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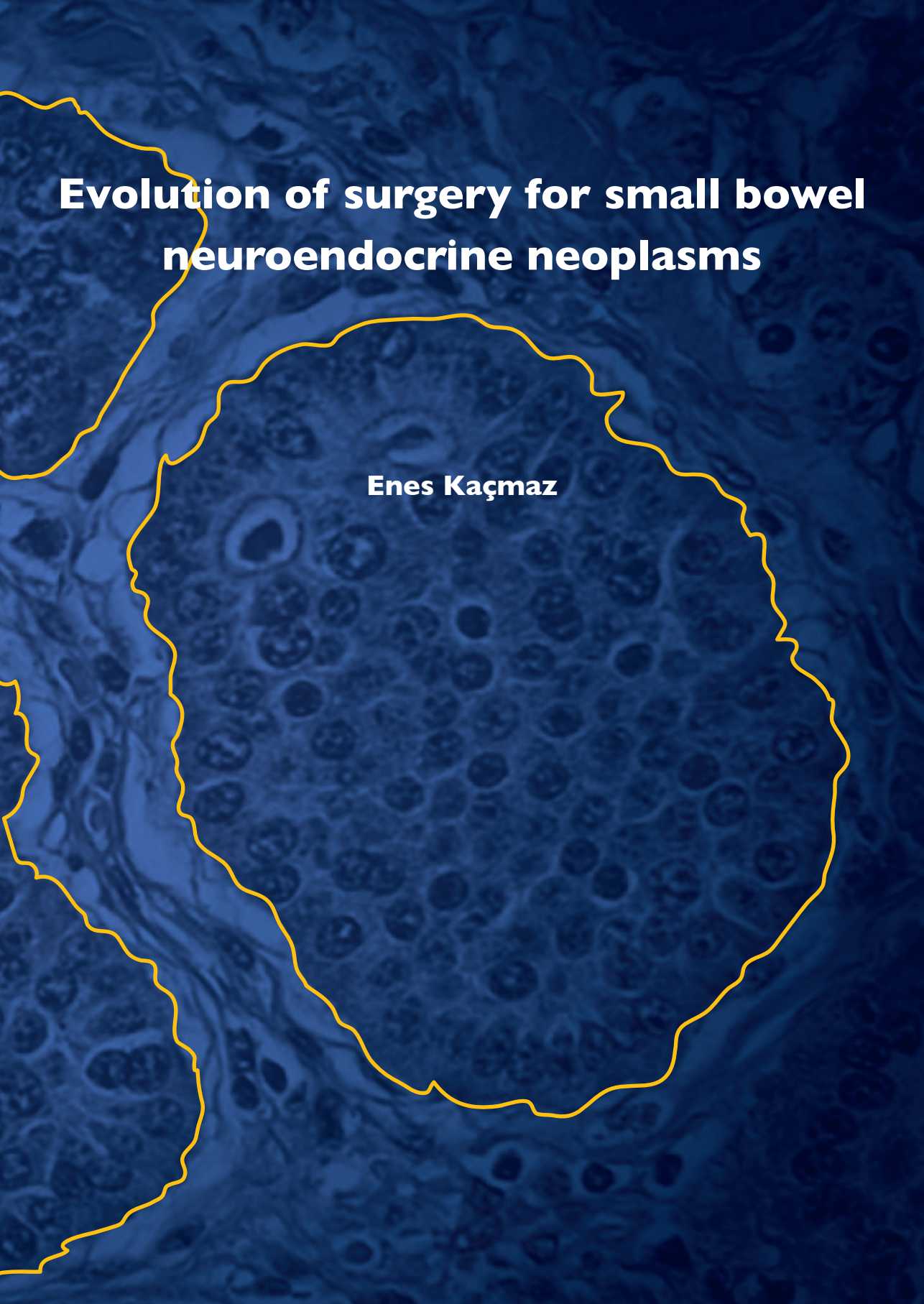

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Enes Kaçmaz

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This thesis was written at the Department of Surgery, Amsterdam UMC, University of Amsterdam, the Netherlands.

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Evolution of surgery for small bowel neuroendocrine neoplasms

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GENERAL INTRODUCTION AND THESIS OUTLINE

The human body is estimated to consist of 37.2 trillion cells (37.200.000.000.000), with a variety of cell types and functions [1]. Neuroendocrine cells are one of those: they are controlled by the brain and produce hormones which are able to travel throughout the body to exert a particular effect. Unfortunately, sometimes, these cells start replicating in an uncontrolled fashion, at which point a neuroendocrine neoplasm (NEN) develops. This thesis will focus on the most common amongst the rarest: small bowel neuroendocrine neoplasms (SB-NEN).

Clinical presentation

The clinical presentation of patients varies based on the location of the primary tumour, and whether it produces hormones. Consequently, the symptomatology varies as well, for example: vague abdominal pain, weight loss, obstructions, or hormone overproduction related symptoms such as vomiting, diarrhoea and hypoglycaemia [2, 3]. At presentation, approximately 40-50% of the patients have distant metastases [4, 5]. This could be explained by the vague symptomatology, resulting in a delay in diagnosis. The metastases are often located in the liver, before travelling across the body. Adequate treatment of patients with stage IV disease is of importance to establish favourable survival outcomes, including for example resection of liver metastases [4, 5].

Epidemiology

Gastroenteropancreatic neuroendocrine neoplasms (GEP-NEN) are a rare kind of malignancy located in the gastrointestinal tract, with an incidence of 860 newly diagnosed patients in 2020 in the Netherlands [6]. The most common localization of GEP-NEN is the small bowel, followed by the pancreas, colon and appendix. A recent study from 2017 found that the incidence of GEP-NEN was 3.56 per 100.000 persons per year in the United States [7]. Survival rates of SB-NEN are relatively high when compared to other cancer localizations. Patients with stage 4 SB-NEN have , median survival of 103 months and 5-year survival rate of 69% [7].

Hormonal activity

As stated earlier, NEN originate from neuroendocrine cells, and have the ability to excrete hormones. The types of hormones that are produced depend on the location at which the NEN develops (e.g. pancreatic NEN often produce insulin). SB-NEN and its metastases are known to excrete serotonin, a type of neurotransmitter with pro-fibrotic effects [8, 9]. Excessive excretion over a prolonged period might result in mesenteric fibrosis. Which is present in 40-55% of SB-NEN patients, and evidenced by a spoke-wheel appearance with radiating strands of soft tissue on imaging [10-12]. Extensive stranding creates multiple problems: (I) they can encase of mesenteric vessels, (II) exert traction on bowel segments, and (III) complicates surgical resection. Unfortunately, part of the patients presenting with abdominal pain and food intolerance are diagnosed at this stage [10, 12-14]. Previous research reported on the presence of IgG4 expressing plasma cells in mesenteric tumour deposits of SB-NENs [13]. This finding might be useful for future development of treatment strategies.

Surgical treatment

All surgical procedures come with a certain risk on complications, which often have a multifactorial etiology such as: setting (emergency/elective), hospital and surgeon volume, extent of disease and concomitant comorbidities [15]. One of the most feared post-operative complication in colorectal cancer surgery is the occurrence of anastomotic leaks, which is reported to occur in up to 30% of patients [16]. Anastomotic leaks do not only result in a longer hospital stay, but is on the long-term, associated with a negative impact on overall survival, cancer-specific survival, disease-free survival and recurrence rates [17]. The majority of patients with SB-NEN are amenable for surgical resection, even in presence of distant metastases [3]. Anastomotic leaks after SB-NEN are reported to occur in up to 2% of patients [18].

In the recent decades, minimally invasive surgery has gained more acceptance in the field of gastrointestinal surgery [19-21]. For colorectal cancer, minimally invasive surgery is in the short term associated with less blood loss, less pain, faster recovery of bowel function, faster return to normal diet and less wound infections, whereas on the long-term it is associated with a decreased risk of small bowel obstruction and incisional hernia without compromising local recurrence rates [22-27]. However, these procedures take a longer time to perform, and are associated with an additional learning curve process for surgeons. Although implementation for other indications is well established, implementation for SB-NEN is lagging behind. This could be explained by: scarce evidence on minimally invasive surgery for SB-NEN [28-32], restrictive advice from guidelines [33, 34], technical difficulties of this technique, and concerns of oncologic adequacy (i.e. removal of all malignant tissue). The technical difficulties are mainly related to lymph node metastases, as these extent to the mesenteric root and are present in more than 80% of patients [33]. To date, three studies comparing open and laparoscopic approach for SB-NEN were published [28, 31, 32]. None reported inferior outcome regarding lymphadenectomy or concerning short-term post-operative outcomes. Nevertheless, research in this feels appears to stagnate to some extent. Safe implementation of minimally invasive surgery for SB-NEN should therefore be seen a multistage rocket, in which multiple studies are necessary to establish the for minimally invasive surgery for this indication.

Fluorescence guided surgery

As stated before, mesenteric lymph node metastases form one of the biggest technical challenges in surgery for SB-NEN. Mesenteric lymph node metastases are present in more than 75% of patients and resection thereof is of particular importance for symptom prevention, locoregional control and survival [33, 35]. These lymph node metastases are not "just" lymph node metastases, as they are often associated with mesenteric fibrosis [36]. This is due to the neuroendocrine cells of which the lymph node metastases exist, and their excretion of serotonin with pro-fibrotic effects. (Extensive) fibrosis is often visible on computed tomography scans, with a spoke-wheel appearance. During surgical resection, dissection is often close to the mesenteric lymph nodes and blood vessels, which is a potential risk for healthy bowel segments. Fluorescence angiography using indocyanine green has been described as a method to assess perfusion during gastro-intestinal surgery [37]. This technique makes use of the binding property of indocyanine green to plasma proteins, which enables visualization of blood flow using a near-infrared camera.

At presentation, up to 40-50% of patients with NEN have distant metastases, of which the majority is located in the liver [4]. Presence of NELM is associated with a poor survival outcome and is associated with excessive hormone production [38]. Resection of NELM is associated with survival benefit. Therefore, resection should always be considered if this is technically possible. Intra-operative ultrasonography is a method to distinguish colorectal liver metastases from normal, healthy liver tissue [39]. As stated earlier, indocyanine green dye can be used to assess perfusion intra-operatively. However, intraoperative fluorescence imaging using indocyanine green dye is also able to identify previously unknown sub-centimetre colorectal liver metastases in up to 24% of patients [40]. After intravenous administration, indocyanine green accumulates in or around the malignant liver tissue [41]. This results in a homogeneous signal or fluorescent rim, depending on tumour morphology and differentiation [41]. One of the characteristics of neuroendocrine neoplasms is that they are hypervascular neoplasms. This feature is used by radiologists to identify lesion that are suspect for neuroendocrine origin, as these show uptake of contrast agents. Similarly, fluorescence angiography performed to assess perfusion could be used to visualize NELM.

Surgical resection of GEP-NEN shows best survival results compared to other modalities, with excellent 5-year overall survival ranging between 85-100% [3]. Important factors during surgical resection are to safely remove tumour tissue (i.e. without damaging healthy (vital) structures), whilst performing an oncologically adequate resection (i.e. complete removal of tumour tissue). Hence, accurate intra-operative identification of tumour tissue would be of great additional value. Currently, a variety of clinical trials are performed assessing safety and efficacy of several different fluorescent dyes for different target tissues [42]. In neuroendocrine neoplasms, the ^{68}Ga-DOTATATE PET-scan is used in order to localize (distant/primary) disease, making use of the overexpressed somatostatin type 2 receptors (SSTR2) on the cell membrane. Targeting SSTR2 results in a high sensitivity and specificity (>90%) of the ^{68}Ga-DOTATATE PET-scan [43].

AIM OF THIS THESIS

The aim of this thesis was to evaluate different aspects of surgical treatment of patients with neuroendocrine neoplasms (NEN), which included treatment of liver metastases, minimally invasive resection of primary tumours of the small bowel (SB-NEN), and the application of fluorescence guided surgery for NEN.

Thesis outline

This thesis is subdivided in three parts. **Part I** gives context and background for the following two parts: **Part II** focuses on surgical management of SB-NEN and **Part III** focuses on application of fluorescence guided surgery for NEN.

Part I. Disease characteristics of small bowel neuroendocrine neoplasms
Chapter 1 is a systematic review and meta-analysis of the available literature regarding different treatment modalities and its effect on survival outcome of neuroendocrine liver metastases (NELM). **Chapter 2** describes a retrospective nationwide cohort study investigating the epidemiology, treatment and survival characteristics of SB-NEN in the Netherlands, with the aim to present more recent data. In **Chapter 3** an exploratory study is presented on the association between the extent of mesenteric fibrosis on pre-operative diagnostic imaging and IgG4 expression in resected specimens.

Part II. Minimally invasive surgery for small bowel neuroendocrine neoplasms
Chapter 4 is a systematic review and meta-analysis of the available literature on post-operative morbidity and mortality after surgical resection of SB-NEN. We performed multiple studies which contributed to the multi stage rocket that is needed for implementation of minimally invasive surgery for SB-NEN. In **Chapter 5** an international survey study amongst surgeons who treat patients with SB-NEN is presented, in order to identify current practice and future perspectives regarding minimally invasive surgery for SB-NEN. **Chapter 6** is a retrospective cohort study which compared short-term post-operative outcomes between minimally invasive and open surgery of SB-NEN, independent from suspected lymph node involvement. Finally, **Chapter 7** is a retrospective nationwide cohort study which evaluated the surgical approach for SB-NEN at a national level considering selection based on patient and tumour characteristics, and identified independent predictors of overall survival.

Part III. Fluorescence guided surgery of neuroendocrine neoplasms
We hypothesised that the use of fluorescence angiography during surgical resection of SB-NEN would be of added value, especially due to the central location and fibrosis associated with mesenteric lymph node metastases. In **Chapter 8** an exploratory study is present which investigated the potential value and post-operative outcomes of intraoperative fluorescence angiography during surgical resection of SB-NEN. Similarly, we hypothesised that fluorescence angiography would be of use for resection of NELM. We performed an exploratory study which investigated the potential value of fluorescence guided surgery using indocyanine green during surgical resection of NELM, which is presented in **Chapter 9. Chapter 10** is the study protocol of the PHOTON trial, which will assess the (pre-) clinical safety, pharmacology and efficacy of a SSTR2 targeted fluorescent tracer.

RESEARCH QUESTIONS ADRESSED IN THIS THESIS

Chapter	Research questions
1	Which treatment modality results in longest overall survival in patients with liver metastases from gastroenteropancreatic neuroendocrine neoplasms?
2	What are the epidemiological, treatment and survival characteristics of patients with grade 1 and 2 small bowel neuroendocrine neoplasms?
3	What is the relationship between immunoglobulin G4 expression and the extent of mesenteric fibrosis from small bowel neuroendocrine neoplasms?
4	What is the morbidity and mortality after resection of small bowel neuroendocrine neoplasms, and how is this affected by hospital volume?
5	What is the current international practice and attitude towards minimally invasive small bowel neuroendocrine neoplasm resection?
6	What are the peri-operative differences between patients who underwent a minimally invasive or open resection for small bowel neuroendocrine neoplasms?
7	What is the most common surgical approach to resect small bowel neuroendocrine neoplasms in the Netherlands?
8	What is the value of fluorescence angiography using indocyanine green during surgical resection of small bowel neuroendocrine neoplasms?
9	What is the value of fluorescence guided resection of neuroendocrine liver metastases using indocyanine green?
10	Is a SSTR2 targeted fluorescent tracer safe to use in humans, and does it effectively delineate tumour tissue from healthy tissue?

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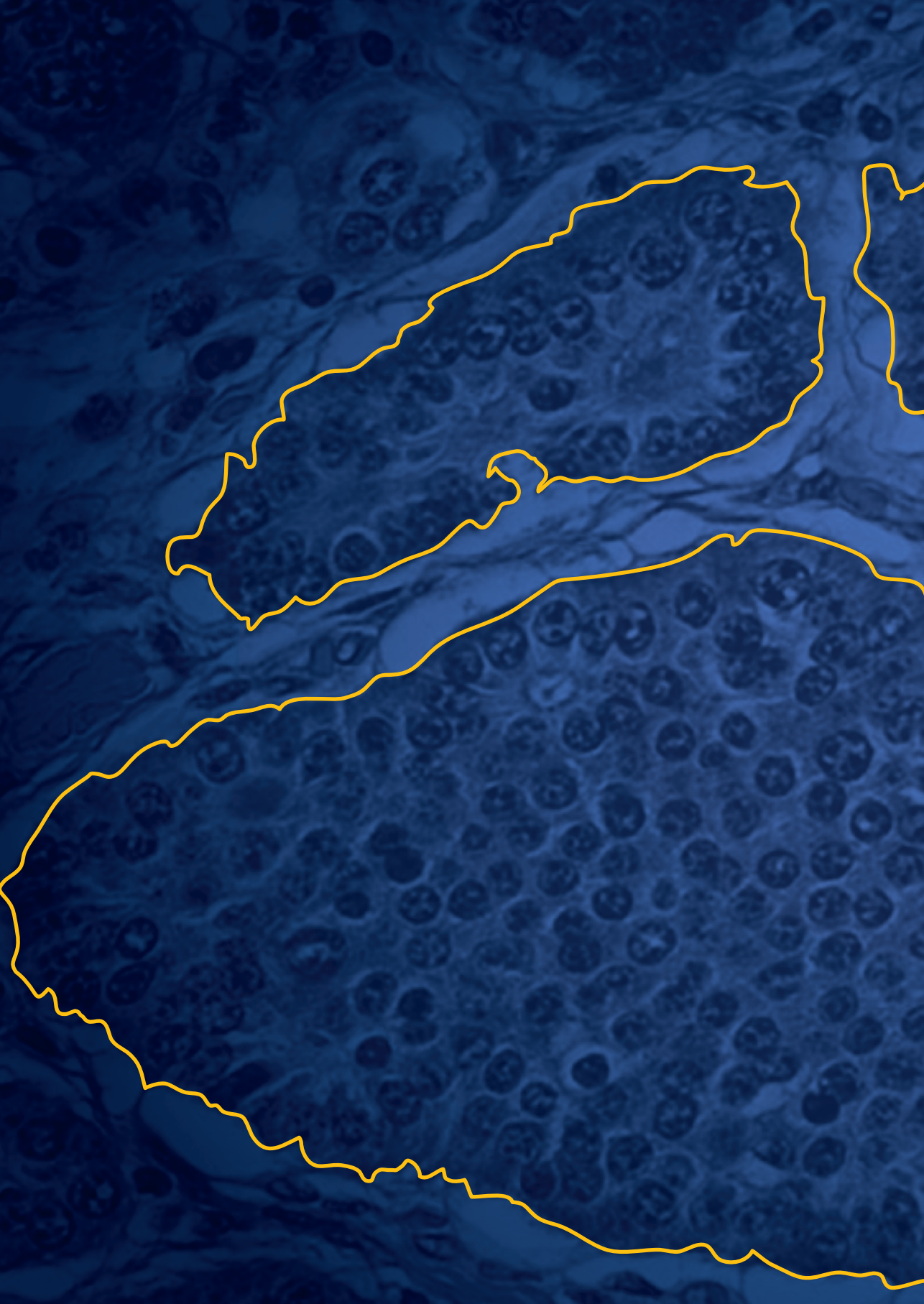

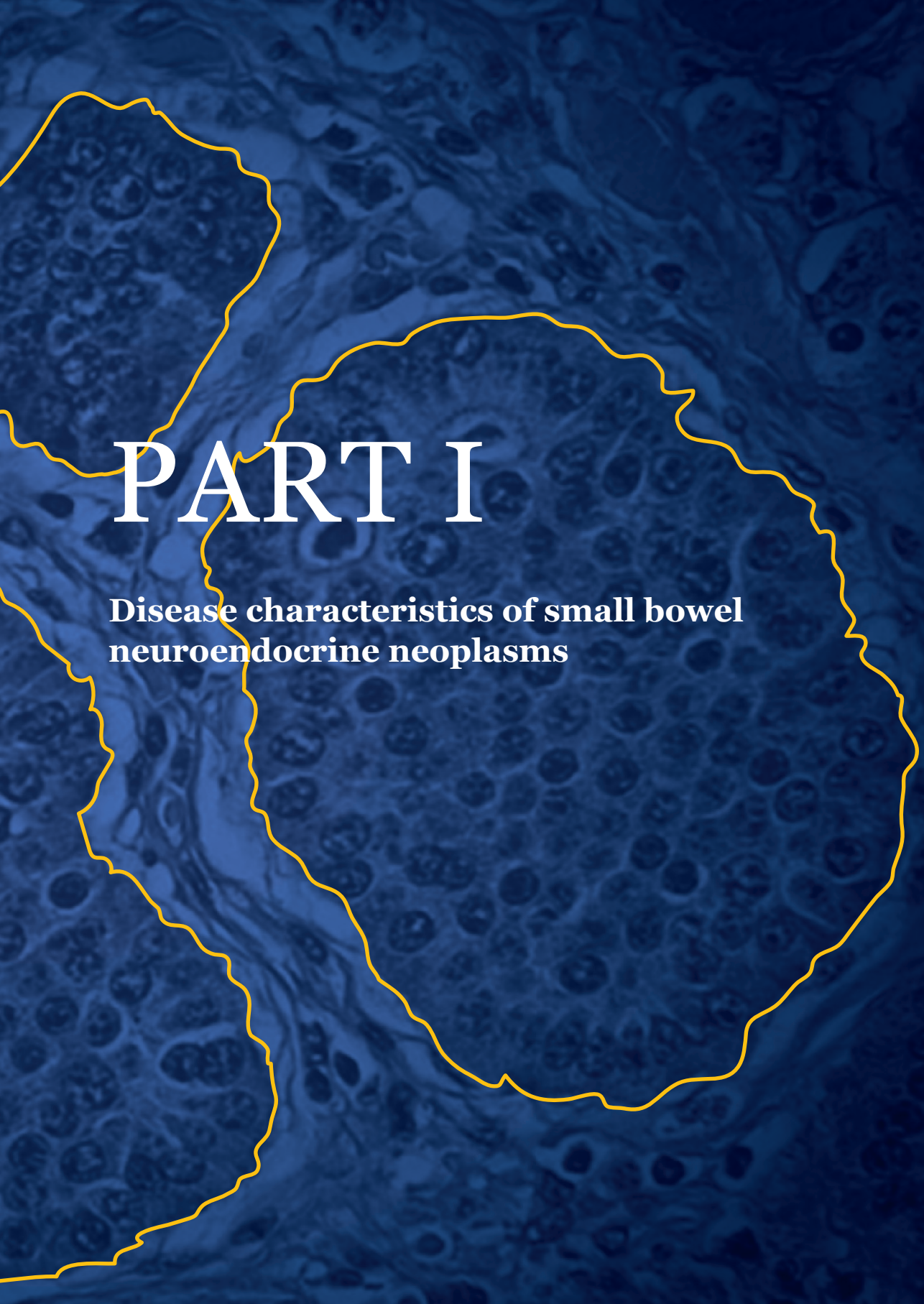

PART I

Disease characteristics of small bowel neuroendocrine neoplasms

CHAPTER 1

Treatment of liver metastases from midgut neuroendocrine tumours: a systematic review and meta-analysis

Enes Kaçmaz, Charlotte M. Heidsma, Marc G.H. Besselink, Koen M.A. Dreijerink, Heinz-Josef Klümpen, Anton F. Engelsman and Els J.M. Nieveen van Dijkum

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ABSTRACT

Background Strong evidence comparing different treatment options for liver metastases (LM) arising from gastroenteropancreatic neuroendocrine tumours (GEP-NET) is lacking. The aim of this study was to determine which intervention for LMs from GEP-NETs shows the longest overall survival (OS).

Methods A systematic search was performed in MEDLINE, Embase and the Cochrane Library in February 2018. Studies reporting on patients with LMs of any grade of sporadic GEP-NET comparing two intervention groups were included for analysis. Meta-analyses were performed where possible.

Results Eleven studies, with a total of 1108, patients were included; 662 patients had LM from pancreatic NETs (pNET), 164 patients from small-bowel NETs (SB-NET) and 282 patients of unknown origin. Improved 5-year OS was observed for surgery vs. chemotherapy (OR 0.05 95% CI [0.01, 0.21] $p < 0.0001$), for surgery vs. embolization (OR 0.18 95% CI [0.05, 0.61] $p = 0.006$) and for LM resection vs. no LM resection (OR 0.15 95% CI [0.05, 0.42] $p = 0.0003$).

Conclusion This is the largest meta-analysis performed comparing different interventions for LMs from GEP-NETs. Despite the high risk of bias and heterogeneity of data, surgical resection for all tumour grades results in the longest overall survival. Chemotherapy and embolization should be considered as an alternative in case surgery is not feasible.

INTRODUCTION

Gastroenteropancreatic neuroendocrine tumours (GEP-NET) represent a heterogeneous group of tumours arising from neuroendocrine cells of the gastro-intestinal tract. The annual incidence of GEP-NETs is estimated to be around 2.88 (European standardized rate, ESR) [1]. In specialized centres, liver metastases (LM) are diagnosed in up to 80–90% of patients with small-bowel NETs (SB-NET) and 60–70% of patients with pancreatic neuroendocrine NETs (pNET) [2]. LM is the strongest predictor for poor survival of patients with GEP-NET regardless of the location of the primary tumour with a 5-year overall survival of 13–54 months for patients with untreated LM [3].

Treatment of patients with LM is aimed at local tumour control and symptom relief. Several treatment modalities for NET-LMs exist, and include resection or debulking of the metastases, radiofrequency ablation (RFA), tumour embolization and pharmacological treatment. Pharmacologic interventions include somatostatin analogues (SSA), targeted therapy, peptide receptor radionuclide therapy (PRRT), chemotherapy and immunotherapy. SSAs reduce hormone associated symptoms in patients, while lengthening progression free survival (PFS) [4,5,6]. The phase 3 NETTER-trial showed improvement in PFS when treating patients with 177-Lu-Dotatate (PRRT) and octreotide with long acting release (LAR) versus octreotide LAR alone in patients with well differentiated metastatic midgut NETs [7]. The protein kinase inhibitor everolimus and sunitinib also increase PFS in patients with advanced NETs [8,9,10]. Hepatic artery embolization (HAE) prolongs survival, whilst being safe and feasible [11]. Current ENETS guidelines state that SSA, octreotide and lanreotide are equally effective in both symptom control and antiproliferative effect [12].

A systematic review published in 2008 by Gurusamy et al. aimed to compare liver resection to other treatment modalities in patients with LMs from GEP-NETs, but were unable to conduct an analysis due to a lack of relevant articles at that time [13]. In the past decade, multiple cohort studies were published. The aim of this systematic review is to determine which treatment modality leads to highest overall survival in patients with LM from GEP-NETs.

METHODS

Search strategy

A systematic search was performed in MEDLINE (PubMed), Embase (Ovid) and the Cochrane Library on 1 February 2018 (Supplementary material 1). The search strategy is presented in Supplementary material 1 and included both keywords and MeSH terms: ‘neuroendocrine tumours’, ‘midgut’, ‘liver metastasis’, ‘pancreatic neoplasms’, ‘duodenal neoplasms’, ‘ileal neoplasms’, ‘jejunal neoplasms’, ‘somatostatin’, ‘interferons’, ‘molecular targeted therapy’, ‘chemotherapy’, ‘surgery’, ‘surgical oncology’, and ‘catheter ablation’. No publication date restriction was used. Studies published in any language other than English were excluded. This study was registered in PROSPERO with the following registration number: CRD42018104328.

In- and exclusion criteria

All randomized controlled trials, cross-sectional, cohort studies and case-series reporting on treatment of GEP-NET related LM with at least 5 patients in a minimum of two compared intervention groups were eligible for inclusion. All grades of GEP-NETs were included. Patients with mixed neuroendocrine or non-neuroendocrine neoplasms (MINEN/MENEN) were excluded. No age limit was applied.

Study selection

All studies identified by the search were screened for eligibility by two independent authors (AE, EK) using Rayyan software (Qatar Computing Research Institute, Doha, Qatar) [14]. After selection based on title and abstract, full texts were analysed for further in- or exclusion. Any conflicts arising from the selection were resolved by consensus. The 5-year overall survival or 5-year disease specific survival after intervention had to be stated in the study, or the data to calculate this had to be available. No strict definition of a curative or palliative resection had to be met. Patients with LM from pancreatic, duodenal, jejunal or ileal NETs were included. In case of publications with overlapping patient cohorts, the study with the largest cohort size was included for analysis.

Data extraction

The following characteristics were extracted: patient characteristics, primary tumour location (pancreas or small bowel), type of therapy for LM, resection of the primary tumour, LM status (resectable/unresectable), uni- or bilobar metastases, extrahepatic disease, WHO (World Health Organization) 2010 grade and follow-up period. The primary outcome was 5-year overall survival. Secondary outcomes included disease free survival (DFS), progression free survival (PFS) and post-operative complications. Subgroups for analysis were defined as resection of primary tumour versus no resection at all, LM resection versus no resection at all, any resection versus chemotherapy, any resection versus embolization and any resection versus LTx (liver transplantation). 'No resection at all' was defined as no LM nor primary resection, 'any resection' was defined as a primary with or without LM resection.

Statistical analysis

For the meta-analysis, outcome data stratified by subgroups were pooled using Review Manager 5.3 (The Nordic Cochrane Centre, The Cochrane Collaboration, Denmark, Copenhagen) and presented in a forest plot. Heterogeneity was assessed by calculating the I2 index. An $I2 < 25\%$ was considered as low and a fixed effects model was used for the meta-analysis using and the Mantel–Haenszel method [15]. An I2 between 25–75% was considered as intermediate and consequently a random effects model was used for the meta-analysis. An $I2 > 75\%$ was considered substantial and no meta-analysis was performed. Funnel plots were made to assess publication bias.

Risk of bias assessment

The ROBINS-I (Risk of Bias in Non-randomized Studies—of Intervention) tool was used to assess risk of bias for the included studies [16].

RESULTS

Description of studies

A total of 712 studies were identified through the electronic search in MEDLINE (PubMed), Embase and the Cochrane Library. After the screening and selection process, 11 studies fulfilled the inclusion criteria (Figure 1) [17,18,19,20,21,22,23,24,25,26,27]. Characteristics of the included studies are presented in Table 1. There were no randomized controlled trials found. The 11 included studies represent a total of 1108 patients, of which 662 patients had pNETs, 164 patients had SB-NETs and 282 patients had a tumour originating from lungs (n = 26), ovaries (n = 1) and unknown primary locations (n = 102) (Table 2). Out of all included studies, five intervention groups were composed: primary tumour resection versus no resection at all, LM resection versus no resection at all, any resection versus chemotherapy, any resection versus embolization and any resection versus LTx.

Figure 1. PRISMA flow chart of the study screening and selection process.

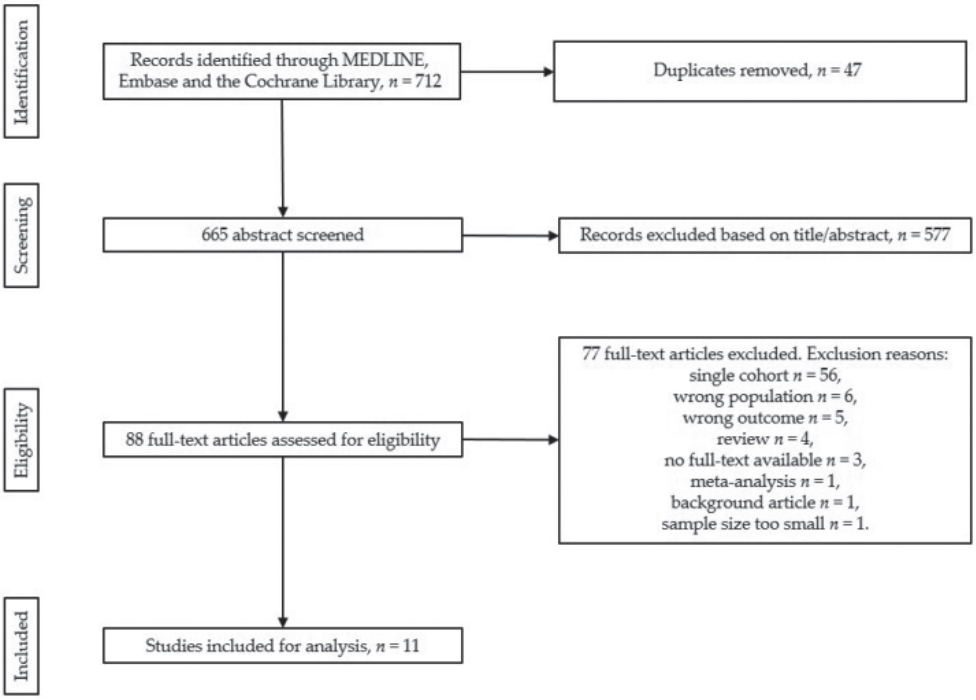


Table 1. Characteristic of included studies.

Author	Country	Design	No. patients (n)	Inclusion criteria per study	Exclusion criteria per study	Intervention groups	Control group
Watzka et al. [27]	DE	Retrospective	204	Patients with LM of NEN.	N/A	Radical LM resection (n = 38)	No resection at all (n = 110)
Partelli et al. [26]	IT	Retrospective	166	Patients with synchronous LM from sporadic pNET.	Patients with extra-abdominal disease as well as those with peritoneal carcinomatosis and those with an inherited syndrome.	Radical LM resection + primary resection (n = 18)	No resection at all (n = 75) (SSA; PRRT; chemotherapy; everolimus or sunitinib)
Citterio et al. [21]	IT	Retrospective	139	≤ 20 mitoses/10 high power field (HPF) and Ki-67 labelling index ≤ 20% at either the primary or metastatic sites; Hormone-secreting status associated with a distinct clinical syndrome (functioning NETs); Performance status (PS) 0-1 at presentation, according to the ECOG[§]	N/A	LM resection (n = 36) (32 were after primary resection)	No resection at all (n = 103) (SSA n = 95, SSA + chemo n = 30, SSA + everolimus n = 14, TACE or RFA + systemic and/ or surgical treatment* n = 25)
Du et al. [24]	CN	Retrospective	130	LM from NET.	N/A	Radical resection of primary tumour (n = 42) LM + primary resection (R0) n=26, LM resection (R0) n=6	No resection at all (n = 56) (TACE (16/18 also received an RFA) n=18, systemic chemotherapy n=9, SSA n=12, no treatment n=17)
						Primary + LM resection n = 26, primary resection n = 42, LM resection n = 6	Chemotherapy (n = 21) chemotherapy (fluorouracil and/or epirubicin and/or doxorubicin and/or etoposide and/or cisplatin, etc.) n = 9, SSA n = 12) TACE (n = 18) (16 also received a RFA)

Author	Country	Design	No. patients (n)	Inclusion criteria per study	Exclusion criteria per study	Intervention groups	Control group
Bertani et al. [17]	IT	Retrospective	121	Patients with synchronous and unresectable pNET LM.	N/A	Resection of primary tumour (n = 62) (n = 59 also received PRRT)	No resection at all (n = 59) (PRRT n = 55, SSA n = 29)
Boyar et al. [18]	NO	Retrospective	114	Patients with (WHO 2010) grade 1 and grade 2 tumours.	N/A	Resection of primary tumour with curative intent (n = 46)	No resection at all (n = 51) (streptozotocin + 5-fluorouracil/doxorubicin; SSA; IFN; embolization; PRRT; M-tor inhibitor)
Chamberlain et al. [19]	US	Retrospective	85	Patients treated for hepatic NET metastases.	The absence of identifiable liver disease, pathologic review at MSKCC[¶] revealing a non-NET or high-grade NET, or a patient decision to seek care elsewhere.	Segmentectomy or enucleation n = 12, lobectomy n=3, extended resection n=19[‡]	Chemotherapy (n = 18) (streptozocin+ 5-FU; streptozocin + doxorubicin; 5-FU + leucovorin or cisplatin + etoposide) HAE, with polyvinyl alcohol particles (n = 33)
Musunuru et al. [25]	US	Retrospective	48	Patients with liver-only metastatic neuroendocrine tumours.	N/A	Anatomical liver resection n = 6, ablation n = 4, resection and ablation n = 3	Chemotherapy (n = 17) (observation, octreotide, and/or systemic chemotherapy) Embolization (n = 18)
Chen et al. [20]	US	Retrospective	38	Patients treated for hepatic NET metastases.	Patients with evidence of extrahepatic disease or unresected known primary tumour.	LM resection (n = 15) (12 were combined with primary resection)	No resection at all (n = 23) (chemoembolization n = 5, chemotherapy and radiation n = 6, chemotherapy only n = 3, radiation only n = 2, no therapy n = 7)
Dousset et al. [23]	FR	Retrospective	34	Patients with metastatic endocrine tumours with bilobar metastases.	N/A	Curative intent resection n=12 Palliative intent n=5[†]	Chemotherapy (n = 8) (streptozotocin + fluorouracil n = 4, chemoembolization n = 4) LTx (n = 9)
Coppa et al. [22]	IT	Retrospective	29	LM from NET, confirmed histological diagnosis.	Non-carcinoid primary tumours, tumours with systemic venous drainage.	Hepatic resection with curative intent (n = 20)	LTx (n = 9)

Table 2. Patient characteristics of included studies.

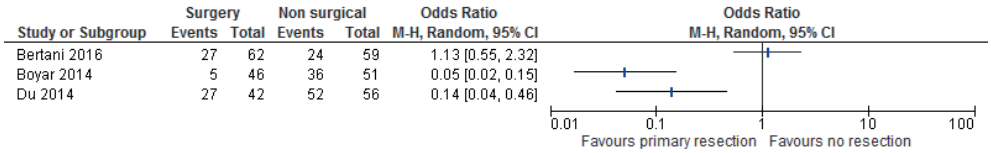
Study	No. Patients (n)	Sex (n, %)		Age (years)	Primary tumour location			LM size in cm (median, range)	Non-functional NETs (n, %)	Resection of primary tumour (n, %)	Resectable/ unresectable LM	Uni-/ Bilobar metastases	Extra-hepatic disease (n, %)	WHO 2010 grade
		Male	Female		Pancreas (n, %)	Small bowel (n, %)	Other/ Unknown (n, %)							
Watzka et al. [27]	204	111 (54)	93 (46)	58 ± 15 (60)*	58 (28)	73 (36)	73 (36)	N/A	123 (60)	165 (81)	Mixed	N/A	N/A	All
Partelli et al. [26]	166	92 (55)	74 (45)	N/A‡	166	0	0	LM resection 0.8 cm (0.3-1.7 cm); no resection at all 3.4 cm (1-7 cm) †	152 (92)	91 (55)	Resectable	Both	N/A	All
Citterio et al. [21]	139	67 (48)	72 (52)	56 (51-55) †	36 (26)	66 (47)	37 (27)	N/A	0	93 (67)	Mixed	N/A	N/A	1-2
Du et al. [24]	130	69 (53)	61 (47)	49.0 ± 12.1 (N/A)*	85 (65)	7 (5)	38 (30)	Mean 4.1 cm (range 3-15 cm)	100 (77)	68 (52)	Mixed	N/A	N/A	All
Bertani et al. [17]	121	66 (55)	58 (45)	54.6 ± 12.6 (54.5)*	121 (100)	0	0	N/A	29 (24)	63 (52)	Unresectable	N/A	28 (23)	All
Boyar et al. [18]	114	61 (54)	83 (46)	57 (32-83)†	111 (97)	0	3 (3)	N/A	89 (78)	46 (40)	Mixed	N/A	51 (45)	1-2
Chamberlain et al. [19]	85	37 (44)	48 (56)	52 (20-79) †	42 (49)	0	43 (51)	N/A	49 (58)	36 (42)	Mixed	Both	45 (53)	1-2
Musunuru et al. [25]	48	30 (63)	18 (37)	N/A	15 (31)	0	33 (69)	Embolization 8.9 ± 6.1 cm; chemotherapy 3.7 ± 2.9 cm; any resection 4.5 ± 2.3 cm*	N/A	12 (25)	Unclear	Both	0	N/A
Chen et al. [20]	38	24 (63)	14 (37)	N/A‡	11 (29)	9 (24)	18 (47)	N/A	9 (24)	12 (32)	Mixed	Bilobar	0	N/A
Dousset et al. [23]	34	18 (53)	17 (47)	49.5 (29-76)†	17 (50)	9 (26)	8 (24)	N/A	5 (15)	21 (62)	Mixed	Bilobar	0	N/A
Coppa 200et al.1 [22]	29	13 (45)	16 (55)	N/A‡	0	0	29§	N/A	N/A	11 (38)	Mixed	N/A	0	N/A

*mean ± SD (median);
†median (range);
‡Age was reported for each subgroup separately;
§21 have a pancreatic or ileal origin, whilst 8 originated in the lung or rectum;
N/A: not available.

Resection of primary tumour versus no resection at all

This intervention group compares primary resection versus no primary resection with LM presence in both groups. Three studies reported outcomes on resection of primary tumour (n = 150) versus no resection of primary tumour (n = 166) with a total number of 365 patients [17,18,24]). High statistical heterogeneity based on an I2 of 92% withheld us from conducting a meta-analysis with these studies (Figure 2).

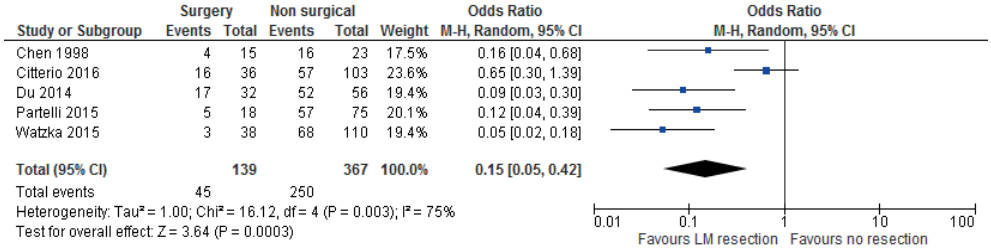
Figure 2. Forest plot for overall survival (OS) after resection of primary tumour versus no resection at all.



LM resection versus no resection at all

Five studies reported outcomes on resection of LM (n = 139) versus no resection (n = 367) with a total number of 506 patients [20,21,24,26,27]). Chen et al. reported a median DFS of 21 months after LM resection [20]. Partelli et al. reported a median DFS of 42, 27 and 15 months after curative, palliative and no surgery, respectively [26]. Statistical heterogeneity amounted to 75% thus a meta-analysis was performed. The meta-analysis resulted in a statistically significant benefit in 5-year OS (overall survival) in favour of LM resection versus no resection at all (OR 0.15 with 95% CI 0.05–0.42, p = 0.0003, Figure 3).

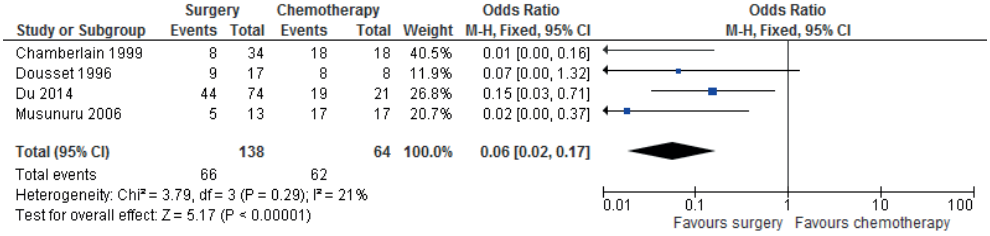
Figure 3. Forest plot for overall survival (OS) after liver metastases (LM) resection versus no resection at all.



Any surgery versus chemotherapy

Four studies reported outcomes on surgery (n = 138) versus chemotherapy (n = 64) with a total number of 202 patients (19, 23–25). Additional therapy was provided for two out of 32 patients in the surgery group with either TACE or RFA in the study by Du et al. [24]. Statistical heterogeneity amounted to 21%, thus a meta-analysis was performed. The meta-analysis resulted in a statistically significant 5-year OS in favour of any surgery versus chemotherapy (OR 0.05 with 95% CI 0.01–0.21, p < 0.0001, Figure 4).

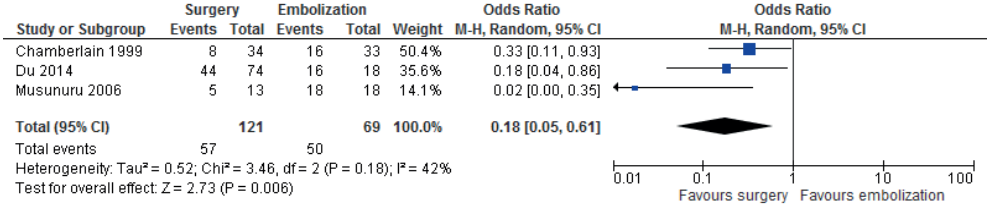
Figure 4. Forest plot for overall survival (OS) after any surgery versus chemotherapy.



Any surgery versus embolization

Three studies reported outcomes on surgery (n = 121) versus embolization (n = 69) with a total number of 190 patients [19,24,25]. Statistical heterogeneity amounted to 42%, thus a meta-analysis was performed. The meta-analysis resulted in a statistically significant OS in favour of any surgery versus embolization (OR 0.18 with 95% CI 0.05–0.61, p = 0.006, Figure 5).

