	0.5

Management and survival of pancreatic neuroendocrine tumors in the Netherlands.		
European Pancreatic Club, Liverpool, England.	2017	0.5
European Neuroendocrine Tumor Society, Barcelona, Spain.	2017	0.5
European-African HPB Association, Mainz, Germany.	2017	0.5
World Congress of Surgery, Basel, Switserland	2017	0.5
Ki67 to Predict Recurrence and Survival of Pancreatic Neuroendocrine Tumors.		
Annual Conference oft he European Neuroendocrine Tumors Society, Barcelona, Spain	2017	0.5
Meta-analysis of recurrence after curative surgery for pancreatic neuroendocrine tumors.		
Annual Conference of the European Neuroendocrine Tumors Society, Barcelona, Spain.	2017	0.5
European-African HPB Association, Mainz, Germany.	2017	0.5
American Association of Endocrine Surgeons, Orlando, USA.	2017	0.5
European Pancreatic Club, Budapest, Hungary.	2017	0.5
Nomogram to predict recurrent disease in grade 1 and 2 non-functional pancreatic neuroendocrine tumors.		
European Society for Surgical Research, Prague, Czech Republic	2016	0.5
Chirurgendagen, Veldhoven, the Netherlands.	2017	0.5
European Pancreatic Club, Liverpool, Engeland.	2017	0.5
European Neuroendocrine Tumor Society, Barcelona, Spain.	2017	0.5
European-African HPB Association, Mainz, Germany.	2017	0.5

(Inter)national conferences

Chirurgendagen, Veldhoven, the Netherlands	2016-2018	4.5
European Neuroendocrine Tumor Society, Barcelona, Spain.	2016-2017	4.0
European-African HPB Association, Mainz, Germany.	2017	2.0
World Congress of Surgery, Basel, Switzerland.	2017	2.0
American Association of Endocrine Surgeons, Orlando, USA	2017	2.5
European Society for Surgical Research, Prague, Czech Republic.	2016	1.5

2. Teaching

	Year	*Workload (ECTS)*
Lecturing		
Writing a scientific abstract; Bachelor Medicine, University of Amsterdam.	2018	0.5
Tutoring/supervising		
E. Ronde, bachelor student AMC	2017	1.0
T. Engel, Medical Informatics Student HvA	2017	1.0
T.M. Mackay, honors student AMC	2016	1.0

Other

NETwerkgroep AMC	2015-2017	1.0
Reviewer for the Amsterdam Medical Students Journal	2016-2017	1.0
Research Fellowship WREN Laboratory, USA	2017	4.0
Organization NET Patient Day AMC	2016	0.5

1. Parameters of Esteem

	Year
Grants, Awards and Prizes	
Lisa Waller Hayes Foundation Research Grant	2017
IPSEN Science Travel Award	2017
Top 10 poster award winner AAES Annual Meeting	2017

DANKWOORD

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CURRICULUM VITAE

Cansu Genç was born in Ankara on March 13th 1986. She moved to the Netherlands at age 6, where she lived in Hilversum. After graduating from the Gemeentelijk Gymnasium in Hilversum in 2004 she studied Psycho-Biology at the University of Amsterdam. In 2005 she was accepted at the Erasmus University in Rotterdam to study Medicine, which she combined with a master in Neuroscience. After her graduation in 2013, she started working as a medical doctor at the department of Surgery at Zaans Medisch Centrum under the supervision of dr. F.C. den Boer. The graduation thesis for her master Neuroscience was her introduction into scientific research and led to an ambition to complete a PhD fellowship. In 2015 she started her current PhD fellowship on pancreatic neuroendocrine tumors at the department of Surgery at the Erasmus Medical Center in Rotterdam under the supervision of prof. dr. C.H.J van Eijck and the department of Surgery at the Academic Medical Center in Amsterdam under supervision of dr. E.J.M. Nieveen van Dijkum. From 2015 to 2017, she managed to complete multiple well received studies which formed the basis for national as well as international protocolisation of the treatment of pancreatic neuroendocrine tumors. Currently she is working as a surgical resident at the Spaarne Gasthuis under supervision of dr. H. Rijna, while still actively involved in her field of research. In January 2019 she will start her surgical training at the Amsterdam University Medical Centers – VU Medical Center.