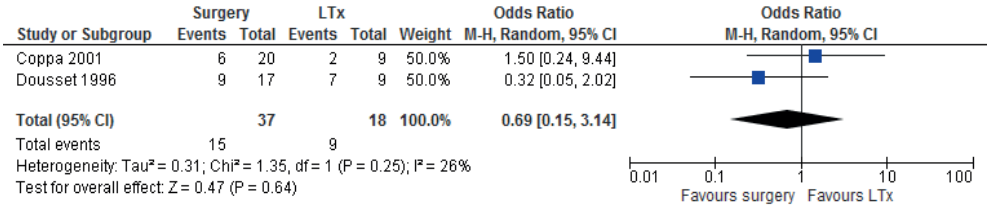
Figure 5. Forest plot for overall survival (OS) after any surgery versus embolization.



Any surgery versus LTx

Two studies reported outcomes on surgery (n = 37) versus LTx (n = 18) with a total number of 55 patients [22,23]. Studies used strict criteria for patients to be eligible for LTx. Statistical heterogeneity amounted to 26%, thus a meta-analysis was performed. The meta-analysis showed no difference in OS regarding any surgery versus LTx (OR 0.69 with 95% CI 0.15–3.14, p = 0.64, Figure 6). Coppa et al. reported a median DFS of 24 months after hepatic resection [22]. Dousset et al. reported a median DFS of 17 months after curative and palliative surgery and 19.5 months after LTx [23].

Figure 6. Forest plot for overall survival (OS) after any surgery versus liver transplantation (LTx).



Risk of bias

In accordance with the ROBINS-I guidelines, the overall risk of bias was scored as critical for all studies (Table 3), the reason being that all studies scored a critical risk of bias in the 'bias due to confounding' domain due to the lack of randomized controlled trials. The funnel plots show that, as expected, some publication bias is present in the included study (Supplementary material 2).

Table 4. Risk of bias in included studies scored with the ROBINS-I tool.

	Bias due to confounding	Bias in selection of participants into the study	Bias in classification of interventions	Bias due to deviations from intended interventions	Bias due to missing data	Bias in measurement of outcomes	Bias in selection of the reported result	Overall bias
Chamberlain et al. [19]	-	+/-	+	+	+/-	+/-	+/-	-
Coppa et al. [22]	-	+/-	+	+	+/-	+/-	+/-	-
Du et al. [24]	-	+/-	+	+	+/-	+/-	+/-	-
Musunuru et al. [25]	-	+/-	+	+	+/-	+/-	+/-	-
Boyar et al. [18]	-	+/-	+/-	+	+/-	+/-	+/-	-
Bertani et al. [17]	-	+/-	+	+/-	+/-	+/-	+/-	-
Chen et al. [20]	-	+/-	+	+/-	+/-	+/-	+/-	-
Citterio et al. [21]	-	+/-	+	+/-	+/-	+/-	+/-	-
Partelli et al. [26]	-	+/-	+	+/-	+/-	+/-	+/-	-
Watzka et al. [27]	-	+/-	+	+/-	+/-	+/-	+/-	-
Dousset et al. [23]	-	+/-	+/-	+/-	+/-	+/-	+/-	-

+ : low
+/- : moderate
- : critical

DISCUSSION

Surgical resection of LM with curative intent is the current standard of care [2]. The aim of this treatment strategy is to prolong OS and maintain quality of life. This systematic review presents the first meta-analysis, involving 11 cohort studies and 1108 patients, comparing surgery with other treatment modalities for GEP-NET related LM. The meta-analysis showed a significantly improved 5-year OS after LM resection versus no resection at all, after any surgery versus chemotherapy and after any surgery versus embolization. No significant benefit of any surgery as compared to LTx was observed.

Although our results are heterogeneous, they are supported by a recent study from Yu et al. [28]. In this study, a systematic review and meta-analysis were performed comparing liver resection with non-liver resection treatments for patients with LM from all grades of pNET. The meta-analysis resulted in a median 5-year OS of 68% in the liver resection group, and 27% in the non-liver resection group. Survival outcomes reached statistical significance for 5-year OS with an OR of 5.30 (95% CI [3.24, 8.67] $p < 0.001$), in favour of liver resection.

A number of studies in this systemic review also reported an improved DFS in favour of surgery versus other treatments. However, because of the limited data reported, no meta-analysis could be performed [20,22,23,25]. Data regarding complications was limited, only two studies reported complications due to hepatic surgery [26,27]. Different from an earlier published Cochrane review, cohort studies were considered for inclusion, which enabled the meta-analysis [13]. Although this study was not able to conduct a meta-analysis comparing primary tumour resection to no primary resection, a trend towards a beneficial effect of primary tumour resection is observed and supported by other studies [29,30]. In addition, performing LTx remains a topic of debate due to the small number of patients reported in the literature [31].

This review also included patients with metastases of WHO grade 3 SB-NETs. These patients showed an improved 5-year OS after resection of the LMs. This supports the ENETS 2012 guideline regarding an indication for resection of LM in WHO grade 3 NETs whenever possible, assessed per individual case [2]. We agree with the ENETS 2012 guideline; however, we also propose that the presence of extrahepatic metastases should not be an exclusion criterion, but that resection should be, again, considered per individual case [2]. Our data supports the updated ENETS 2016 guideline, stating that ablative therapies should be considered when surgery is contraindicated in LM from grade 1 and grade 2 NETs (Figure 5) [12].

This systematic review and meta-analysis have a number of limitations, mainly due to the rarity of the disease and limited conducted interventional studies, with a lack of randomized controlled trials (RCT). This resulted in inclusion of 11 retrospective cohort studies, resulting in a low level of evidence (level C) [32]. As a consequence, drawing conclusions is challenging due to a high risk of selection bias, but hypothesis generating remains possible. Moreover, the included studies have small cohort sizes on subgroup level, interventions were performed on different tumour grades and the studies used a variety of types of individual interventional approaches. It is also unfortunate that no quality-of-life data were reported in the included studies. Because

our analyses are based only on published data, there is also a risk of publication bias. Despite the obvious drawbacks of this study, it is at present the best available evidence.

Even though a systematic approach was used in this study, the data is of limited quality and the question of which intervention yields the most benefit for OS in patients with LM from pNET/SB-NET remains unanswered. Randomized trials would generate evidence of great quality, but the execution of such a study is challenging (due to the long follow-up time needed and financial burden, among other things). Therefore, further prospective multi-centre research should address this question, for example by collaboration of multiple ENETS Centers of Excellence. Dousset et al. and Partelli et al. also report underestimation of liver disease by preoperative imaging studies, indicating room for improvement [23,26]. Watzka and colleagues reported on the largest included cohort of LM from GEP-NET [27].

In multivariate analysis, occurrence of synchronous or metachronous LM, hormonal activity and the site of the primary tumour were not independent significant prognostic factors, whereas tumour grade and resection margin status were. These prognostics factors should be taken into account when designing new studies.

Currently, a randomized trial is being conducted, comparing the resection of primary tumours vs. no resection of primary tumours in asymptomatic patients with unresectable LM from SB-NET (NCT03442959). However, survival analyses are not expected soon.

Surgical resection of LMs from all grades GEP-NETs should be considered if possible, and chemotherapy and embolization should be considered as an alternative in case surgery is not feasible. We therefore advocate that all patients with LM from pNET/SB-NET should be discussed in referral centers with specialized multidisciplinary meetings for NETs, preferably in ENETS Centers of Excellence.

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Author contributions

Conceptualization: EK, EJMNvD, and AFE.
Data curation: EK.
Formal analysis: EK and AFE.
Supervision: CMH, MGHB, HJK, EJMNvD, and AFE.
Validation: EK, CMH, HJK, EJMNvD, and AFE.
Writing—original draft: EK.
Writing—review and editing: EK, CMH, KMAD, HJK, EJMNvD, and AFE.

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SUPPLEMENTARY MATERIALS

Supplementary material 1. Search strategy.

MEDLINE (Pubmed) search strategy

(Neuroendocrine tumors [mesh] OR neuroendocrine tumo* [tiab])
AND
(mnet* [tiab] OR midgut [tiab] OR metastas* [tiab])
AND
(Liver [mesh] OR liver neoplasms [mesh] OR liver* [tiab])
AND
(Pancreatic neoplasms [mesh] OR pancreatic neoplasm* [tiab] OR Duodenal neoplasms [mesh] OR duodenal neoplasm* [tiab] OR Ileal neoplasms [mesh] OR ileal neoplasm* [tiab] OR jejunal neoplasms [mesh] OR jejunal neoplasm* [tiab])
AND
(Somatostatin [mesh] OR Somatostatin analog* [tiab] OR Octreotide [mesh] OR octreotide [tiab] OR lanreotide [Supplementary Concept] OR lanreotide [tiab] OR Interferons [mesh] OR interferon* [tiab] OR Molecular targeted therapy [mesh] OR molecular targeted therap* [tiab] OR Radioligand assay [mesh] OR radioligand [tiab] OR Radioimmunoassay [mesh] OR radioimunnoassay [tiab] OR Peptide receptor radioligand therap* [tiab] OR Chemotherapy adjuvant [mesh] OR Consolidation chemotherapy [mesh] OR maintenance chemotherapy [mesh] OR cytotoxic chemotherapy [tiab] OR Immunotherapy [mesh] OR immunotherapy* [tiab] OR surgical procedures, operative [mesh] OR resection [tiab] or surgical oncology [mesh] OR High-Intensity Focused Ultrasound Ablation [mesh] OR High-Intensity Focused Ultrasound Ablation [tiab] OR catheter ablation [mesh] OR catheter ablation [tiab] OR radiofrequency ablation [tiab])
AND
("Randomized Controlled Trial" [Publication Type] OR "Review" [Publication Type] OR "Cross-Sectional Studies"[Mesh] OR "Meta-Analysis" [Publication Type] OR "Cohort Studies"[Mesh] OR case-series [tiab] OR random* [tiab] OR systematic review* [tiab] OR cross-sectional* [tiab] OR meta-analy* [tiab] OR cohort* [tiab])

Embase (Ovid) search strategy

Neuroendocrine tumor/ OR neuroendocrine tumo$.ti,ab,kw
AND
Midgut/ OR liver metastasis/ OR midgut$.ti,ab,kw OR liver metasta$.ti,ab,kw
AND
Liver/ OR liver cancer/ OR liver tumor/ OR liver$.ti,ab,kw
AND
Pancreas cancer/ OR pancreas carcinoma/ OR pancreas tumor/ OR pancreatic$.ti,ab,kw OR duodenum cancer/ OR duodenum tumor/ OR duodenal$.ti,ab,kw OR ileum cancer/ OR ileum tumor/ OR ileal$.ti,ab,kw OR jejunum cancer/ OR jejunum tumor/ OR jejunal$.ti,ab,kw
AND
Somatostatin/ OR somatostatin analog$.ti,ab,kw OR octreotide/ OR angiopeptin/ OR lanreotide$.ti,ab,kw OR interferon/ OR Molecular targeted therapy/ OR radioassay/ OR

radioligand assay$.ti,ab,kw OR radioimmunoassay/ OR adjuvant chemotherapy/ OR consolidation chemotherapy/ OR maintenance chemotherapy/ OR cytotoxic chemotherapy.ti,ab,kw OR surgical/ OR surgery/ OR high intensity focused ultrasound/ OR cathether ablation/ OR radiofrequency ablation/
AND
Randomized controlled trial/ OR random$.ti,ab,kw. OR review/ OR review$.ti,ab,kw OR systematic$.ti,ab,kw. OR cross-sectional studies/ OR cross-sectional$.ti,ab,kw. OR meta-analysis/ OR meta-analy$.ti,ab,kw. OR cohort studies/ OR cohort$.ti,ab,kw. OR case series.ti,ab,kw.

Cochrane search strategy
(Neuroendocrine tumors [mesh] OR neuroendocrine tumo* [tiab])
AND
(mnet* [tiab] OR midgut [tiab] OR metastas* [tiab])
AND
(Liver [mesh] OR liver neoplasms [mesh] OR liver* [tiab])
AND
(Pancreatic neoplasms [mesh] OR pancreatic neoplasm* [tiab] OR Duodenal neoplasms [mesh] OR duodenal neoplasm* [tiab] OR Ileal neoplasms [mesh] OR ileal neoplasm* [tiab] OR jejunal neoplasms [mesh] OR jejunal neoplasm* [tiab])

Supplementary material 2. Funnel plots.

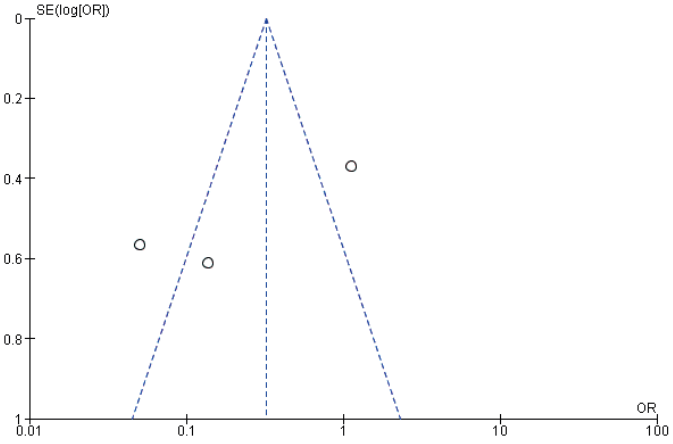

Figure 1. Funnel plot for overall survival (OS) after resection of primary tumour versus no resection at all.

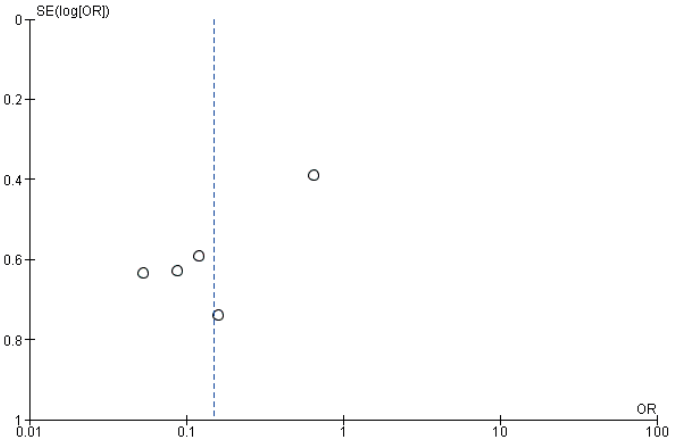

Figure 2. Funnel plot for overall survival (OS) after liver metastases (LM) resection versus no resection at all.

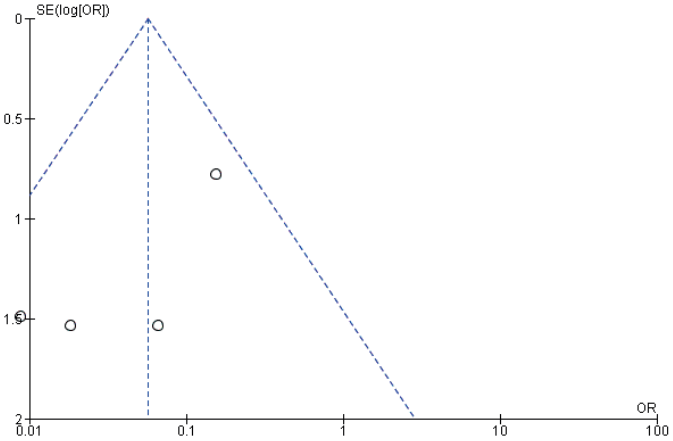

Figure 3. Funnel plot for overall survival (OS) after any surgery versus chemotherapy.

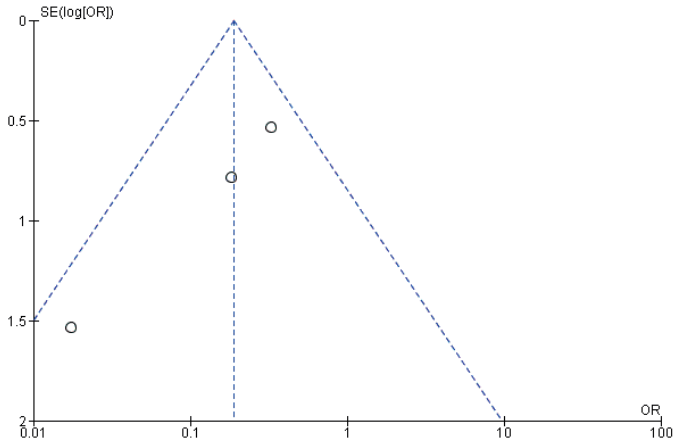

Figure 4. Funnel plot for overall survival (OS) after any surgery versus embolization.

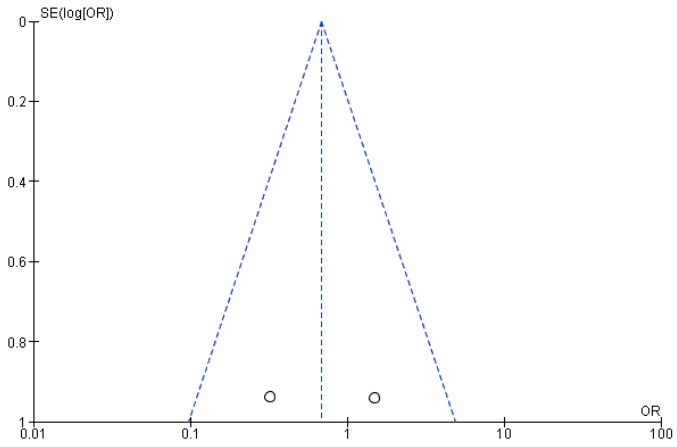

Figure 5. Funnel plot for overall survival (OS) after any surgery versus liver transplantation (LTx).

CHAPTER 2

Update on incidence, prevalence, treatment and survival of patients with small bowel neuroendocrine neoplasms in the Netherlands

Enes Kaçmaz, Arantza Farina Sarasqueta, Susanne van Eeden, Koen M. A. Dreijerink, Heinz-Josef Klümpen, Pieter J. Tanis, Els J. M. Nieveen van Dijkum and Anton F. Engelsman

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ABSTRACT

Background Small bowel neuroendocrine neoplasms (SB-NEN) are rare cancers, population-based studies are needed to study this rare indolent disease. The aim of this study was to explore trends in epidemiology, treatment and survival outcomes of patients with SB-NEN based on Dutch nationwide data.

Methods Patients with grade 1 or 2 SB-NEN diagnosed between 2005 and 2015 were retrieved from the Netherlands Cancer Registry and linked to The Nationwide Network and Registry of Histo- and Cytopathology in the Netherlands. Age-adjusted incidence rates were calculated based using the direct standardization method. Survival analyses were performed with the Kaplan–Meier method.

Results A total of 1132 patients were included for epidemiological analyses. The age-adjusted incidence rate of SB-NEN increased from 0.52 to 0.81 per 100.000 person-years between 2005 and 2015. Incidence was higher for males than females (0.93 vs. 0.69 in 2015). Most patients had grade 1 tumours (83%). Surgery was performed in 86% of patients, with resection of the primary tumour in 99%. During the study period, administration of somatostatin analogues (SSAs) increased from 5 to 22% for stage III and from 27 to 63% for stage IV disease. Mean follow-up was 61 (standard deviation 38) months. Survival data were complete for 975/1132 patients and five-year overall survival was 75% for stage I-II, 75% for stage III and 57% for stage IV.

Conclusion This study shows an increase in the incidence of SB-NEN in the Netherlands. A predominant role of surgery was found in all disease stages. Use of SSAs has increased over time.

INTRODUCTION

Small bowel neuroendocrine neoplasms (SB-NEN) are classified as a rare cancer type based on the incidence of <4/100.00 persons per year [1]. Despite its rarity, it represents 40% of all neoplasms of the small bowe00l [2], while simultaneously being the most common site of origin of gastroenteropancreatic neuroendocrine neoplasms (GEP-NEN) (incidence 1.05 per 100.000 person-years) [1].

Patients present with non-specific symptoms (e.g. abdominal pain) in 40% of the cases. Patients experience symptoms related to excessive hormone secretion (e.g. diarrhoea, flushing) in 20–30% of the cases [3]. Survival rates of SB-NEN are relatively high compared to other NENs, despite the delay (caused by non-specific symptoms) in diagnosis of these patients [1]. Two-thirds of the patients have locoregional disease (stage I-III) with a corresponding 5-year overall survival ranging between 97 and 100% [3, 4]. The remaining one-third has distant metastases (stage IV) with a reported 5-year overall survival of approximately 85%. This favourable outcome in the metastatic setting as compared to other malignancies might be due to the fact that some patients (with liver only metastases) are still eligible for curative intent surgery [3].

Recently, an increase in incidence and prevalence of GEP-NENs was observed in a study from the United States of America (USA) based on Surveillance, Epidemiology and End Results (SEER) data [1]. The most recent epidemiological evaluation of SB-NENs in the Netherlands was based on data between 1980 and 1997 [5]. The aim of this study was to provide an update of these Dutch data and to explore trends in epidemiology, treatment and survival outcomes of patients with grade 1 and 2 SB-NEN between 2005 and 2015.

METHODS

Study design

All patients with grade 1 and 2 SB-NEN diagnosed between 2005 and 2015 were retrieved from the Netherlands Cancer Registry (NCR). This registry contains all cases of cancer in the Netherlands (mean total population of 16.9 million during the study period) based on hospital records, and pathology reports, treatment and survival data. Full pathology reports were requested from The Nationwide Network and Registry of Histo- and Cytopathology in the Netherlands (PALGA; Pathologisch-Anatomisch Landelijk Geautomiseerd Archief) [6]. This study is performed in accordance with the STROBE guidelines [7].

Study population

Patients with pathologically proven grade 1 and 2 SB-NEN of any stage were included. The diagnosis was based on the International Classification of Disease-Oncology (ICD-O-3) morphology codes according to the World Health Organisation classification [8]. Exclusion criteria were duodenal NENs, autopsy and cytology data, benign neoplasms and non-neuroendocrine neoplasms. Neuroendocrine carcinoma, mixed adenoneuroendocrine

carcinoma (MANEC) and patients with multiple primary cancers (e.g. adenocarcinoma of the colon/breast cancer/lymphoma and SB-NEN) were only used to calculate incidence rates. Patients with high-grade tumours (NET G3, neuroendocrine carcinomas and MANEC) were excluded from survival analyses. Autopsy data were excluded because those patients died of other reasons than cancer-related, and cytology data were excluded because histology is considered the standard to diagnose SB-NENs (3).

Data collection

Primary tumour location was classified as jejunum (C17.1), ileum (C17.2) or small bowel not otherwise specified (C17.9), according to the ICD-O-3 codes. C17.9 reports were checked manually for tumour location. Tumour grade was based on the Ki67 index or mitoses index reported in the pathology reports, whichever was higher [9]. Tumour stage was reported based on the pathological tumour-node-metastasis (TNM) classification at the time of registration (6th edition during 2003–2009 and 7th edition during 2010–2016), supplemented with the clinical TNM classification [10, 11]. A one-digit summary stage (Extent of Disease) was recorded in patients without pathological confirmation of cancer [12]. The Extent of Disease code is used for patients who had no TNM stage available.

Data in both NCR and PALGA databases correspond based on unique NCR-codes. This feature was used to couple both datasets. Data regarding topography (site of primary), differentiation grade, resection margins, TNM staging, tumour positive lymph nodes reported by the NCR were cross-checked with the full pathology reports provided by PALGA. Morphology codes (cell of origin) were used in case of a mismatch in differentiation grade [13]. Data from PALGA prevailed, in case of disagreement between both datasets, because the pathology reports are more detailed. Tumour grading was based on the WHO 2010 classification. Finally, all tumours were restaged according to the 8th edition of the TNM classification to avoid differences between different TNM classifications [14]. The study period was divided into three time periods (2005–2007, 2008–2011 and 2012–2015), based on the publication date of the ENETS guidelines to compare different treatment strategies, stratified for disease stage [15, 16]. NCR only includes treatments 9 months before or after diagnosis.

In case of multiple pathology reports (e.g. one biopsy followed by resection), the first date was used for survival analyses. Time to treatment analyses could not be performed because the diagnosis was based on pathology data, which was often the date of surgery. Survival was defined as the time between date of diagnosis and date of death or censored at last follow-up date. Records of patients with pathologically proven recurrences were assessed for possible tumour dedifferentiation (i.e. tumour grade change from G1 to G2). Incidental diagnosis was defined as a patient whose first pathology report describes a resection with signs of ileus/stenosis/perforation, without previous biopsy available.

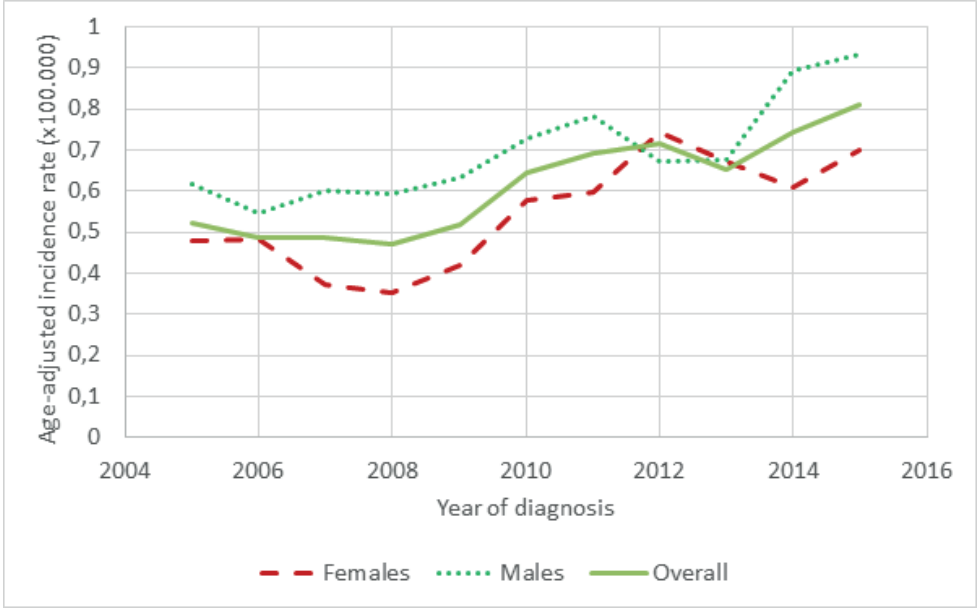
Statistical analysis

Study populations were categorized into five age groups (<20, 20–40, 40–65, 65–80 and ≥80) according to Statistics Netherlands (CBS). Age-adjusted incidence rates were calculated, as this enables comparison with other countries, based on population data from CBS and were age-adjusted to the European Standard Population (ESP) of 2010 using the direct standardization method [17]. Baseline and treatment characteristics were compared between regional and university hospitals. Survival analyses were performed using the Kaplan–Meier method and compared with the Log-Rank test. To analyse differences in survival outcomes over the years, overall survival was calculated by stratifying for periods at which different versions of the ENETS guidelines were published (2005–2007, 2008–2011 and 2012–2015). Univariable and multivariable Cox proportional hazards regression models were used to estimate hazard ratios (HR) with 95% confidence intervals (95% CI) to identify factors associated with survival. A two-sided P value ≤0.05 was considered statistically significant. Data were analysed using the Statistical Package for Social Sciences (SPSS) version 26.0 (IBM Corp. Armonk, NY, USA).

RESULTS

A total of 1451 patients were identified, of whom 1132 were eligible for epidemiological analysis. The age-adjusted incidence rate increased from 0.52 to 0.81 per 100.000 persons years between 2005 and 2015 (Figure 1). Males had higher incidence rates than females throughout the years, with an incidence of 0.93 versus 0.69 per 100.000 persons in 2015.

Figure 1. Age-adjusted incidence rates of patients diagnosed with SB-NEN between 2005 and 2015 in the Netherlands, stratified for sex.



Patients demographics

After excluding multiple primary cancers (N=122), high-grade tumours (N=28) and MANEC (N=7), 975 patients were left for survival analyses. Mean age at diagnosis was 63 (SD ±12) years. Baseline characteristics are summarized in Table 1. Patients from university hospitals were significantly younger and had significantly more often multiple primary SB-NEN than patients from primary centres. All other patient and tumour characteristics were similar for the two types of hospitals. Mean follow-up was 61 (SD ±38) months, and all-cause mortality was 33%. Most patients had a grade 1 tumour (83%) (WHO 2010). Lymph node metastases (either pN1 or pN2) were present in 84% of G1 and 89% of G2 tumours (P=0.26). Distant metastases were more frequent in G2 (56%) than G1 (34%) tumours (P<0.001), and in node-positive than node-negative tumours (36% vs. 26%) (P=0.030).

Table 1. Patient and tumour characteristics stratified for centre of diagnosis.

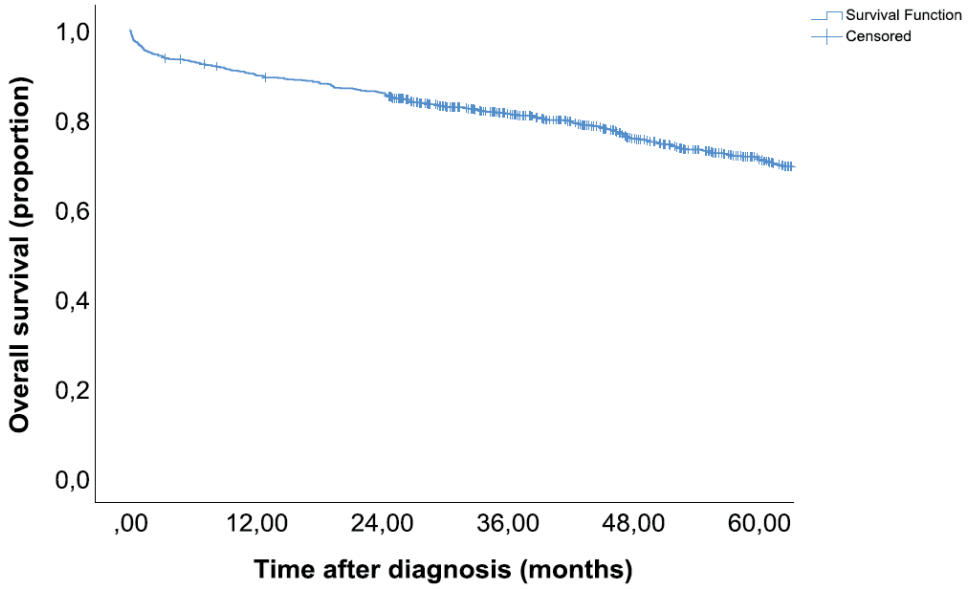
Characteristics	Missing	Total (*N* = 974)	Diagnosis at:		
			Regional hospital (*N* = 788)	University hospital (*N* = 186)	*P* Value
Sex					
Male	0	511 (52)	414 (53)	97 (52)	0.92
Age	0				
<20		1 (0)	0 (0)	1 (1)	**0.002**
20 up to 40		26 (3)	20 (3)	6 (3)	
40 up to 65		482 (49)	372 (47)	110 (59)	
65 up to 80		386 (40)	324 (41)	62 (33)	
≥80		79 (8)	72 (9)	7 (4)	
Clinical disease stage	431 (44)		358 (83)	73 (17)	
Stage I-II		40 (4)	34 (8)	6 (5)	0.63
Stage III		119 (12)	93 (21)	26 (23)	
Stage IV		384 (39)	303 (71)	81 (72)	
Pathological TNM-stage	184 (19)		155 (84)	29 (16)	
pT					
T1		49 (5)	40 (6)	9 (6)	0.45
T2		92 (9)	79 (13)	13 (8)	
T3		386 (40)	303 (48)	83 (53)	
T4		263 (27)	211 (33)	52 (33)	
Multiple tumours	*	172 (18)	128 (17)	44 (24)	**0.031**
pN	256 (26)		214 (84)	42 (16)	
N0		109 (11)	90 (16)	19 (13)	0.71
N1		510 (52)	404 (70)	106 (74)	
N2		99 (10)	80 (14)	19 (13)	
pM1	*				

Characteristics	Missing	Total (*N* = 974)	Diagnosis at:		
			Regional hospital (*N* = 788)	University hospital (*N* = 186)	*P* Value
pM1a		209 (21)	168 (57)	41 (56)	0.78
pM1b		129 (13)	104 (36)	25 (34)	
pM1c		28 (3)	21 (7)	7 (10)	
Prognostic stage group	128 (13)		111 (87)	17 (13)	
Stage I-II		70 (7)	57 (8)	13 (8)	0.95
Stage III		410 (42)	327 (48)	83 (49)	
Stage IV		366 (38)	293 (43)	73 (43)	
Tumour grade	11 (1)		10 (91)	1 (9)	
Grade 1		800 (82)	652 (84)	148 (80)	0.22
Grade 2		163 (17)	126 (16)	37 (20)	
Recurrence	*				
Dedifferentiation		9/79 (11)	7/61 (12)	2/18 (11)	0.08

Survival outcomes

Five-year overall survival of the entire cohort was 67% (Figure 2A). There were no significant differences in overall survival for patients diagnosed in different years (5-year overall survival of 62% in 2005–2007, 67% in 2008–2011 and 62% in 2012–2015, P=0.39), or diagnosed in academic (66%) or regional hospitals (67%) (P=0.74). Differences in survival outcomes between different types of hospitals and stratified for disease stages were present but were not significant: stage I-II 72 versus. 89%, stage III 76 versus. 67%, stage IV 56 versus. 62% for regional vs. academic hospitals, respectively. Five-year overall survival was 70% for G1, which was significantly higher (P=0.002) than the 64% survival rate for G2 tumours (Figure 2B). Stratified for stage, 5-year overall survival was 75% for stage I-II, 75% for stage III and 57% for stage IV (Figure 2C). Stage I-II and III disease showed significantly better survival compared to stage IV disease, with an absolute difference in mean survival of at least 21 months (P=0.019 and P<0.001, respectively). Presence or absence of multifocal primary SB-NEN did not affect survival (P=0.75). Pathologically proven recurrence was present in 80/975 (8%) patients, and 9/80 (11%) had tumour dedifferentiation.

Figure 2. Overall survival of (A) all patients, (B) patients with different tumour grades, (C) patients based on tumour stage, (D) patients stratified for different treatments.
Figure 2A. Overall survival of all patients.

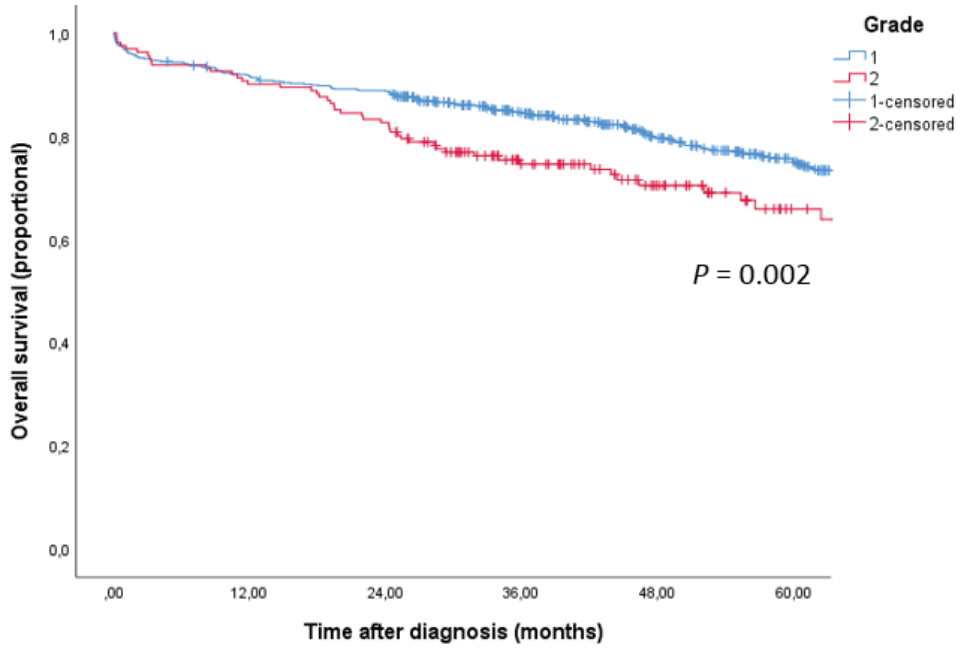


Number of patients at risk.

Time (mo)	0	12	24	36	48	60
All patients	968	866	828	681	542	432

Survival (mo)	Mean OS (95% CI)	Median OS (95% CI)	5-year OS
All patients	103.3 (99.1-136.7)	Not reached	67%

Figure 2B. Overall survival of patients with different tumour grades.

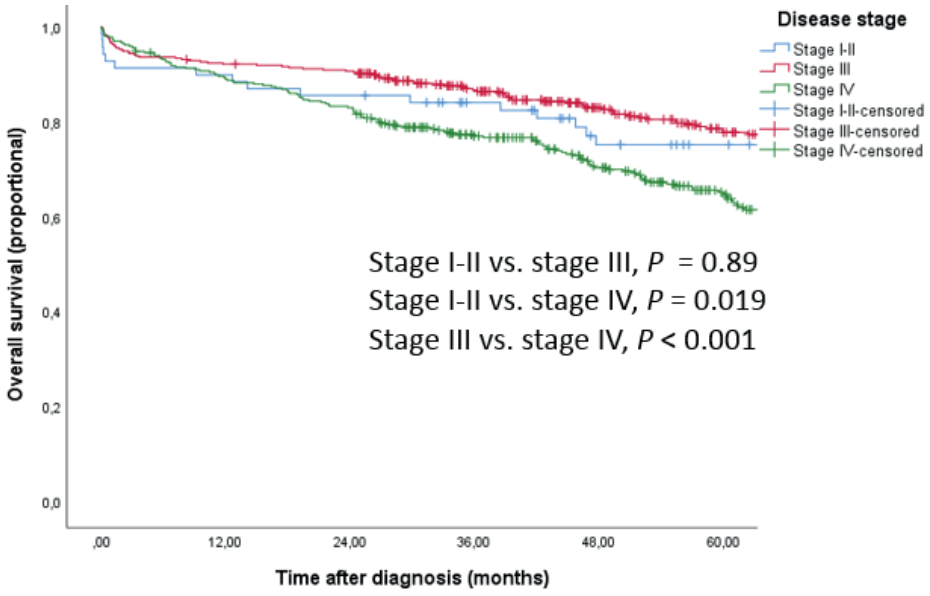


Number of patients at risk.

Time (mo)	0	12	24	36	48	60
Grade 1	683	622	603	508	408	331
Grade 2	161	145	133	88	60	34

Survival (mo)	Mean (95% CI)	Median (95% CI)	5-year OS
Grade 1	108.5 (103.5-113.4)	Not reached	70%
Grade 2	82.8 (72.6-92.9)	97.3 (71.5-123.1)	64%

Figure 2C. Overall survival of patients based on tumour stage.

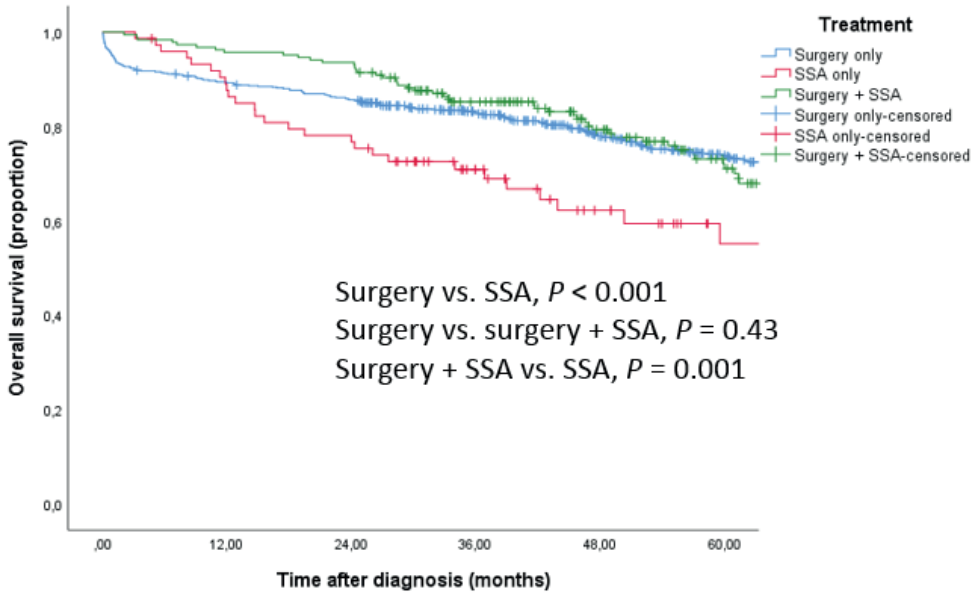


Number of patients at risk.

Time (mo)	0	12	24	36	48	60
Stage I-II	69	62	59	51	40	34
Stage III	406	373	366	304	241	193
Stage IV	365	323	301	241	193	145

Survival (mo)	Mean (95% CI)	Median (95% CI)	5-year OS
Stage I-II	113.9 (99.4-128.4)	Not reached	75%
Stage III	114.8 (108.6-121.0)	Not reached	75%
Stage IV	92.5 (85.7-99.4)	94.9 (79.5-110.3)	57%

Figure 2D. Overall survival of patients stratified for different treatments.



Number of patients at risk.

Time (mo)	0	12	24	36	48	60
Surgery	643	571	547	469	386	319
SSA	74	64	57	37	24	13
Surgery + SSA	187	179	175	137	101	71

Survival (mo)	Mean (95% CI)	Median (95% CI)	5-year OS
Surgery	108.0 (103.0-113.0)	Not reached	69%
SSA	63.0 (53.3-72.7)	72.4 (52.1-92.7)	51%
Surgery + SSA	99.9 (89.7-110.2)	113.5 (84.9-142.2)	68%

Treatment strategies

The majority of the patients underwent surgery (86%), which comprised resection of the primary tumour in 99% (Table 2). The R0 resection rates increased over the years and was 84%, 81% and 62% in stage I-II, stage III and stage IV disease (Table 2). Findings that suggest an incidental diagnosis were ileus, stenosis and perforation, which were reported in 2–3% of the pathology reports. SSA use was significantly higher in university hospitals 90/223 (40%) patients, compared to 98/613 (16%) patients in regional hospitals (P<0.001).

Table 2. Trends in treatment for patients with SB-NEN in the Netherlands, according to postoperative disease stage.

Stage	Treatment	2005-2007, No. (%)	2008-2011, No. (%)	2012-2015, No. (%)
Stage I-II	Total patients	13 (100)	25 (100)	32 (100)
	Primary resection[a]	13 (100)	25 (100)	32 (100)
	R0	10 (77)	20 (80)	27 (84)
	R1/2	2 (15)	2 (8)	2 (6)
	SSA	-	1 (4)	4 (13)
Stage III	Total patients	73 (100)	142 (100)	195 (100)
	Primary resection[a]	70 (96)	136 (96)	190 (97)
	R0	46 (65)	100 (74)	154 (81)
	R1/2	12 (17)	20 (15)	20 (11)
	SSA	4 (5)	17 (12)	43 (22)
	PRRT	-	1 (1)	-
	Systemic therapy	-	-	1 (1)
	No therapy	-	2 (1)	3 (2)
Stage IV	Total patients	73 (100)	123 (100)	170 (100)
	Primary resection[a]	61 (84)	89 (72)	116 (68)
	R0	31 (51)	61 (69)	72 (62)
	R1/2	15 (25)	18 (20)	24 (21)
	Metastasectomy	11 (15)	23 (19)	20 (12)
	SSA	20 (27)	54 (44)	107 (63)
	Systemic therapy	2 (3)	2 (2)	2 (1)
	RFA	1 (1)	1 (1)	-
	PRRT	1 (1)	2 (2)	-
	Embolization	-	2 (2)	3 (2)
	Radiotherapy	-	1 (1)	-
	No therapy	6 (8)	2 (2)	9 (5)

Abbreviations: PRRT: peptide receptor radioligand therapy, RFA: radiofrequency ablation, SSA: somatostatin analogues. Treatments are reported in a range of 9 months before or after diagnosis.

[a] Resection margins do not add up to 100% due to missing variables.

Survival was not different for stage IV disease after primary or simultaneous resection of primary and metastases (Supplementary Fig. 1). Survival after surgery and after surgery combined with somatostatin analogues (SSAs) were significantly longer than survival after SSA alone (P<0.001, P=0.001) (Figure 2D). A similar effect is observed in the presence of distant metastases (Supplementary Fig. 2). Surgery, SSA or a combination of both were included in the survival analyses, since the other treatment groups were too small.

In Table 2, time trends in treatment modalities for SB-NEN are presented. All patients with stage I-II disease underwent resection. Throughout the years, the resection rate for stage I-III disease remained high (96–100%) and administration of SSAs increased from 5 to 22% for stage III between 2005 and 2015. In patients with stage IV disease, the primary tumour resection rate decreased, while administration of SSAs more than doubled during the study period from 27 to 63%. Complete (R0) resections were performed in 57/62 (90%) patients with stage I-II disease, 300/352 (85%) patients with stage III disease and 164/221 (74%) patients with stage IV disease. All treatments that are reported in the NCR database took place within 9 months from diagnosis.

Factors associated with survival

Male sex, age between 20 and 40, 65–80 and ≥80 years, stage I-II and III disease, grade 2 tumours, surgery and SSA use showed an association with a shorter overall survival in univariable analysis. In multivariable analysis, male sex (HR 1.39, 95% CI 1.09–1.78, P=0.008), age between 65 and 80 years (HR 2.93, 95% CI 2.23–3.87, P<0.001), age ≥80 years (HR 9.99, 95% CI 6.61–15.11, P<0.001), stage III disease (HR 0.51, 95% CI 0.38–0.69, P<0.001 with stage IV as reference), grade 2 tumours (HR 1.48, 95% CI 1.09–2.02, P=0.013) and not having surgery (HR 1.50, 95% CI 1.07–2.09, P=0.018) all showed a significant association with a shorter overall survival. The results of univariable and multivariable analyses for overall survival are shown in Table 3.

Table 3. Univariable and multivariable survival analyses of patients with SB-NEN in the Netherlands.

Risk factors	Univariable analysis		Multivariable analysis	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Sex				
Male	1.19 (0.96-1.48)	**0.12**	1.39 (1.09-1.78)	**0.008**
Female	1 [Reference]		1 [Reference]	
Age				
<20	-		-	
20 up to 40	0.20 (0.03-1.41)	**0.11**	0.27 (0.04-1.95)	0.19
40 up to 65	1 [Reference]		1 [Reference]	
65 up to 80	2.81 (2.19-3.60)	**<0.001**	2.93 (2.23-3.87)	**<0.001**
≥80	6.34 (4.52-8.89)	**<0.001**	9.99 (6.61-15.11)	**<0.001**
Multiple primary SB-NEN				
Yes	1.03 (0.77-1.38)	0.83	-	
No	1 [Reference]		-	
Disease stage				
Stage I-II	0.56 (0.34-0.91)	**0.020**	0.63 (0.37-1.07)	0.08
Stage III	0.55 (0.42-0.70)	**<0.001**	0.51 (0.38-0.69)	**<0.001**
Stage IV	1 [Reference]			
Tumour grade				
Grade 1	1 [Reference]		1 [Reference]	
Grade 2	1.49 (1.12-1.99)	**0.006**	1.48 (1.09-2.02)	**0.013**
Resection margin				
R0	1 [Reference]		-	
R1/2	1.14 (0.81-1.60)	0.44	-	
Surgery				
Yes	1 [Reference]		1 [Reference]	
No	1.99 (1.53-2.62)	**<0.001**	1.50 (1.07-2.09)	**0.018**
SSA				
Yes	1.25 (0.98-1.60)	**0.07**	1.09 (0.81-1.46)	0.57
No	1 [Reference]		1 [Reference]	

DISCUSSION

This population-based study observed an increase in incidence of grade 1 and 2 SB-NEN between 2005 and 2015, and surgery remained the mainstay of treatment. The most remarkable changes were seen for stage IV SB-NEN, with a reduced rate of surgery and substantial increase in the use of SSAs. Survival did not change over time. Five-year overall survival rate of 75% for stage I-II disease was relatively low and similar to survival of patients with stage III SB-NEN.

The increase in incidence of SB-NEN in the Netherlands (56% in the last 10 years (0.81 per 100.000 persons) can be explained by more clinical awareness and increased utilization of cross-sectional imaging for any reason, even including screening. Such imaging might reveal asymptomatic liver lesions or lymph node metastases in the mesentery, that eventually turn-out to originate from SB-NEN. Furthermore, SB-NEN might increasingly be diagnosed as incidentalomas by pathologists in resection specimens after surgery for other diagnoses. The incidence has also risen compared to the prior study conducted in the Netherlands, which dates from 2001 [5]. Previous population-based studies conducted in Europe reported comparable increased incidence rates: 0.29 in Austria (2004–2005, grade 1 and 2 only), 0.30 in Italy (1981–2005, grades not reported), 0.80 in Iceland (2000–2014, grades not reported) and 0.81 in Norway (1993–2004, grades not reported), per 100.000 persons (18–21). Another explanation for the increase in incidence is an ageing population.

While some studies report relatively high 5-year overall survival rates for stage IV disease (69–85%), this trend is not seen in the Netherlands where survival rates are lower (57%) [1–22]. Similarly, 5-year overall survival in stage I-II was relatively low (75%) compared to the literature [3]. This could be due to differences in patient populations regarding competing risks of death, besides pathological classification, inclusion criteria (because only grade 1 and 2 were included), statistical methods, treatment differences between countries, and different inclusion periods. Survival outcomes differ between treatment strategies (Figure 2D), but this is probably due to the imbalance in disease stages among the different treatment modalities (Table 2). We also observed a relatively low pathologically proven recurrence rate (8%), which is most likely an underestimation, as other studies report recurrence rates as high as 31–64% [23, 24]. The low recurrence rate might be explained by the high frequency of lack of histological confirmation of recurrent disease and subsequently under-reporting in the PALGA database. Indeed, Cives et al. diagnosed macroscopic recurrence by imaging or surgical exploration, and Le Roux et al. diagnosed recurrence in asymptomatic patients with imaging during follow-up monitoring [23, 24].

Multifocal SB-NEN were only present in 18% of the patients, which differs from the literature (45–54%) [25, 26]. However, our data show that tumour multifocality is not associated with overall survival [26]. A Swiss population-based study investigating treatment sequences in NENs found that 80% of SB-NEN patients received surgery (either with or without subsequent therapy), which is a similar rate as found by the present study (86%) [27]. An increase in SSA administration was seen for all stages, with a doubling for stage IV SB-NEN. This is probably a consequence of the positive effects of SSAs that have been reported: reduction of excessive hormone secretion by (liver) metastases, prolonged progression-free survival and anti-proliferative effects [28, 29].

Surprisingly, no significant differences in neither clinical or pathological TNM stages were observed between university and regional hospitals. Hence, patients were not referred for surgical resection to either one of those centres based on cTNM stages between 2005 and 2015. It is likely that centralization improves patient outcomes as choosing the right treatment strategy is evenly, if not more, challenging than executing the treatment itself (except for

complex surgery). Nevertheless, current data did not show any survival difference between academic and regional hospitals. Probably, clinicians should focus first on discussing all patients in a multidisciplinary team (MDT) meeting in a specialized centre for NENs. Taken together, an international, multicentre registry with data on patient level is needed to carefully investigate diagnostic, treatment and outcome variables.

Long-term nationwide population-based data were used for this study, making it more representative than cohort studies and enable description of trends over the years. However, the findings of this study should be seen in light of some limitations. Comorbidity data were missing which might have influenced survival. To reduce this effect, patients with multiple primary cancers were excluded from survival analyses. Second, pTNM stage was used as a stratifying factor although in real-life treatment strategies are chosen based on cTNM stage. Third, pathological classification of NENs according to the WHO has changed over the years. This could lead to wrongly classified lesions. Fourth, imaging data during follow-up were not present, which is especially useful to give insights in disease recurrence and therefore the actual incidence is probably higher than reported by the NCR. Finally, treatment is only registered within 9 months from diagnosis, and the dataset lacks details on several specific local treatments of metastatic disease, such as peptide receptor radionuclide therapy, embolization, stereotactic radiotherapy and thermal ablation. This limits evaluation of all types of treatments given during complete follow-up.

In conclusion, this study showed an increase in the incidence of grade 1 and 2 SB-NEN, which is not uniformly reported in Europe. Surgery is still the cornerstone of treatment. An increase in use of SSAs was observed in stage IV disease over time. Stage-dependent survival was relatively low compared to the literature and remained similar over time.

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Author contributions

Conceptualization: EK, EJMNvD, and AFE.
Data curation: EK.
Formal analysis: EK and AFE.
Supervision: AFS, SvE, KMAD, HJK, PJT, EJMNvD, and AFE.
Validation: EK, AFS, SvE, KMAD, HJK, PJT, EJMNvD, and AFE.
Writing—original draft: EK.
Writing—review and editing: EK, AFS, SvE, KMAD, HJK, PJT, EJMNvD, and AFE.

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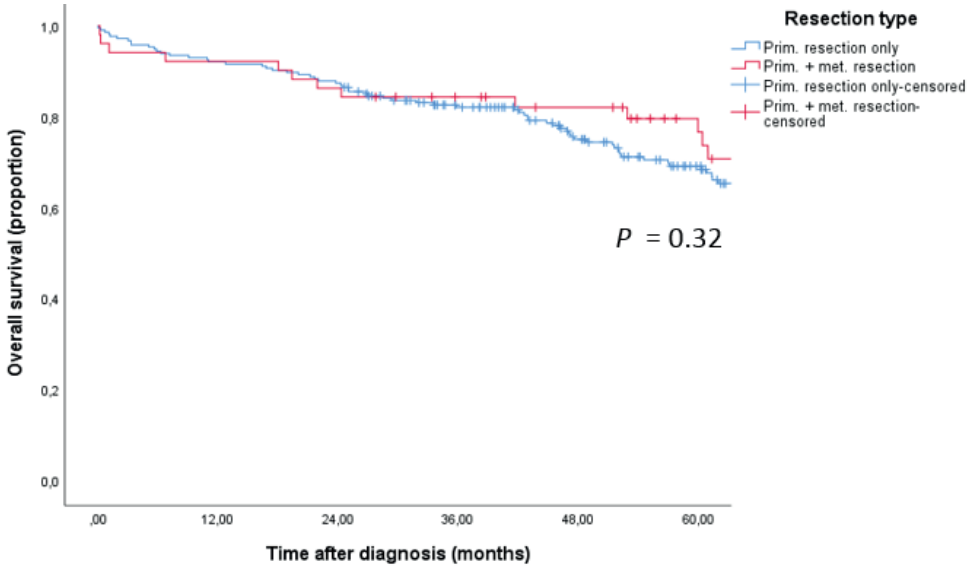
SUPPLEMENTARY MATERIALS

Supplementary figure 1. Survival of patients with stage IV disease after primary tumour resection only or resection of primary tumour and metastases.

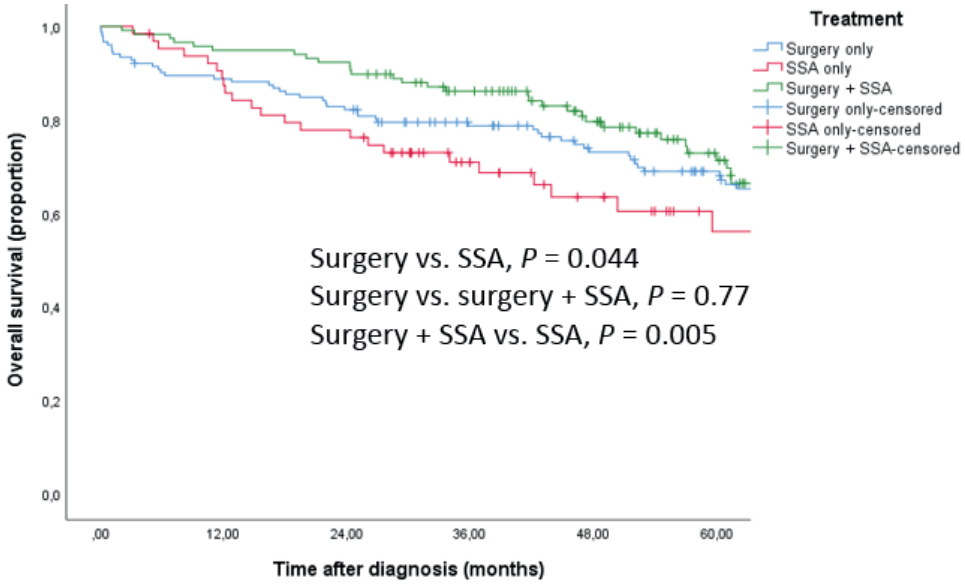


Number of patients at risk.

Time (mo)	0	12	24	36	48	60
Primary	214	197	187	156	123	94
Primary + metastases	51	47	44	39	35	27

Survival (mo)	Mean (95% CI)	Median (95% CI)	5-year OS
Primary	98.9 (90.2-107.6)	Not reached	60%
Primary + metastases	104.4 (88.2-120.6)	Not reached	67%

Supplementary figure 2. Survival of patients with stage IV disease after different treatment strategies.



Number of patients at risk.

Time (mo)	0	12	24	36	48	60
Surgery	152	134	124	105	89	75
SSA	64	55	49	31	23	13
Surgery + SSA	117	111	108	91	70	47

Survival (mo)	Mean (95% CI)	Median (95% CI)	5-year OS
Surgery	99.3 (89.0-109.5)	Not reached	58%
SSA	63.6 (53.3-73.9)	72.4 (51.8-93.0)	52%
Surgery + SSA	98.8 (86.6-111.0)	101.7 (72.4-131.0)	66%

CHAPTER 3

IgG4 expression in small bowel neuroendocrine neoplasms with radiological signs of mesenteric fibrosis and the introduction of the mesenteric fibrosis score; a new tool for quantifying mesenteric fibrosis

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Submitted

ABSTRACT

Background Mesenteric fibrosis in patients with small bowel neuroendocrine neoplasm (SB-NEN) might eventually lead to ischemia or bowel obstruction. The aim of this study was to investigate the relationship between IgG4 expression, the extent of mesenteric fibrosis and other clinicopathological features.

Methods This retrospective study included patients who underwent resection of a SB-NEN. Imaging data from preoperative scans were assessed, and mesenteric fibrosis was quantified. Formalin fixed paraffin embedded resection material of these patients was selected for additional IgG/IgG4 immunostaining. Subgroup analyses were performed for patients with high and low mesenteric fibrosis scores using a novel tool specifically designed for this purpose.

Results A total of fourteen patients with a mean age of 64 years were included. The median (interquartile range) mesenteric fibrosis score was 54 (39-62). Ten out of fourteen samples had IgG4 positive plasma cells surrounding the tumour cells. The mean IgG/IgG4 ratio was lower in the group with mesenteric fibrosis score <54 (24%) compared to the ≥54 group (36%). Tumours were grade 2 in 60% of patients with IgG/IgG4 ratios over 40%, and in 22% with IgG/IgG4 ratios less than 40%. Higher mean IgG/IgG4 ratios were seen in stage IV vs. stage III patients (34 vs. 21%) and in symptomatic vs. asymptomatic patients (32 vs. 21%).

Conclusion There is a trend towards a higher IgG/IgG4 ratio in patients with more extensive mesenteric fibrosis, higher grade tumours, higher stage and symptomatic disease. Further research is warranted to translate these findings to clinical practice and to further validate the mesenteric fibrosis score.

INTRODUCTION

Hormonal active neuroendocrine neoplasms (NENs) may trigger carcinoid syndrome, which is known to have fibroproliferative effects, leading to cardiac valvulopathy, and mesenteric fibrosis in case of small bowel NEN (SB-NEN) [1]. At presentation, about 40 to 55% of SB-NEN patients have signs of mesenteric fibrosis on imaging [2, 3]. Typical features are the spoke-wheel appearance with radiating strands of soft tissue, which might look like IgG4-related sclerosing mesenteritis (Figure 1) [4, 5].

Mesenteric fibrosis can lead to encasement of adjacent mesenteric vessels, edema, (segmental) intestinal ischaemia, venous stasis, intestinal obstruction which in turn result in (postprandial) abdominal pain, progressive food intolerance and eventually cachexia [2, 4-6]. Hence, care should be taken in resection of SB-NEN with mesenteric fibrosis, as extensive mesenteric fibrosis is associated with postoperative morbidity [6]. Careful follow-up of patients with small bowel NEN and mesenteric fibrosis should also focus on the progression of the fibrosis and indications of timely surgical interventions. Because of the prevalence of mesenteric fibrosis in SB-NEN and the additional surgical challenges, methods to downsize or minimize the extent of mesenteric fibrosis preoperatively could be of added value in the surgical and clinical management (i.e. fewer ischemic complaints) of these patients. Previous research reported IgG4 expressing plasma cells in mesenteric tumour deposits of SB-NENs [5]. If a causal relationship exists, patients with mesenteric fibrosis and IgG4 expressing plasma cells could benefit from preoperative immunosuppressive therapy, similarly to IgG4 related gastrointestinal disease [7]. However, the relation between the IgG/IgG4 ratio and IgG4 positive plasma cells and the extent of mesenteric fibrosis in patients with SB-NEN has yet to be elucidated. Therefore, we aim to investigate the relation between the IgG/IgG4 ratio in tumour deposits and the extent of mesenteric fibrosis in SB NEN patients, and secondly investigate the relationship between the IgG/IgG4 ratio in tumour deposits and other relevant clinicopathological features of SB-NEN.

METHODS

Patients

This retrospective cohort study included consecutive patients who underwent resection of an histopathologically confirmed SB-NEN between 2009 and 2019 at Amsterdam UMC, University of Amsterdam with available archival Formalin-Fixed Paraffin-Embedded (FFPE) resection material. Patients were identified from an institutional database. All patients gave written informed consent to re-use their data and perform additional immunohistochemistry (IHC) on available FFPE archival material. This study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments.

Outcome parameters

The primary outcome was the correlation between the IgG/IgG4 ratio and the extent of mesenteric fibrosis. Secondary outcomes included: the correlation of IgG/IgG4 ratio to other

clinicopathological characteristics: sex, tumour grade, symptomatic disease and usage of somatostatin analogues.

Preoperative imaging

All preoperative imaging (68Ga-DOTATATE positron emission-/computed tomography, PET/CT) scans were independently assessed by an expert radiologist and a researcher (E.K. and J.C.C.K). In case of discrepancy, consensus was reached by discussion. The following data were extracted from PET/CT-scans: long and short axis of the mesenteric deposit, number of strands and their thickness, all in mm. Fibrotic strands were defined as radiating soft-tissue strands (i.e. with a 'spoke-wheel' appearance) (Figure 1). Measurements were made using the build-in measuring tool of Enterprise Imaging XERO Viewer (Agfa HealthCare, Mortsel, Belgium), in either coronal or transverse planes, whichever was better to make measurement in. Decimals were rounded to the nearest whole number.

To enable quantitative analysis, a formula was developed to translate imaging data to a single value, which we called 'mesenteric fibrosis score' (dimensionless number). The mesenteric fibrosis score was calculated by adding the mean size of the mesenteric deposit (long + short axis divided by 2) to the sum of the amount of strands (N * 1 mm strands + N * 2 mm strands + N * 3 mm strands + N * 4 mm strands + N * 5 mm strands). Using patient 1 as an example, the formula is as follows: (22+33/2) + (1*1 + 7*2 + 2*3 + 0*4 + 0*5) = 27.5 + 21 = 48.5.

Pathology

Haematoxylin & eosin (H&E) slides were reviewed by a blinded GEP-NEN expert pathologist (A.F.S.) and sections were selected for additional immunohistochemical stainings with IgG and IgG4 antibodies. See Supplementary Table 1 for used materials. The IgG/IgG4 ratio was calculated by counting the number of IgG4-positive and IgG positive cells was manually, which was performed by E.K. and A.F.S. in the area with the most IgG4 positive cells by light microscopy (Olympus, Tokyo, Japan). We decided to use IgG/IgG4 ratio, as the count of individual cells is highly dependent on the density of lymphoplasmocellular infiltrates, and therefore the ratio gives a more comprehensive indication of the plasma cell subtype following the European guideline on IgG4-related disease [7]. Digital images were made using the Olympus BX51 microscope with the Olympus UC90 camera and Olympus CellSens Entry 2.2 software (Olympus, Tokyo, Japan). According to the recently reviewed European Guidelines for the diagnosis of IgG4 related disease [7], the following data were scored: number of IgG and IgG4 positive plasma cells, ratio of IgG/IgG4 positive plasma cells, the presence or absence of sclerotic stroma (prominent / occasional / none), the presence of absence of lymphoplasmacytic infiltration (prominent / occasional / none), the presence or absence of storiform fibrosis (present / absent), and the presence or absence of obliterative phlebitis (present / absent). A cut-off of 40% IgG/IgG4 ratio was used to group patients as determined by the European Guidelines for the diagnosis of IgG4 related disease [7]. The tumour grade (Ki67) was extracted from the diagnostic reports.

Statistical analysis

Categorical data is reported as percentages (counts divided by the number of patients who had data that could be evaluated). Continuous data is reported as means with standard deviations (SD) or medians with interquartile ranges (IQR) (rates are based on patients who had data that could be evaluated), depending on the distribution. Correlations were tested with the Spearman's Rank correlation test, or Pearson correlation test, depending on the distribution of the data. The median of the mesenteric fibrosis score was used as a cut-off value to categorize low and high groups. Post-hoc superiority power analyses was performed using Sealed Envelope [8]. A two-sided *P*-value less than 0.05 was considered statistically significant. Data was analysed using the Statistical Package for Social Sciences (SPSS) version 26.0.0.1. (IBM Corp. Armonk, NY, USA).

RESULTS

We identified 33 patients who were operated because of SB-NEN. Evaluable imaging studies and paraffin tissue were available in 14 (42%) patients, and those patients could be included in the present study. Of those 14 patients, the mean (±SD) age was 64 (±9) years, and half of the patients were male (Table 1).

Radiological features

Radiological assessment of the extent of mesenteric fibrosis was performed on PET-CT images in eight patients and CT images only in the remaining six patients (Figure 2, left panels). The mean (±SD) length of the short and long axis of mesenteric fibrosis was 24 (± 9) mm and 36 (±12) mm, respectively. Fibrotic strands were present in all patients, with a mean (± SD) of 8 (±6) strands, with a median (IQR) thickness of 2 (2-3) mm. The median (IQR) mesenteric fibrosis score was 54 (39-62) (Table 1), so the two subgroups created had a mesenteric fibrosis score of <54 or ≥54. Individual values of the measurements are presented in Supplementary Table 2.

IgG4 positivity

Eleven of fourteen tissue samples showed IgG4 positive plasma cells (Figure 2, middle and right panels). Stainings of 3 patients were IgG4 negative (Figure 2F). Features of IgG4 related fibrosis as described by the European guidelines were scored as follows: prominent sclerotic stroma present in 55% (6/11) of the patients, no lymphoplasmacytic infiltration in 57% (8/14), occasional lymphoplasmacytic infiltration in 46% (5/11), prominent lymphoplasmacytic infiltration present in 18% (2/11), storiform fibrosis was present in 18% (2/11) and obliterative phlebitis was present in 27% (3/11) of the patients.

IgG/IgG4 ratio and mesenteric fibrosis

The IgG/IgG4 ratio was lower, although statistically not significant, in the group of patients with mesenteric fibrosis score <54 (in 7/14 patients) compared to the 7/14 patients with mesenteric fibrosis score >54 (mean 24% vs. 36%, *P* = 0.37 (Figure 3). Five patients had IgG/

IgG4 ratios over 40%, and those patients had larger mean (SD) tumour size as compared to the 9 patients with ratios less than 40%, although not reaching statistical significance: 35 mm (±13) versus 27 mm (±7), respectively (P = 0.17). The mean (SD) number of strands were lower in the group with an IgG/IgG4 ratio higher than 40% (5 strands ± 2) compared to patients with a ratio lower than 40% (10 strands ± 6), neither reaching significance (P = 0.16).

Table 1 Patient, imaging and pathology characteristics.

Patient	Sex	Age	Ki67	MF-score	IgG/IgG4 ratio	Sclerotic stroma	Lymphoplasmacytic infiltration	Storiform fibrosis	Obliterative phlebitis
Patient 1	F	64	5%	48.5	0	None	None	Present	Absent
Patient 2	M	55	2%	55	44%	Occasional	Occasional	Absent	NA
Patient 3	F	72	10%	58.5	24%	Prominent	None	Absent	Present
Patient 4	M	70	5%	56	76%	Prominent	Occasional	Absent	Present
Patient 5	F	52	1%	35	0	Occasional	None	Absent	Absent
Patient 6	M	73	10%	29.5	52%	Prominent	None	Absent	Absent
Patient 7	F	45	2%	63.5	45%	Occasional	Prominent	Present	Absent
Patient 8	F	66	2%	42	20%	Occasional	None	Absent	Absent
Patient 9	M	66	1%	63	34%	Prominent	Occasional	Absent	Absent
Patient 10	F	64	2%	40	8%	None	None	Present	Absent
Patient 11	M	72	2%	61.5	0	None	None	Absent	Absent
Patient 12	M	55	14%	52	53%	Prominent	Prominent	NA	Present
Patient 13	F	72	1%	73	27%	Prominent	Occasional	Absent	Absent
Patient 14	M	72	2%	34.5	35%	Occasional	None	Absent	Absent

Abbreviations: MF: mesenteric fibrosis, NA: not available

Figure 2. (A) CT-scan of patient 4 **(B)** H&E staining of patient 4, stage IV, grade 2 (Ki67 5%) **(C)** IgG4 staining of patient 4, IgG4 positive cells: 57, IgG/IgG4 ratio: 76% **(D)** Coronal slide of a CT-scan of patient 11 showing a mesenteric deposit, mesenteric fibrosis is not visible on this specific slide **(E)** H&E staining of patient 11, stage 3, grade 1 (Ki67 2%) **(F)** IgG4 staining of patient 11, IgG4 negative **(G)** Coronal slide of a CT-scan of patient 14 **(H)** H&E staining of patient 14, stage 3, grade 1 (Ki67 2%) **(I)** IgG4 staining of patient 14, IgG4 positive cells: 14, IgG/IgG4 ratio: 35%. All images were made with a 40x magnification.

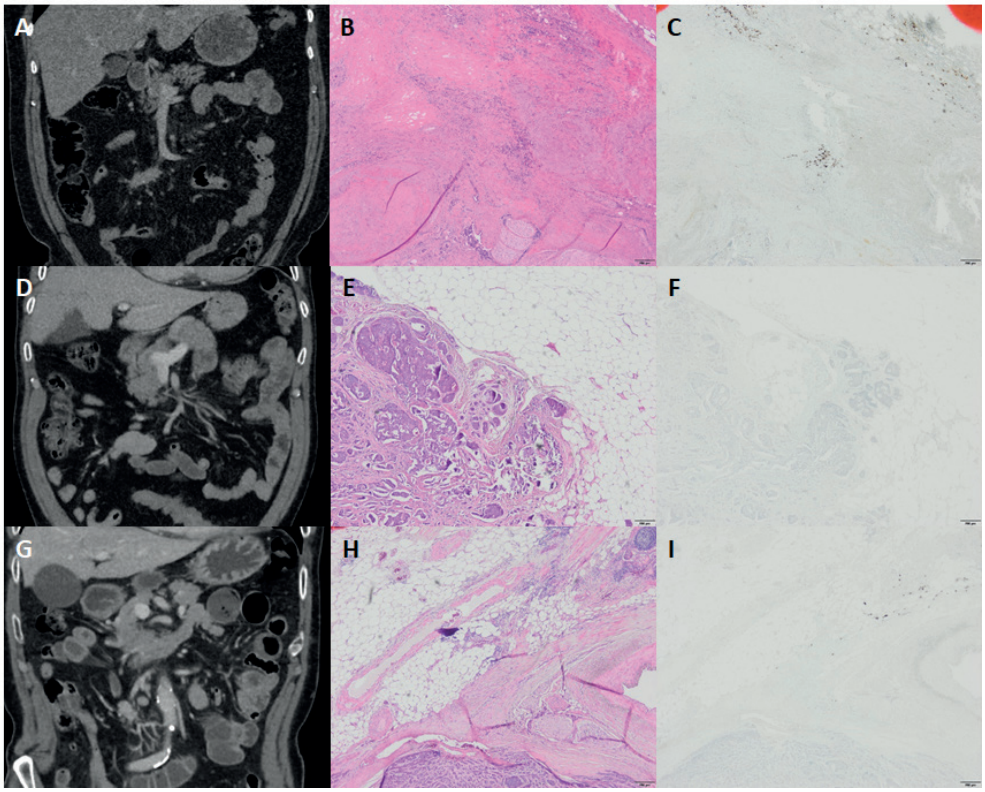
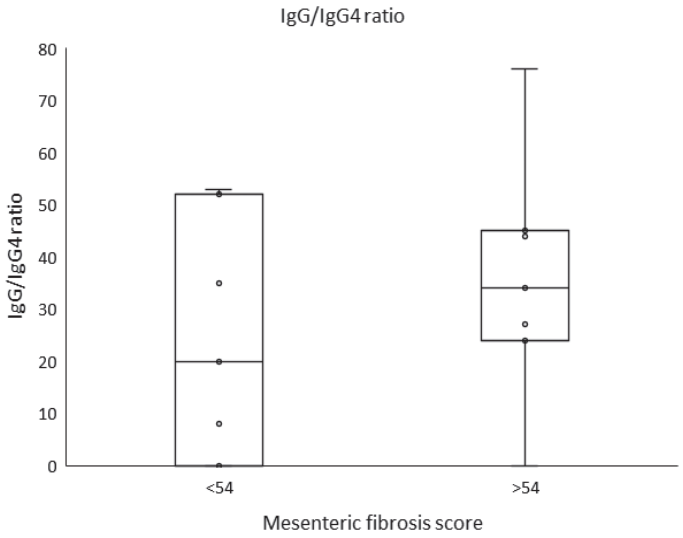


Figure 3. Boxplot of mesenteric fibrosis score (MF) and IgG/IgG4 ratio.



IgG/IgG4 ratio and clinicopathological features

Male patients had a significantly higher mean (SD) IgG/IgG4 ratio compared to females (42 ± 23% vs. 18 ± 16%, $P = 0.042$). Tumours were grade 2 in 3/5 patients (60%) with IgG/IgG4 ratios over 40%, and in 2/9 (22%) patients with IgG/IgG4 ratios less than 40% ($P = 0.16$). Higher mean (SD) IgG/IgG4 ratios not reaching statistical significance were seen in stage IV vs. stage III patients (34 ± 26% vs. 21 ± 16%, $P = 0.29$), in symptomatic vs. asymptomatic patients (32 ± 23% vs. 21 ± 26%, $P = 0.48$), and in patients received somatostatin analogues vs. patients without somatostatin analogues (32 ± 21% vs. 28 ± 25%, $P = 0.76$).

DISCUSSION

The present study explores the relation between IgG4 positive plasma cells in mesenteric tumour deposits in patients with SB-NEN and mesenteric fibrosis. Patients with a greater mesenteric fibrosis score had a higher IgG/IgG4 ratio, although this association did not reach statistical significance. This hypothesis generating finding needs to be confirmed. Based on the current results, 116 patients are required to have an 80% chance of detecting, as significant at the 5% level, an increase of the mean IgG/IgG4 ratio of 24% to 36%.

One unanticipated finding of the current study was the suggested association between an IgG/IgG4 ratio greater than 40% and grade 2 SB-NEN. This finding was not reported by Roberts *et al.* which is to our knowledge one of the first publications investigating the relationship between SB-NEN and IgG4 [5]. A possible explanation for this could be that the aim of that study was to investigate histological and immunophenotypic overlap with IgG4-related sclerosing mesenteritis, in which the association between tumour grade and IgG/IgG4 ratio was not described.

Males had significantly higher IgG/IgG4 ratios compared to females, which is consistent with literature for IgG4 related disease [7]. Patients with higher IgG/IgG4 ratios had more often grade 2 tumours, stage IV and symptomatic disease, although these associations did not reach statistical significance. Hence, it seems that a higher IgG/IgG4 ratio is associated with advanced disease, which was not previously observed for SB-NEN. These observations do not allow for definitive conclusions and warrant future studies.

We did not use the scoring system of mesenteric fibrosis previously proposed by Pantongrag-Brown *et al.*, because we wanted to express the extent of mesenteric fibrosis on a continuous objective scale for analysis purposes, whereas Pantongrag-Brown uses terms as "thin/thick" strands, which in our opinion are subjective and not fully reproducible [9]. To overcome this problem, we developed a new scoring system to quantify the extent of mesenteric fibrosis. Although the scoring system is a promising easy-to-use tool on preoperative imaging, it has yet to be validated. Ideally, this would involve multiple radiologists, and a comparison with the first known classification system of Pantongrag-Brown [9]. After validation and optimisation, this tool could be of added value in decision making regarding surgical management of these patients like operability and surgical approach [10]. Furthermore, the location of the lymph nodes could also be taken into account as previously proposed by Ohrvall et al. [11]. The validation and optimisation of the scoring model is however beyond the scope of this paper.

The tumour microenvironment of SB-NEN is complex and contains a multitude of pro-fibrotic factors such as: transforming growth factor beta, connective tissue growth factor, platelet-derived growth factor, insulin-like growth factor 1, epidermal growth factor, and transforming growth factor alpha, which can for example be targeted by tyrosine kinase inhibitors [12]. The rationale behind the present study was to determine whether IgG4 could be added to this list by investigating the relation between IgG4 expression and the extent of mesenteric fibrosis in SB-NEN patients. Like fibrosis within the IgG4 related disease, SB-NEN patients could benefit from immunosuppressive therapy (e.g. prednisone) before surgery to downsize extent of mesenteric fibrosis, but also to avoid complaints related to ischemia in inoperable patients.

This is an exploratory study in a rather small cohort of patients and although the association between IgG4 and mesenteric fibrosis was not statistically significant, this might form the basis for further research to validate our new quantitative mesenteric fibrosis scoring system, and subsequently correlate this with IgG/IgG4 ratios and serum IgG4 levels. Such a study would ideally be conducted using large institutional databases or biobanks, because 116 patients are necessary to have sufficient statistical power.

The present study has some limitations; firstly and most important is the small sample size, although this was an expected limitation due to the exploratory nature of the study. Secondly, there is an inherent amount of referral bias as we are a tertiary referral centre for SB-NEN. Finally, the mesenteric fibrosis score has yet to be externally validated, as it was specifically designed for the purpose of this study.

In conclusion, we describe here a possible relationship between a higher IgG/IgG4 ratio and more extensive mesenteric fibrosis, higher tumour grade, higher stage and symptomatic disease. These correlations need further research. If IgG4 is ultimately proven to play an important role, potential therapeutic interventions might aim for downsizing of mesenteric fibrosis and limiting ischemic complaints in the future.

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Author contributions

Conceptualization: EK, AFE, JCCK, EJMNvD, and AFE.
Data curation: EK, JCC, PJT, AFS.
Formal analysis: EK.
Supervision: AFE, JCCK, KMAD, HJK, PJT, AFS, EJMNvD.
Validation: EK, AFE, JCCK, KMAD, HJK, PJT, AFS, EJMNvD.
Writing—original draft: EK.
Writing—review and editing: EK, AFE, JCCK, KMAD, HJK, PJT, AFS, EJMNvD.

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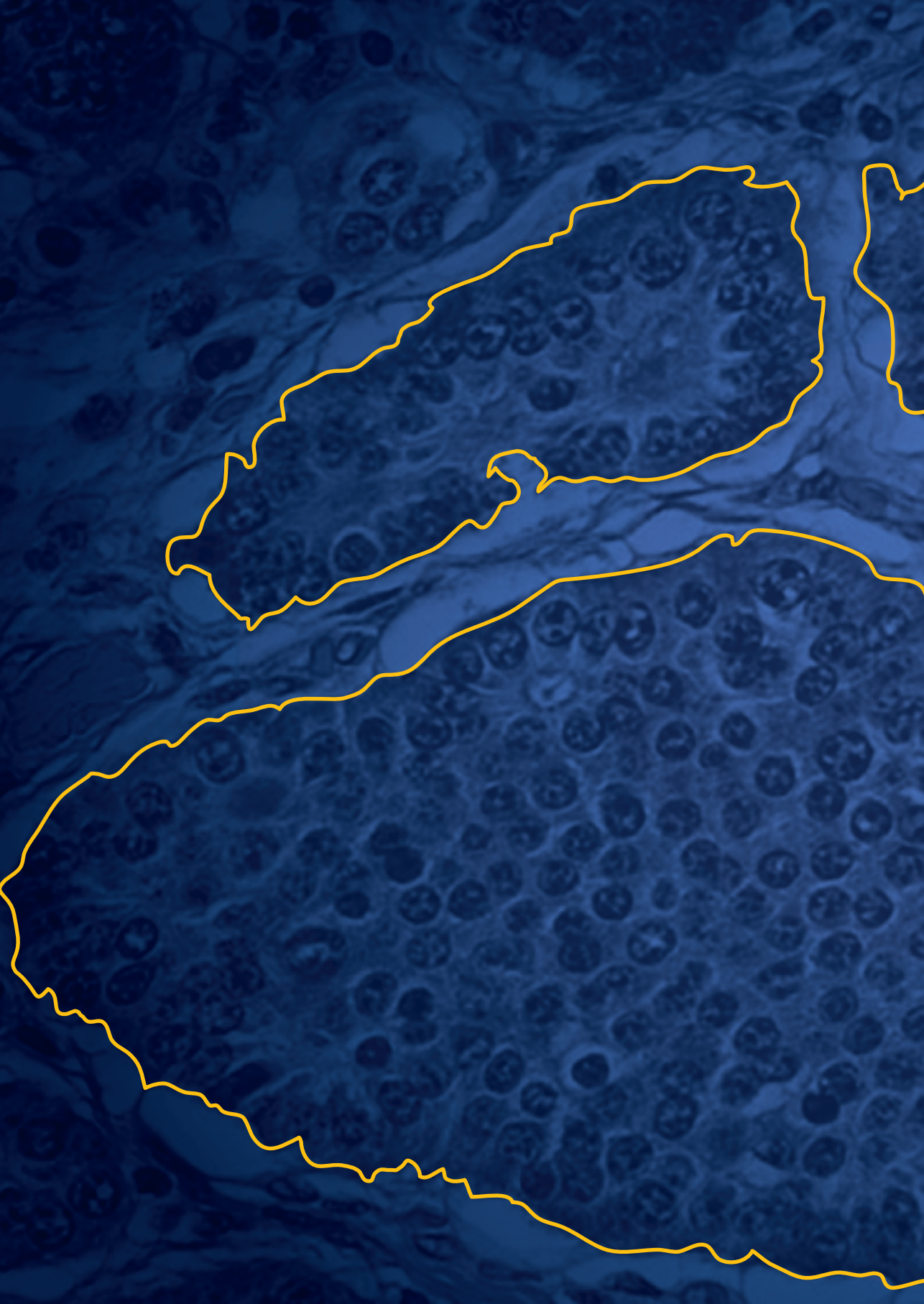
SUPPLEMENTARY MATERIALS

Supplementary table 1. Materials used for immunohistochemistry.

Material	Company	Description
Ventana (automated slide staining machine)	Roche, Basel, Switzerland	Used for automated antibody staining.
IgG	Invitrogen, Waltham, Massachusetts, USA	Used at 1:500 dilution.
IgG4	Invitrogen, Waltham, Massachusetts, USA	Used at 1:500 dilution.

Supplementary table 2. Individual values of the radiological measurements.

Patient	Mass short axis, mm	Mass long axis, mm	Mean size, mm	No. strands	No. 1 mm strands	No. 2 mm strands	No. 3 mm strands	No. 4 mm strands	No. 5 mm strands	Strand score	MF-score
Patient 1	22	33	27.5	10	1	7	2	0	0	21	48.5
Patient 2	36	42	39	7	1	3	3	0	0	16	55
Patient 3	22	33	27.5	13	0	10	2	0	1	31	58.5
Patient 4	17	43	30	7	0	3	0	0	4	26	56
Patient 5	23	25	24	4	0	1	3	0	0	11	35
Patient 6	17	28	22.5	2	0	0	1	1	0	7	29.5
Patient 7	46	63	54.5	4	0	3	1	0	0	9	63.5
Patient 8	24	28	26	5	0	0	4	1	0	16	42
Patient 9	22	34	28	15	5	5	0	5	0	35	63
Patient 10	26	28	27	5	0	3	1	1	0	13	40
Patient 11	31	56	43.5	7	0	3	4	0	0	18	61.5
Patient 12	25	31	28	7	0	1	2	4	0	24	52
Patient 13	15	33	24	23	0	22	0	0	1	49	73
Patient 14	15	22	18.5	6	0	3	2	1	0	16	34.5



PART II

Minimally invasive surgery for small bowel neuroendocrine neoplasms

CHAPTER 4

Postoperative morbidity and mortality after surgical resection of small bowel neuroendocrine neoplasms: a systematic review and meta-analysis

Enes Kaçmaz, Jeffrey W. Chen, Pieter J. Tanis, Els J. M. Nieveen van Dijkum and Anton F. Engelsman

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ABSTRACT

Background Although small bowel resection is generally considered a low risk gastrointestinal procedure, this might not be true for small bowel neuroendocrine neoplasms (SB-NEN) as a result of potential central mesenteric involvement. We aimed to determine the reported morbidity and mortality after resection of SB-NEN in the literature and assess the effect of hospital volume on postoperative morbidity and mortality.

Methods A systematic review was performed by searching MEDLINE and Embase in March 2021. All studies reporting morbidity and/or mortality after SB-NEN resection were included. Pooled proportions of overall morbidity (Clavien-Dindo I-IV), severe morbidity (Clavien-Dindo III-IV), 30-day mortality, 90-day mortality and in-hospital mortality were calculated, as well as the association with hospital volume (high volume defined as the fourth quartile).

Results Thirteen studies were included, with a total of 1087 patients. Pooled proportions revealed an overall morbidity of 13% (95% confidence interval [CI] = 7%-24%, I^2 = 90%), severe morbidity of 7% (95% CI = 4%-14%, I^2 = 70%), 30-day mortality of 2% (95% CI = 1%-3%, I^2 = 0%), 90-day mortality of 2% (95% CI = 2%-4%, I^2 = 35%) and in-hospital mortality of 1% (95% CI = 0%-2%, I^2 = 0%). An annual hospital volume of nine or more resections was associated with lower overall and severe morbidity compared to lower volume: 10% vs 15% and 4% vs 9%, respectively. Thirty-day mortality was similar (2% vs 1%) and 90-day mortality was higher in high-volume hospitals: 4% vs 1%.

Conclusion This systematic review with meta-analyses showed severe morbidity of 7% and low mortality rates after resection of SB-NEN. The currently available literature suggests a certain impact of hospital volume on postoperative outcomes, although heterogeneity among the included studies constrains interpretation.

INTRODUCTION

Small bowel neuroendocrine neoplasms (SB-NEN) are rare tumours of the small bowel with an incidence of one to four per 100,000 person years [1,2]. Patients are often amenable for surgery, in either a curative or palliative setting (50% stage I-III, 40% patients with liver metastases) [2-4]. Surgery consists of a partial small bowel resection or right hemicolectomy with mesenteric lymphadenectomy, and is sometimes combined with resection or debulking of liver or peritoneal metastases. Open surgery is still considered the standard approach, although minimally invasive surgery is emerging as an alternative technique in selected patients [5-10]. The timing of the resection is still a subject of debate (i.e., prevent or relieve obstructive symptoms) and remains unanswered by recent guidelines [11].

One of the challenges of SB-NEN surgery is the safe and complete resection of mesenteric lymph nodes, which are present in > 80% of patients [2,12]. Because mesenteric tumour masses can have a close relationship with the main mesenteric trunks, vascularisation of the small bowel may be at risk during central mesenteric dissection. Other potential complications after surgery for SB-NEN include postoperative haemorrhage, surgical site infections, abscess and anastomotic leakage [13].

It is a common assumption among healthcare providers that clinics with higher volumes of specific procedures have lower morbidity and mortality rates; for example, as reported for pancreatic and colorectal surgery [14,15]. Besides surgical experience, anesthesia management might also be relevant. This is especially the case for patients undergoing surgery for hormonally active NEN because intra-operative carcinoid syndrome develops in up to 55% of patients, regardless of preoperative prophylactic octreotide infusions [16]. Recently, Hallet et al. [17] investigated the association between anaesthesiologist volume and postoperative morbidity after complex gastrointestinal surgery. Interestingly, cases performed by high-volume anaesthesiologists had significantly less complications with a Clavien-Dindo grade III-V.

Because of the low incidence of SB-NEN, there is a restricted amount of literature compared to other high incidence gastrointestinal malignancies, and evidence is mostly based on observational studies. The aim of the present systematic review and meta-analyses was to determine the incidence of morbidity and mortality in patients with SB-NEN who undergo resection of the primary tumour, and to assess any potential association with hospital-volume.

METHODS

This systematic review and meta-analysis was performed in accordance with the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) statement and the Meta-analysis of Observational Studies in Epidemiology guideline [18,19]. The protocol of this study was registered in PROSPERO (www.crd.york.ac.uk/prospero) under registration number CRD42020185001.

Eligibility criteria

We aimed to identify all studies reporting on morbidity and mortality after SB-NEN resection. Both prospective and retrospective studies that were published in English after the year 2000 were included. Case reports, conference abstracts and reviews were excluded. In the case of a mixed population (ie, pancreatic NEN and SB-NEN), studies were excluded if no separate data were reported for patients with SB-NEN. Studies including patients with concomitant hepatectomies in more than 20% of the patients were excluded from analyses to limit heterogeneity. In the case of publications with overlapping patient cohorts, the study with the largest cohort size was included for analysis.

Literature search strategy

A search was performed in MEDLINE (PubMed) and Embase (Ovid) on 8 March 2021. The key words and Medical Subject Headings (MeSH) terms used for both databases were: ileal/jejunal neoplasms, neuroendocrine tumours, surgery, postoperative complications, morbidity and mortality. The complete search string is provided in (Table S1). Additional hand screening was performed with respect to the reference lists of included articles.

Study selection

Study selection was performed according to the PRISMA statement. Abstracts were screened for eligibility by two independent researchers (EK and JWC), using Rayyan software (Qatar Computing Research Institute, Doha, Qatar) [20]. Any discrepancies were resolved by discussion. Subsequently, two independent researchers (EK and JWC) screened full texts and selected studies for inclusion in the systematic review and meta-analysis.

Data collection and outcome parameters

Data collection was performed by one author (EK). Collected data included study characteristics (author, country, publication year, inclusion period), patient characteristics (age, sex, disease stage), operative characteristics (type of operation, surgical approach) and postoperative events. Outcome parameters were overall morbidity, severe morbidity, 30-day mortality, 90-day mortality and in-hospital mortality.

Overall morbidity was defined as Clavien-Dindo grade I-IV and severe morbidity was defined as grade III-IV [21]. All study authors were contacted to complete and correct extracted data. Low volume centres were defined as an annual case load equal or below the third quartile, whereas high-volume centres were defined as those with an annual case load higher than the third quartile.

Risk of bias

Risk of bias was assessed by one author (EK) using the Joanna Briggs Institute ([JBI] Faculty of Health Sciences, The University of Adelaide, South Australia) checklist for case series. The predefined criteria for each of the 10 questions in the JBI checklist (low, unclear or high risk of bias) were modified to suit the present study and are provided in (Table S2). A risk-of-bias graph displays overall risk of bias for each item on the JBI checklist across all included studies.

Statistical analysis

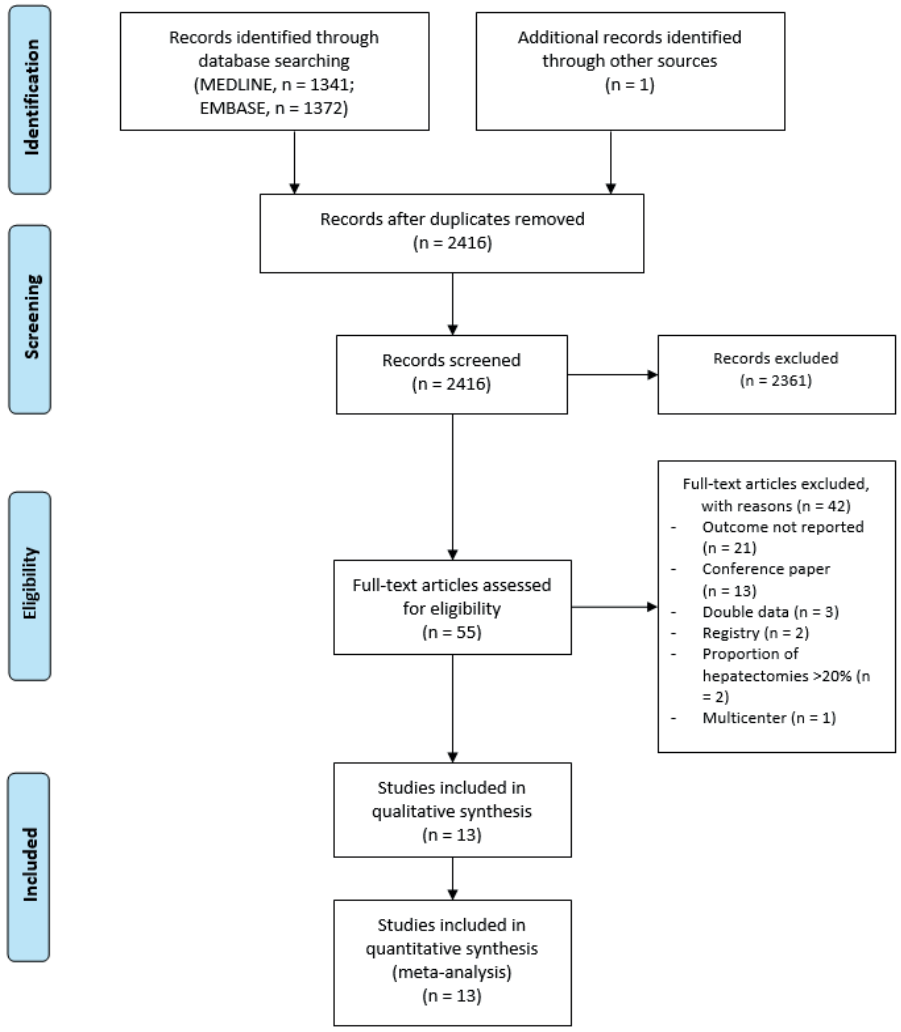
Postoperative events were classified according to the Clavien-Dindo classification in case the study authors did not already do so [21]. The annual hospital volume was estimated per publication using the formula: total number of patients/inclusion period in years. Subgroup analyses were performed for studies reporting outcomes after minimally invasive surgery. Categorical values are presented as numbers with percentages, whereas continuous data as presented as the mean ± SD or the median with interquartile range (IQR). Reported medians were converted to means using the method described by Wan et al [22]. Pooled proportions were calculated for the different outcome parameters. The results are presented in forest plots, providing an estimate of the mean proportion with a 95% confidence interval (CI). Heterogeneity was assessed using the I^2 statistic. $I^2 > 50\%$ was considered to indicate a moderate amount of heterogeneity, which resulted in use of the random effects model, and $I^2 > 75\%$ was considered to indicate a substantial amount of heterogeneity, for which a meta-analysis was not performed. Funnel plots were made to estimate publication bias. Meta-analyses were performed with a random effects model using the meta package, version 4.15-1 in Rstudio, version 1.2.5033 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Study selection

A total of 2416 studies were identified through the electronic search (without duplicates). After the screening and selection process, 13 studies comprising a total of 1087 patients were included (Figure 1) [8, 23-35].

Figure 1. PRISMA flow chart



Study characteristics

Four (29%) studies had a prospective design [27, 28, 32, 33], while the others had retrospective design studies [7, 8, 23, 25, 26, 29, 31, 34, 35]. Thirty-three percent of the patients had stage III disease, and 62% of the patients had stage IV disease (Table 1). Segmental bowel resections were performed in 76% of the cases, and were combined with concomitant liver resections or cholecystectomy in some cases (Table 2). These additional procedures were performed besides the resection of the primary tumour and/or metastases: Horwitz et al. performed the small bowel resections after endovascular embolization of encased mesenteric vessels [33],

Reissman et al. performed a prophylactic cholecystectomy to avoid future cholecystitis caused by somatostatin analogue usage or peptide receptor radiotherapy [29], and Wang et al. secured gel foam strips soaked with 5-fluorouracil in the mesenteric tumour resection site in 86/189 (46%) patients [34]. Minimally invasive surgery was performed in 60/1087 (1%) patients.

Post-operative morbidity

Overall morbidity was reported in 12 studies (901 patients) with a pooled overall morbidity rate of 13% with high heterogeneity (95% CI 7-24%, random effects model; I^2 = 90%). Severe morbidity was reported in 11 studies (589 patients), with a pooled severe morbidity rate of 7% (95% CI 4-13%, random effects model, I^2 = 70%) (Figure 2A). Seven studies (313 patients) reported details on the type of post-operative complications that occurred (Supplementary Table 3) [8, 23, 26, 27, 29, 33]. The two most common post-operative complications were intra-abdominal bleeding (9/313, 3%) and ileus (8/313, 3%). Reoperations were performed in 6 of 313 (2%) patients (Supplementary Table 3).

Post-operative mortality

Thirty-day mortality was reported in all studies, accounting for 1087 patients. The pooled 30-day mortality rate of these studies was 2% (95% CI 1-3%, fixed effects model, I^2 = 0%) (Figure 2B). Ninety-day mortality was reported in 12 studies, including 775 patients. The pooled 90-day mortality rate of these studies was 2% (95% CI 2-4%, fixed effects model, I^2 = 0%) (Figure 2C). In-hospital mortality was reported in 10 studies with a total of 400 patients. The pooled in-hospital mortality rate of these studies was 1% (95% CI 0-2%, fixed effects model, I^2 = 0%) (Figure 2D).

Table 1. Study characteristics.

Author	Country	Inclusion period	Patients (N)	Annual volume (N/ year)	Age, years, mean	Males, N (%)	Disease stage, N (%)		
							I-II	III	IV
Addeo et al. [23]	France	1997-2018	44	2	63	20 (45)	0	0	44 (100)
Evers et al. [35]	Germany	2000-2020	65	3	61	38 (58)	11 (17)	54 (83)	0
Figueiredo et al. [8]	France	1996-2012	73	5	56	40 (55)	NR	NR	43
Fisher et al. [32]	USA	2001-2018	17	1	57	9 (53)	0	0	17 (100)
Folkestad et al. [31]	Norway	1998-2018	186	9	65	101 (54)	23 (12)	101 (54)	61 (33)
Horwitz et al. [33]	USA	2014-2018	14	4	64	7 (50)	0	7 (50)	7 (50)
Kaçmaz et al. [7]	Netherlands	2003-2019	34	2	67	21 (62)	0	16 (47)	17 (53)
Norlen et al. [25]	Sweden	1985-2010	312	12	63	NR	NR	NR	NR
Pasquer et al. [26]	France	2000-2013	107	8	62	62 (58)	NR	NR	75 (70)
Pasquer et al. [27]	France	2013-2015	21	11	55	11 (52)	0	8 (38)	13 (62)
Pedrazzani et al. [28]	Italy	2014-2019	5	1	70	0	0	3 (60)	2 (40)
Reissman et al. [29]	Israel	2002-2012	20	2	60	8 (40)	0	10 (50)	10 (50)
Wang et al. [34]	USA	2003-2012	189	21	NR	80 (42)	0	0	189 (100)
Total			1087	4 (2-9)[a]	62	397 (51)[b]	34 (6)[b]	199 (33)[b]	478 (62)[b]

Abbreviations: MIS: minimally invasive surgery, NR: not reported, USA: United States of America

[a] median (interquartile range).

[b] proportions are calculated for studies who presented these variables.

Table 2. Surgical characteristics

Author	Surgical approach	Emergency resection	Procedure, no. (%)				Hospital stay, days, mean (±SD)
			Total	Segmental resection	Ileocolic resection	Right hemicolectomy	
Addeo et al. [23]	Open	NR	44	18 (41) [a]	0	26 (59)	NR
Evers et al. [35]	NR	NR	65	24 (37)	0	41 (63)	NR
Figueiredo et al. [8]	61 (84) open, 12 (16) MIS	9 (12)	73	45 (62) [b]	25 (38)	0	NR
Fisher et al. [32]	NR	NR	17 [c]	NR	NR	NR	NR
Folkestad et al. [31]	Open	45 (24)	186 [d]	112 (60)	33 (18)	35 (19)	NR
Horwitz et al. [33]	Open	0	14	7 (50)	7 (50)	0	13 (±21)
Kaçmaz et al. [7]	11 (32) open, 23 (68) MIS	4 (12)	34	20 (59)	8 (24)	6 (17)	8 (±6)
Norlen et al. [25]	Open	NR	312	312 [e]	NR	NR	NR
Pasquer et al. [26]	Open	NR	107	58 (54)	9 (8) [f]	40 (18) [f]	NR
Pasquer et al. [27]	Open	NR	21	21 (100)	0	0	NR
Pedrazzani et al. [28]	MIS	NR	5	0	0	5 (100)	7 (±6)
Reissman et al. [29]	MIS	NR	20	20 (100) [g]	NR	NR	6 (±NR)
Wang et al. [34]	Open	NR	189	189 (100)	NR	NR	NR
Total			1087	826 (76)	82 (8)	153 (14)	9

[a] 3/18 resections were combined with a major liver resection.

[b] 7/45 were segmental resection + Ileocolectomy.

[c] Ileal resection, right hemicolectomy, or an extended right hemicolectomy.

[d] 6/186 procedures were not described.

[e] A distal small bowel resection was often combined with a right hemicolectomy, but unknown in how many cases.

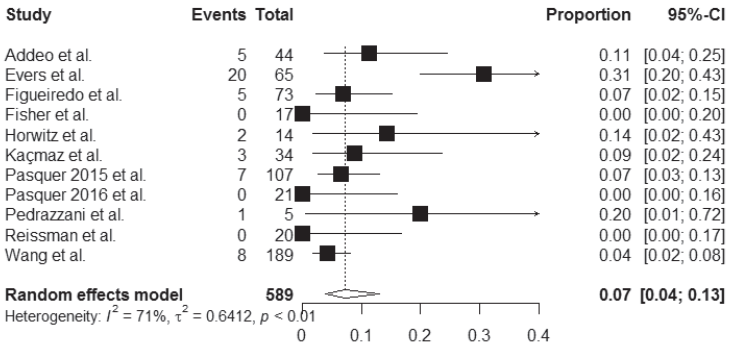
[f] Ileocolectomy and hemicolectomies were combined in an unknown amount of procedures with segmental resections.

[g] All patients had consequent cholecystectomy.

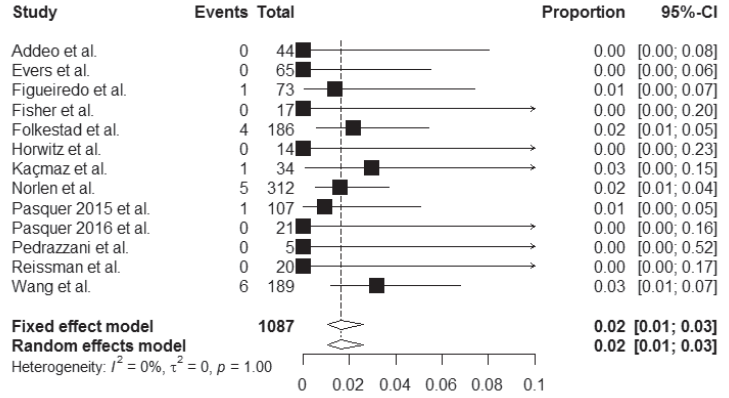
[h] 86/103 had 5-fluorouracil gel foam strips sutured in the mesentery.

Figure 2. Pooled proportions

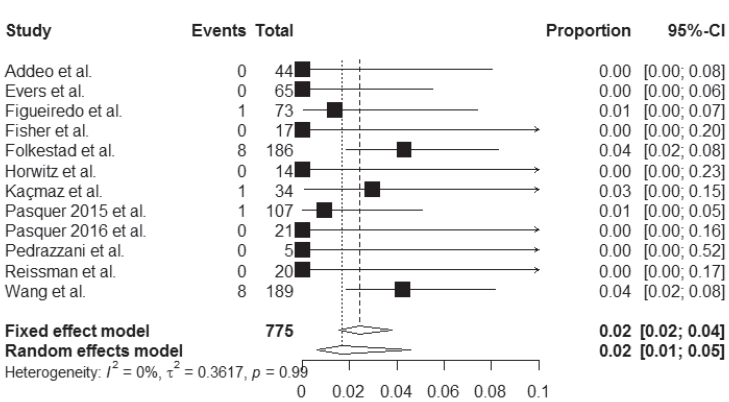
2A

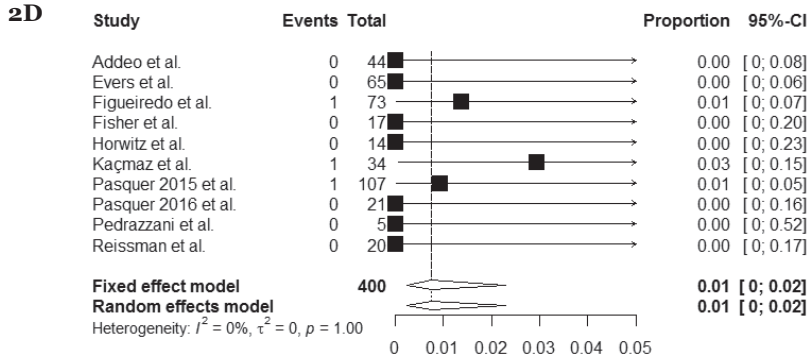


2B



2C





(A) severe morbidity, Clavien Dindo grade III-IV, **(B)** 30-day mortality, **(C)** 90-day mortality, and **(D)** in-hospital mortality.

Hospital volume and minimally invasive surgery

The median (IQR) annual hospital volume of SB-NEN resection was 4 (2-9) and the fourth quartile constituted 9 or more resections per year (defined as high-volume). Thirty-day mortality was similar (2% vs. 1%) and 90-day mortality rates were higher in high volume centers (4% vs 1%) (Table 3, Supplementary Figure 1A-1D). High annual volume was associated with lower overall and severe morbidity compared to low volume: 10% vs. 15% and 4% vs. 9%, respectively (Supplementary Figure 1F-1I). Funnel plots estimating publication bias are presented in Supplementary Figure 5A-5D. Herein, a skewed distribution is observed in the low volume hospitals, whereas outcomes in high volume hospitals are more centred. Pooled overall and severe morbidity rates were 20% (95% CI 12-32%, fixed effects model, I^2 = 0%) and 7% (95% CI 3-16%, fixed effects model, I^2 = 0%), respectively after minimally invasive surgery (Supplementary Figure 2A and 2B).

Table 3. Pooled proportions for post-operative outcomes, stratified for median no. procedures per year

Outcomes [a]	Procedures per year	
	8 or less	9 or more
30d mortality	1% (95% CI 0-2%), I^2 = 0%	2% (95% CI 1-3%), I^2 = 0%
90d mortality	1% (95% CI 0-2%), I^2 = 0%	4% (95% CI 2-6%), I^2 = 0%
In-hospital mortality	1% (95% CI 0-2%), I^2 = 0%	N/A [b]
Overall morbidity	15% (95% CI 6-31%), I^2 = 89%	10% (95% CI 5-20%), I^2 = 81%
Severe morbidity	9% (95% CI 6-16%), I^2 = 0%	4% (95% CI 2-7%), I^2 = 0%

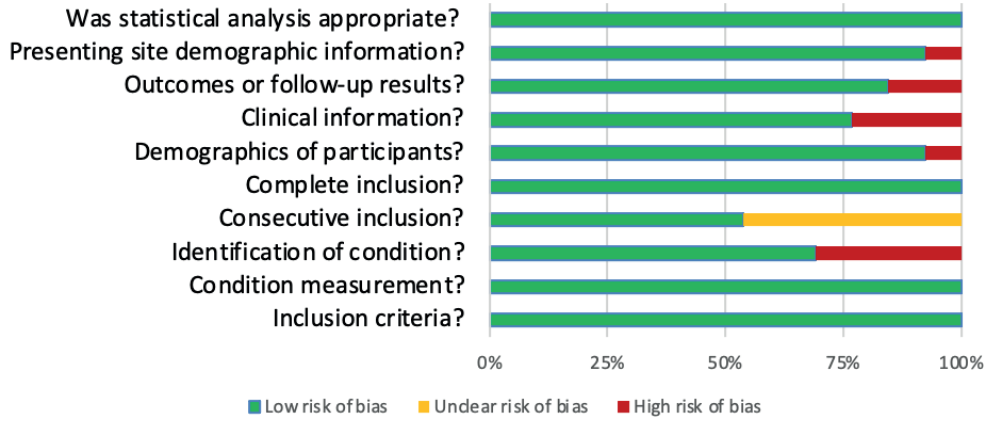
[a] Forest plots of individual analyses are presented in Supplementary Figure 4.

[b] This proportion could not be calculated as only one study reported this outcome.

Critical appraisal and risk of bias

Figure 3 presents the overall risk of bias for each item of the JBI checklist across all included studies. The study-level risk of bias for each individual study is presented in Supplementary Table 4. The majority of the studies were retrospective [7, 8, 23, 25, 26, 29, 31, 34, 35]. A high risk of bias (i.e. incomplete data) was present for clinical information in 3/13 studies (23%) [8, 23, 25] and for post-operative outcomes in 2/13 (15%) studies [29, 32]. Funnel plots estimating publication bias are presented in Supplementary Figure 3A-3D and 4A-4B. The in-hospital mortality analysis was particularly skewed, and all but one study remained within the 95% confidence intervals (severe morbidity analysis).

Figure 3. Risk of bias graph. Overall risk of bias across all included studies.



DISCUSSION

This systematic review with meta-analysis on morbidity and mortality after resection of SB-NEN consisted of 13 studies with a total of 1087 patients. The meta-analyses revealed a severe morbidity rate of 7%, 30-day mortality rate of 2%, 90-day mortality rate of 2%, and an in-hospital mortality rate of 1%. Analysis of annual hospital volume revealed that high volume centers appeared to have lower morbidity rates, but higher 90-day mortality rate that probably reflects differences in case-mix and methodological issues.

Albers et al. recently published a paper in which they analysed data of post-operative complications using the EUROCRINE registry, a European online endocrine surgical quality registry [13]. They included 133 patients across 23 centers from 9 different countries who underwent resection of a SB-NEN. Severe morbidity occurred in 11% of the patients, which is slightly higher than observed by the present study (6%), and mortality in 1%, which is similar. Underreporting of complications might be one of the explanations for observed differences, while mortality is a more reliable outcome parameter in general. Only a minority of studies in the present review had a prospective design, illustrating the risk of underestimation of morbidity.

Current analyses show that overall and severe morbidity was lower in centers with a higher annual volume. Remarkably, 30- and 90-day mortality was slightly higher in high volume centers, 1% and 3% respectively, compared to low volume centers. This might be explained by the studies that could be included for the different endpoints. Wang et al. included only stage IV patients and was the proportionally most weighed study for both 30- and 90-day mortality analyses [34]. Other factors that might have resulted in discrepancies between the different endpoints might be related to differences in the quality of the reported data or populations characteristics (i.e. patient comorbidities, tumour stage, type and extensiveness of surgery) among the eligible studies for each of the meta-analyses. This hypothesis is supported by the funnel plots presented in Supplementary Figure 5A-5D: substantial publication bias is present in the low volume hospital papers, whereas outcomes are around the estimated effect size in high volume centers. Therefore, the reported mortality rates in low volume hospitals might not reflect the true mortality rates. Indication for surgery differs between clinics, in which some prefer to operate electively. Others prefer to delay the resection to a later stage with an increased risk for an emergency resection due to obstruction, perforation or ischemia. In a retrospective cohort study, Folkestad et al. found that 24% of the patients underwent an emergency resection [31]. The diagnosis SB-NEN was unknown in 58% of emergency resection cases, and significantly more post-operative deaths due to surgical complications occurred compared to an elective resection (9% vs. 0%, respectively).

Morbidity and mortality rates after minimally invasive surgery did not differ from the overall group (i.e. including MIS patients). A comparison between open and MIS was performed by two studies, in which one study found less complications after MIS whilst the other found no differences [7, 8]. Well-designed prospective studies might be able to elucidate the differences between open and MIS regarding post-operative morbidity and mortality.

Pooling of data and excluding studies with >20% concomitant hepatectomies the current study more representative than individual cohort studies. However, the findings of this study should be seen in the light of some limitations. Although excluding studies with >20% concomitant hepatectomies limited (some) heterogeneity, it failed to do so in the severe morbidity analyses, which had an I^2 of 71%. Some moderately sized studies reported no severe morbidities, while some smaller did. This suggests that differences between centers exist (e.g. different expertise, surgical approach, or complex surgical oncology units). Also, variables that could potentially have an influence on post-operative outcomes (i.e. individual surgeon volume, location of mesenteric mass, body mass index, Charlson Comorbidity Index, American Society of Anaesthesiologists score) were not readily available or could not be deduced, and hence could not be corrected for or taken into account while interpreting the data. Ideally, a random effects meta-regression could have been considered to assesses such sources of heterogeneity across included studies. Similarly, several details about surgical treatment were not uniformly available. The results of the in-hospital mortality rate should be interpreted with caution, as the funnel plot (Supplementary Figure 1D) is skewed, which could represent presence of reporting bias. Also, the majority of the publications had a retrospective design and did not report on consecutive cases, which might have introduced selection bias. Finally, the periods for which post-operative morbidity was reported by studies was only known for 4/13 studies, which makes comparison of reported outcomes less comparable.

The most common post-operative complication was ileus, which could be attributable to extensive manipulation of the small bowel and the mesentery for lymphadenectomy. We have previously described techniques to prevent (potential) ischemic complications with the use of fluorescence angiography as a consequence of mesenteric lymphadenectomy [36]. The complications that followed were intra-abdominal bleeding, wound/bladder infections and anastomotic leaks, which are relatable to gastrointestinal surgery in general, and hence multifactorial in etiology.

We recommend that surgical studies clearly report morbidity/mortality outcomes. To achieve this, reporting of morbidity/mortality outcomes could be added to reporting guidelines, or made a mandatory condition for publication in journals. Future studies should also include the indication for surgery, whether patients were operated in a progressive disease stage with or without abdominal complaints or if they were operated in a stable disease stage as a more pre-emptive resection of the primary tumour to prevent future complications of the primary tumour and/or mesenteric metastases. Centralization of care for this rare disease has potential advantages, because quality improvement programmes, innovation and clinical research requires a certain volume in general. Although the present review does not clearly indicate a certain volume-outcome relationship with contradictory associations regarding morbidity and mortality, probably because of several methodological issues. The authors plan to set-up an international surgical registry of SB-NEN surgery to elucidate the contradictory finding regarding morbidity and mortality, and investigate post-operative complications using standardized definitions, assessed at pre-defined time-points.

This systematic review with meta-analyses showed relatively low morbidity and mortality rates after resection of SB-NEN. Contradictory associations of morbidity and mortality with hospital volume were found, probably related to heterogeneity among eligible studies for different endpoints.

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Author contributions

Conceptualization: EK, PJT, EJMNvD, and AFE.
Data curation: EK, JWC.
Formal analysis: EK.
Supervision: PJT, EJMNvD, and AFE.
Validation: EK, JWC, PJT, EJMNvD, and AFE.
Writing—original draft: EK.
Writing—review and editing: EK, JWC, PJT, EJMNvD, and AFE.

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SUPPLEMENTARY MATERIALS

Supplementary table 1. Search string for MEDLINE (PubMed) and Embase (Ovid).

MEDLINE (PubMed)

(Ileal neoplasms [Mesh] OR Ileal neoplasm* [Tiab] OR ileum [tiab] OR ileal [tiab] OR jejunal neoplasms [Mesh] OR jejunal neoplasm* [Tiab] OR jejunum [tiab] OR jejunal [tiab] OR Small bowel [tiab] OR small intestine [tiab] OR midgut [tiab])

AND

(neuroendocrine Tumors [Mesh] OR neuroendocrine Tumo* [Tiab] OR Neuroendocrine Tumo* [Tiab] OR neuroendocrine [tiab] OR Gastro-enteropancreatic neuroendocrine tumor [Supplementary Concept] OR gastroenteropancreatic [tiab])

AND

(Cytoreduction Surgical procedures [Mesh] OR Cytoreduction Surgical procedures [Tiab] OR Surgical Procedures, Operative [Mesh] OR Surgical Procedures, Operative [Tiab] OR surgical oncology [Mesh] OR surgical oncology [Tiab] OR Surgical [Mesh] Or Surgical [Tiab] OR resection [tiab] or surgery [tiab])

AND

(eng [la])

NOT

(case reports [publication type] OR non-neuroendocrine [ti] OR nonneuroendocrine [ti] OR case report [ti] OR melan* [ti] OR Guideline [Publication Type] OR Editorial [Publication Type] OR Letter [Publication Type] OR News [Publication Type] OR Comment [Publication Type] OR Historical Article [Publication Type] OR Anecdotes as Topic [Mesh] OR letter* [ti] OR comment* [ti] OR abstracts [ti])

Embase (Ovid)

1. exp Ileum tumor/ OR exp ileum cancer/ OR ileum*.ti,ab,kw. OR exp ileum/ OR exp jejunum tumor/ OR exp jejunum cancer/ OR jejunum*.ti,ab,kw OR jejunum/ OR exp small intestine/ OR small bowel.ti,ab,kw OR small intestine.ti,ab,kw
2. exp neuroendocrine tumor/ OR neuroendocrine tumo#r.ti,ab,kw OR neuroendocrine neoplasm*.ti,ab,kw
3. exp small intestine resection/ OR exp surgery/
4. 1 and 2 and 3
5. animal/ not (animal/ and human/)
6. 4 not 5
7. (child/ or child*.ti,ab,kw.) and adult/
8. 6 not 7
9. "review"/ or "systematic review"/ or exp practice guideline/ or editorial/ or letter/ or literature/ or (letter* or comment* or abstracts).ti.
10. 8 not 9
11. limit 10 to (english)
12. limit 11 to yr= "2000-2020"

Supplementary Table 2. JBI criteria

Were there clear inclusion criteria?	**Low**: SB-NEN **Unclear**: Not mentioning primary tumour location **High**: Gastroenteropancreatic NEN
Was the condition measured in a standard, reliable way for all participants?	**Low**: Histopathologic prove of SB-NEN **Unclear**: Histopathology not mentioned **High**: no criteria described
Were valid methods used for identification of condition for all participants?	**Low**: Definition mortality including period (i.e. 30 day, 90 day) and description of morbidity including classification according to Clavien-Dindo or comparable **High**: Missing one of above
Did the case series have consecutive inclusion of participants?	**Low**: Consecutive or all mentioned **Unclear**: Consecutive or all missing **High**: selected cases
Did the case series had complete inclusion of participants?	**Low**: Follow up and/or drop-outs described **High**: Missing follow up and/or drop-outs
Was there clear reporting of the demographics of the participants?	**Low**: includes age, sex specific for group of interest **High**: Missing age or sex
Was there clear reporting of clinical information of the participants?	**Low**: TNM classification/WHO stage **High**: Missing one of above
Were the outcome or follow up results of cases clearly reported?	**Low**: mortality and morbidity (subdivided per complication) as described in methods **High**: Missing one of above
Was there clear reporting of the presenting site(s) demographics information?	**Low**: Single center or in multicenter described per center **Unclear**: Multicenter and not described per center
Was statistical analysis appropriate?	**Low**: statistical analysis described **Unclear**: not described **High**: inappropriate test used

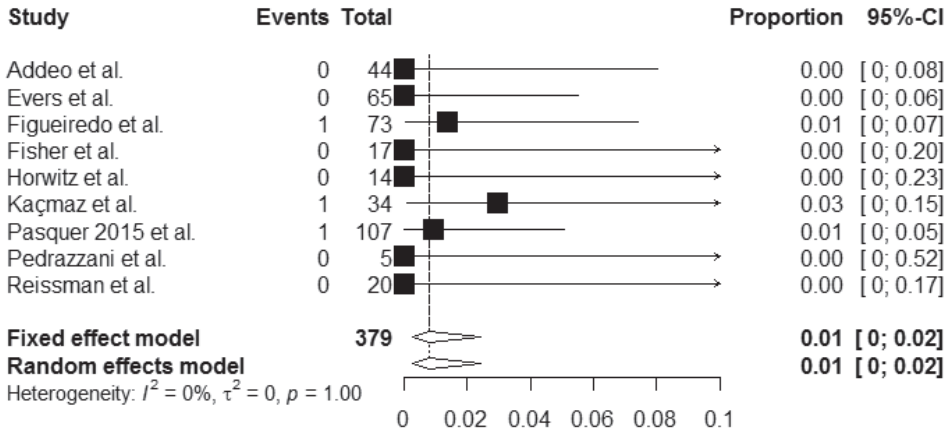
Supplementary Table 3. Reported complications

Complications within 30 days	Total	Addeo [19]	Figueiredo [21]	Horwitz [30]	Kaçmaz [33]	Pasquer 2015 [23]	Pasquer 2016 [24]	Reissman [26]
Ileus	8			1	2	4		1
Intra-abdominal bleeding	7		3			4		
Cystitis	6					6		
Reoperation	6	1	3		2			
Abdominal wall dehiscence		1			1			
Abdominal wall hematoma			1					
Anastomotic leak				1	1			
Intra-abdominal bleeding			2					
Wound infection	6				5			1
Sepsis	5				1	4		
Anastomotic leak	4	0		2	1	1		
Wound abscess	3					3		
Abdominal wall dehiscence	2	1			1			
Gastropareses	2				2			
Pneumonia	2				1	1		
Scar defects	2					2		
Abdominal abscess	1					1		
Abdominal wall hematoma	1		1					
Acute pancreatitis	1					1		
Cholecystitis	1					1		
Chyle leak	1			1				
Gastric ulcer	1					1		
Lymphorrhea	1						1	
Rectal bleeding due to haemorrhoids	1						1	

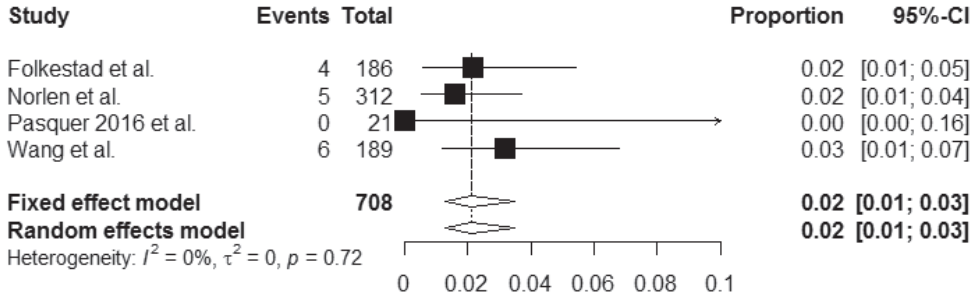
Supplementary Table 4. Risk of bias in individual studies.

Author	Inclusion criteria?	Condition measurement?	Identification of condition?	Consecutive inclusion?	Complete inclusion?	Demographics of participants?	Clinical information?	Outcomes or follow-up results?	Presenting site demographic information?	Was statistical analysis appropriate?
Addeo [19]	low	low	low	low	low	low	high	low	low	low
Evers [32]	low	low	low	low	low	low	low	low	low	low
Figueiredo [21]	low	low	low	unclear	low	low	high	low	low	low
Fisher [29]	low	low	high	unclear	low	low	low	high	low	low
Folkestad [28]	low	low	low	unclear	low	low	low	low	low	low
Horwitz [30]	low	low	low	unclear	low	low	low	low	low	low
Kaçmaz [33]	low	low	low	low	low	low	low	low	low	low
Norlen [22]	low	low	low	low	low	high	high	low	high	low
Pasquer 2015 [23]	low	low	low	low	low	low	low	low	low	low
Pasquer 2016 [24]	low	low	high	low	low	low	low	low	low	low
Pedrazzani [25]	low	low	high	unclear	low	low	low	low	low	low
Reissman [26]	low	low	high	low	low	low	low	high	low	low
Wang [31]	low	low	low	unclear	low	low	low	low	low	low

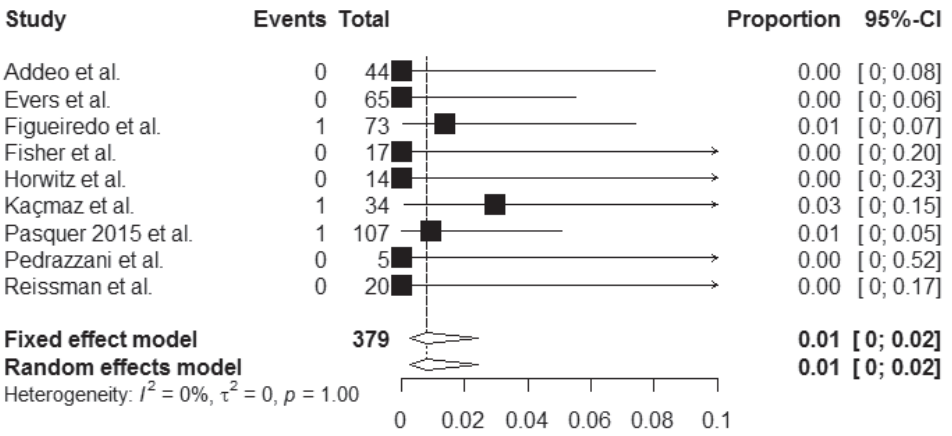
Supplementary Figure 1A. Pooled 30-day mortality in low volume centers



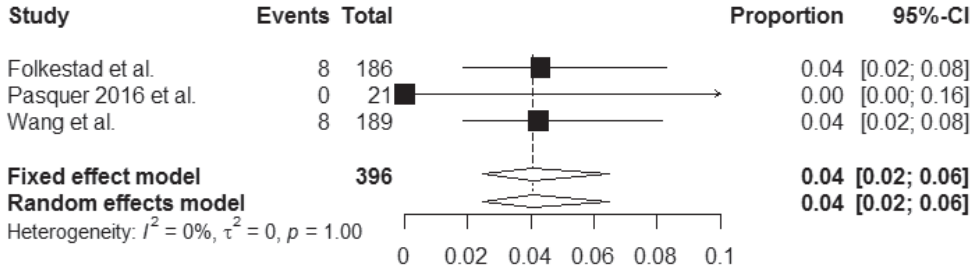
Supplementary Figure 1B. Pooled 30-day mortality in high volume centers



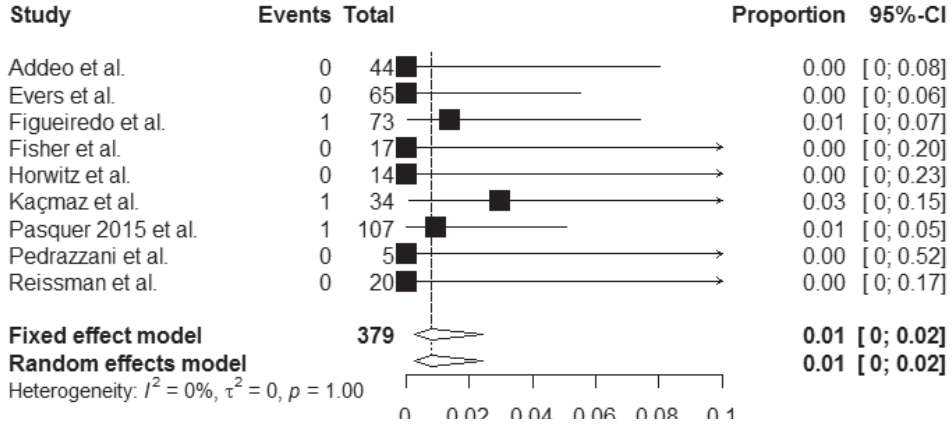
Supplementary Figure 1C. Pooled 90-day mortality in low volume centers



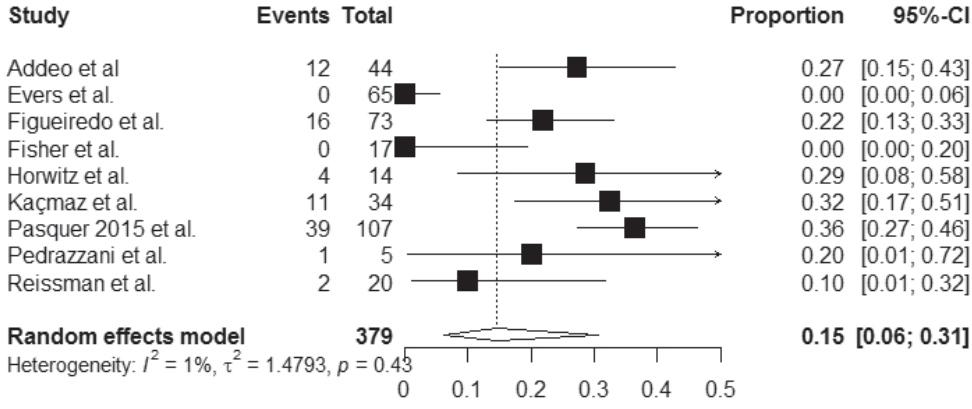
Supplementary Figure 1D. Pooled 90-day mortality in high volume centers



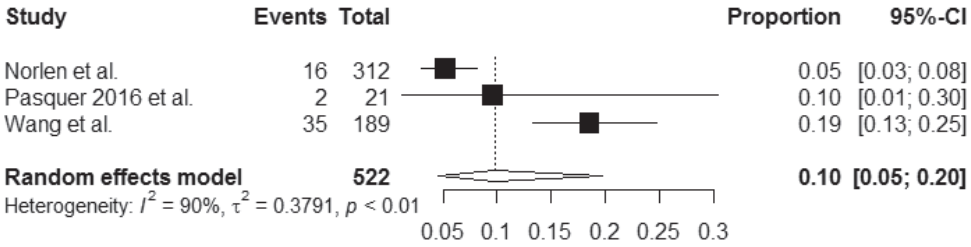
Supplementary Figure 1E. Pooled in-hospital mortality in low volume centers



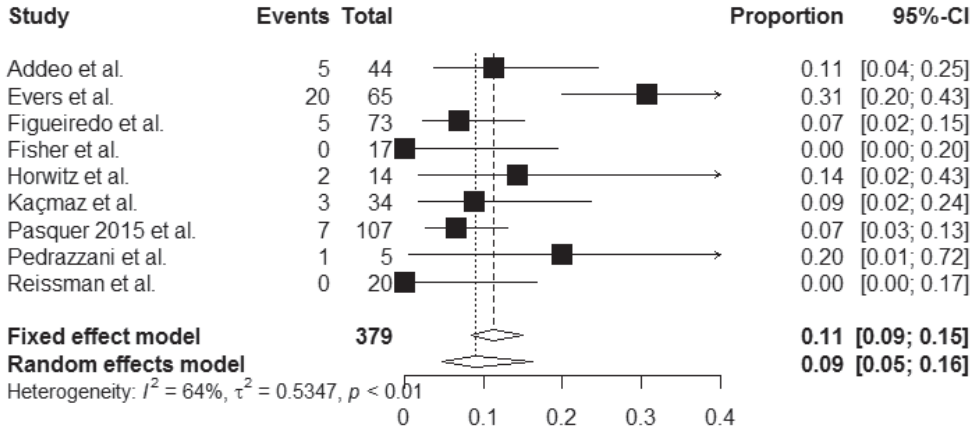
Supplementary Figure 1F. Pooled overall morbidity in low volume centers



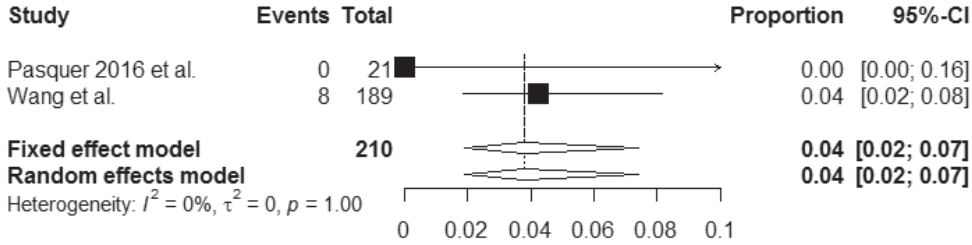
Supplementary Figure 1G. Pooled overall morbidity in high volume centers



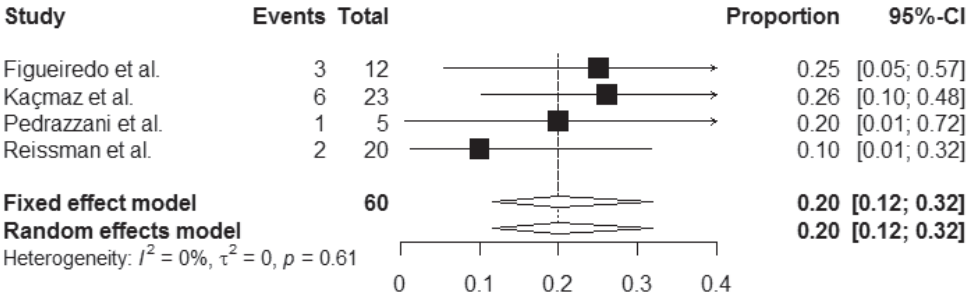
Supplementary Figure 1H. Pooled severe morbidity in low volume centers



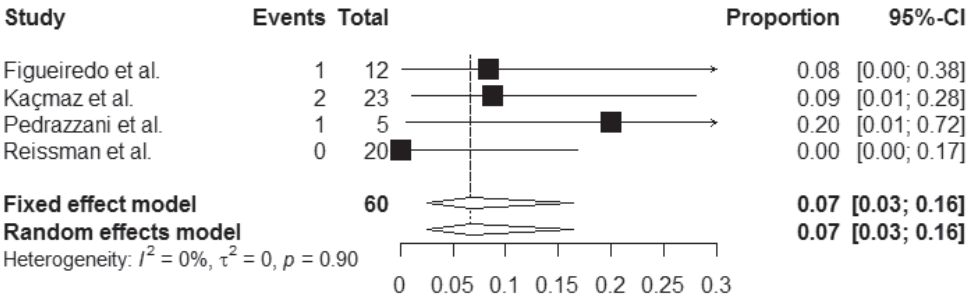
Supplementary Figure 1I. Pooled severe morbidity in high volume centers



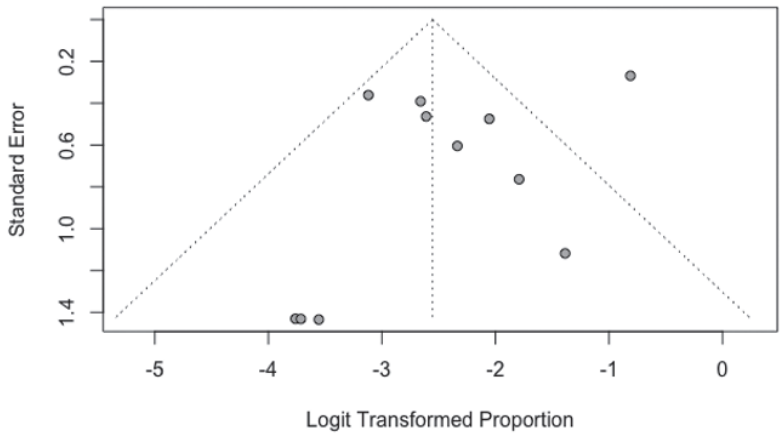
Supplementary Figure 2A. Pooled overall morbidity rates for MIS



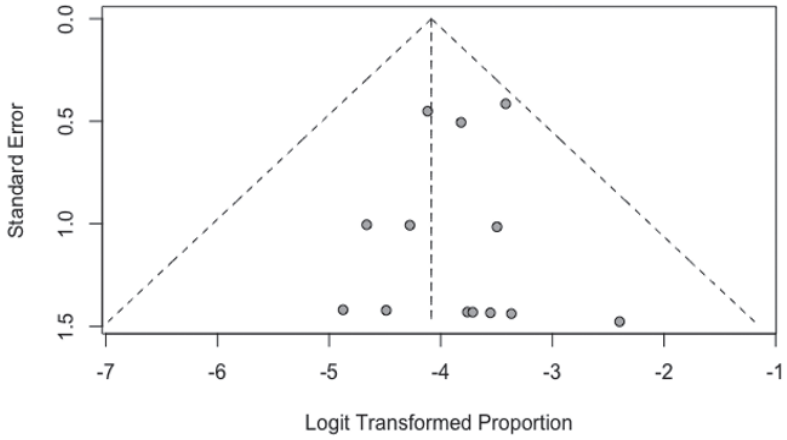
Supplementary Figure 2B. Pooled severe morbidity rates for MIS



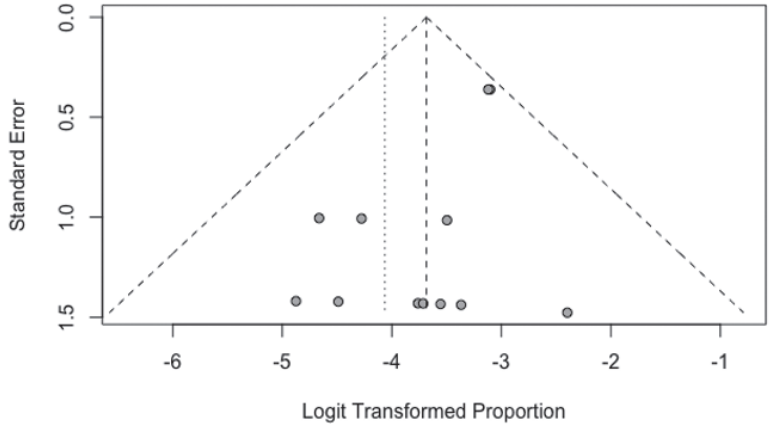
Supplementary Figure 3A. Funnel plot of severe morbidity, Clavien Dindo grade III-IV.



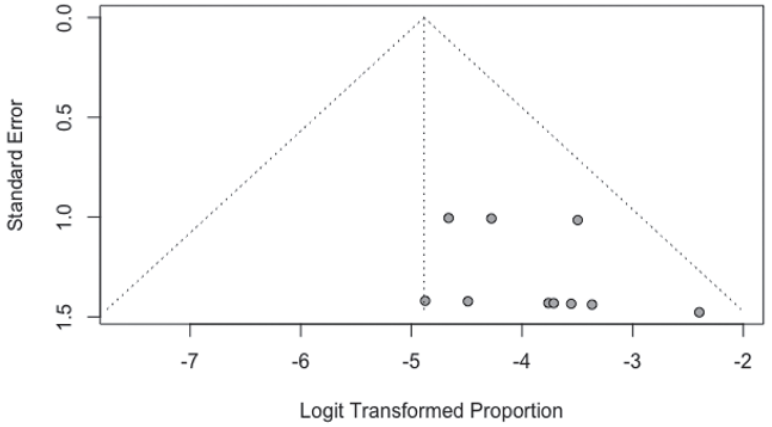
Supplementary Figure 3B. Funnel plot of 30-day mortality



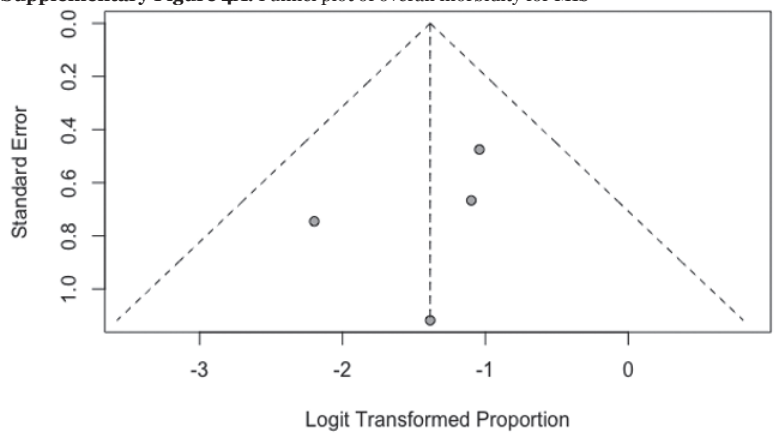
Supplementary Figure 3C. Funnel plot of 90-day mortality



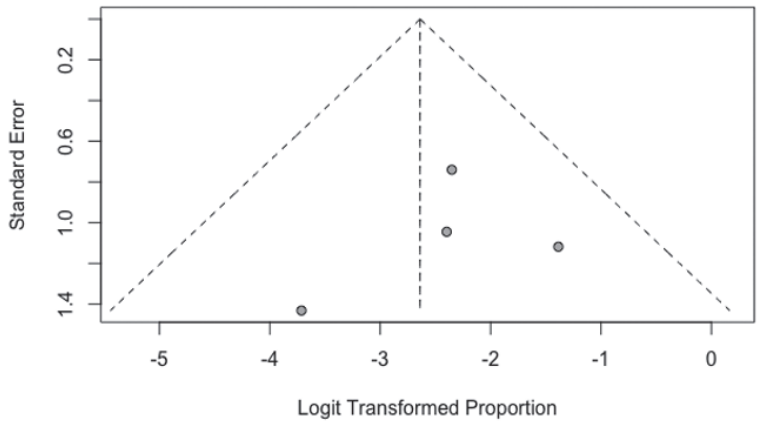
Supplementary Figure 3D. Funnel plot of in-hospital mortality



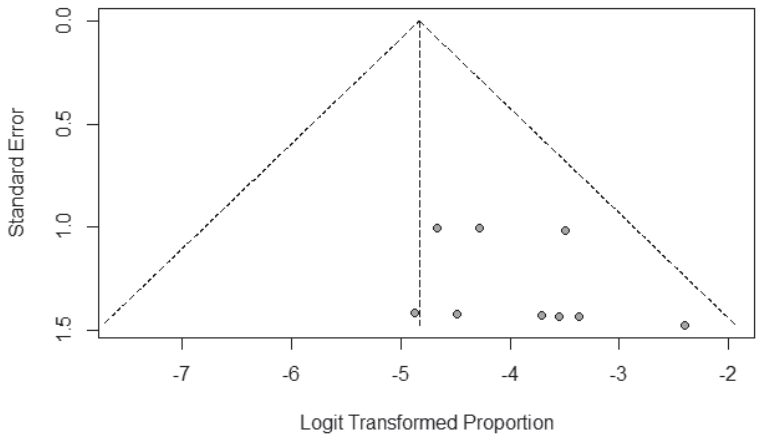
Supplementary Figure 4A. Funnel plot of overall morbidity for MIS



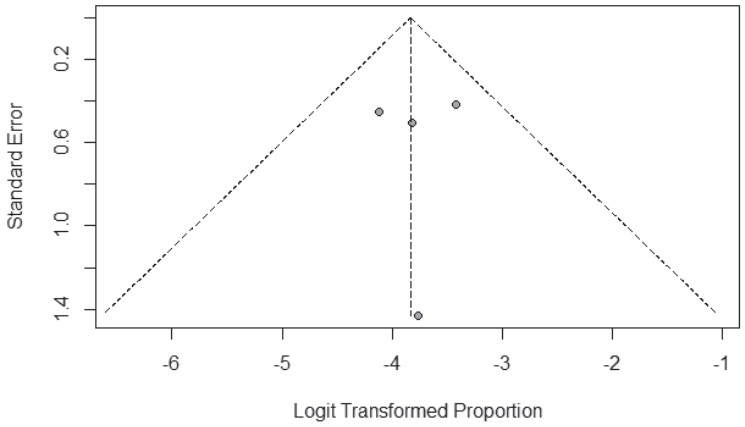
Supplementary Figure 4B. Funnel plot of severe morbidity for MIS



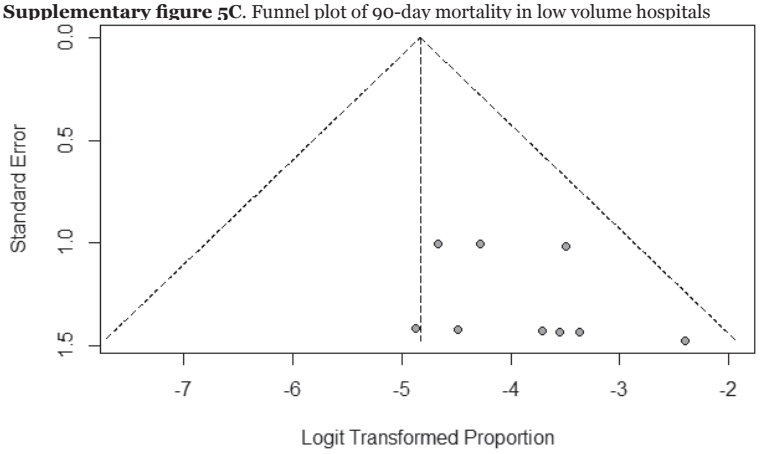
Supplementary figure 5A. Funnel plot of 30-day mortality in low volume hospitals



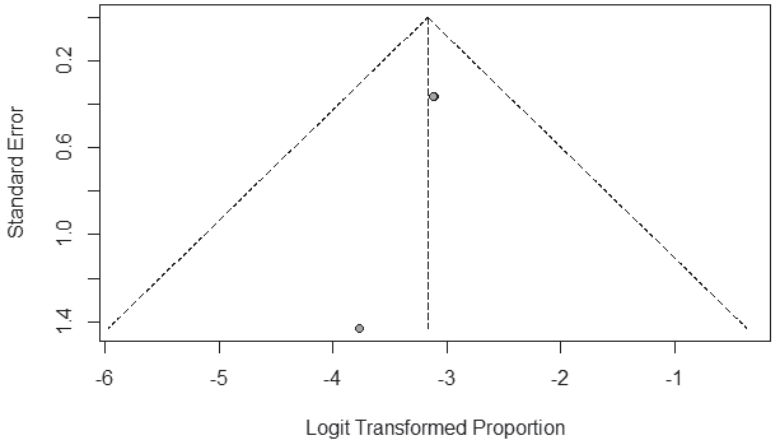
Supplementary figure 5B. Funnel plot of 30-day mortality in high volume hospitals



Supplementary figure 5C. Funnel plot of 90-day mortality in low volume hospitals



Supplementary figure 5D. Funnel plot of 90-day mortality in high volume hospitals



CHAPTER 5

International survey on opinions and use of minimally invasive surgery in small bowel neuroendocrine neoplasms

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ABSTRACT

Introduction Although minimally invasive surgery is becoming the standard technique in gastrointestinal surgery, implementation for small bowel neuroendocrine neoplasms (SB-NEN) is lagging behind. The aim of this international survey was to gain insights into attitudes towards minimally invasive surgery for resection of SB-NEN and current practices.

Methods An anonymous survey was sent to surgeons between February and May 2021 via (neuro)endocrine and colorectal societies worldwide. The survey consisted of questions regarding experience of the surgeon with minimally invasive SB-NEN resection and training.

Results A total of 58 responses from five societies across 20 countries were included. Forty-one (71%) respondents worked at academic centers. Thirty-seven (64%) practiced colorectal surgery, 24 (41%) endocrine surgery and 45 (78%) had experience in advanced minimally invasive surgery. An open, laparoscopic or robotic approach was preferred by 23 (42%), 24 (44%), and 8 (15%) respondents, respectively. Reasons to opt for a minimally invasive approach were mainly related to peri-operative benefits, while an open approach was preferred for optimal mesenteric lymphadenectomy and tactile feedback. Additional training in minimally invasive SB-NEN resection was welcomed by 29 (52%) respondents. Forty-three (74%) respondents were interested in collaborating in future studies, with a cumulative median (IQR) annual case load of 172 (86-258).

Conclusions Among respondents, 69% applies minimally invasive surgery for resection of SB-NEN. Arguments for specific operative approaches differ, and insufficient training in advanced laparoscopic techniques seems to be a barrier. Future collaborative studies can provide better insight in selection criteria and optimal technique.

INTRODUCTION

Although minimally invasive surgery has several generally acknowledged applications in the treatment of gastrointestinal malignancies, its use for small bowel neuroendocrine neoplasms (SB-NEN) is not yet widely accepted. This could be explained by the rarity which limits clinical exposure, and the fact that surgeons treating SB-NEN are not necessarily those with experience in advanced laparoscopic surgery. One of the technical challenges specific for SB-NEN are the nodal metastases, as these often extent to the mesenteric root and are present in more than 80% of patients [1]. Dissection of the superior mesenteric vessels has the risk of bleeding, and there are concerns about inappropriate oncological clearance of all macroscopic tumour if using a minimally invasive approach.

The lacking evidence for minimally invasive SB-NEN resection is probably also related to restricted advice regarding minimally invasive SB-NEN resection by The North American Neuroendocrine Tumor Society and European Neuroendocrine Tumor Society [1, 2]. Arguments against a minimally invasive approach are mainly based on risk of missing multifocal primary tumours and challenging vascular dissection due to large mesenteric masses.

These arguments against the laparoscopic approach for SB NEN are mainly based on expert opinion, as there are only a few studies reporting on minimally invasive SB-NEN resection [3-8]. A comparison between minimally invasive and open resection would be of added value, but is currently impossible due to the lack of comparative studies [3, 7, 9, 10]. This could be explained by low volume, the hampers sufficient accrual in such trials, as well as lack of equipoise with some surgeons advocating that open surgery is still standard of care for SB-NEN resection.

For the purpose of this study, a survey was developed, with the aim to give insights in current practice concerning minimally invasive SB-NEN resection, existing attitudes/future prospects towards minimally invasive SB-NEN resection, and to explore interest and willingness among surgeons to participate in future studies regarding minimally invasive SB-NEN resection.

METHODS

Survey

An invitation to participate to the study was sent to surgeon members of 32 (neuro)endocrine and colorectal societies between 16th February 2021 and 3rd May 2021. The survey was conducted anonymously using Google Forms (Mountain View, California, USA), and was adapted from a survey regarding minimally invasive surgery for pancreatic cancer [11]. Responders were given the option to leave their contact information (irrespective of given answers) to receive the study results, and to be contacted for future collaborative studies. Due to a possible overlap in the membership databases of the associations and their confidentiality requirements, the total amount of invited respondents is unknown.

Investigated parameters

Investigated parameters included demographic characteristics (e.g. country, age, hospital type), experience of the surgeon (e.g. scope of practice, years of experience), minimally invasive SB-NEN resection (e.g. attitudes and possible contraindications), and training (e.g. type of necessary training for these procedures). The full survey can be found in Supplementary material 1.

Definitions

Minimally invasive surgery was defined as laparoscopic or robot-assisted surgery. Advanced gastrointestinal minimally invasive surgery was defined as any minimally invasive procedure of the gastrointestinal tract, excluding cholecystectomy, appendectomy or inguinal hernia repair surgery. Consensus was defined as ≥80% agreement, and moderate consensus was defined as 60 to 80% agreement.

Statistical analysis

Categorical data are presented as number of cases and percentages, whilst continuous data are presented as either mean with standard deviation (SD) or median with interquartile range (IQR), depending on the data distribution. Incomplete surveys were excluded from analyses. The authors did not fill in the survey to prevent investigator bias. Sensitivity analyses were performed to investigate the influence of hospital type and experience in advanced minimally invasive surgery. Data was analysed using the Statistical Package for Social Sciences (SPSS) version 26 (IBM Corp. Armonk, NY, USA).

RESULTS

Participants

Five of 32 societies accepted to disseminate the survey without charge (European Society of Endocrine Surgeons, European Neuroendocrine Tumour Society, Spanish Group of Neuroendocrine and Endocrine Tumors, German Society of Coloproctology, and the Colorectal Surgical Society of Australia and New Zealand). This resulted in 58 responses across 20 countries, of which 27 (46%) surgeons were from Europe and 22 (38%) from Oceania (Figure 1). Forty-one (71%) respondents worked at academic centers, 11 (19%) at non-academic referral centers, and 6 (10%) at regional hospitals. The scope of practice was colorectal in 37 (64%), endocrine in 24 (41%), and hepatopancreatobiliary in 9 (16%) respondents. Forty-five (78%) respondents had experience in advanced minimally invasive surgery with a median of 10 (5-15) years (Table 1).

Figure 1. Response from countries

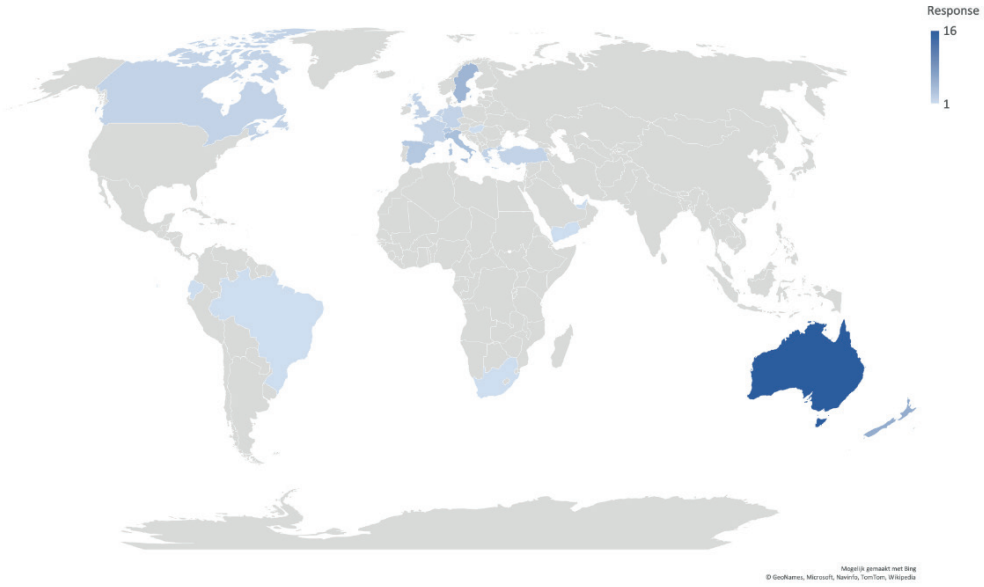

Table 1. Characteristics of participating surgeons

Characteristics, No. (%)	Total (*N* = 58)	Academic hospitals (*N* = 41)	Experience in advanced MIS (*N* = 45)
Sex			
Male	46/56 (82%)	34	35
Age, years, mean (SD)	50 (9)	50 (10)	48 (9)
Type of hospital			
Academic	41 (71)	41	33
Non-academic, referral center	11 (19)	0	8
Regional	6 (10)	0	4
Scope of surgical practice [a]			
Colorectal	37 (64)	23	16
Endocrine	24 (41)	16	29
HPB	9 (16)	7	8
General	3 (5)	3	3
Experience as an attending surgeon, years, mean (SD)	17 (10)	18 (11)	16 (7-23)
Performs advanced MIS	45 (78)	33	10 (5-15)
Experience in advanced MIS, years, median (IQR)	10 (5-15)	10 (5-15)	-

[a] multiple answers were possible, cumulative percentage may exceed 100%. IQR: interquartile range, MIS: minimally invasive surgery, SD: standard deviation.

Preferred surgical approach

An open, laparoscopic or robotic approach was preferred by 23 (42%), 24 (44%), and 8 (14%) of the surgeons, respectively (Table 2). Reasons to prefer an open approach were tactile feedback and better lymphadenectomy (consensus, >80%). Reasons to prefer a laparoscopic approach were less post-operative pain (consensus, > 80%), shorter length of stay and time to functional recovery (moderate-consensus, 60-80%). Reasons to prefer a robotic approach were enhanced dexterity and better ergonomics (consensus, 80%).

Table 2. Preferred surgical technique and reasons for this technique, multiple answers

	Total (*N* = 58)			**Academic hospitals (*N* = 41)**			**Experience in advanced MIS (*N* = 45)**		
Reasons, No. (%) [a]	**Laparoscopic (N = 24/55, 44%)**	**Robot (N = 8/55, 14%)**	**Open (N = 23/55, 42%)**	**Laparoscopic (N = 14/41, 34%)**	**Robot (N = 7/41, 17%)**	**Open (N = 17/41, 41%)**	**Laparoscopic (N = 24/45, 53%)**	**Robot (N = 6/45, 13%)**	**Open (N = 15/45, 33%)**
Because better/increased									
Dexterity	1 (4)	**7 (88)**	6 (26)	1 (7)	**6 (86)**	5 (29)	1 (4)	**5 (83)**	5 (33)
Ergonomics	3 (12)	**7 (88)**	1 (4)	3 (21)	**6 (86)**	1 (6)	3 (13)	**5 (83)**	1 (7)
Life expectancy	0	0	1 (4)	0	0	1 (6)	0	0	0
Lymphadenectomy	4 (17)	2 (25)	**20 (87)**	3 (21)	2 (29)	**17 (100)**	4 (17)	2 (33)	**14 (93)**
R0 rate	1 (4)	0	9 (39)	0	0	7 (41)	1 (4)	0	5 (33)
Tactile feedback	8 (33)		**20 (87)**	4 (29)	0	**16 (94)**	8 (33)	0	**14 (93)**
Tumour staging	0	0	1 (4)	0	0	1 (6)	0	0	1 (7)
Visibility	12 (50)	*6 (75)*	9 (39)	6 (43)	*5 (71)*	7 (41)	12 (50)	*4 (67)*	5 (33)
3D vision	3 (12)	*6 (75)*	5 (22)	3 (21)	*5 (71)*	5 (29)	3	*4 (67)*	3 (20)
Because less/decreased									
Blood loss	8 (33)	3 (38)	2 (9)	3 (21)	2 (29)	2 (12)	8 (33)	1 (17)	0
Cost	8 (33)	0	6 (26)	4 (29)	0	5 (29)	8 (33)	0	3 (20)
Length of stay	*18 (75)*	3 (38)	2 (9)	*10 (71)*	2 (29)	0	*18 (75)*	1 (17)	0
Pain after surgery	**20 (83)**	*5 (63)*	2 (9)	*11 (78)*	4 (57)	0	**20 (83)**	3 (50)	0
Post-operative complications	11 (46)	3 (38)	5 (22)	5 (36)	2 (29)	3 (18)	11 (46)	1 (17)	1 (7)
Set-up time	6 (25)	0	10 (43)	3 (21)	0	8 (47)	6 (25)	0	5 (33)
Time to functional recovery	*16 (67)*	3 (38)	3 (13)	*9 (64)*	2 (29)	1 (6)	*16 (67)*	1 (17)	1 (7)

[a] multiple answers were possible, cumulative percentage may exceed 100%. Consensus statements (>80%) are presented in bold, moderate consensus (60-80%) in italic.

Minimally invasive SB-NEN resection

The median annual volume of SB-NEN resection for individual surgeons was 4 (2-6) (Table 3). Forty (69%) surgeons performed minimally invasive SB-NEN resection, with a mean (SD) annual volume of 4 (3) resections. The most common reasons for only performing open resection by the remaining 18 (31%) surgeons were: lack of training in this technique, lack of scientific evidence, lack of time in surgical schedules and no supporting guidelines (no consensus).

Table 3. Minimally invasive SB-NEN resection

Characteristics, No. (%)	**Total (*N* = 58)**	**Academic hospitals (*N* = 41)**	**Experience in advanced MIS (*N* = 45)**
Annual SB-NEN resections, median (IQR)			
Total performed at hospital	10 (5-15)	10 (5-18)	6 (5-14)
Total performed by surgeon	4 (2-6)	4 (2-6)	3 (2-6)
Performs minimally invasive SB-NEN resection	40/58 (69)	27 (66)	38 (84)
Minimally invasive SB-NEN resections per year, mean (SD)	4 (3)	4 (2)	3 (3)
Type of MIS SB-NEN resection [a]			
Laparoscopic dissection, open bowel transection	*25/40 (63)*	*12/27 (44)*	*24/38 (63)*
Fully laparoscopic	18/40 (45)	2/27 (7)	17/38 (45)
Hand-assisted minimally invasive	7/40 (18)	1/27 (4)	7/38 (18)
Fully robot-assisted	2/40 (5)	15/27 (56)	1/38 (3)
Laparoscopic dissection, with robot-assisted dissection	1/40 (3)	4/27 (15)	1/38 (3)
Does not perform MIS SB-NEN resection	18/58 (31)	14 (34)	6 (13)
Reasons not to perform MIS SB-NEN resection [a]			
Lack of training in this technique	9/18 (50)	7/14 (50)	2/6 (33)
Lack of scientific evidence	7/18 (39)	5/14 (36)	3/6 (50)
Lack of time in surgical schedules	6/18 (33)	5/14 (36)	1/6 (17)
No guidelines by the societies are published on this topic	5/18 (28)	4/14 (29)	3/6 (50)
Difficulty of the surgical technique	4/18 (22)	3/14 (21)	2/6 (33)
Other surgeon(s) perform this procedure in our center	3/18 (17)	2/14 (14)	1/6 (17)
Institutional culture discourages it	2/18 (11)	2/14 (14)	0
The costs are too high	2/18 (11)	1/14 (7)	0
Not relevant in my center	1/18 (6)	0	1/6 (17)
Patient preference for open approach	0	0	0

[a] multiple answers were possible, cumulative percentage may exceed 100%. Consensus statements (>80%) are presented in bold, moderate consensus (60-80%) in italic. IQR: interquartile range, MIS: minimally invasive surgery, SD: standard deviation.

Opinions

The current value of minimally invasive SB-NEN resection was thought to be superior to open resection by 24 (48%) surgeons, and 58% expects this to rise in the future (Table 4). Patients without pN2 lymph nodes or with distal lymph nodes and no encasement of the main mesenteric vessels are thought to be eligible for a minimally invasive resection (moderate consensus, 60-80%). In general, respondents indicated that patients are expected to benefit from a minimally invasive resection if performed by an experienced surgeon (consensus, >80%). A risk of incomplete resection (R1/R2) is believed to be a contraindication (moderate consensus, 60-80%) (Table 5).

Table 4. Opinions on MIS SB-NEN resection

Characteristics, No. (%)	Total respondents (*N* = 58)	Academic hospitals (*N* = 41)	Experience in advanced MIS (*N* = 45)
Current overall value of MIS compared to open approach			
Inferior value of MIS	9/50 (18)	7/33 (21)	5/40 (13)
Equivalent value of MIS	17/50 (34)	10/33 (30)	14/40 (35)
Superior value of MIS	24/50 (48)	16/33 (48)	21/40 (53)
Future value of MIS compared to open approach			
Inferior value of MIS	7/50 (14)	6/33 (18)	5/40 (13)
Equivalent value of MIS	14/50 (28)	8/33 (23)	9/40 (23)
Superior value of MIS	29/50 (58)	19/33 (58)	*26/40 (65)*
Patients without pN2 lymph node metastases are amenable for MIS	*39/51 (76)*	*26/35 (74)*	**35/42 (83)**
Guidelines should give clear criteria for patients selection in MIS	31/55 (56)	*18/28 (64)*	*24/37 (65)*
Patients with distal lymph nodes, without encasement of mesenteric vessels are amenable for MIS	*42/55 (76)*	*27/38 (71)*	*34/43 (79)*
In general, patients benefit from MIS when performed by an experienced surgeon	**44/51 (86)**	*27/34 (79)*	**37/41 (90)**
Expected effect on quality of life after MIS compared to open			
Better quality of life after MIS	25/48 (52)	14/32 (44)	22/40 (55)
Equal quality of life after MIS	23/48 (48)	18/32 (56)	18/40 (45)
Worse quality of life after MIS	0	0	0

[a] multiple answers were possible, cumulative percentage may exceed 100%. Consensus statements (>80%) are presented in bold, moderate consensus (60-80%) in italic.

Table 5. Contraindications for MIS SB-NEN resection

Contraindications, No. (%) [a]	Total respondents (N= 58)	Academic hospitals (*N* = 41)	Experience in advanced MIS (*N* = 45)
Risk of incomplete resection (R1/2)	*39 (67)*	*30 (75)*	*32 (71)*
Arterial involvement of the tumour	32 (55)	22 (55)	26 (58)
Venous involvmeent of the tumour	30 (52)	*24 (60)*	25 (56)
Large size of mesenteric metastases (pN2, >2cm)	27 (47)	18 (45)	19 (42)
Multiple primary tumours	25 (43)	19 (48)	20 (44)
Prior laparotomy	12 (21)	5 (13)	7 (16)
Risk of intra-operative bleeding	7 (12)	5 (13)	6 (13)
Morbid obesity (BMI >30)	4 (7)	3 (8)	3 (7)
None	4 (7)	3 (8)	3 (7)
ASA score >3	2 (3)	1 (3)	2 (4)
Advanced age	0	0	0

[a] multiple answers were possible, cumulative percentage may exceed 100%. Consensus statements (>80%) are presented in bold, moderate consensus (60-80%) in italic.

Training and education

Specific training in advanced minimally invasive surgery is thought to be essential to be able to perform minimally invasive SB-NEN resection (moderate consensus, 60-80%) (Table 6). Twenty-nine (52%) surgeons stated that they would potentially benefit form additional training in minimally invasive SB-NEN resection, irrespective of previous training (Table 7). Ideally this would be in the form of video-training (moderate consensus, 60-80%). Implementation of a credentialing system was not supported by the respondents.

Table 6. Essentials in MIS SB-NEN resection

Characteristics, No. (%)	Total respondents (*N* = 57)	Academic hospitals (*N* = 41)	Experience in advanced MIS (*N* = 45)
Specific training in advanced MIS	*35 (61)*	23/40 (58)	26/44 (59)
Multidisciplinary asessment of patients for MI SB-NEN resection	33 (58)	*25/40 (63)*	*27/44 (61)*
High volume NEN center	28 (49)	*22/40 (55)*	20/44 (45)
High volume advanced MIS center	24 (42)	16/40 (40)	22/44 (50)
Specific training in open SB-NEN resection	21 (37)	*18/40 (45)*	15/44 (34)
Specific training in MI SB-NEN resection	18 (32)	13/40 (33)	12/44 (27)
At least two surgeons with experience in MI SB-NEN resection	10 (18)	6/40 (15)	5/44 (11)
Specific accreditation for MI SB-NEN resection	1 (2)	1/40 (3)	1/44 (3)

[a] multiple answers were possible, cumulative percentage may exceed 100%. Consensus statements (>80%) are presented in bold, moderate consensus (60-80%) in italic.

Table 7. Training and education in MIS SB-NEN resection

Characteristics, No. (%)	Total respondents (*N* = 58)	Academic hospitals (*N* = 41)	Experience in advanced MIS (*N* = 45)
No. MI SB-NEN resections needed to complete the learning curve, median (IQR)	10 (10-20)	10 (10-20)	10 (5-20)
Proportion MI SB-NEN resection at own hospital ten years from now, mean (SD)	54% (32)	53% (32)	59 (29)
Did you receive training in advanced MIS	33 (57)	25	29 (64)
Would you benefit from (adittional) training in MIS SB-NEN resection?	29/56 (52)	17/39 (44)	26 (58)
Training form should be [a]			
Video-training	*33/55 (60)*	21/38 (55)	*27/43 (63)*
Proctoring	28/55 (51)	20/38 (53)	23/43 (53)
Central-training, e.g. in surgical laboratory	26/55 (47)	18/38 (47)	20/43 (47)
Formalized residency	21/55 (38)	13/38 (34)	15/43 (35)
Credentialling should be implemented	13 (22)	8/41 (20)	8 (18)
Credentialling should include: [a]			
Training in advanced minimally invasive surgery	*9/13 (69)*	*6/8 (75)*	*6/8 (75)*
Trianing in open SB-NEN resection	*9/13 (69)*	*6/8 (75)*	*5/8 (63)*
Training in minimally invasive SB-NEN resection	*9/13 (69)*	*5/8 (63)*	*5/8 (63)*
Participation in registry for minimally invasive SB-NEN resection	*8/13 (62)*	*5/8 (63)*	*6/8 (75)*
Minimum number of cases under proctorship	5/13 (38)	2/8 (25)	1/8 (13)
Video review of procedure	4/13 (31)	1/8 (13)	2/8 (25)

[a] multiple answers were possible, cumulative percentage may exceed 100%. Consensus statements (>80%) are presented in bold, moderate consensus (60-80%) in italic. IQR: interquartile range, MIS: minimally invasive surgery, SD: standard deviation.

Sensitivity analyses

Academic hospitals

In academic hospitals (41 respondents), the median annual personal case load was 4 (2-6), and 27 (66%) of the surgeons performed minimally invasive SB-NEN resection (Table 3). The most common reasons not to choose this was lack of scientific evidence (50%), lack of supporting guidelines (36%) and lack of training in this technique (36%) (Table 3). Moderate consensus (60-80%) was reached regarding eligibility of patients without N2 lymph nodes for a minimally invasive reresection (Table 4). Patients without N2 lymph nodes or with distal lymph nodes and no encasement of the main mesenteric vessels are thought to be eligible for a minimally invasive resection (moderate consensus, 60-80%). Guidelines should give clear criteria for patient selection (moderate consensus, 60-80%). Contraindications were: risk of incomplete resection and venous involvement (moderate consensus, 60-80%) (Table 5).

Experience in advanced minimally invasive surgery

Of the surgeons with prior experience in advanced minimally invasive surgery (N=45), 38 (84%) stated to perform minimally invasive SB-NEN resection (Table 3). The preferred technique was laparoscopic dissection, followed by open bowel transection (moderate consensus, 60-80%). Patients without N2 lymph nodes are deemed amenable for a minimally invasive resection (consensus, >80%), as well as lymph nodes without encasement of the mesenteric vessels (moderate consensus, 60-80%) (Table 4). The most important contraindication was risk of incomplete resection (moderate consensus, 60-80%) (Table 5). Despite experience in advanced minimally invasive surgery for other indications, 58% of the surgeons stated that they would benefit from additional training in minimally invasive SB-NEN resection, ideally via video-training (moderate consensus, 60-80%) (Table 7).

DISCUSSION

This international survey study aimed to give insights in experience and attitudes towards minimally invasive surgery for treatment of SB-NEN. A laparoscopic, robotic or open resection was the preferred technique by 44%, 14% and 42% of the respondents, respectively. In patients with lymph node involvement but without N2 disease or encasement of main mesenteric vessels, consensus was reached among respondents that minimally invasive surgery is the preferred surgical approach in those patients. Insufficient training appeared to be one of the barriers for using a minimally invasive approach, besides lack of supporting evidence and guideline recommendations.

Reasons to opt for a laparoscopic approach were benefits related to post-operative pain, time to functional recovery, length of stay. The scarcely available literature reports median length of stay of 7-8 days after open resection and 4-6 days after minimally invasive resection of SB-NEN [3, 7, 9]. Evidence to verify the remaining arguments (post-operative pain and time to functional recovery) in the setting of SB-NEN resection is currently not present, but is expected to be beneficial for the laparoscopic approach, similar to colon cancer surgery [12].

Reasons to opt for an open resection were related to better lymphadenectomy and tactile feedback. An adequate lymphadenectomy is of particular importance, as presence of lymph nodes have a negative impact on survival, irrespective of presence of liver metastases, and is complex in case of N2 nodes [8, 13]. However, it should be noted that no differences between the number of resected lymph nodes were reported between minimally invasive and open resection by any of the comparative studies, and that R0 resection rates were higher in the minimally invasive group [3, 7, 9, 10]. This is probably a consequence of adequate patient selection. The argument of tactile feedback is expected to be related to the importance of palpating the small bowel to find and resect multiple primaries that are potentially missed on pre-operative imaging [2]. However, palpation of the entire small bowel is also possible in minimally invasive surgery, because the small bowel can be externalized through the extraction site, which was indeed performed as such by 63% of the respondents. Using this specific technique, Mahuron et al. was able to find a similar number of multifocal tumours (41% minimally invasive resection vs. 36% open resection, P = 0.70) [7]. Contrary to these

results, Ethun et al. described significantly less multifocal tumours after a minimally invasive resection (21% minimally invasive vs. 50% open, P = 0.03), but the operative technique was not described in detail [10].

Risk of incomplete resection was the only contraindication reaching moderate consensus. Appropriate Long-term outcome data for minimally invasive resection are still not available. Further studies are required to determine the risk of incomplete resection in minimally invasive SB-NEN resection for different tumour stages and whether this impacts on long-term survival. But oncological safety should not be compromised for the sake of short-term benefits during the years that this evidence has to be obtained. Arterial and venous involvement of the tumour was the second and third most common contraindication stated by the respondents. This is indeed a specific challenge for these procedures, as up to 40% of the patients present with this advanced nodal stage [9]. Surgeons could make use of fluorescence angiography with indocyanine green to help aid safe resection, either during a minimally invasive or open procedure [14].

Regarding selection criteria, patients without N2 lymph node metastases and without encasement of the mesenteric vessels were deemed eligible for a minimally invasive resection. In sensitivity analyses for previous experience in advanced minimally invasive surgery and academic hospitals, it was also stated that guidelines should give clear criteria for this. The classification system of mesenteric metastases proposed by Ohrvall et al. could be considered for this purpose [15]. Herein, the location of mesenteric metastases are staged from I to IV, in which stage I consists of nodal disease with a close proximity to the intestine (i.e. distal) and stage IV constitutes metastases extending retroperitoneally or peri-pancreatic, or encasing the superior mesenteric vessels (i.e. proximal).

Essential items to consider when conducting minimally invasive SB-NEN resection according to the respondents were either previous training in advanced minimally invasive surgery (for other indications), and multidisciplinary assessment to discuss eligibility of patients for a minimally invasive resection. These findings did not differ in sensitivity analyses. Multidisciplinary assessment could be performed during regular tumour board meetings, or can be part of specific technical meetings.

Regarding additional training, 52-58% of the respondents stated that they would potentially benefit from this. Video-training was the preferred way to do this. Our group has previously published two video vignettes describing the operative technique, with use of intra-operative fluorescence angiography using indocyanine green [16, 17]. Video-training only might not be sufficient to learn to perform such a complex procedure. An initial wet-lab training focused on laparoscopic D3 dissection might be of benefit, and is something that could be investigated via the ISGSS. Subsequently, a number of cases should ideally be proctored, and this might be tailored to the experience level of the surgeon to be trained and the efficiency of gaining additional skills as perceived by the proctor.

The findings of this study should be seen in light of some limitations. Bias might be introduced due to personal preference of surgeons, which is inherent to qualitative research. Also, three large endocrine and colorectal societies from the United States and Europe did not participate, hence comparison of experiences and attitudes within continents was not possible. Finally, based on the answers as to why certain approaches are preferred (Table 2), respondents might have given the "right" answers to some questions, instead of genuine thoughts or considerations.

Forty-three (74%) of the respondents were interested in collaboration for conducting future studies. We are currently giving shape to this collaboration by setting up the International Study Group of small bowel neuroendocrine Surgery (ISGSS, www.ISGSS.org). Based on the survey, the median (IQR) annual cumulative case-load of ISGSS is estimated to be 172 (86-258) resections of which 129 (86-215) are minimally invasive. With these numbers, more solid evidence for guidelines could be generated, and studies that were previously thought to be impossible could be performed (e.g. randomized trials). The evidence generate by the international study group can be used to validate the arguments given by the respondents. Furthermore, this infrastructure can be used to organize training for minimally invasive SB-NEN resection.

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Author contributions

Conceptualization: EK, AFE, PJT, EJMNvD.
Data curation: EK.
Formal analysis: EK.
Supervision: AFE, WAB, PJT, EJMNvD.
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SUPPLEMENTARY MATERIALS

Supplementary material 1. Survey.

1. Are you a surgeon? (1 option)
   a. Yes
   b. No
2. What is your sex (1 option)
   a. Female
   b. Male
   c. Prefer not to say
   d. Other:
3. What is your age? (open question)
4. In which country do you work? (open question)
5. In what type of hospital do you work? (1 option)
   a. Academic
   b. Regional
   c. Non-academic, referral
6. How many years have you been working as an attending surgeon (post-surgical training/ fellowships included) (open question)
7. What type of surgery do you perform mostly? (e.g. colorectal, endocrine, HPB etc.) (open question)
8. Do you perform advanced MIS resections? (1 option)
   a. Yes
   b. No
9. How many years of experience do you have in advanced MIS? (open question)
10. Which of these techniques do you consider superior? (1 option)
    a. Open
    b. Robot-assisted
    c. Laparoscopic (incl. handport, and laparoscopic dissection with open bowel transection)
11. I consider this technique superior, because (multiple answers)
    a. Tactile feedback
    b. Less expensive
    c. Faster set-up time compared to robot or laparoscopic
    d. Better lymphadenectomy
    e. Higher margin-free rate (R0)
    f. Enhanced dexterity due to more degreees of freedom
    g. Improved visibility
    h. 3D vision
    i. Better ergonomics
    j. Less blood loss during surgery
    k. Decreased time to functional recovery
    l. Less pain after surgery

m. Decreased length of hospital stay
n. Fewer post-operative complications
o. Increased life expectancy
p. Other:

12. I consider this superior, because (select most important argument) (1 option)
    a. Tactile feedback
    b. Less expensive
    c. Faster set-up time compared to robot or laparoscopic
    d. Better lymphadenectomy
    e. Higher margin-free rate (R0)
    f. Enhanced dexterity due to more degreees of freedom
    g. Improved visibility
    h. 3D vision
    i. Better ergonomics
    j. Less blood loss during surgery
    k. Decreased time to functional recovery
    l. Less pain after surgery
    m. Decreased length of hospital stay
    n. Fewer post-operative complications
    o. Increased life expectancy
    p. Other:
13. How many SB-NEN resections are performed in your center annually? (total number, i.e. MIS and open) (open question)
14. How many SB-NEN resections do you personally perform as primary (attending) surgeon annually (procedures In which you supervise a fellow/resident included)? (open question)
15. Do you perform MIS SB-NEN resection as primary (attending) surgeon? (1 option)
    a. Yes (go to question 16)
    b. No (go to question 18)
16. How many MIS SB-NEN resections do you perform annually? (open question)
17. Which type of MIS SB-NEN resection do you perform (multiple answers) (go to question 20)
    a. Fully laparoscopic
    b. Fully robot-assisted
    c. Laparoscopic dissection, with robot-assisted dissection (i.e. vessels)
    d. Laparoscopic dissection, open bowel transection
    e. Hand-assisted minimally invasive
    f. Other:
18. Why are you not performing MIS SB-NEN resection? (multiple answers)
    a. Lack of scientific evidence
    b. No guidelines by the societies are published on this topic
    c. Lack of training in this technique
    d. Lack of time in surgical schedules
    e. Not relevant in my center

f. Other surgeon(s) perform this procedure in our center
g. Difficulty of the surgical technique
h. The costs are too high
i. Institutional culture discourages it
j. Patient preference for open approach
k. Other:

19. Choose your most important reason for not performing MIS SB-NEN resection (1 option)
    a. Lack of scientific evidence
    b. No guidelines by the societies are published on this topic
    c. Lack of training in this technique
    d. Lack of time in surgical schedules
    e. Not relevant in my center
    f. Other surgeon(s) perform this procedure in our center
    g. Difficulty of the surgical technique
    h. The costs are too high
    i. Institutional culture discourages it
    j. Patient preference for open approach
    k. Other:
20. In your opinion, what is currently the overall value of MIS SB-NEN resection compared to an open approach in patients eligible for both approaches? (1 option)
    a. Inferior value of MIS SB-NEN resection
    b. Equivalent value of MIS SB-NEN resection
    c. Superior value of MIS SB-NEN resection
    d. Other:
21. In your opinion, what will be the overall value of MIS SB-NEN resection compared to an open approach in patients eligible for both approaches? (1 option)
    a. Inferior value of MIS SB-NEN resection
    b. Equivalent value of MIS SB-NEN resection
    c. Superior value of MIS SB-NEN resection
    d. Other:
22. In your opinion, do you think that patients without large lymph node metastases (i.e. pN2, >2cm), are amenable for MIS SB-NEN resection? (1 option)
    a. Yes
    b. No
    c. Other:
23. In your opinion, do you think that guidelines should give clear criteria for which patients are amenable for MIS SB-NEN resection? (1 option)
    a. Yes
    b. No
    c. Other:

24. In your opinion, do you think that patients with distal lymph nodes, i.e. without encasing the mesenteric vessels are amenable for MIS SB-NEN resection? (1 option)
    a. Yes
    b. No
    c. Other:
25. In your opinion, do patients in general benefit from MIS SB-NEN resection, when performed by a surgeon who is experienced in these procedures? (1 option)
    a. Yes
    b. No
    c. Other:
26. In your opinion, what kind of effect on quality of life has MIS SB-NEN resection compared to open SB-NEN resection? (1 option)
    a. Better quality of life after MIS SB-NEN resection
    b. Equal quality of life after MIS SB-NEN resection
    c. Worse quality of life after MIS SB-NEN resection
    d. Other:
27. In your opinion, which of the following is a contraindication for proceeding with MIS SB-NEN resection? (multiple answers)
    a. Large size of mesenteric metastases (pN2, >2cm)
    b. Multiple primary tumours
    c. Venous involvement of the tumour
    d. Arterial involvement of the tumour
    e. Risk of intra-operative bleeding
    f. Risk of incomplete resection (R1/2)
    g. Morbid obesity (BMI >30)
    h. Advanced age
    i. Prior laparotomy
    j. ASA score > 3
    k. None
    l. Other:
28. In your opinion, which of the following is a contraindication for proceeding with MIS SB-NEN resection? (choose the most important reason) (1 option)
    a. Large size of mesenteric metastases (pN2, >2cm)
    b. Multiple primary tumours
    c. Venous involvement of the tumour
    d. Arterial involvement of the tumour
    e. Risk of intra-operative bleeding
    f. Risk of incomplete resection (R1/2)
    g. Morbid obesity (BMI >30)
    h. Advanced age
    i. Prior laparotomy
    j. ASA score > 3
    k. None
    l. Other:

29. Which items are in your opinion essential (i.e. absolutely required) for performing MIS SB-NEN resection? (multiple answers)
    a. Specific training in open SB-NEN resection
    b. Specific training in advanced MIS
    c. Specific training in MIS SB-NEN resection
    d. High volume NEN center
    e. High volume advanced MIS center
    f. At least two surgeons with experience in MIS SB-NEN resection
    g. Multidisciplinary assessment of patients for MIS SB-NEN resection
    h. Specific accreditation for MIS SB-NEN resection
    i. Other:
30. Which items are in your opinion essential (i.e. absolutely required) for performing MIS SB-NEN resection? (choose the most important item) (1 option)
    a. Specific training in open SB-NEN resection
    b. Specific training in advanced MIS
    c. Specific training in MIS SB-NEN resection
    d. High volume NEN center
    e. High volume advanced MIS center
    f. At least two surgeons with experience in MIS SB-NEN resection
    g. Multidisciplinary assessment of patients for MIS SB-NEN resection
    h. Specific accreditation for MIS SB-NEN resection
    i. Other:
31. In your opinion, how many MIS SB-NEN resections are necessary to complete the learning curve? (open question)
32. Did you receive specific training in advanced MIS? e.g. fellowship (1 option)
    a. Yes
    b. No
    c. Other:
33. Do you think you would benefit from specific training in MIS SB-NEN resection? (1 option)
    a. Yes
    b. No
    c. Other:
34. How should training for MIS SB-NEN resection ideally be organized? (multiple answers)
    a. Central training, e.g. in a surgical laboratory
    b. Proctoring
    c. Video-training
    d. Through formalized residency and/or fellowship only
    e. Other:
35. How should training for MIS SB-NEN resection ideally be organized? (choose most important item) (1 option)
    a. Central training, e.g. in a surgical laboratory
    b. Proctoring

c. Video-training
d. Through formalized residency and/or fellowship only
e. Other:

36. Do you think there should be a credential process for MIS SB-NEN resection?
    a. Yes (go to question 37)
    b. No (go to question 39)
    c. Other:
37. What should credentialing include? (multiple answers)
    a. Training in open SB-NEN resection
    b. Training in general advanced MIS
    c. Training in MIS SB-NEN resection
    d. Minimum number of cases done under proctorship
    e. Video review of operation
    f. Participation in a surgical registry, specific for MIS SB-NEN
    g. Other:
38. What should credentialing include? (choose most important item) (1 option)
    a. Training in open SB-NEN resection
    b. Training in general advanced MIS
    c. Training in MIS SB-NEN resection
    d. Minimum number of cases done under proctorship
    e. Video review of operation
    f. Participation in a surgical registry, specific for MIS SB-NEN
    g. Other:
39. What percentage of SB-NEN resection do you believe will be performed via a minimally invasive approach in your center, ten years from now? (open question)
40. Can we approach you for future collaborative studies regarding surgery for SB-NEN or do you want to receive a single update upon publication of the study results? (1 option)
    a. Yes
    b. No (go to question 42)
41. Please fill in your e-mail address (open question)
42. You can write any comments you have below, if not, thank you for your help! (open questions)

CHAPTER 6

Value of laparoscopy for resection of small bowel neuroendocrine neoplasms including central mesenteric lymphadenectomy

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Diseases of the Colon and Rectum 2021

ABSTRACT

Background Literature on laparoscopic resection of small bowel neuroendocrine neoplasms (SB-NEN) consists of single case descriptions or small selected case-series only, likely because of challenging mesenteric lymphadenectomy. This study evaluated an institutional change in approach from open to laparoscopic resection of SB-NEN independent from lymph node involvement.

Methods This is a retrospective comparative cohort study, conducted at a tertiary referral center. Patients with SB-NEN who underwent a laparoscopic or open segmental bowel resection with central mesenteric lymphadenectomy were included. The complexity of lymphadenectomy was assessed by determining distance between suspect lymph nodes and main mesenteric branches on preoperative CT. Number of (tumor-positive) lymph nodes, conversion to open surgery, post-operative complications according to Clavien-Dindo and length of stay.

Results A total of 34 patients were identified, of whom 11 (32%) underwent open and 23 (68%) laparoscopic surgery. Distances between lymph nodes and main mesenteric branches and number of examined and tumor-positive lymph nodes did not differ significantly. Laparoscopy was converted in 7 patients (30%). Major post-operative complications (grade 3-5) occurred in one (9%) patient in the open surgery group (grade 5) and 2 (9%) patients in the laparoscopic surgery group (grade 3b). The LOS was 8 (range 6-18) days in the open surgery group and 4 (4-8) days in the laparoscopic group ($p = 0.036$).

Conclusion Long-term outcomes could not reliably be assessed due to the relatively short follow-up of the laparoscopy group. Laparoscopic bowel resection with central mesenteric lymphadenectomy for SB-NEN seems safe and associated with similar pathological outcome and shorter length of stay in the setting of a tertiary referral center.

INTRODUCTION

Small bowel neuroendocrine neoplasms (SB-NEN) have an average incidence of 1/100.000 person years [1]. Reported survival rates vary substantially, with 5-year overall survival of 70-80% for stage I-IIIa, and 35-80% for stage IV [2]. According to the European Neuroendocrine Tumor Society (ENETS) 2016 guidelines, radical resection of SB-NEN is indicated for stage I-III disease by means of local radical open or laparoscopic resection of the primary tumor with concomitant lymph node resection, which is associated with prolonged survival [2-5].

There is a lack of data on laparoscopic surgery for SB-NEN, which might be explained by the technical difficulties that can be faced during a minimally invasive approach of SB-NEN. Laparoscopy is especially challenging because of nodal metastases, as these often extent to the mesenteric root and are present in more than 80% of patients [6]. This is why laparoscopy for SB-NEN is generally restricted to highly selected patients.

There is little evidence regarding the place of laparoscopic surgery for SB-NEN and guidelines are restrictive in their recommendations regarding minimally invasive surgery. The North American Neuroendocrine Tumor Society (NANETS) 2017 guideline states that laparoscopic surgery should be reserved for patients with extensive, inoperable liver metastases. Similarly, the ENETS 2016 guideline states that laparoscopic surgery should be reserved for selected patients. Limitations of laparoscopic surgery include identification of sub-centimeter primary lesions (uni- or multifocal), debulking of peritoneal metastases (present in 20% of patients) and extensive mesenteric fibrosis [3, 6-8]. It is commonly thought that laparoscopic resection of SB-NEN will result in inadequate mesenteric lymphadenectomy with risk of uncontrollable vascular injury at the level of the mesenteric root, thereby compromising oncological outcome. In colorectal cancer, laparoscopic surgery is associated with less blood loss, less pain, faster recovery of bowel function, faster return to normal diet and less wound infections in the short term when compared to open surgery, and with decreased risk of small bowel obstruction and incisional hernia in the long-term [9-13]. It is likely that these benefits can also be achieved when applying laparoscopic surgery for SB-NEN.

Current literature on laparoscopy for SB-NEN consists of single case descriptions or small selected case series only. At our tertiary referral center, minimally invasive surgery was gradually implemented and became the routine approach for resection of SB-NEN from 2015 onwards, being performed by two surgeons with an extensive experience in advanced laparoscopic surgery. Therefore, the aim of this study was to compare post-operative outcomes between laparoscopic and open surgery in a cohort of SB-NEN that reflects an institutional change of surgical approach independent from suspected lymph node involvement.

METHODS

Patients

This retrospective cohort included all consecutive patients undergoing open or laparoscopic resection of a histopathologically confirmed primary SB-NEN between 2003 and 2019 at the Amsterdam University Medical Centers (UMC), University of Amsterdam. Patients were identified from a prospective institutional database. All patients were discussed in a multidisciplinary meeting. Patients were included regardless of tumor grade or stage. Resection for recurrent SB-NEN was an exclusion criterion. Patients had to give written informed consent to extract their data from the hospital records. This study was approved by the data protection officer of the Amsterdam UMC, University of Amsterdam in November 2018. We report this study in accordance with the STROBE guidelines [14].

Outcome parameters

The primary outcome was postoperative hospital stay. Secondary outcomes included conversion to open surgery (defined as any midline incision made for surgical dissection and not just specimen extraction), number of (tumor-positive) lymph nodes (LN), post-operative complications using the Clavien-Dindo classification, reoperation rate and 90-day mortality rate [15].

Data extraction and definitions

The following data were extracted from hospital records: patient data (sex, age, body mass index, American Society of Anaesthesiologists Classification, comorbidities, somatostatin analogue usage, serum Chromogranin A, symptoms and presence of liver metastases), preoperative CT imaging data (imaging modality, location of primary lesion, number of visible LN, diameter of largest LN, shortest distance between LNs and main mesenteric branches and duodenal wall, number of visible LNs within 2 cm of mesenteric branches), surgical intervention data (emergency/elective setting, type of resection, blood loss and duration of surgery, conversion), pathology characteristics (tumor grade, TNM classification, stage, length of resected bowel, largest tumor size, resection margins, number of examined LNs, number of tumor-positive LNs, immunohistochemical stainings) and postoperative outcomes (complications, length of hospital stay, 90-day mortality). Postoperative complications were assessed for 90-days postoperatively and graded using the Clavien-Dindo classification [15]. Minor complications were defined as grade 1 or 2, and major complications as grade 3 to 5. Tumor grading and staging was based on the 2016 ENETS guideline and the 8th edition American Joint Committee on Cancer (AJCC) manual [2, 16]. Postoperative care was according to Enhanced Recovery After Surgery (ERAS), which was implemented at our unit preceding the LAFA trial (inclusion 2005-2009), although some patients from the beginning of the present cohort might have received not all elements of ERAS [17].

Preoperative imaging

All preoperative imaging (both CT and PET) were assessed independently by the surgeon (PJT) and the nuclear medicine physician (JCCK) retrospectively. Any discrepancies were resolved

by discussion. The distances between the mesenteric LN suspected for lymph node metastasis and the superior mesenteric vein (SMV) and superior mesenteric artery (SMA) were measured in the coronal plane. The distance between the mesenteric LN and the duodenal wall were measured in the sagittal plane. Reconstructions were made if necessary. All distances were measured from the outermost edges (e.g. outermost edge of the duodenal wall and outermost edge of the mesenteric LN), in mm, using the build-in measuring tool of Enterprise Imaging XERO Viewer (Agfa HealthCare, Mortsel, Belgium).

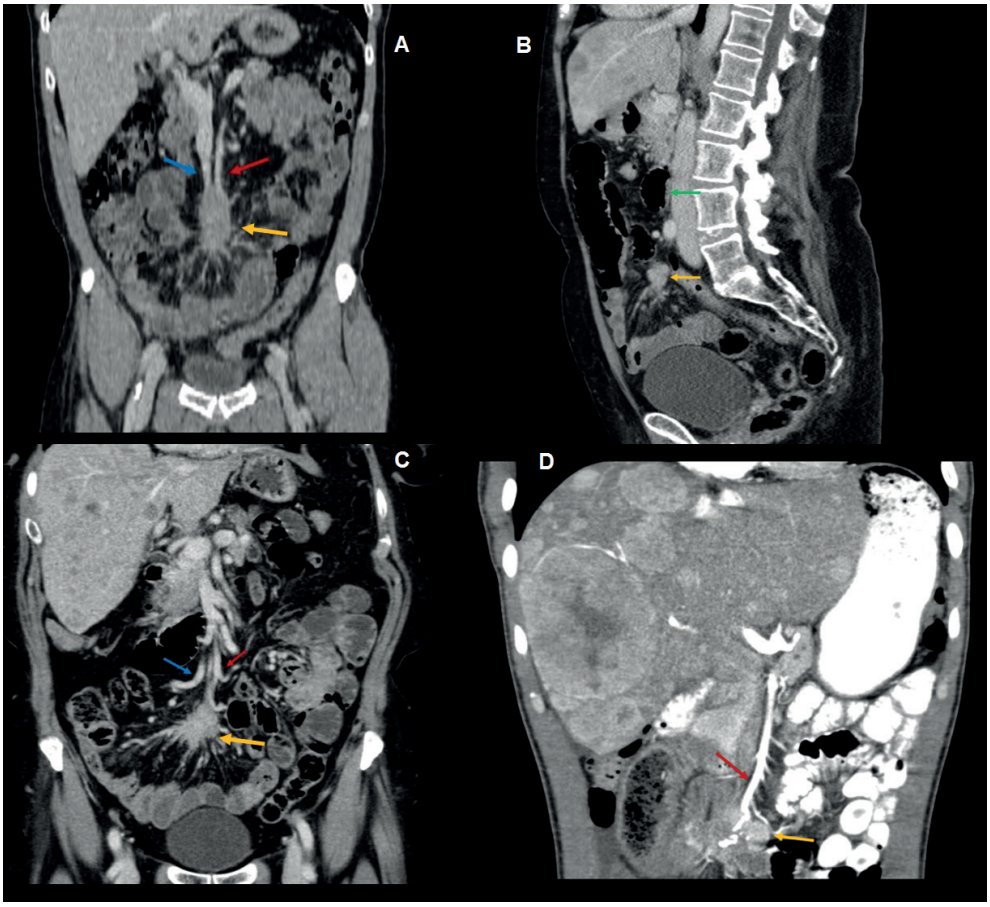
Surgical technique

For a laparoscopic procedure, the patient was in French position (legs split). The procedure started with complete inspection of the small bowel for identification of the primary tumor and potential multifocal lesions, followed by identification of pathologically enlarged mesenteric nodes and inspection of the peritoneum. The caudal to cranial dissection was initiated in Trendelenburg position by opening the peritoneum at the level of the terminal ileum, followed by complete retroperitoneal dissection of the mesenteric root up to the ventral surface of the duodenum and pancreas. The procedure was then continued in reverse-Trendelenburg, and the transverse mesocolon was cranially retracted. Depending on the location of the most central enlarged LNs and relationship to vascular structures on preoperative CT (Figure 1), the superior mesenteric artery (SMA) and vein (SMV) were carefully dissected. Subsequently, the segmental branches of the SMA/SMV were divided just proximal to the most centrally located pathological LN. Hereafter, the mesentery was further transected towards the proximal and distal side of the primary tumor, thereby including all mesentery belonging to the involved small bowel segment and visually/palpable enlarged nodes. In case of primary tumor location in the terminal ileum with central ligation of the ileocolic vessels, the ascending colon was also mobilized, similar to a laparoscopic right hemicolectomy. Conversion to a 10 cm midline laparotomy above the umbilicus was performed if there was inadequate exposure or bleeding of the mesenteric root. If the procedure was not converted for mesenteric dissection, a 10 cm midline laparotomy above the umbilicus was made and the involved bowel extracted. At this point, palpation was performed for identification of additional sub-centimeter lesions in the remaining bowel or mesentery. Based on blueish discoloration and identification of the vascular watershed, the small bowel was transected with oncologically safe margins. Subsequently, an entero-enterostomy was made, and the mesenteric window closed. Parts of the technique have been previously described in more detail [18].

Statistical analysis

Patients were stratified according to the intended surgical approach (laparoscopic or open). Converted laparoscopic procedures were analyzed in the laparoscopic group. Patient, intervention and pathology characteristics were tabulated using descriptive statistics. Continuous data was reported in medians with an interquartile range (IQR) or mean with standard deviation (SD), and compared using the Mann-Whitney U test or unpaired t-test, depending on the distribution. Categorical data was reported as number of cases with percentages, and compared using the chi-square test. Statistical significance was defined as a two-sided P value less than 0.05. Data was analyzed with Statistical Package for the Social Sciences (SPSS) version 24.0 (IBM Corp. Released 2016, Version 24.0. Armonk, NY).

Figure 1. Examples of patients with central mesenteric lymph node metastases.



(A) close relationship between central lymph node metastasis and the superior mesenteric artery and vein, **(B)** sagittal image with the duodenum and lymph node, **(C)** lymph node with typical fibrotic strands, **(D)** encasement of a main branch of the superior mesenteric artery; blue arrows depict (branches of) the superior mesenteric vein. Red arrows depict (branches of) the superior mesenteric artery, yellow arrows depict the lymph node, green arrows depict the duodenum.

RESULTS

A total of 34 consecutive patients who underwent surgery for primary SB-NEN were included. Mean age was 68 (SD 9), and 21 (62%) were male. An open resection was performed in 11 (33%) patients, and 23 (67%) underwent an intentionally laparoscopic resection. Liver metastases were present in 17 (50%) of the patients: nine did not receive a resection, two were resected during a separate operation (both R0, one laparoscopic, one open), five received embolization/ radio frequency ablation and one peptide receptor radionuclide therapy. All patients had a single small bowel resection (i.e. one segmental resection). The median (IQR) year of surgery was 2010 (2008-2014) in the open group and 2017 (2012-2018) in the laparoscopy group (p = 0.001). Treatment with somatostatin analogues was given preoperatively to 11 patients

(33%), and postoperatively to 17 patients (50%). Patient characteristics did not differ significantly between the two surgical approaches and are displayed in Table 1. Abdominal pain was the most prevalent symptom at presentation (56%).

Preoperative imaging by either CT (all with intravenous contrast) and/or ^{68}Ga-DOTATATE positron emission tomography (PET)-CT scan was performed in all patients, with relatively more 68-Gallium DOTATATE PET-CT scans in the laparoscopy group 13 (56%) vs. 2 (18%); $p = 0.064$). The number of visible LNs in the drainage area, diameter of the largest LN, and shortest distance between LNs and SMA, SMV, and duodenal wall did not differ significantly between both groups.

Pathology reports of the resected specimens were available for 33/34 (97%) patients (Table 2). Stage III disease was present in 16 (47%) patients and stage IV in 17 (50%) patients. There were no patients with stage I-II disease. Tumor grade, TNM-classification, ENETS stage, length of resected bowel, largest tumor size, resection margin, number of examined LNs, number of tumor-positive LNs and LN ratio did not differ significantly between both groups.

Seven (30%) laparoscopic procedures were converted due to inadequate exposure of the mesenteric root. Blood loss and duration of surgery were comparable, and no intra-operative complications occurred. Median length of stay (LOS) amounted to 8 (6-18) days in the open surgery group and 4 (4-8) days in the laparoscopic surgery group ($p = 0.036$). Opioid (including epidural) usage in the postoperative period did not differ significantly between both intervention groups.

Median follow-up in the open group was 98 (IQR 52-131) months, and 26 (7-70) months in the laparoscopy group.

In the open surgery group, 4/11 (36%) patients had grade 1-2 complications and 1/11 (9%) a grade 3-5 complication (Table 3). Corresponding complication rates in the laparoscopic group were 4/23 (17%) and 2/23 (9%), respectively. Wound infections requiring antibiotic treatment (grade 2) occurred in three patients, one in the open group, and two in the laparoscopic group. Two patients required reoperation, 1/11 (9%) in the open surgery group and 1/23 (4%) in the laparoscopic group. In the open surgery group, the patient had an anastomotic leakage, and deceased due to a septic shock with multi-organ failure (grade 5). Re-intervention in the laparoscopic group was indicated for fascial dehiscence (grade 3b).

Table 1. Baseline patient characteristics

Characteristics	Surgery, No. (%)		P Value
	Open (n = 11)	Laparoscopic (n = 23)	
Sex			
Male	6 (55)	15 (65)	0.709[a]
Age, mean (SD), y	66 (9)	67 (9)	0.788[b]
BMI, mean (SD), kg/m^2	26.7 (6.7)	26.6 (5.0)	0.962[b]
ASA classification			
ASA 1	1 (13)	3 (14)	1[a]
ASA 2	5 (63)	12 (57)	
ASA 3	2 (25)	6 (29)	
Comorbidities			
Diabetes mellitus	0 (0)	3 (13)	0.296[a]
Cardiovascular	8 (73)	12 (52)	0.223[a]
Serum Chromogranin A, median (IQR), µg/L	428 (88-749)	162 (74-841)	0.139[b]
Preoperative SSA	5 (46)	6 (26)	0.345[a]
Symptoms			
Diarrhea	4 (44)	5 (25)	0.396[a]
Flush	2 (22)	3 (16)	1[a]
Abdominal pain	5 (71)	14 (74)	1[a]
Fatigue	1 (9)	2 (9)	0.701[a]
Liver metastases			
No liver metastases	3 (27)	13 (59)	0.199[a]
Unilobar liver metastases	3 (27)	5 (23)	
Bilobar liver metastases	5 (46)	4 (18)	
Preoperative imaging			
Modality			
CT	9 (82)	15 (65)	0.437[a]
68Ga-DOTATATE PET-CT	2 (18)	13 (56)	0.064[a]
Primary lesion identified			
No	6 (55)	0 (0)	0.000[a]
Yes	4 (36)	19 (95)	
Yes, multiple primary tumors	1 (9)	1 (4)	
Lymph nodes			
Visible LN in drainage area, mean (SD)	4 (2)	3 (3)	0.390[b]
Diameter of largest LN, mean (SD), mm	32 (20)	34 (16)	0.764[b]
Distance suspected LN and SMV, mean (SD), mm	28 (24)	25 (23)	0.742[b]
Distance suspected LN and SMA, mean (SD), mm	35 (27)	34 (27)	0.910[b]
Distance suspected LN and duodenal wall, mean (SD), mm	21 (19)	25 (19)	0.548[b]

Characteristics	Surgery, No. (%)		P Value
	Open (n = 11)	Laparoscopic (n = 23)	
Suspected LN within 2 cm of SMA/SMV			
0	6 (55)	11 (52)	0.784[a]
1	4 (36)	6 (29)	
>1	1 (9)	4 (19)	
Surgical procedure			
Emergency surgery	1 (9)	3 (13)	1[a]
(extended) Ileocecal resection	2 (18)	6 (26)	0.605[a]
(extended) hemicolectomy	1 (9)	5 (22)	
Segmental small bowel resection	8 (73)	12 (52)	
Postoperative therapy			
SSA	8 (73)	9 (39)	0.141[a]
Chemotherapy	1 (9)	2 (9)	1[a]
PRRT	1 (9)	3 (13)	1[a]

Abbreviations: ASA, American Society of Anesthesiologists; BMI, body mass index (calculated as weight in kilograms divided by height in in meters squared); IQR, interquartile range; PET, positron emission tomography; CT, computed tomography; LN, lymph nodes; PRRT, peptide receptor radionuclide therapy; SMA, superior mesenteric artery; SMV, superior mesenteric vein; SSA, somatostatin analogues.

[a] X^2 test applied

[b] Independent-Samples T Test applied

Table 2. Pathology

Characteristics	Surgery, No. (%)		P Value
	Open (n = 11)	Laparoscopic (n = 23[c])	
Tumor grade			
Grade 1	7 (64)	15 (68)	1[a]
Grade 2	4 (36)	7 (32)	
TNM classification			
T1	0 (0)	1 (4)	1[a]
T2	4 (46)	8 (36)	
T3	6 (55)	11 (50)	
T4	1 (9)	2 (9)	
N0	0 (0)	0 (0)	0.443[a]
N1	6 (55)	15 (68)	
N2	5 (45)	7 (32)	
M0	4 (36)	12 (55)	0.465[a]
M1	7 (64)	10 (45)	
Single tumors	7 (64)	17 (77)	0.681[a]
Multiple tumors	4 (36)	5 (23)	
Disease stage			
Stage III	4 (36)	12 (55)	0.472[a]
Stage IV	7 (64)	10 (45)	
Length of resected bowel, mean (SD), cm	49 (21)	51 (25)	0.519[b]
Largest tumor size, mean (SD), mm	24 (9)	23 (16)	0.814[b]
Resection margin			
R0	8 (73)	18 (82)	1[a]
R1[d]	2 (18)	3 (14)	
R2	1 (9)	1 (4)	
Lymph nodes			
Number of examined LNs, mean (SD)	17 (11)	13 (7)	0.280[b]
Diameter of largest LN, mean (SD), mm	35 (27)	34 (18)	0.974[b]
Number of tumor positive LNs, median (IQR)	4 (4)	5 (6)	0.593[b]
Immunohistochemistry			
Synaptophysin	7 (64)	17 (85)	0.210[a]
Chromogranin A	8 (73)	15 (75)	1[a]
CD56/NCAM	6 (55)	3 (18)	0.095[a]

Abbreviations: ENETS, European Neuroendocrine Tumor Society; SD, standard deviation; LN, lymph node; HPF, high power fields; NCAM, neural cell adhesion molecule.

[a] X^2 test applied.

[b] Independent-Samples T Test applied

[c] Pathology data could not be retrieved for one patient.

[d] One patient in the laparoscopic group had an R1 resection of the primary tumor, the remaining 5 R1 resections concern the central mesenteric lymph node dissection.

Table 3. Intra- and post-operative outcomes

Characteristics	Surgery, No. (%)		P Value
	Open (n = 11)	Laparoscopic (n = 23)	
Blood loss, median (IQR), mL	400 (75-750)	400 (300-1400)	0.724[a]
Conversion to laparotomy	-	7 (30)	-
Duration of surgery, mean (SD), min	145 (48)	191 (69)	0.053[b]
Length of hospital stay, median (IQR), d	8 (6-18)	4 (4-8)	0.036[a]
Complications			
Minor morbidity (Clavien-Dindo grade 1-2)	4 (36)	4 (17)	0.391
Major morbidity (Clavien-Dindo grade 3-5)	1 (9)	2 (9)	1
90-day mortality	1 (9)	-	-

[a] Mann-Whitney U Test applied

[b] Independent-Samples T Test applied

[c] X^2 test applied

DISCUSSION

The present comparative cohort study reports on a gradual institutional switch in surgical approach from open to laparoscopy for the resection of SB-NEN, independent from the findings on preoperative imaging regarding the need for central mesenteric lymphadenectomy. The technical difficulties that might be experienced during laparoscopic dissection of the mesenteric root in SB-NEN are reflected by the 30% conversion rate. Hospital stay was 4 days shorter after laparoscopic resection in this historical comparison. Other pathological and postoperative outcomes were not significantly different, although patient numbers were small.

Figueiredo et al. analyzed a cohort of 73 patients with SB-NEN, of whom 12 (16%) underwent laparoscopic resection [19]. Patients in the open surgery group had a significantly higher number of clinically manifest LNs at diagnoses (52 vs. 3 nodes, $p < 0.001$), significantly more liver metastases (42 vs. 1 metastases, $p<0.001$) and significantly longer small bowel resection specimens (48 vs. 19 cm, $p = 0.009$). Patient selection was therefore in compliance to the ENETS guideline, but essentially different from the present study, in which the surgical approach was chosen based on a gradual shift towards laparoscopy and independent from the extent of disease or any other factors, as demonstrated by the absence of significant baseline and preoperative imaging differences.

In a similar cohort published by Ethun et al., resection of SB-NEN started as intentional laparoscopic procedure in 36 of 93 (39%), with conversion to open in 9 patients (25%) hand-assisted procedure in 21 (58%), and entire laparoscopic resections in 6 patients (17%) [20]. In line with Figueiredo et al., case selection for laparoscopy was suggested based on less obstructive symptoms (4% vs. 24%) and less metastatic disease (19% vs. 44%). Multifocal localization was significantly less frequent in the laparoscopy patients (21% vs. 50%), while a smaller non-significant difference was found in our series (23% vs. 36%). In contrast, the study of Ethun et al. showed a similar number of resected lymph nodes (13 vs. 12), while our study revealed a tendency towards lower number of resected lymph nodes with laparoscopy (13 vs. 17). We

agree with Ethun et al. that it is difficult to determine whether observed differences can only be attributed to patient selection, or if identification of primary NETs or lymph node harvest is suboptimal using laparoscopy. It is important to note that palpation of the entire small bowel during laparoscopy is still feasible after exteriorizing the small bowel through the extraction site.

Long-term oncological follow-up is probably the most important outcome. Reissman et al. report a series of 35 laparoscopically resected patients without local or regional recurrence during a mean follow-up period of 41 months (range 3-96) [21]. Follow-up of the present series was relatively short in the laparoscopy group to allow for any meaningful conclusion, but only incidental recurrences occurred so far.

Similar to our study, Fiqueiredo et al. and Ethun et al. reported that laparoscopic surgery was associated with a shorter hospital stay compared to open surgery (6 vs. 8 days) [19, 20]. It is important to note that hospital stay is always difficult to interpret in a non-randomized comparison and should be interpreted with caution, due to the historical changes in peri-operative care. Furthermore, hospital stay does not accurately reflect patients' recovery. Prospective studies on surgical approach often choose time to functional recovery as endpoint [22]. However, this parameter is difficult to determine retrospectively.

Thomaschewski et al. state that a mini-laparotomy would enable an easy resection of a SB-NEN, although a definition or size of such a mini-laparotomy was not reported [23]. In selected patients without clinical suspicion of LN metastases (stage I and II), a mini-laparotomy might be sufficient. However the majority of SB-NEN cases present with pathological lymph nodes on preoperative imaging, often extending towards the mesenteric root [24]. Resection of LN metastases prolongs survival and can prevent future mesenteric ischemia due to encasement of the mesenteric trunk, which significantly impairs quality of life [3-5, 25]. Laparotomy is still considered the standard of care by guidelines for such patients, for the purpose of optimal mesenteric lymphadenectomy.

However, in the current era, dissection of the supplying mesenteric vessels close to their origin at the level of the mesenteric root is feasible through laparoscopy, similar to for example laparoscopic D3 lymphadenectomy for colon cancer [25, 26]. If performed by surgeons with extensive experience in advanced laparoscopy, adequate lymphadenectomy can be achieved, given the similar number of positive LNs among the two groups (Table 3). Furthermore, no uncontrollable bleeding was experienced and no intraoperative complications occurred. The relatively high conversion rates reflect the complexity of the procedure [20, 27]. Even in these cases, the full caudal to cranial mobilization of the retroperitoneum at the beginning of the procedure allows for a more limited upper midline laparotomy for just the dissection of the mesenteric root.

Approaching SB-NEN laparoscopically requires good preoperative evaluation of the available imaging regarding location of suspicious lymph nodes and defining the arterial and venous vasculature at that level. Relevant surgical expertise has to be gained in diseases with higher prevalence such as colorectal cancer. Experience in laparoscopic dissection of vascular structures and control of bleeding is a prerequisite before undertaking central lymphadenectomy for SB-NEN, especially considering the additional complexity related to

mesenteric fibrosis that is associated with this disease. Robotic surgery might facilitate these difficult laparoscopic procedures, but this needs future studies.

Besides requirements considering surgical expertise, it is also important that these patients are discussed in multidisciplinary meetings with specific focus on NEN. Therefore, centralization of care for patients with SB-NEN seems to play a vital role in order to optimize the management of this rare disease. To confirm our findings, multicenter prospective survival studies are necessary. Currently, no clinical trial addressing this question is registered.

This study has several limitations, one of which is the retrospective design of the study with inherent risk of allocation bias. Even though our institute is a tertiary referral center for SB-NEN, only 34 patients could be identified in a 16 year time period, restricting the statistical power of the comparative analysis. Furthermore, long-term outcomes could not reliably be assessed due to the relatively short follow-up of the laparoscopy group. Due to a gradual predilection towards laparoscopic surgery from 2015 onwards, bias has likely been introduced regarding hospital stay as the primary outcome because of historical changes in perioperative care. It also seems that ^{68}Ga-DOTATATE PET-CT scans were more often performed in laparoscopy group. This is merely a consequence of the combination of the gradual predilection towards laparoscopic surgery and implementation of ^{68}Ga-DOTATATE PET-CT scan in clinical practice. Although our unit adopted ERAS principles early on, some patients from the beginning of the present cohort might have received not all elements of ERAS. Despite methodological shortcomings, the present study is considered to be of additional value, given the scarcity of literature on the topic.

In conclusion, this study shows that a laparoscopic approach is feasible in the treatment of SB-NEN, even when preoperative imaging shows suspect lymph nodes at the level of the mesenteric root. A tendency towards lower proportion of multiple primary NETs after laparoscopy emphasizes the need for meticulous palpation of the small bowel using the extraction site. Advanced laparoscopic skills and experience in dissection of the superior mesenteric vessels seems to be essential. As this is one of the few reports of a laparoscopic approach for SB-NEN, studies from other institutes have to define the external validity of the findings, especially long-term oncological outcomes.

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Conceptualization: EK, EJMNvD, AFE, PJT.
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CHAPTER 7

Evaluating nationwide application of minimally invasive surgery for treatment of small bowel neuroendocrine neoplasms

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ABSTRACT

Background Open resection of small bowel neuroendocrine neoplasms (SB-NEN) is still considered standard-of-care, mainly because of frequently encountered multifocality and central mesenteric masses. The aim of this study was to evaluate surgical approach for SB-NEN at a national level and determine predictors for overall survival.

Methods Patients with SB-NEN who underwent resection between 2005-2015 were included from the Netherlands Cancer Registry. Patient and tumour characteristics were compared between laparoscopic and open approach. Overall survival was assessed by Kaplan-Meier and compared with the Log-rank test. Independent predictors were determined by Cox proportional hazards model.

Results In total, 482 patients were included, of whom 342 (71%) underwent open and 140 (29%) laparoscopic resection. The open resection group had significantly more multifocal tumours resected (24% vs. 14%), pN2 lymph nodes (18% vs. 7%) and stage IV disease (36% vs. 22%). Overall survival after open resection was significantly shorter compared to laparoscopic resection (3-year: 81% vs. 89%, 5-year: 71% vs. 84%, $p = 0.004$). In multivariable analysis, age above 60-years (60-75, HR 3.38 (95% CI 1.84-6.23); >75 years, HR 7.63 (95% CI 3.86-15.07)), stage IV disease (HR 1.86 (95% CI 1.18-2.94)) and a laparoscopic approach (HR 0.51 (95% CI 0.28-0.94)) were independently associated with overall survival, whereas multifocal primary tumour, grade and resection margin status were not.

Conclusion Laparoscopic resection was the approach in 29% of SB-NEN at a national level with selection of the more favourable patients. Laparoscopic resection remained independently associated with better overall survival besides age and stage, but residual confounding cannot be excluded.

INTRODUCTION

Small bowel neuroendocrine neoplasms (SB-NEN) are a rare type of gastrointestinal cancer and constitute 15% of all neoplasms of the jejunum and approximately 60% of the ileum, making it the most common gastroenteropancreatic NEN [1, 2]. Patients with stage I-III disease are amenable for curative resection, as well as selected stage IV patients with liver metastases [3, 4]. Resection remains the main treatment modality for these patients, resulting in relatively high 5-year overall survival rates of 70-80% for stage I-III and 35-80% for stage IV disease [3].

The majority of patients with SB-NEN already present with mesenteric lymph node metastases, and multifocal primary tumours can be found in up to 25-44% [5]. These disease characteristics make SB-NEN resection challenging. Although minimally invasive surgery is increasingly gaining acceptance as a standard approach for other gastrointestinal malignancies, minimally invasive surgery is still thought to potentially compromise oncological safety in SB-NEN, thereby potentially worsening survival outcomes [5]. Because of this, guidelines advise laparoscopic resection only in patients in which an appropriate intraoperative assessment of the bowel with proper segmental resection and adequate lymphadenectomy can be performed [3, 5].

Considering the evolution in the application of advanced laparoscopic resection for more complex oncological disease, and more specifically the experience with D3 mesenteric lymphadenectomy [6], application of minimally invasive surgery in SB-NEN might have increased as well. However, there are no population based data or prospective studies on this topic.

The primary aim of this study was to evaluate surgical approach for SB-NEN at a national level considering selection based on patient and tumour characteristics. Secondarily, the aim was to identify independent predictors of overall survival.

METHODS

Study design

Data from all patients with SB-NEN diagnosed between 2010 and 2015 were extracted from the Netherlands Cancer Registry (NCR). The NCR contains all cases of cancer in The Netherlands (i.e. total population of 17.4 million), mainly based on notification by the digital pathology archive and the national registry of hospital discharge diagnoses. Independent data-managers collect data on baseline and tumour characteristics as well as treatment and survival data in each Dutch hospital based on hospital records. Full histopathology reports were requested from The Nationwide Network and Registry of Histo- and Cytopathology in The Netherlands (PALGA) [7]. This registry contains histopathology reports from all Dutch pathology laboratories, including all histopathological examined tissues. All histopathology laboratories are connected to PALGA via a special network that enables collection of the histopathology reports. Both NCR and PALGA are independent organizations, funded by the Dutch government. This study is reported in accordance with the STROBE guidelines [8].

Study population

Patients with histopathologically proven SB-NEN of any stage and differentiation grade were included. The diagnosis was based on the International Classification of Disease-Oncology (ICD-O-3) topography and morphology codes [9]. Surgical approach (open/laparoscopic) is registered in the NCR since 2010, hence only patients with a diagnosis between 2010 and 2015 were included for the present study. Exclusion criteria were: grade 3 NEN, mixed neuroendocrine-non-neuroendocrine tumours (MiNEN), duodenal NENs, double tumours (e.g. concomitant SB-NEN and adenocarcinoma of the colon), autopsy and cytology data, benign neoplasms and non-neuroendocrine neoplasms. Grade 3 NEN were excluded because of the essentially different prognosis and rarity for small bowel localization, therefore it should be considered a separate disease entity.

Data collection

Primary tumour location was classified as jejunum (C17.1), ileum (C17.2) or small bowel not otherwise specified (C17.9), according to the ICD-O-3 codes. Missing TNM stage was assessed using supplementary data on "extend of disease" present in the NCR database.

Data in both NCR and PALGA databases correspond based on unique NCR-codes. This feature was used to couple both datasets. Data regarding topography, differentiation grade, resection margins, TNM staging and tumour positive lymph nodes were extracted from the full histopathology reports provided by PALGA. Morphology codes were used in case of a mismatch in differentiation grade [10]. Data from PALGA prevailed, in case of disagreement between both datasets. Finally, all tumors were restaged according to the 8th edition of the TNM classification [11].

In case of multiple histopathology reports (e.g. two biopsies followed by a resection), the first date was used as 'date of diagnosis'. Time to treatment analyses could not be performed because the diagnosis was based on pathology data, which was often the date of surgery. Overall survival was defined as the time between date of diagnosis and date of death or censored at the end of follow-up.

Statistical analysis

Categorical data are presented as number of cases and percentages, whilst continuous data are presented as either mean with standard deviation (SD) or median with interquartile range (IQR), depending on the data distribution. Overall survival analyses were performed using the Kaplan-Meier method and compared with the Log-Rank test. Univariable and multivariable Cox proportional hazards regression models were used to estimate hazard ratios (HR) with 95% confidence intervals (CI) to identify factors associated with overall survival. Factors with a P value < 0.2 in univariable analyses were added to multivariable analyses in a forward stepwise fashion. The study period was divided into two time periods (2010-2012 and 2013-2015), and added to the Cox proportional hazards regression model to correct for historical improvements in outcomes. A two sided P value ≤ 0.05 was considered statistically significant. Data were analysed using the Statistical Package for Social Sciences (SPSS) version 26 (IBM Corp. Armonk, NY, USA).

RESULTS

In total, 482 patients were included over a period of six years (2010-2015), of whom 342 (71%) underwent open and 140 (29%) laparoscopic resection (Table 1). There was a minor increase in the proportion of laparoscopic resections during the study period: 46% (2010-2012) vs. 54% (2013-2015). Academic centers performed less often laparoscopic resections than regional hospitals (24/121 (20%) vs. 111/339 (33%), $p = 0.012$). Patients undergoing open resection were more often male (58% vs. 43%, P = 0.003) and older (64 vs. 60 years, $p = 0.009$) compared to patients undergoing laparoscopic resection. Emergency procedures constituted a minority of patients, with a slightly skewed distribution towards more emergencies in the open group: 5% vs. 3% obstruction ($p = 0.36$) and 2% vs. 0% perforation ($p = 0.07$), respectively.

Patients in the open resection group had a significantly higher clinical stage of NEN with higher proportions of cN1-2 and cM1 stage. Also pathological outcomes were significantly different between the two surgical approaches, with higher pT, pN and pM stages in the open group, as well as a higher percentage of multifocal tumours and larger size of the (largest) primary tumour. A trend towards more positive resection margins in the open resection group was observed (19% vs. 11%, $p = 0.06$).

Conversion rate was only available for the year 2015, in which 8 of 30 (27%) laparoscopic procedures were converted. Although no strict reasons for conversion were documented, the following outcomes were observed: pT4 tumours were present in 5/8 (63%) patients, multifocal tumours in 3/8 (38%), pN2 lymph node metastases in 2/8 (25%) and R1/2 resection margin in 1/8 (13%) patients. Mean (SD) tumour size was 27 (9) in the converted cases and 21 (9) mm in the non-converted cases ($p = 0.10$).

Within 30 days postoperatively, 16 patients (5%) died in the open group and 3 patients (2%) after laparoscopic resection ($p = 0.19$). Estimated 5-year overall survival of the entire cohort (i.e. patients amenable for resection) was 74%. Without correction for confounders, patients undergoing laparoscopic resection had significantly higher 5-year overall survival rates compared to open resection: 84% vs. 71% ($p = 0.004$), respectively (Figure 1). Survival rates were also separately analysed for stage III and stage IV disease (Figure 2). A statistically significant higher 5-year overall survival was found after laparoscopic surgery in stage III patients (88% vs. 77%; $p = 0.041$), while there was no significant difference between the two surgical approaches for stage IV (59% vs. 63%; $p = 0.59$).

In univariable analysis, age above 60 years, multifocal tumours, stage IV disease and laparoscopic resection showed an association with overall survival (Table 2). In multivariable analyses, age between 60-75 years (HR 3.39, 95% CI [1.85-6.25], $p < 0.001$) and ≥75 years (HR 7.69, 95% CI [3.89-15.18], $p < 0.001$), stage IV disease (HR 1.89, 95% CI [1.20-2.99], $p = 0.006$), and laparoscopic resection (HR 0.52, 95% CI [0.28-0.95], $p = 0.032$) remained significantly associated with overall survival. The results of univariable and multivariable analyses for overall survival are shown in Table 2.

Table 1. Patient and pathology characteristics

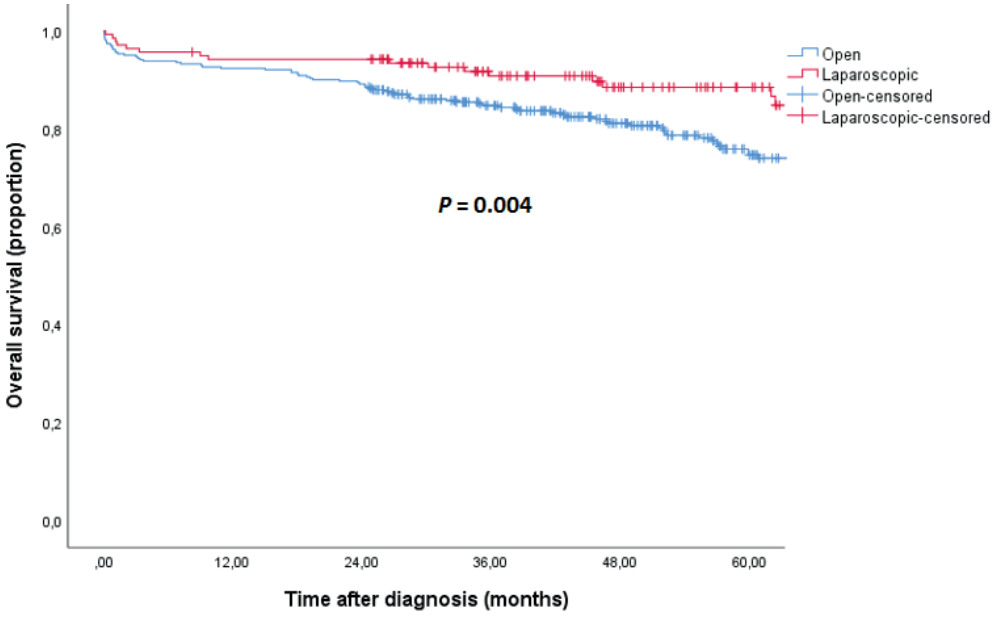
Characteristics, No. (%)	Surgery, No. (%)[a]		
	Open (n = 342)	Laparoscopic (n = 140)	P Value
Diagnosis year			
2010-2012	169 (49)	65 (46)	0.55
2013-2015	173 (51)	75 (54)	
Treatment center [b]	335	135	
Regional hospital	238 (71)	111 (82)	**0.012**
Academic center	97 (29)	24 (18)	
Sex			
Male	197 (58)	60 (43)	**0.003**
Age, years, mean (SD)	64 (12)	60 (12)	**0.009**
Tumor grade: no evaluable	341	138	
Grade 1	270 (79)	110 (80)	0.90
Grade 2	71 (21)	28 (20)	
Clinical TNM classification [b]			
registered cT stage	88	33	**<0.001**
cT4	26 (30)	4 (12)	
registered cN stage	259	109	**<0.001**
cN1-2	165 (64)	37 (34)	
registered cM stage	341	139	**<0.001**
cM1	140 (41)	28 (20)	
Pathological T classification [b]	320	133	
pT1	8 (2)	20 (15)	**<0.001**
pT2	31 (10)	20 (15)	
pT3	169 (53)	56 (42)	
pT4	112 (35)	37 (28)	
Pathological N classification [b]	296	123	
pN0	45 (15)	24 (20)	**0.018**
pN1	198 (67)	90 (73)	
pN2	53 (18)	9 (7)	
Pathological M classification [b]			
pM1	112 (33)	28 (20)	**0.005**
Multifocal tumors	81 (24)	19 (14)	**0.014**
Size of (largest) primary tumor, mm, mean (SD)	21 (10)	18 (10)	**0.007**
Lymph nodes			
Number of examined LNs, mean (SD)	12 (10)	11 (7)	0.81
Number of tumor positive LNs, mean (SD)	3 (4)	3 (3)	0.43
Disease stage [b]	312	124	

Characteristics, No. (%)	Surgery, No. (%)[a]		
	Open (n = 342)	Laparoscopic (n = 140)	P Value
Stage I-II	25 (8)	17 (14)	**0.012**
Stage III	175 (56)	79 (64)	
Stage IV	112 (36)	28 (22)	
Resection margin	300	118	
R0	244 (81)	105 (89)	0.06
R1/2	56 (19)	13 (11)	
Conversion rate [c]	-	8/22 (36)	-
30-day mortality	16 (5)	3 (2)	0.19

SD Standard Deviation, mm millimeter, LN Lymph node

[a] Unless stated otherwise; [b] Data is reported for evaluable cases; [c] Conversion rates were only reported in 2015, during which 22 laparoscopic resections were performed.

Figure 1. Overall survival of patients who underwent an open or laparoscopic resection.

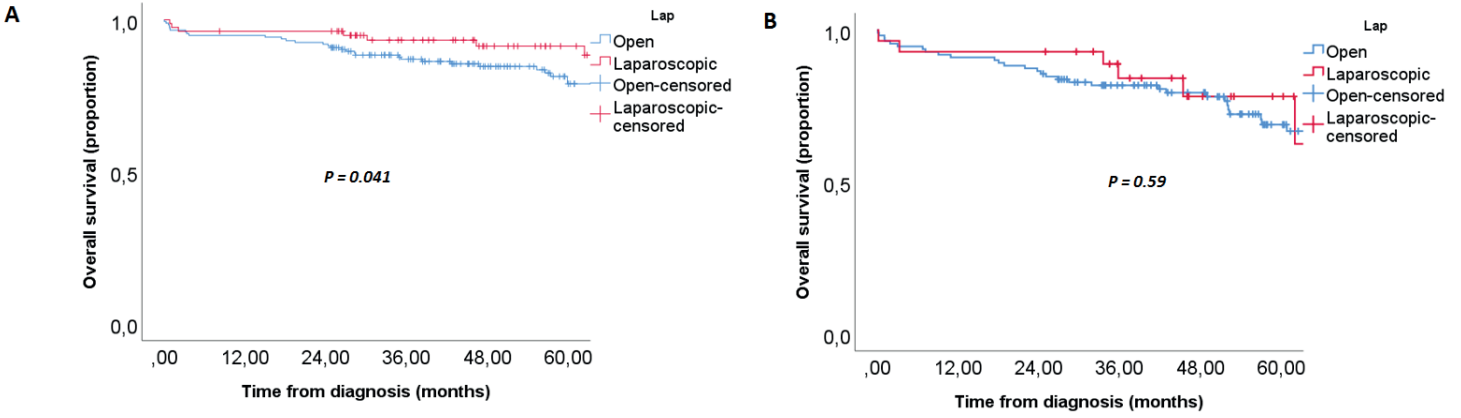


Number of patients at risk.

Time (months)	0	12	24	36	48	60
Open	340	314	303	240	180	122
Laparoscopic	138	129	129	97	71	53

Survival (months)	Mean OS (95% CI)	Median OS (95% CI)	5-year OS
Open	74.8 (71.3-83.1)	N/A	71%
Laparoscopic	82.9 (78.6-87.3)	N/A	84%

Figure 2. Overall survival of patients who underwent an open or laparoscopic resection, **(A)** stage III patients, **(B)** stage IV patients.
Figure 2A.

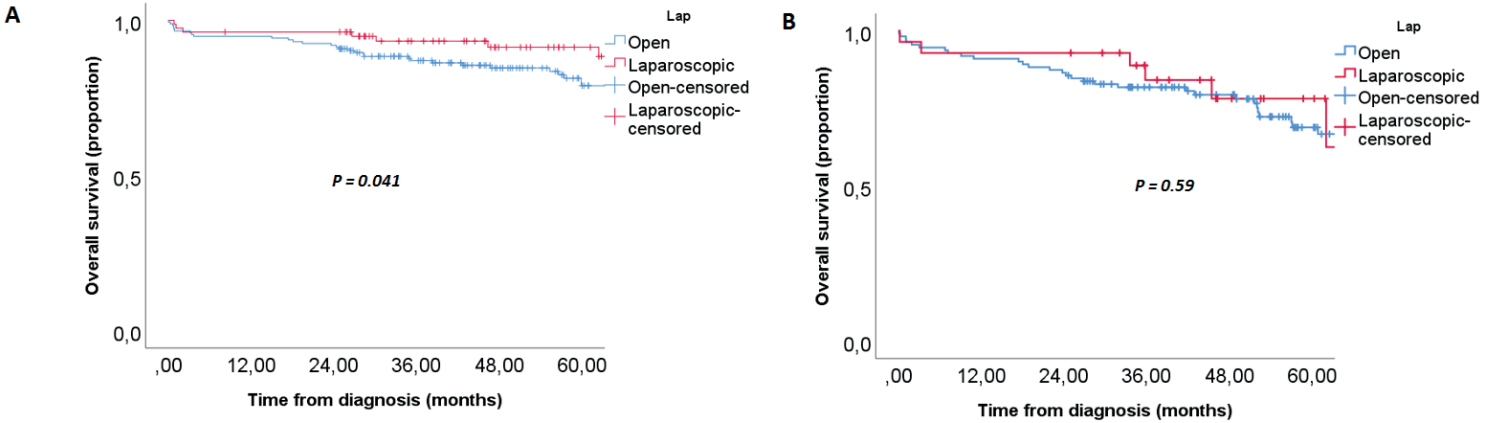


Number of patients at risk.

Time (months)	0	12	24	36	48	60
Open	174	165	160	126	93	66
Laparoscopic	79	75	75	56	42	32

Survival (months)	Mean OS (95% CI)	Median OS (95% CI)	5-year OS
Open	78.6 (74.1-83.1)	N/A	77%
Laparoscopic	81.1 (76.4-85.7)	N/A	88%

Figure 2B.



Number of patients at risk.

Time (months)	0	12	24	36	48	60
Open	111	101	96	76	62	35
Laparoscopic	28	26	26	18	11	7

Survival (months)	Mean OS (95% CI)	Median OS (95% CI)	5-year OS
Open	69.7 (63.4-79.9)	N/A	59%
Laparoscopic	70.8 (58.6-83.1)	N/A	63%

Table 2. Uni- and multivariable survival analyses of patients with SB-NEN in The Netherlands

Risk factors	Univariable analysis		Multivariable analysis	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Diagnosis year				
2010-2012	1.47 (0.93-2.32)	**0.10**	1.33 (0.82-2.15)	**0.25**
2013-2015	1 [Reference]		1 [Reference]	
Sex				
Male	1 [Reference]		-	
Female	0.84 (0.57-1.25)	0.40	-	
Age				
<60	1 [Reference]			
60-75	3.20 (1.81-5.66)	**<0.001**	3.38 (1.84-6.23)	**<0.001**
≥75	6.86 (3.68-12.82)	**<0.001**	7.63 (3.86-15.07)	**<0.001**
Multifocal tumours				
No	1 [Reference]		1 [Reference]	
Yes	1.40 (0.90-2.19)	**0.14**	1.25 (0.78-2.00)	0.35
Disease stage				
Stage I-II	1.60 (0.73-2.50)	0.24	-	
Stage III	1 [Reference]		1 [Reference]	
Stage IV	1.96 (1.25-3.06)	**0.003**	1.86 (1.18-2.94)	**0.043**
Tumor grade				
Grade 1	1 [Reference]		-	
Grade 2	1.33 (0.83-2.14)	0.24	-	
Resection margin				
R0	1 [Reference]		-	
R1/2	1.16 (0.67-1.99)	0.60	-	
Surgery				
Open	1 [Reference]		1 [Reference]	
Laparoscopic	0.47 (0.28-0.80)	**0.005**	0.51 (0.28-0.94)	**0.032**

DISCUSSION

The main finding of this nationwide study was that 29% of the patients with SB-NEN were planned for a laparoscopic approach. There was a slight but non-significant increase in laparoscopic resection rate over time. Case selection was clearly seen, with less favourable tumours in patients who underwent open resection, as reflected by significantly higher stage, larger size, and more multifocal tumours. Academic centers performed less laparoscopic resections as compared to regional hospitals, likely reflecting tertiary referral of more advanced cases. With the available variables in the dataset, the association between surgical approach and overall survival was corrected for confounding as much as possible. The multivariable

model revealed better overall survival after a laparoscopic approach, with age and stage as the other independent predictors. Potential prognostic factors such as margin status, grade and multifocal tumour location were not found to be associated with overall survival in this patient cohort.

The application of a laparoscopic approach for resection of SB-NEN is mainly determined by the extensiveness of mesenteric lymph node metastases. Ohrvall et al. proposed a classification of these metastases, ranging from resectable stage I (close to the intestine) to irresectable stage IV (retroperitoneal, peri-pancreatic or encasement of the mesenteric artery with involvement of proximal jejunal arteries) [12]. In this study, 38% of lymph node metastases extended along the superior mesenteric artery without encasement, whilst 16% were irresectable. Depending on the laparoscopic experience, one would expect that at least 40% of patients are amenable to a laparoscopic approach based on these data. The 29% laparoscopic resection rate as found in the present study suggests a still restricted application.

The recent European Society of Medical Oncology guideline states that patients with SB-NEN often present in the emergency setting [13]. An emergency resection without prior knowledge of the presence or nature of a small bowel neoplasm might lead to oncologic inferior resections. Interestingly, emergency resections for obstruction or perforation were performed in only a small minority (8%) of this nationwide cohort. This finding suggests that patients might have been offered 'up-front' resection to prevent bowel obstruction and/or ischemia [13]. However, the value of 'up-front' resection for SB-NEN has been debated in literature, as this is associated with significantly more reoperations, rather than yielding a survival advantage [14].

A common misunderstanding of laparoscopic resection is the inability of palpating the small bowel. However, after completion of the lymph node dissection, almost the entire small bowel can be exteriorized through an umbilical extraction site, enabling meticulous palpation [15]. Identification of multifocal primary disease is regarded a critical step during resection, although recent analyses suggest that the presence of multifocal disease does not affect overall survival [5, 16]. Therefore, we carefully hypothesize that multifocality is not a contraindication for laparoscopic resection. Nevertheless, multifocal primary tumors were more often found in the open resection group, although there might not be a causal relationship, but rather a reflection of case selection and more advanced tumor stage.

The conversion rate (36%) was only reported in the last registration year (2015) and was higher compared to previous studies (25-30%) [15, 17]. In contrast, the conversion rate for laparoscopic D3 lymphadenectomy for colon cancer was 5% in a randomized clinical trial [18]. The substantially higher conversion rate reflects the level of complexity that might be encountered during resections for SB-NEN. The essential difference between central mesenteric lymph node metastases that originate from colon cancer or SB-NEN is related to the infiltrative growth with sometimes extensive mesenteric fibrosis and vascular encasement in the latter tumor type. Likely, D3 lymphadenectomy requires even more skills if performed for SB-NEN than for colon cancer. A handport-assisted laparoscopic procedure can sometimes be an alternative for conversion.

Pedrazzani et al. described a case series of nine patients undergoing laparoscopic right hemicolectomy with complete mesocolic excision for terminal ileum/right colon/appendix NEN [19]. Although it comprises a small cohort, peri-operative and long-term survival outcomes were promising: 1/9 had a Clavien-Dindo grade III complication, no mesenteric locoregional recurrence and all patients with an R0 resection were disease free after a median follow-up of 18 months (range 6-50). One randomized trial of laparoscopic versus open D3 lymphadenectomy has been published in stage II-III colon cancer, which revealed beneficial short-term outcomes for minimally invasive surgery (less blood loss, shorter time to pass first flatus, decreased use of postoperative analgesics and shorter hospital stay), without compromising 5-year overall survival [18, 20]. Interestingly, a recent meta-analyses that pooled the results of this trial with comparative cohort series, suggested that laparoscopic resection was even associated with better oncological outcomes for colon cancer [21], similar to the present study. However, the methodological issues of non-randomized comparisons do not allow for definitive conclusions on this observed association. There is a high risk of bias, and laparoscopic resection might just be a reflection of treatment by more specialized surgeons in dedicated centers with optimized peri-operative care.

Long-term nationwide population-based data were used for this study, making it more representative than cohort studies. However, the findings of this study should be seen in light of some limitations. The NCR database is primarily focused on oncologic characteristics, which limits analysis of (peri-) operative characteristics (indication, conversion rate, post-operative morbidity) and imaging data such as postoperative CT or PET scanning to assess the completeness of mesenteric lymphadenectomy. Time to recurrence, which is a relevant marker of "surgical success" in regard to lymphadenectomies, could not be reported, as recurrent disease is often not diagnosed with biopsies. The most important missing data concern the reasons for choosing an open or laparoscopic approach and whether surgery was performed in the emergency setting.

We propose that guidelines should adapt their recommendations regarding selection criteria for laparoscopic resection, for example using the classification as proposed by Ohrvall et al. [12]. It seems that more SB-NENs are eligible for laparoscopic resection, especially in hands of colorectal surgeons with experience in D3 lymphadenectomies. Also, less emphasis on multifocality as a reasons not to perform laparoscopic resection should be given.

Further work is required to establish the role of laparoscopic resection for SB-NEN. Ideally, this would be a multicenter international randomized clinical trial with stratification for extent of lymph node metastases. However, such a trial would be challenging because of the rarity of the disease and potential lack of equipoise. To overcome this issue, a prospective international cohort study with clearly documented inter-hospital variability in surgical practice could also provide relevant data. To really be of added value, such a study should include variables that reflect "surgical success", which are part of standard follow-up protocols (pathology reports of recurrent lesions, blood tests, PET/CT) [3].

In conclusion, this study showed that laparoscopic resection of SB-NENs was performed in 29% of patients in the Netherlands. Current data do not raise major concern regarding oncologic adequacy of laparoscopic resection in selected cases.

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Author contributions

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Validation: EK, HJK, WAB, EJMNvD, AFE, PJT.
Writing—original draft: EK.
Writing—review and editing: EK, HJK, WAB, EJMNvD, AFE, PJT.

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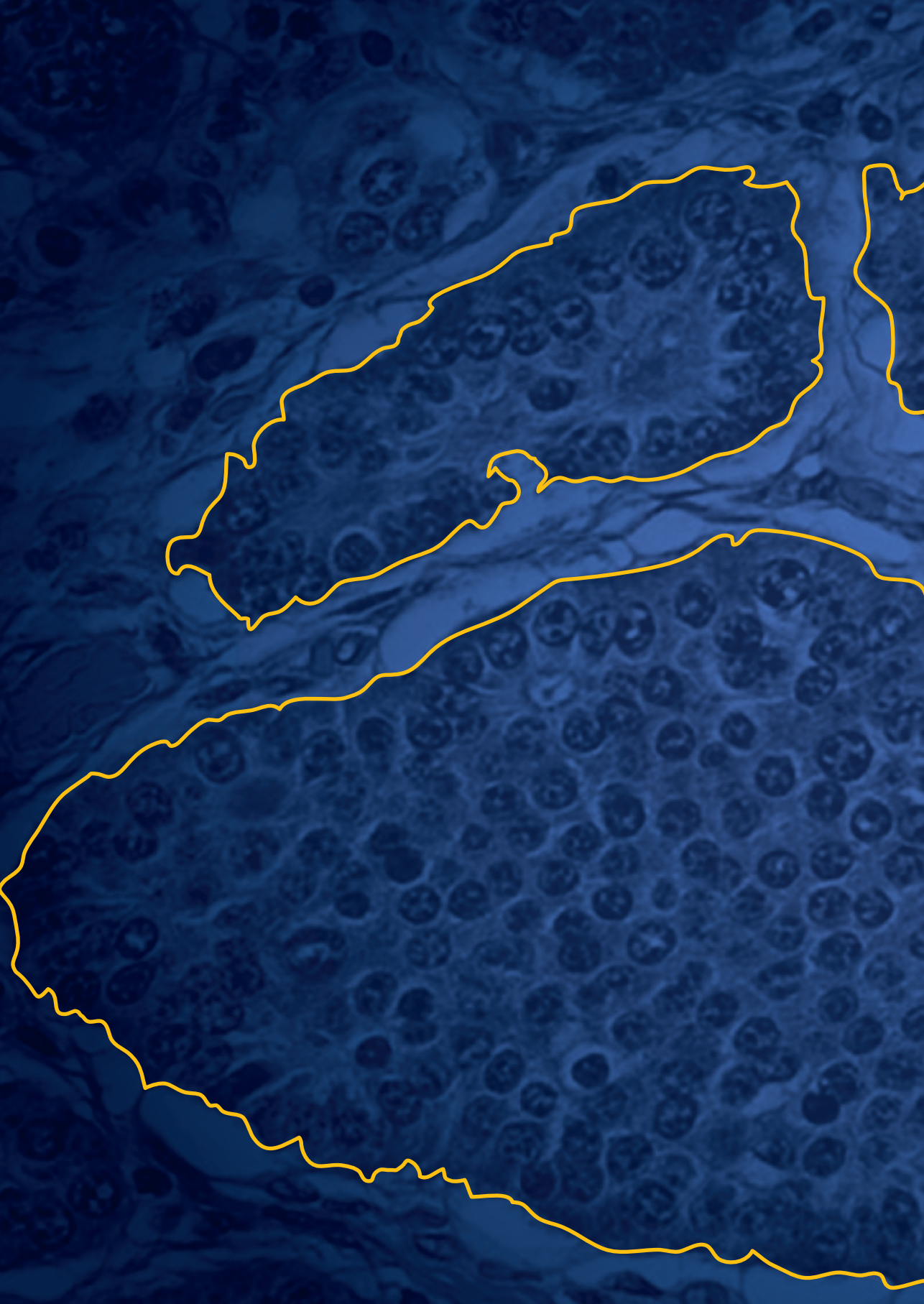

PART III

Fluorescence guided surgery of neuroendocrine neoplasms

CHAPTER 8

Fluorescence Angiography Guided Resection of Small Bowel Neuroendocrine Neoplasms with Mesenteric Lymph Node Metastases

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ABSTRACT

Background Surgery for small bowel neuroendocrine neoplasms (SB-NEN) might result in vascular compromise of the remaining bowel due to resection of lymph node metastases in close proximity to main mesenteric vessels. Fluorescence angiography (FA) has been described as a safe technique to assess perfusion during gastro-intestinal surgery. This study aimed to evaluate the potential value of intraoperative FA during surgery for SB-NEN.

Methods This proof-of-concept-study included patients undergoing surgery for SB-NEN of any stage. The planned level of transection was marked by the surgeon, after which FA using indocyanine green (ICG) was performed. The primary study outcome was change in management due to FA.

Results Ten consecutive patients with SB-NEN were included, all with metastatic lymph nodes close to main mesenteric vessels. FA use led to management changes in eight patients (80%); four patients had less bowel resected with a preserved length of 5 to 35 cm. The other four patients had more extended bowel resections with an additional length varying from 3 to 25 cm. The median postoperative stay was 4 days (interquartile range 4-6). No anastomotic leakage occurred.

Conclusion This is the first known series describing preliminary results of FA during SB-NEN surgery. FA led to a management change in 80% of patients with better tailoring the extent of resection of small bowel. Structural implementation of FA during small bowel resection for small bowel NET seems to improve outcome, either by preserving small bowel or resecting ill-perfused small bowel.

INTRODUCTION

Small bowel neuroendocrine neoplasms (SB-NEN) have a relatively high overall survival rate, amounting 95-100% at 5-years following radical resection of stage I-III SB-NEN [1]. Mesenteric lymph node metastases (MLM) are present in up to 75% of patients and resection of MLM is of particular importance for symptom prevention, locoregional control and survival [2].

Ohrvall et al. developed a classification system for MLM, describing the location of MLM from stage I (close to the small bowel) to stage IV (16-22%, involvement of the trunk of the superior mesenteric artery (SMA)) [3, 4]. The added value of such a classification system is to consistently categorize patients into potential high-risk procedures regarding vascular compromise [4].

Surgeons need to balance preservation of bowel length to prevent a short bowel syndrome with adequate mesenteric lymphadenectomy for optimal oncological results and prevention of vascular encasement of progressive central node metastases in the nearby future. Besides bowel length, extent of mesenteric dissection can also influence complication rates such as anastomotic leakage and small bowel ischemia due to insufficient perfusion [5]. Anastomotic leakage and small bowel ischemia are severe complications after gastrointestinal surgery. This is demonstrated by significant associated morbidity, prolonged hospital stay, considerable extra healthcare costs, and increased short- and long-term mortality [6-8].

Currently, the transection level is decided upon visual inspection of the perfusion of the small bowel and arterial palpation. State-of-the-art near infrared fluorescence angiography (FA) with indocyanine green (ICG) is able to evaluate perfusion intraoperatively during gastrointestinal surgery and is a user friendly medical device [9]. After intravenous administration, ICG is rapidly distributed by binding to plasma proteins. This property minimizes leakage to the interstitial space, making it an ideal marker for perfusion [10].

FA has been reported to delineate vascular confines and thereby decrease anastomotic leakage rates in gastrointestinal surgery [11-14]. Vascularisation of the small bowel might get compromised after resection of MLM, as half of them are located within close proximity to main mesenteric vessels (< 2 cm) [15]. The aim of this study was to assess the potential value of intraoperative FA during resection of SB-NEN, and to evaluate the postoperative outcomes.

METHODS

This is a single-centre prospective study cohort study, conducted at a tertiary referral centre (Amsterdam UMC). All patients with age above 18 years undergoing surgery for SB-NEN with restoration of continuity between July 2018 and July 2020 were eligible for inclusion. Patients were included irrespective of tumour grade or presence of distant metastases. Exclusion criteria were allergy to ICG, iodide or sodium iodide, hyperthyroidism, benign thyroid tumour, thyroid examination using radioactive iodide within one week, or breast feeding. The study

was approved by the Institutional Review Board of Amsterdam UMC and informed consent was obtained from all participating patients.

Procedure

The first step of the surgical procedure was to dissect the central part of the mesentery containing the draining lymph nodes of the primary SB-NEN, with ligation of the relevant mesenteric branches. Subsequently, the mesenteric dissection was continued distally towards the efferent and afferent bowel segments. The planned level of transection for the proximal and distal bowel ends were then marked by the surgeon [16]. Hereafter, ICG was administered intravenously (0.01 mg/kg/bolus). FA was performed using the laparoscopic PINPOINT or hand-held Spy-phi fluorescence imaging system (Stryker, Kalamazoo, Michigan, United States of America). Segments with a signal within 90 seconds after injection of ICG were deemed viable, based on previous experience for other indications [17]. During FA, case record forms were used to collect data.

Outcome parameters

The primary outcome was change in management due to FA, defined as the length of additional resected or spared small bowel. Degree of perfusion was based on a combination of the intensity of the fluorescent signal and time to enhancement thereof.

Secondary outcomes included mean arterial pressure (MAP), heart rate, inotropic/vasopressive drug use during FA; intra- and post-operative complications within 30 days according to Clavien-Dindo classification (with attention for anastomotic leakage and ischemia) and post-operative hospital stay. The following time points were recorded for the calculation of time to fluorescence: time of ICG injection, time of first fluorescent enhancement in the small bowel, time of fluorescent enhancement in planned anastomotic site, and the difference between enhancement of both bowel ends. Differences between fluorescent time-points were calculated in seconds. Anastomotic leakage was defined as abnormal amounts of free air and/or fluid or extravasation of contrast on CT within 30 days postoperatively.

Potential high-risk procedures were identified by measuring distance between MLM and SMA/SMV and classifying MLM using the classification system described by Ohrvall et al. [3]. Proximity to main branches of the SMA/SMV were measured on pre-operative computer tomography (CT) scans with intravenous contrast, as previously described[16].

Statistical analysis

Patient, intervention and pathology characteristics were tabulated using descriptive statistics. Due to the small sample size of the study, continuous data is reported in medians with interquartile range (IQR). Categorical data is reported as number of patients with percentages. Data analyses were performed using the Statistical Package for Social Sciences (SPSS) version 26.0 (IBM Corp. Armonk, New York, United States of America).

RESULTS

In total, 11 consecutive patients were assessed for eligibility. One patient was excluded due to an iodine allergy, leaving 10 patients for inclusion. Baseline patient characteristics are presented in Table 1. The median age was 68 years (IQR 64-72). Liver metastases were present in three patients. The median distance between MLM and the SMA/SMV was 23 mm (IQR9-26). Overall, two and eight patients were classified as MLM stage I and II, respectively. During surgery, a median of 44 cm of bowel (IQR 18-62) was resected (Table 2). All patients were operated laparoscopically, with conversion to open dissection of the mesenteric lymph nodes in two patients.

Table 1. Baseline characteristics

Characteristics, No. (%)	Overall (n = 10)
Male	6 (60%)
Age[a], years	68 (64-72)
BMI[a], kg/m^2	26.7 (23.1-31.0)
ASA 3-4	4 (40%)
Preoperative SSA	3 (30%)
Symptomatic	9 (90%)
MLM to SMA/SMV main branch[a], mm	23 (9-26)
MLM stages	
Stage I	2 (20%)
Stage II	8 (80%)
Disease stage	
Stage III	6 (60%)
Stage IV	4 (40%)

Abbreviations: ASA, American Society of Anesthesiologists; BMI, body mass index (calculated as weight in kilograms divided by height in in meters squared).

[a] continuous variables are reported in medians (IQR)

Table 2. Surgical characteristics

Characteristic, No. (%)	Overall (n = 10)
Surgical procedure	
Segmental small bowel resection	8 (80%)
(extended) Ileocoecal resection	2 (20%)
Duration of surgery[a], min	236 (163-259)
Blood loss[a], mL	50 (20-850)
Length of resected bowel[a], cm	44 (18-62)
Intra-operative complications[b]	0
Postoperative hospital stay[a]	4 (4-6)

Abbreviations: cm, centimetre; min, minutes.

[a] continuous variables are reported in medians (IQR)

[b] Intraoperative complications included uncontrollable bleeding, serosal bowel lesions, bowel perforation, etc.

Fluorescence Angiography

FA led to change in management in eight patients (80%) (Table 3). Four patients had bowel preservation with a length varying from 5 to 35 cm (Figure 1). The other four patients had an additional resection, with a length ranging from 3 to 25 cm (Figure 2).

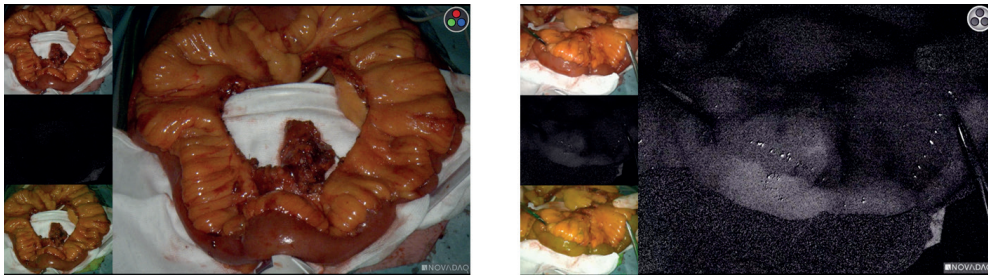
The first fluorescent signal in the small bowel was seen after a median (IQR) of 14 (17-45) seconds after ICG injection. The median time from first enhancement to the anastomotic site was 66 seconds (IQR 29-132). The difference between fluorescent enhancement of both bowel ends was a median of 14 seconds (IQR 6-24)). During FA, the median MAP was 78 mmHg ((IQR) 70-88) and vasopressive drugs (noradrenalin) were infused in four patients, with a mean of 300 μg/hour (standard deviation 145-380). Due to the small sample size, FA could not be correlated to vasopressive drug infusion.

Table 3. Fluorescence angiography characteristics

Characteristics	Overall (n = 10)
Change of management	8 (80%)
Additional resection, No. (%)	4 (40%)
Length of additional resection, cm (range	11 (3-25)
Sparing resection, No. (%)	4 (40%)
Length of preserved bowel, cm (range)	10 (5-35)
Fluorescence angiography times[a] (sec)	
ICG injection – 1st signal	14 (17-45)
ICG injection – signal at anastomosis	67 (29-132)
1st signal – signal at anastomosis	32 (10-116)

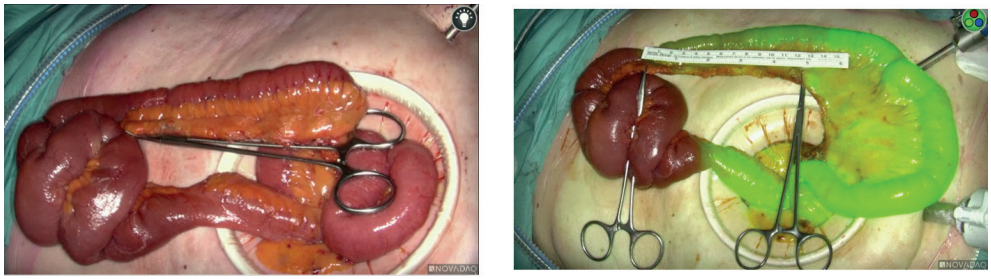
Abbreviations: cm, centimetre; NA, not available; sec, seconds.

Figure 1. Example of a sparing resection.



The small bowel is visible after complete central mesenteric dissection (left image), ICG shows the vascularization to be fully intact up to the tumour localization in the small bowel (right image).

Figure 2. Example of an additional resection.



The part of the small bowel containing the tumour is clearly poor perfused (left image), which is supported by ICG. Also, it appeared that a section of the small bowel was poorly perfused (marked with the ruler).

Post-operative outcomes

No major (Clavien-Dindo grade ≥3) complications occurred postoperatively, and median postoperative stay was 4 days (IQR 4-6). Two patients developed Clavien Dindo grade 2 complications. The first patient (patient A) suffered from postoperative abdominal angina. This patient had an intra-operative management change, with 35 cm of small bowel preserved based on FA. Time from injection to first signal, time from injection to planned anastomotic site and time from first signal to planned anastomotic site was 14, 119 and 105 seconds, respectively. CT angiography was performed, which did not show signs of bowel ischemia or any other abnormalities. Complaints compatible with abdominal angina resolved spontaneously after three months.

Another patient (patient B) had a paralytic ileus for which he received total parenteral nutrition. This patient also had a peri-operative management change based on the FA result, with an additional bowel resection of 25cm, as the planned anastomotic site had no ICG uptake. Time from injection to first signal was 46 seconds.

DISCUSSION

This is the first known proof-of-concept study describing FA during resection of SB-NENs. Eight out of ten (80%) patients had a change in management due to FA, and no major post-operative complications occurred. The median postoperative hospital stay was 4 days.

Previous studies focussing on colorectal surgery report much lower rates of change in management (6-8%) [18, 19]. The high rate of change in management might be a reflection of the complexity of the procedure as a consequence of the central location of MLM with concomitant mesenteric fibrosis as a typical finding in SB-NEN. Small bowel perfusion is generally considered to be better than colonic perfusion. But after dividing central branches close to the mesenteric root, bowel perfusion gets largely dependent on collateral circulation. The appropriateness of the alternative routes of small bowel perfusion is not always clearly visible during surgery, with gradual reduction of perfusion. Without a clear watershed visible,

it might be difficult to determine the level of most appropriate bowel transection. This decision also entails a weighed balance between the risk of anastomotic leakage and remaining bowel length. It is therefore more relevant to discuss change in management for both subtypes: additional resection and sparing resection.

In half of the patients with a change in management, additional small bowel was resected due to absence of ICG perfusion. This finding, although preliminary, suggests that FA might be of value to optimize anastomotic perfusion. However, a recent study analysing European data concluded that anastomotic leaks occur in 1.6% of GEP-NEN patients [20]. The clinical relevance of routine perfusion assessment with FA is therefore debatable. The added value in upper gastro-intestinal and colorectal surgery seems to be more relevant, as the anastomotic leak rates are higher (5-20%) [14, 21].

Simultaneously, a sparing resection was performed in four patients, which is a good example of "primum non nocere" (i.e. "first, do no harm"). It is import to note that a radical resection of the tumour and lymph node metastases is the primary aim during the procedure. A sparing resection is only performed if vasculature is intact and there is no risk of oncological inadequacy. Again, evidence regarding the clinical relevance of a sparing resection remains unknown, especially given the relatively short bowel length that could be preserved. Prevention of a short bowel syndrome does not seem to be an issue in patients without prior bowel resections, because such extensive resections (i.e. more than half of the small bowel) are to our knowledge rarely performed for SB-NENs [22].

No major post-operative complications occurred, including anastomotic leakage. Lack of post-operative complications is probably attributable to the small study size. However, previous studies have shown that severe complications (Clavien Dindo grade III or higher) can occur in up to 11% of the patients [20].

The assessment of FA in target tissue can be divided into three categories: (I) rapid distribution of ICG, (II) delayed distribution of ICG and (III) no ICG uptake. The clinical consequence of category (I) and (III) are straightforward; a sparing or additional resection should be performed, respectively. Category II signals are probably a consequence of arterial diffusion through the small bowel/mesentery or venous outflow obstruction [23]. Larger prospective studies are needed to correlate this category II signal of FA with clinical factors such as vasopressive drug use during FA, or vascular anomalies (i.e. atherosclerosis).

One patient in this study had transient abdominal angina, which resolved three months postoperatively. This was the first patient who had per-operative FA. A total of 35 cm of small bowel was spared, and due to the category II ICG distribution, time to enhancement of the anastomosis was 119 seconds. After this first case, our protocol changed and only bowel segments that lit up shortly after ICG injection (i.e. category I) were deemed viable, and thus spared. We did not observe similar outcomes thereafter. Another patient developed a paralytic ileus, which is probably a consequence of the central mesenteric lymph node dissection.

The findings of this study should be seen in light of some limitations. Since this study describes a series from a tertiary referral center, selection bias might be present, even though all consecutive patients were included in the study. Also, due to the rather modest study population, extrapolating postoperative outcomes to a broader patient population is limited. Finally, some observer-expectancy bias might have been introduced due to the proof-of-concept design of the study.

FA seems to be of added value, as it has the potential to optimise the assessment of small bowel perfusion intraoperatively, without adding too much surgical time. Although it is fairly simple to use technique, routine use during procedures is preferred to gain experience and pass the learning curve. Application should especially be considered for patients with MLM located near the SMA/SMV and their main branches. Standard use of FA has the potential to decrease post-operative complications related to tailored resection with sufficient perfusion of the remaining small bowel. Future studies should focus on quantifying FA signals and investigate the association with postoperative outcomes in larger cohorts.

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Author contributions

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CHAPTER 9

Indocyanine green fluorescence guided resection of neuroendocrine liver metastases: a proof-of-concept study

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Submitted

ABSTRACT

Background Even though the relative indolent character of neuroendocrine neoplasms, untreated NELM have a detrimental impact on survival outcomes. Therefore, complete resection should be considered if technically possible. The aim of this study was to assess the value of indocyanine green (ICG) guided fluorescence guided surgery of neuroendocrine liver metastases.

Methods This is a proof-of-concepts study including patients who underwent resection of NELM of any grade, and patients who underwent liver resection for other tumour types to compare ICG uptake. Patients received an intravenous bolus of 10 mg ICG approximately 24 hours prior to surgery. Resection of liver metastases were performed using guidance of fluorescence cameras and intra-operative ultrasonography. All resected lesions underwent histopathological assessment by an expert pathologist.

Results Six patients with liver metastases were included in the study, three with NELM and three with colorectal liver metastases (CRLM). All liver metastases showed uptake of ICG. The fluorescence pattern of NELM and CRLM was comparable. There were no adverse events related to use of ICG fluorescence. All resection margins were negative (R0).

Conclusion This is to our knowledge the first proof-of-concept study describing ICG fluorescence guided resection of NELM. Fluorescence guided resection of NELM using ICG is feasible, and uptake of ICG by NELM is comparable to CRLM.

INTRODUCTION

Up to 40-50% of patients with neuroendocrine neoplasms (NEN) present with metastases, of which the majority is located in the liver (neuroendocrine liver metastases, NELM) [1]. Surgical resection of NELM is the standard of care, and should especially be considered, for grade 1 and grade 2 NENs [2]. Even though the relative indolent character of NEN, untreated NELM has a poor prognosis, with a 5-year overall survival of 13-54% [3].

Intraoperative ultrasonography (IOUS) aids in identifying additional lesions and parenchyma preserving resection of colorectal liver metastases [4]. However, IOUS can only be performed by an experienced surgeon or a radiologist. Therefore, an easier system capable of identifying additional lesions would be desirable, as pre-operative imaging often underestimates the number of metastases.

Intraoperative fluorescence imaging using indocyanine green (ICG) dye aids in differentiating between normal, benign and malignant liver tissue, and identifies previously unknown sub-centimetre colorectal liver metastases in up to 24% of patients [5]. ICG is registered by the Food and Drug administration and the European Medicine Agency, and is safe to use as toxicity and allergic reactions occur rarely.

Previous studies have shown that after intravenous administration, ICG accumulates around the liver metastases in the form of a fluorescent 'rim', aiding the surgeon in detecting malignant lesions [6]. The fluorescent rim is a consequence of ICG accumulation in bile due to outflow obstruction caused by malignant tissue [7].

Neuroendocrine neoplasms are approximately 10-fold more vascularized compared to adenocarcinomas [8]. This property creates an characteristic pattern on CT/MR imaging to detect NELM, due to the hypervascular appearance in the arterial phase and wash-out in the late phase, which is present in 70% of the patients with NELM [9]. We therefore hypothesized that visualization of NELM with ICG fluorescence should be possible. The aim of this study was to assess the feasibility of ICG-guided fluorescence guided surgery of NELM.

METHODS

This is a single-centre prospective cohort study, conducted at a tertiary referral centre (Amsterdam UMC). All patients with age above 18 years undergoing surgery for GEP-NEN between November 2019 and July 2021 were eligible for inclusion. Patients who underwent resection of NELM were included. Patients who underwent resection of colorectal liver metastases (CRLM) were included to compare fluorescence patterns of NELM with non-NELM. Exclusion criteria were allergy to ICG, iodide or sodium iodide, hyperthyroidism, benign thyroid tumour, thyroid examination using radioactive iodide within one week prior to surgery, or breast feeding. Informed consent was obtained from all participating patients. The study was approved by the Institutional Review Board of Amsterdam UMC and registered in the Netherlands Trial Register (NL8802).

Procedure

Patients received a single intravenous bolus of 10 mg ICG approximately 24h prior to liver surgery. All procedures were minimally invasive. After entry into the abdominal cavity, inspection of the peritoneum, liver and adjacent structures was performed with white-light and fluorescence imaging. Hereafter, the resection phase started with adequate mobilization of the liver. Liver metastases were demarcated using cautery, aided by fluorescence imaging and intra-operative ultrasonography. For fluorescence imaging, the PINPOINT Endoscopic Fluorescence Imaging System (Stryker, Kalamazoo, MI, USA) and da Vinci Fluorescence Imaging Vision System (Intuitive Surgical, Inc., Sunnyvale, CA, USA) were used, which are CE-marked systems for detection of ICG. Case-record forms were used during the procedure to collect data.

RESULTS

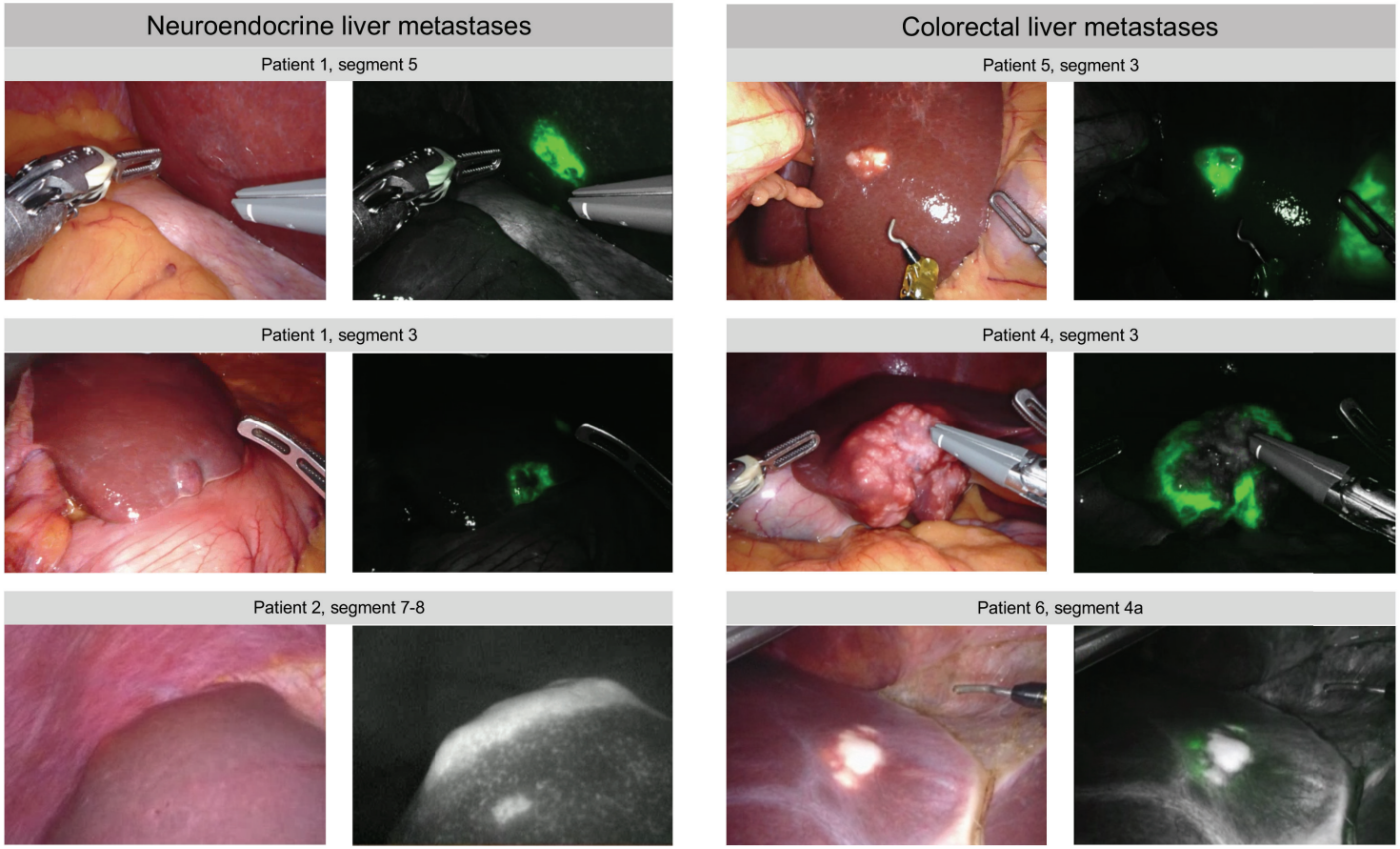
In total, six patients (2 female and 4 male) were included, the median (IQR) age was 56 (55-70) years (Table 1). Patients 1-3 had NELM and patient 4-6 had CRLM. Median (IQR) surgical time and blood loss was 130 (115-149) minutes and 75 (13-100) mL. None of the procedures were converted to laparotomy. All patients had uptake of ICG in the liver metastases (Figure 1). The NELM patients showed a fluorescence pattern without a distinct fluorescent rim pattern. This pattern is well shown in patient 2, in which also a satellite lesion appeared on fluorescence. The patients with CRLM (patients 4-6) showed the distinct fluorescence rim feature. We decided not to perform a resection in patient 2 intra-operatively, due to unforeseen extensive bilobar metastases as assessed by IOUS and ICG fluorescence. No ICG-related adverse events occurred. Histopathological assessment showed grade 1 NELM in patients 1-3 and adenocarcinoma in patients 4-6. All resection margins were negative (R0).

Table 1. Patient characteristics

Patients	Pt. 1	Pt. 2	Pt. 3	Pt. 4	Pt. 5	Pt. 6
Sex	M	F	F	M	M	M
Age, years	56	30	54	56	75	69
Primary tumour	Pancreas	Pancreas	Pancreas	Colon	Colon	Colon
Surgical procedure	Robot	Diagnostic laparoscopy	Laparoscopic resection	Robot	Robot	Robot
Tumour site	S2/3, S4a, S5	S7/8	S3, S4a, S4b, S7	S4b	S3	S4a
Histopathology	NET G1	NET G1	NET G1	Adeno-carcinoma	Adeno-carcinoma	Adeno-carcinoma
Resection margins	R0	N/A	R0	R0	R0	R0

Abbreviations: HCC: hepatocellular carcinoma

Figure 1. Fluorescence images



Intra-operative images of patient 3 are not available because they were accidentally not saved.

DISCUSSION

This is to our knowledge the first proof-of-concept study describing ICG fluorescence guided resection of NELM. Fluorescence guided resection of NELM using ICG is feasible, and is especially useful to apply during minimally invasive procedures. We observed different uptake patterns for NELM, compared to CRLM. For instance, the rim effect described for CRLM is not evidently present in NELM (patient 2, homogeneous uptake). Administration of ICG 24 hours prior to surgery seems to be sufficient to visualize NELM.

Abo et al. is the only previous study investigating the usefulness of ICG for resection of liver tumours in a cohort of patients including a single patient with NELM [10]. Some differences with the present study were that ICG was administered multiple times at different moments and a different camera system was used, but uptake of ICG was strong. This supports the idea that ICG-guided resection of NELM is possible due to its hypervascular properties.

One unexpected finding in the current study was the fluorescent rim observed in one of the NELM (patient 1, segment 3), which is more commonly described for the appearance of CRLM. A heterogeneous tumour morphology could potentially explain this, but there is no evidence in literature that supports this hypothesis.

We performed a small (n=3) proof-of-concept study in order to assess the efficacy of fluorescent visualization of NELM using ICG. The current findings support performing future studies, which should focus on surgical and oncological outcomes after fluorescence guided resection of NELM, and further optimize timing of ICG infusion. Development of a NEN-specific fluorescent tracer would be of added value to visualize malignant tissue more accurately.

Author contributions

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CHAPTER 10

Tumour-specific fluorescence-guided surgery for gastroenteropancreatic neuroendocrine neoplasms using PHT001: a phase 0, open-label, single-arm, microdosing study – PHOTON-trial

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Submitted

ABSTRACT

Introduction Currently, preoperative imaging of well-differentiated gastroenteropancreatic neuroendocrine neoplasms (GEP-NENs) is conducted using [68Ga]Ga-DOTATATE, which makes use of the overexpression of somatostatin type 2 receptors (SSTR2) on cell surfaces of GEP-NENs. An intraoperative counterpart that could provide visual guidance during surgery would be highly valuable. Conjugation of the near-infrared dye IRDye800 to the SSTR2-targeting peptide TOC was performed using a novel linker known as the multimodality chelator (MMC). The resulting agent MMC(IRDye800CW)-TOC is able to localize SSTR2-expressing tumours in animal models with high selectivity and clearly delineate tumour boundaries *in vivo*. Similar results are shown with *ex vivo* staining of human biospecimens of NENs and indicate strong translational potential. PHT001 is a successor of MMC(IRDye800CW)-TOC with better fluorescence performance. Successful implementation of PHT001 in clinical practice is therefore expected to aid in identification of lymph node and distant metastases and complete removal of tumour cells. The aim of this study is to produce and implement an SSTR2-targeted fluorescent tracer and assess its safety to accurately identify GEP-NEN during surgical resection.

Methods This is a phase 0, open-label, single-arm, microdosing study investigating safety of the newly developed fluorescent tracer PHT001. Non-clinical safety studies will be performed according to ICH M3(R2). Patients undergoing surgical resection of GEP-NEN will be included. PHT001 will be administered with a dose of 100 μg in three patients to assess the safety profile.

Discussion The phase 0 PHOTON trial will assess the safety profile of PHT001, a SSTR2-targeted fluorescent tracer for clinical use in patients with GEP-NEN. We expect that results of the phase 0 trial will aid future phase I/II clinical trials at higher doses.

INTRODUCTION

Gastroenteropancreatic neuroendocrine neoplasms (GEP-NEN) have a high tendency to metastasize to mesenteric lymph nodes and the liver. Surgical resection shows best results compared to other modalities (e.g. embolization) with excellent 5-year overall survival ranging between 85-100% [1, 2]. Also, complete removal of liver metastases is of particular importance as patients are often symptomatic due to hormonal overproduction. We already observed the added value of fluorescence angiography during surgery to aid in perfusion assessment [3]. A logical next step is to translate this to a tumour specific fluorescent tracer to increase sensitivity and specificity of fluorescence guided surgery in GEP-NEN.

Currently, preoperative imaging of well-differentiated GEP-NENs is conducted using [68Ga] Ga-DOTATATE, which makes use of the overexpressed somatostatin type 2 receptors (SSTR2) on cell surfaces of GEP-NENs. [68Ga]Ga-DOTATATE PET is known to have a high sensitivity and specificity (both >90%) [4], which make it an ideal model for development of a fluorescent derivative. Since the octreotide analogue TOC and the near-infrared fluorescent dye IRDye800CW have a well-characterized clinical performance, they were coupled to each other *via* the multimodality chelator (MMC) to produce the bioactive SSTR2-targeted fluorescent agent MMC(IRDye800CW)-TOC for intra-operative optical imaging. The MMC enables direct radiolabelling of the fluorescent agent (e.g., 68Ga for PET), which was used in preclinically in cells to show retention of agonist properties in the low nanomolar range [5] and in a proof-of-concept studies in xenografted mice for tumour-specific fluorescence imaging [6, 7]. Validation of MMC(IRDye800CW)-TOC binding *in* vivo was performed by *ex vivo* immunohistochemical staining of the SSTR2-positive tumours, SSTR2-negative tumours and normal tissues, and comparing findings with fluorescent confocal microscopy. *Ex vivo* staining of frozen sections from human NEN biospecimens showed tracer binding that correlated with SSTR2 expression and allowed clear delineation of tumour boundaries. PHT001 is a successor of MMC(IRDye800CW)-TOC with better fluorescence performance. Successful implementation of PHT001 in clinical practice is therefore expected to aid in adequate and complete removal of tumour cells, identify malignant lymph nodes and distant metastases.

METHODS

Study objectives

The primary objective of this study is to produce and implement a SSTR2-targeted fluorescent tracer during surgical resection of GEP-NEN, with the aim to assess the safety of the tracer by evaluation of the number of (serious) adverse events and suspected unexpected serious adverse reactions. Secondary objectives depend on the visualization capacity of the IMP at microdosing levels: (I) *ex vivo* validation of targeted uptake by tumour tissue by histopathology, (II) comparison of the no. of additionally identified metastatic GEP-NENs due to PHT001 compared to number of pre-operative [68Ga]-DOTATATE PET imaging, and (II) complete removal of tumour tissue.

Study design

The PHOTON trial is a phase 0, open-label, single-arm, microdosing study. The flow chart of the study is presented in Figure 1.

Ethical considerations

This trial will be conducted according to Good Clinical Practice guidelines and the principles of the declaration of Helsinki.

Study population

Inclusion criteria are:

- Patients undergoing surgery for a primary well-differentiated GEP-NEN (liver, stomach, duodenum, ampulla of Vater, pancreas, jejunum, ileum, colon or rectum), of any stage, and intent (i.e. curative/palliative) or metastases of a GEP-NEN;
- SSTR2-positive disease, as proven by a [68Ga]-DOTATATE PET scan pre-operatively (conducted at location AMC and part of standard care);
- Age of 18 years and older;
- Written informed consent.

Exclusion criteria are:

- Pregnant or breast-feeding women;
- Known hypersensitivity to the IMP or any of its components;
- Patients with known allergies to intravenous radiographic contrast agents;
- Patients who have not provided a signed informed consent form to participate in the study, prior to the start of any protocol related activities;
- Patients who, within the last 30 days, have participated in any clinical study of a therapeutic agent which may interfere with the safety or efficacy analysis of the IMP;
- Serious non-malignant disease (e.g. psychiatric infectious, autoimmune, metabolic, renal, hepatic, cardiovascular or hematological), that may interfere with the objectives of the study or with the safety of the subject, as judged by the investigator;
- A marked baseline prolongation of QT/QTc interval (e.g., repeated demonstration of a QTc interval >450 ms);
- A history of additional risk factors for torsade de pointes (e.g., heart failure, hypokalaemia, family history of Long QT Syndrome);
- The use of concomitant medications that prolong the QT/QTc interval.

Figure 1. Study flow chart

Examination/ evaluation			Treatment									Follow-up		
Time point	Days	-90 to -1 days	0							1 d	Discharge from hospital	30 d	60 d	90 d
	Hours		Pre-IMP	IMP	0 - 0.5 h	0.5 - 2 h	±4 h	2 - 5 h	5 - 12 h					
Informed consent		X												
Review inclusion / exclusion criteria		X												
General														
Medical history		X												
Physical examination		X	X		X	X		X	X	X	X	X		X
Vital signs		X	X		X	X		X	X	X	X	X		X
Blood analysis		X			X	X		X	X	X	X	X		X
Urinalysis		X			X	X		X	X	X	X	X		X
Pregnancy test		X												
12-lead ECG		X	X											
Current biopsy		X												
Tumour histology		X												
EORTC QLQ-C30 and GI.NET21		X	X									X	X	X
Medication use		X	X											X
Adverse events			X	X	X	X	X	X	X	X	X	X	X	X
Treatment														
PHT001 infusion				X										
Surgery							X							

Informed consent procedure

Patients meeting all eligibility criteria stated above will be informed on the trial at the outpatient clinic by a member of the research team. Written informed consent will be obtained for participation in the trial.

Investigational medicinal product

Summary of findings from clinical studies

In a comparable clinical research setting, Li et al. have developed [68Ga]Ga-IRDye800CW-BBN (bombesin, peptide) [8]. Implementation of this tracer led to improved intraoperative tumour visualization aiding a safe and adequate resection. We therefore expect equal results with our new tracer, MMC(IRDye800CW)-TOC, albeit for a different indication.

Summary of known and potential risks and benefits

Potential risks include an allergic reactions as a result of the administration of the IMP, similar to any contrast agent [9]. One study with [68Ga]Ga-DOTATATE observed only 3 minor adverse events after conducting scans in 97 patients, which all resolved spontaneously (oxygen desaturation, minor itching on injection site and tachycardia) [10]. Furthermore, severe adverse events are rather uncommon in low molecular weight optical tracers [11]. In case of a peri-operative allergic reaction to the IMP, local allergic reaction protocols have to be followed. Potential benefits that patients are: identification and removal of previously unknown (micro-) metastases, complete removal of tumour tissue.

Description of route of administration and dosages

The IMP will be administered intravenously as a slow bolus infusion, similar to the toxicology studies performed in rodents. The prespecified dose level is 100 μg, which will be administered as a single bolus infusion.

Preparation of the investigational medicinal product

Preparation of the tracer will be done according to Ghosh *et al.* [5]. A batch of PHT001 will be produced under GLP conditions to perform non-clinical safety studies, in accordance with ICH M3(R2) Approach 1 "Microdosing". This consists of an extended single dose toxicity studies in rats which will be performed by a contract research organization. The first-in-human clinical trial will start after receiving favourable results from the non-clinical safety studies. PHT001 for use in patients will be produced on a single patient basis and under GMP conditions at Tracer Center Amsterdam (Amsterdam UMC, Amsterdam, the Netherlands) and obtained as a sterile, isotonic and pyrogen-free solution, ready for intravenous injection.

Administration of the IMP

PHT001 will be administered 4 hours before surgery. Vital parameters will be monitored throughout the infusion process, for at least two hours after administration of PHT001. Blood and urine will be collected at different time points to assess the pharmacokinetic profile of PHT001.

Surgical procedure

During surgery, fluorescent images will be made at four stages: directly after access to the abdomen, after the tumour is exposed, when lymph nodes were encountered, and after completion of resection (i.e., resection-bed imaging). Images will be made with three modalities: bright-field (i.e. conventional image), fluorescence (black-white) and fluorescence colour overlay (black-white, with coloured fluorescent signals). Imaging will be performed using the OnLume NIRF camera system (OnLume, Inc., Madison, Wisconsin). Previously unknown lesions with a fluorescent signal will be biopsied or removed at the surgeon's discretion. After resection, tissue will be imaged on a back table using the OnLume NIRF camera system.

Pathological evaluation

After intraoperative imaging, tissue will be fixed overnight in formalin. Tissue cassettes will be imaged using the OnLume NIRF camera system. Hereafter 5 μm sections will be obtained to confirm the localisation of the IMP in tumour tissue using the Eclipse Ti2 inverted microscope (Nikon, Tokyo, Japan). Images acquired with fluorescence microscopy will be compared with haematoxylin & eosin (H&E) staining to confirm tumour positivity. Immunohistochemistry will be performed using the Ventana autostainer (Roche, Basel, Switzerland) to assess expression of SSTR2 and co-localization of the fluorescence signal (SSTR2 antibody, Thermo Fisher Scientific, Waltham, Massachusetts, USA). All histopathological examinations will be performed by an expert GEP-NEN pathologist, who is blinded to the NIRF images.

Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

Safety reporting

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

QT/QTc interval prolongation

In accordance with The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) E14: "Clinical evaluation of QT/QTc interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs", electrocardiograms will be acquired throughout the study period to determine whether the IMP has a threshold pharmacologic effect on cardiac repolarization, as detected by QT/QTc prolongation. The

threshold level of regulatory concern, is around 5 ms as evidenced by an upper bound of the 95% confidence interval around the mean effect on QTc of 10 ms.

Adverse events

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the investigational product. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded. Adverse events are classified using the Common Terminology Criteria for Adverse Events (CTCAE) v5.0. Serious adverse events are equivalent to grade 3-5 AE according to CTCAE. An elective hospital admission will not be considered as a serious adverse event.

Monitoring

Due to the high-risk profile of the study (as determined by the risk assessment tool developed by the clinical research unit of AMC), a data safety monitoring board will be established to perform ongoing safety surveillance and to perform interim analyses on the safety data, this committee is an independent committee. Monitoring will be conducted by the Clinical Monitoring Center (Clinical Research Unit, AMC).

Sample size calculation

A formal sample size calculation is not possible due to the experimental nature of the study. We plan to include 3 patients.

Statistical analysis

All categorical data will be presented as number of cases and percentages, whilst continuous data will be presented as either mean ± standard deviation (range) or as median and interquartile range (IQR), depending on the data distribution. Data will be analysed using the Statistical Package for Social Sciences (SPSS) of IBM Statistics, version 26.0 (IBM Corp. Armonk, New York, USA).

Data handling and monitoring

Data will be handled confidentially. As long as it is necessary to be able to trace data to an individual subject, a subject identification code list will be used to link the data to the subject. The code is not based on the patient initials and birth-date. The key to the code will be safeguarded by the investigator. Data will be saved for 15 years and destroyed thereafter. Data will be collected using Castor EDC (Amsterdam, the Netherlands). The handling of personal data complies with the EU General Data Protection Regulation and the Dutch Act on Implementation of the General Data Protection Regulation.

Public disclosure and publication policy

This study is registered in the Netherlands Trial Register database (NL9298), EudraCT (2021-000940-23) and Central Committee on Research Involving Human Subjects (NL76946). The results of the study and preclinical studies will be submitted to a peer-reviewed open-access journal regardless of outcomes. Co-authorship will be based on the international ICMJE guidelines.

DISCUSSION

The aim of the PHOTON trial is to develop and implement a SSTR2-targeted fluorescent tracer for clinical use in patients with GEP-NEN. We hypothesise that such a fluorescent tracer will improve complete resection of tumour tissue, result in detection of occult metastases and aid dissection in order to preserve vital structures.

Although survival outcomes of GEP-NEN is relatively better than other gastro-intestinal malignancies (e.g. pancreatic, stomach or esophageal cancer), it does depend on the completeness of the surgical resection. In grade 1 and 2 GEP-NEN, the five year overall survival after a R0/1 resection ranges between 89-92%, which drops to 49-77% after a R2 resection [12]. The same is observed for NELM, in which R2 resection results in significantly worse survival outcomes compared to R0/R1 resections [13]. As expected, R1 status has a negative impact on recurrence free survival after resection of pancreatic NENs (HR 1.8, 95% CI 1.2-2.7, P = 0.002) [14].

Although NEN are known for their overexpression of SSTR2, they are not the only neoplasms with this feature [15]. Lee and colleagues analysed data from The Cancer Genome Atlas to identify other neoplasms with SSTR2 expression. Pheochromocytoma, paraganglioma and normal kidney tissue acted as reference tissues to define high-SSTR2 neoplasms. Data of 9960 primary tumour and 739 normal tissue samples were included. Low grade glioma had the highest proportion (51%) of high-SSTR2 tumours, followed by breast invasive carcinoma (16%). This finding adds to the importance of an SSTR2-targeted fluorescent tracer for clinical use.

Development of fluorescent tracers has made progress in recent years, which is reflected by the vast amount of (predominantly) phase I trials which are currently conducted [16]. Herein, the central theme is to accurately identify tumour tissue and its boundaries with surrounding healthy tissue. Recently, Dijkstra and colleagues developed Ac-Lys0(IRDye800CW)Tyr3-octreotate (800CW-TATE), and successfully visualized SSTR2-positive lung carcinoma xenografted mice and human biospecimens of meningioma's [17]. TATE and TOC represent the targeting components of two FDA-approved radiopharmaceuticals that are known to have near identical performance for targeting GEP-NEN.

In conclusion, previous studies have shown that fluorescence molecular imaging with targeted tracers is able to improve tumour delineation, detect lymph node metastases and occult tumour lesions. PHT001 is hypothesised to be a good candidate for intra-operative fluorescence molecular imaging of GEP-NEN and other SSTR2 expressing tumours. For this reason, the PHOTON trial will investigate the toxicity profile and optimum dose for clinical use.

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Author contributions

Conceptualization: EK, AA, BW, DV, AFE.
Supervision: AA, PJT, EJMNvD, HH, BW, DV, AFE.
Validation: EK, AA, PJT, EJMNvD, HH, BW, DV, AFE.
Writing—original draft: EK.
Writing—review and editing: EK, AA, PJT, EJMNvD, HH, BW, DV, AFE.

Role of the funding source

The PHOTON trial is an investigator-initiated study. The funding sources had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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SUMMARY AND DISCUSSION

SUMMARY

The general aim of this thesis was to evaluate different aspects of surgical treatment of patients with neuroendocrine neoplasms (NEN), which included treatment of liver metastases, minimally invasive resection of primary tumours of the small bowel (SB-NEN), and the application of fluorescence guided surgery. The first part of this thesis focuses on treatment of neuroendocrine liver metastases (NELM), epidemiological and disease characteristics of SB-NEN and describes a new radiological and immunological association for mesenteric fibrosis. The second part evaluated application of minimally invasive surgery for SB-NEN. The third part investigated the added value of fluorescence guided surgery of SB-NEN, NELM and gastroenteropancreatic tumours (GEP-NEN). A summary of these parts and their chapters is presented below.

PART I: DISEASE CHARACTERISTICS OF SMALL BOWEL NEUROENDOCRINE NEOPLASMS

Chapter 1 is a systematic review and meta-analysis and assesses which treatment modality of NELM results in the longest overall survival (OS). In total, 712 studies were screened for eligibility, of which 11 studies comprising 1108 patients were included for analysis. NELM originated from the pancreas, small bowel or other location in 662 (60%), 164 (15%) and 282 (25%) patients, respectively. Surgical resection of NELM was associated with better survival outcomes, compared to other treatment modalities (e.g. embolization, chemotherapy). Results from this study suggest that resection of NELM results in the longest OS for patients with GEP-NEN, which should therefore be considered in all patients in a case-by-case fashion.
Chapter 2 is a retrospective cohort study which analyses epidemiological, treatment and survival outcomes of patients with grade 1 and 2 SB-NEN in the Netherlands between 2005-2015. Data was extracted from the Netherlands Cancer Registry (NCR) and The Nationwide Network and Registry of Histo- and Cytopathology in the Netherlands (PALGA). A total of 1132 patients were included in the epidemiological analyses, which showed an increase in incidence from 0.52 in 2005 to 0.81 per 100.000 persons per year. Eighty-two percent of the patients had a grade 1 tumour and 17% grade 2. The majority of the patients underwent surgical resection (86%), followed by use of somatostatin analogues (30% overall, 50% of stage IV patients). Data regarding survival outcomes was present for 975/1132 (86%) of the patients. Five year overall-survival rates were 75% for stage I-II, 75% for stage III and 57% for stage IV disease. This study has shown that the incidence of SB-NEN is, as expected, rising, that surgical resection is a cornerstone in the treatment strategy, and that survival rates are relatively high.

In the national cohort presented in **Chapter 2**, 62% of the patients had lymph node metastases. One of the sequelae of lymph node metastases from SB-NEN is the development of mesenteric fibrosis. Presence of (extensive) mesenteric fibrosis results in a challenging surgical resection, especially if performed via a minimally invasive approach. **Chapter 3** is an exploratory study investigating the association between IgG4 expression in mesenteric tumour deposits and the extent of mesenteric fibrosis seen on preoperative imaging. To do this, we developed a novel scoring system to quantify the extent of mesenteric fibrosis. IgG/IgG4 stainings were

performed on formalin fixed paraffin embedded tissue, and manually scored by calculating the IgG/IgG4 ratio in the area with the most IgG4 positive cells. A total of 14 patients were included in the study. As hypothesized, the IgG/IgG4 ratio was higher in the group with more extensive mesenteric fibrosis. Also, a higher IgG/IgG4 ratio was seen in grade 2 tumours and in stage IV disease. These findings could potentially result in a new indication for pre-operative administration of corticosteroids (e.g. prednisone) or development and application of (novel) biologicals.

PART II: MINIMALLY INVASIVE SURGERY FOR SMALL BOWEL NEUROENDOCRINE NEOPLASMS

One of the limitations of **Chapter 2** was the unavailability of data on surgical outcomes of SB-NEN. As a consequence of this limitation, we performed in **Chapter 4** a systematic review and meta-analysis to assess post-operative morbidity and mortality after surgical resection of SB-NEN. After screening 2416 articles, 13 were included in the meta-analysis. This resulted in a study population of 1087 patients, of which 62% had stage IV disease, and 76% underwent a segmental small bowel resection. Pooled severe morbidity (Clavien-Dindo grade III-IV) was 7% (95% CI 4-13%, I2 = 71%), pooled 30-day mortality was 2% (95% CI 1-3%, I2 = 0%), pooled 90-day mortality was 2% (95% CI 2-4%, I2 = 0%), and pooled in-hospital mortality was 1% (95% CI 0-2%, I2 = 0%). Severe morbidity (Clavien-Dindo grade III-IV) was lower in hospitals with an annual volume > 9 resections, whereas 90-day mortality was higher. Future research should focus on the effect that hospital and surgeon volume have on post-operative morbidity and mortality.

Chapter 5 is an international survey study among surgeons who treat patients with SB-NEN. The aim was to assess international practice regarding minimally invasive surgery for SB-NEN, current attitudes and future prospects towards minimally invasive surgery for SB-NEN, and finally to set-up an international study group. An anonymous survey was disseminated via international colorectal and (neuro-) endocrine tumour societies. In total, 58 responses from 20 countries were included. Sixty-nine percent of the respondents stated to perform minimally invasive surgery for SB-NEN. Overall, a minimally invasive approach was preferred due to short-term peri-operative benefits, whilst an open approach was preferred for better lymphadenectomy and tactile feedback. Regardless of previous experience in advanced minimally invasive surgery, 52% of the respondents stated to potentially benefit from additional training for this technique. In response to the findings of this survey, we have set up the *International Study Group for Small bowel neuroendocrine neoplasm Surgery* (www.ISGSS.org) to be able to conduct international multicenter research in large research populations.

Chapter 6 is a retrospective cohort study which evaluated an institutional change from open to laparoscopic resection of SB-NEN, irrespective of the location of mesenteric lymph node metastases. Patients who underwent a surgical resection of SB-NEN between 2003 and 2019 were screened for inclusion. Thirty-four patients were included, of which 11 (32%) underwent open resection and 23 (68%) a laparoscopic resection. There were no significant baseline or pathology differences. The primary tumour was identified pre-operative in 95% of the patients in the laparoscopic group, and 36% of the open group (P < 0.001). Median

length of hospital stay was 4 days in the laparoscopic group and 8 days in the open group (P = 0.036). There were no differences in post-operative morbidity or mortality. Hence, it seems that laparoscopic resection of SB-NEN, as performed in our tertiary referral center, results in similar pathological outcomes and shorter hospital stay. Future research should focus on the long-term oncological outcomes of laparoscopic resection, and how the hospital or surgeon volume affects this.

In **Chapter 6,** only peri-operative short-term outcomes could be compared between laparoscopic and open resection of SB-NEN. However, long-term survival data was lacking. Therefore, in **Chapter 7**, we performed a retrospective cohort study in order to assess long-term survival outcomes after laparoscopic resection of SB-NEN. Data concerning patients who underwent a surgical resection between 2005 and 2015 was collected from the NCR and PALGA. A total of 482 patients were included, of whom 342 (71%) had an open resection and 140 (29%) a laparoscopic resection. Histopathological examination showed that patients in the open resection group had significantly more multifocal tumours resected, had more pN2 lymph nodes and stage IV disease. Independent predictors of shorter OS was age above 60 years and stage IV disease, whereas a laparoscopic resection predicted a longer OS. Tumour grade, resection margins and presence of multifocal tumours did not affect OS. Future studies including more detailed peri-operative data are needed to confirm the oncologic safety of a laparoscopic SB-NEN resection.

PART III: FLUORESCENCE GUIDED SURGERY OF NEUROENDOCRINE NEOPLASMS

Chapter 6 and 7 have shown that a laparoscopic resection of SB-NEN is technically feasible, and does not raise concerns of oncologic adequacy. And, as described in **Chapter 2 and 3**, presence of mesenteric metastases (and fibrosis) make surgical resection challenging. Therefore, in **Chapter 8,** we performed an exploratory study to evaluate the potential value of intra-operative fluorescence angiography using indocyanine green (ICG) during surgical resection of SB-NEN. We hypothesized that use of ICG might be of added value, due to the close proximity of mesenteric metastases to mesenteric vessels. Due to the exploratory nature of the study, we pragmatically included 10 patients. Fluorescence angiography was performed after mobilization of the small bowel and marking of the transection level by the surgeon. Hereafter, a bolus infusion of ICG with a dose of 0.1 mg/kg was administered. Change in management was defined as performing the transection at a different level than previously selected by the surgeon. The use of fluorescence angiography lead to change in management in eight patients. Four patients underwent a more extensive resection (3 to 25 cm), and small bowel could be spared in four patients (5 to 35 cm). Median post-operative stay was 4 days, without any major complications (Clavien-Dindo grade 3 or higher). Although these results are promising, future research with larger cohorts should be conducted to confirm this, with a focus on the efficacy of a sparing resection.

As presented earlier in **Chapter 1**, surgical resection of NELM results in the longest OS, and complete removal of NELM contributes to favourable oncological outcomes. **Chapter 9** is a proof-of-concept study which evaluated the efficacy of fluorescence guided surgery for NELM using ICG, as we hypothesized that this would be possible due to the hypervascular appearance of NELM on computer tomography scans. Patients received a bolus infusion of ICG with a dose of 10 mg approximately 24 hours before surgery. To compare the ICG signal, patients with colorectal liver metastases (CRLM) were included. During the study period (2019-2021), three patients with NELM were included, one of which did not receive a resection due to unforeseen extensive bilobar metastases. Three patients with CRLM were included to compare the ICG signal. Uptake of ICG was present in all patients with NELM, and was either rim-shaped or with homogeneous uptake. There were no major complications (Clavien-Dindo grade 3 or higher) related to use of ICG for this purpose. Future studies including large cohorts are needed to assess the efficacy of ICG guided resection of NELM, especially in terms of achieving radical resection margins.

The previous chapters have shown that surgical resection of NEN plays a key to ascertain good survival outcomes (**Chapter 1, Chapter 2 and Chapter 7**), while some technical challenges are faced: due to the size or proximity of mesenteric metastases (**Chapter 6**), presence of mesenteric fibrosis (**Chapter 3**), or by using a minimally invasive approach (**Chapter 5**). Accurate identification of tumour tissue has the potential to alleviate concerns and challenges discussed in the aforementioned chapters. **Chapter 10 is** the study protocol of the PHOTON trial, in which we develop a fluorescent tracer for intra-operative identification of GEP-NEN. The PHOTON trial includes pre-clinical safety assessment of PHT001, a novel somatostatin receptor type 2 targeted fluorescent tracer, and a clinical trial in order to provide first-in-human data. Pre-clinical safety assessment will be performed according to ICH M3(R2), approach 1: with a total dose ≤ 100 μg. This includes an extended single dose study in rats. All pre-clinical safety assessment studies will be performed by a contract research organization. The clinical trial will be conducted at Amsterdam UMC, consisting of 3-5 patients and will supply data to conduct future phase I/II clinical trials.

Summary of research questions and main findings

Chapter	Research questions
1	Which treatment modality results in longest overall survival in patients with NELM? *Surgical resection of NELM results in longest overall survival, compared to no resection, chemotherapy, embolization and liver transplantation.*
2	What are the epidemiological, treatment and survival characteristics of patients with grade 1 and 2 small bowel neuroendocrine neoplasms? *The incidence of SB-NEN has risen to 0.81 per 100.000 persons per year in 2015 and treatment often consists of surgical resection (86% of patients). Five year overall survival rates of stage I-II and III disease were 75%, and 57% for stage IV disease.*
3	What is the relationship between immunoglobulin G4 expression and the extent of mesenteric fibrosis from small bowel neuroendocrine neoplasms? *The IgG/IgG4 ratio was higher in patients with more extensive mesenteric fibrosis, although this relationship was not proven to be statistically significant.*
4	What is the morbidity and mortality after resection of small bowel neuroendocrine neoplasms, and how is this affected by hospital volume? *Severe morbidity and 30-/90-day mortality after surgical resection of SB-NEN is 7% and 2%, respectively. Severe morbidity rates were lower in high-volume centers. Interestingly, the converse was observed for mortality rates.*
5	What is the current international practice and attitude towards minimally invasive SB-NEN resection? *In the investigated cohort, 69% of the respondents stated to perform minimally invasive SB-NEN resection. This approach was preferred due to peri-operative benefits, whilst an open approach was preferred due to concerns of oncologic adequacy (of a minimally invasive resection), and in order to manually palpate the entire small bowel.*
6	What are the peri-operative differences between patients who underwent a laparoscopic or open resection for SB-NEN? *Patients who underwent a minimally invasive resection or open resection had similar baseline characteristics. Hospital stay was significantly shorter in the laparoscopic group. Post-operative morbidity and pathological outcomes did not differ.*

7 What is the most common surgical approach to resect small bowel neuroendocrine neoplasms in the Netherlands?
In this cohort, 71% of the patients underwent an open resection of SB-NEN, whilst 29% underwent a laparoscopic resection. Patients who underwent an open resection had significantly more multifocal tumours resected, pN2 lymph nodes and stage IV disease.

8 What is the value of fluorescence angiography using indocyanine green during surgical resection of small bowel neuroendocrine neoplasms?
Indocyanine green (ICG) fluorescence angiography is able to detect differences in vascularization of the small bowel after mobilization. Leading to change in management by means of a more extensive or sparing resection.

9 What is the value of fluorescence guided resection of NELM using indocyanine green?
Indocyanine green (ICG) accumulates in NELM, and therefore aids intra-operative visualization of tumour tissue and differentiation with healthy liver tissue. Uptake appears either as a rim-shape or with homogeneous distribution.

10 Is MMC(IRDye800CW)-TOC safe to use in humans, and does it effectively delineate tumour tissue from healthy tissue?
We currently do not know the exact answer to this question. However we do not expect adverse outcomes of this compound and expect that it will perform well in clinical trials.

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

This thesis focuses on different aspects of surgical treatment of patient with small bowel neuroendocrine neoplasms (SB-NEN), including treatment of liver metastases, and application of minimally invasive and fluorescence guided surgery. Besides observational studies to assess the status quo and outcomes of (minimally invasive) surgical treatment, we also performed exploratory and experimental studies to investigate the association of mesenteric fibrosis with IgG4 expression and application of fluorescence guided surgery. The latter two studies can potentially facilitate further implementation of minimally invasive surgery in SB-NEN. Potential effectiveness of induction therapy to reduce mesenteric fibrosis would ease dissection, whilst fluorescence guided surgery is a helpful tool in assessing anastomotic perfusion and with the future potential to guide the resection with tumor-targeting. Hence, the individual chapters should be regarded as steppingstones in application of minimally invasive surgery for SB-NEN.

PART I: DISEASE CHARACTERISTICS OF SMALL BOWEL NEUROENDOCRINE NEOPLASMS

A multitude of therapeutical modalities are currently available for patients with NENs, and other types of cancers in general, including neoadjuvant treatment (e.g. to downsize tumour size), advanced surgical techniques (e.g. minimally invasive or fluorescence guided surgery) to adjuvant treatment (e.g. chemo- or targeted therapy). The decision on the strategy from which the patient will benefit most is ideally made in a multidisciplinary team, consisting of at least: surgeons, medical oncologists, radiologists, endocrinologists, nuclear physicians and pathologists. **Chapter 1** showed that surgical resection of neuroendocrine liver metastases (NELM) resulted in best overall survival. Deciding which treatment strategy to follow is not as straight forward as it may seem, as there are some contra-indications for surgical resection (e.g. presence of extra-abdominal metastases, NENs with poor differentiation, extent of metastases, behaviours) [1]. This is exactly why discussing patients with complex and/or rare diseases in a multidisciplinary team meeting is of importance.

Koco *et al.* performed a systematic review to assess the effect of a multidisciplinary team meeting on clinical practice and outcomes for colorectal, lung, prostate and breast cancer [2]. Discussion of colorectal cancer patients in a multidisciplinary team meeting resulted in: change in management (average 16%), significantly better survival (in 6 out 8 studies that reported this outcome), reduction in primary tumour resection (in all studies that reported this outcome), and a significant effect on the type of surgical resection (in all studies that reported this outcome). The same effects can be expected for patients with NENs. Formal analyses of these effects could potentially be analysed by studies focussing on the effects of multidisciplinary team meetings for patients with NENs.

At least two findings reported in **Chapter 2** should be emphasized: the rising incidence of SB-NEN and similarities between academic and regional hospitals. Regarding the rising incidence, possible explanations could be more clinical awareness, increased diagnostic

imaging or more incidental findings during surgery. Another factor that apparently has contributed to a rising incidence is implementation of the colorectal cancer screening program in the Netherlands. Analysis of the bowel cancer screening programme of the United Kingdom shows that 11 (terminal ileum) SB-NENs are diagnosed per 100.000 colonoscopies [3]. As the authors have stated, the true diagnostic rate is possibly higher, as the terminal ileum is not routinely visualized during a colonoscopy. Fifteen out of 28 (54%) patients diagnosed via this route had T3/T4 tumours, 85% (23/27) had N1 disease and 36% (4/11) had M1 disease. In the Netherlands, the colorectal cancer screening programme was introduced in January 2014 [4]. In the period of 2014-2016, 68 colorectal NENs were detected as a consequence of screening, which is 20% of all colorectal NENs diagnosed during that period [5]. The question that arises now is whether gastroenterologists should be asked to visualize the terminal ileum during a screening colonoscopy. The balance between the "number needed to diagnose" and complications of extensive colonoscopy procedures, including the clinical relevance of diagnosis of this (often) indolent tumour could be an interesting topic for future research.

Secondly, the similarities that were present between academic and regional hospitals: similar tumour characteristics and similar survival outcomes. Although this may seem surprising at first, it does need some nuance. Ideally, this comparison would be made based on the expertise of a center. In that case, the comparison would be expert center vs. non-expert center. However, due to the small number of hospitals in the Netherlands, this comparison could not be made due to confidentiality of data. Interestingly, histopathological revision of specimen by NEN expert centers resulted in changes in 36% of the cases (mainly based on Ki67 differences) [6]. It is therefore likely that centralization will result in improved pathological diagnosis and thus better survival outcomes (similar to for example pancreatic surgery) [7]. This question remains unanswered for now. An alternative approach to this question is to focus on the caseload at which differences in survival outcomes are observed, which we will attempt to answer via the International Study Group for Small bowel neuroendocrine Surgery (ISGSS).

Two interesting multimodality treatment strategies are neoadjuvant treatment and induction therapy. The aim of neoadjuvant treatment is to optimize survival outcomes, whereas induction therapy aims to reduce tumour size pre-operatively. Application of neoadjuvant therapies for treatment of patients with NEN is not as standardized as for other cancers (e.g. oesophageal cancer). Several studies report use of peptide receptor radioligand therapy (PRRT) in the neoadjuvant setting, which resulted in disease stabilization/reduction of size in some patients with NEN [8]. Blazevic *et al.* published a retrospective cohort of 530 patients with SB-NEN, of which 132 received neoadjuvant PRRT [9]. Although 13% showed objective response (≥30% size reduction of all lesions combined), only 4% of the mesenteric masses had a size reduction of ≥30%. So, PRRT (alone) is not effective in reduction of the size of mesenteric metastases.

Roberts *et al.* investigated the expression of IgG4 expression in tumour deposits and observed that the IgG/IgG4 ratio was higher in patients with larger mesenteric tumours deposits. This finding attributes to the knowledge on mesenteric fibrosis and might eventually result in a new treatment. **Chapter 3** has shown that grade 2 tumours, stage IV disease and patients with symptomatic disease show a significantly higher IgG/IgG4 ratio as compared to patients

with grade 1 or non-metastatic disease. Methods to downsize or limit the size of mesenteric metastases/extent of fibrosis is desirable, as they make dissection more complex due to vascular involvement, and is associated with a shorter survival [10]. Currently we do not know if and what pathophysiological similarities mesenteric fibrosis and IgG4-related disease have. If there are indeed similarities, existing therapies for IgG4-related disease could be investigated for efficacy in SB-NEN (e.g. glucocorticoids or rituximab) [11, 12].

PART II: MINIMALLY INVASIVE SURGERY FOR SMALL BOWEL NEUROENDOCRINE NEOPLASMS

All surgical procedures are associated with a risk of complications. Some occur more often than others, but rarely occurring complications might have detrimental effects on patient survival and quality of life outcomes. Knowing the rate at which post-operative complications occur serves multiple purposes, such as informing patients with accurate estimates of the post-operative period, whilst it also enables comparison between different centers. The most recent ENETS guideline for surgery of SB-NEN stated that the maximally acceptable rate of post-operative morbidity and mortality is 30% and 1.5%, respectively [13]. The systematic review in **Chapter 4** shows that the overall morbidity rate is 13%, which is significantly lower than the accepted rate according to the ENETS guidelines. Severe complications occur even less often (7%), and a 30- and 90-day mortality rate is 2%. Indeed, analysis of our own cohort in **Chapter 6** showed that overall (including minimally invasive and open resection) severe complications occur in 9% of the patients and mortality in 3% (slightly higher numbers may be attributable to case-mix). Taken together, there seems to be enough room for improvement regarding post-operative morbidity for this procedure. (Further) centralization of care for these patients might be able to accomplish this.

Minimally invasive surgery is readily applied in gastro-intestinal surgery, including for example high-complex upper-GI and hepatobiliary procedures. However, application of minimally invasive surgery for SB-NEN is not yet widely accepted. Multiple factors play a role, amongst others the complexity of the procedure, concerns of oncologic adequacy, low case-load, and a limited amount of researchers in this specific field. Only a very few studies investigated the role of minimally invasive techniques for surgical treatment of SB-NEN. Consequently, these factors limit the generation of evidence which is needed for scientific committees to make clinical guidelines. Guidelines commonly advice open surgery with only limited application of minimally invasive surgery, which in turn hampers generation of new evidence.

The ENETS and NANETS guidelines have concerns that multifocal tumours are missed and that the vascular dissection is very challenging [13, 14]. Diagnosing multifocal disease in SB-NEN pre-operatively is hard, and often missed by imaging, and by up to 33% of the surgeons during intra-operative palpation [15-17]. Pathologic examination plays a key role in classifying SB-NEN as multifocal. The relevance of this argument is topic of debate, as some studies show no effect on survival in the presence of multifocality [17-19], and other studies do show an effect on survival. [20, 21]. Adequate identification and resection of multiple primary lesions in published cohort series with open surgery might explain this absence of a survival impact.

The general opinion is that there is a negative impact on survival, as it is in other diseases [18]. **Chapter 5** indeed confirms these concerns, as risk of incomplete resection (R1/2), vascular involvement, large mesenteric metastases (pN2, >2 cm), and multifocal tumours were the top four perceived contra-indications. We are currently in the set-up phase of the International Study Group for Small bowel neuroendocrine neoplasm Surgery (www.ISGSS.org). The ISGSS aims to bring together international researchers and facilitate multicenter research, with a focus on surgical treatment of SB-NEN. The first project is a large registry including patients who underwent a surgical resection from 2010 onwards. Due to the large number of participating centers worldwide, studies can be performed to investigate and define "textbook outcome", and even perform phase III surgical trials which are challenging to conduct in rare diseases.

Evidence for a minimally invasive approach for SB-NEN is scarce. **Chapter 6** presents the results of our institutional switch from a predominantly open to a minimally invasive approach. Main reasons to prefer a minimally invasive approach are the peri-operative advantages associated with this procedure, as was shown in **Chapter 5**. Hospital stay was significantly shorter after a minimally invasive procedure, and post-operative morbidity and mortality was similar. Due to the lack of comparative studies, we were unable to compare the two surgical approaches in **Chapter 4**. A sub-group analysis was possible, and did not show higher morbidity or mortality rates compared to the pooled ratio of all included studies. Nevertheless, implementation should be performed carefully, without compromising patient's recovery and oncological outcomes. We expect that the ISGSS registry will shed light on this topic, with sufficient statistical power.

Based on findings presented in **Chapter 4** and **Chapter 6,** minimally invasive surgery for SB-NEN has, compared to the open approach, similar peri-operative morbidity and mortality rates, shorter hospital stay and similar pathological outcomes. The question that could not be answered in **Chapter 6** was the long-term survival outcome, which is addressed in **Chapter 7**. This nationwide population-based study shows that, after correction for age and disease stage, a laparoscopic approach is associated with better survival outcomes. This is a positive finding, but it is somewhat hard to imagine that a surgical technique itself results in better survival outcomes. It is probably a reflection of differences in patient populations, indeed: patients had significantly more multifocal tumours, pN2 lymph nodes and stage IV disease in the open resection group. However, this confounding factor is precluded (to some extent) as a minimally invasive resection was associated with significantly better survival outcomes compared to the open approach in stage III patients (no survival differences between both approaches were observed in stage IV patients). An important aspect that should be mentioned is that tumour multifocality was not identified as an independent predictor for survival, which was also observed in **Chapter 2,** and by other research groups [18, 19]. Some other studies contradict these findings [20, 21]. The expectation that multifocality predicts poorer outcome might be due to the known negative effect of multifocality in other tumour types, as was hypothesized by Choi *et al.* [18]. Collaborative research projects are needed to generate evidence that is widely agreed upon, ideally including researchers who are in favor of minimally invasive surgery for SB-NEN and researched who are skeptical.

PART III: FLUORESCENCE GUIDED SURGERY OF NEUROENDOCRINE NEOPLASMS

Since the FDA approval of ICG, a multitude of studies were performed examining the use of fluorescence imaging for different purposes, for example lymph node mapping and perfusion assessment [22, 23]. **Chapter 8** is a proof-of-concept study which investigated efficacy of ICG perfusion assessment during SB-NEN resection. The rationale for this study was that the mesenteric lymph node dissection poses vascular risks for patients. Hence, perfusion assessment would be a helpful tool to identify patients with poorly perfused bowel ends, before creating an anastomosis. Due to the promising results of this study, we incorporated use of ICG fluorescence as a standard step for surgical resection of SB-NEN, and have used this in 20 patients until now.

Although ICG was initially developed for perfusion assessment, several studies were performed to identify its efficacy in tumour boundary delineation (e.g. colorectal liver metastases) [24]. Similarly, **Chapter 9** describes the first application of ICG fluorescence to identify NELM. Although ICG fluorescence is already described for colorectal liver metastases, application for NELM was never investigated [24, 25]. Colorectal liver metastases appear hypovascular on contrast enhanced computer tomography scans, whilst NELM show a hypervascular pattern [26, 27]. We hypothesized that there could be a difference in ICG signal between the two groups, and therefore performed this proof-of-concept study for NELM. Fluorescence with ICG was indeed safe and possible: NELM showed uptake of ICG which appeared as homogeneous or a rim-shaped signal. Hence, ICG fluorescence is a good alternative for visualization of NELM, until targeted tracers for NENs are available. The obvious advantage of a targeted tracer is tumour specific binding, which could be used in all patients with NEN undergoing surgical (or endoscopic) resection.

The abundant expression of SSTR2 receptors by NENs is made use of for positron emission tomography scans (e.g. 68Ga-DOTATATE). This is a major advantage in development of a fluorescent tracer, as the target receptor (SSTR2) is well described in literature. In **Chapter 10** the study protocol of the PHOTON trial is presented, in which we will develop and investigate the safety and efficacy of PHT001, a fluorescent tracer targeting SSTR2. A Phase 0 microdosing study (max. dose 100 mcg) will be performed in 3 patients, followed by a Phase I/II clinical trials. We expect that, if the fluorescent tracer for NENs is safe and effective, fast implementation for other SSTR2 expressing neoplasms is possible [28, 29].

FUTURE PERSPECTIVES

This thesis sheds light on some important aspects of SB-NEN, most importantly in: characterization of IgG4 expression by mesenteric tumours deposits, establishing a foundation for application of minimally invasive surgery for this indication, assessment of the value of fluorescence guided surgery for NEN and starting the development of a NEN-specific fluorescent tracer. Each project was able to answer the corresponding research questions, whilst they also led to new questions.

The chapters presented in this thesis are quite comprehensive and form a foundation for future research in the field of (SB-)NEN. In the following sections I would like to elaborate on some topics that I believe have high potential and are to date not well described in literature. Specific future directions for research were already discussed by each individual chapter.

Minimally invasive surgery is a technically demanding procedure, which is complicated even more by mesenteric fibrosis caused by SB-NEN. Investigating outcomes alone of this technique is not sufficient for safe and wide implementation for SB-NEN. It is also necessary to limit the size and extent of mesenteric metastases/fibrosis and give surgeons tools to successfully perform this procedure. This was the rationale to investigate the association of IgG4 expression in mesenteric tumour deposits, and investigate applicability of fluorescence guided surgery. Future studies should focus on creating induction therapies to help the surgeon achieve radical resection margins, whilst adjuvant treatment schemes could be developed to optimize survival and recurrence outcomes. The abundant expression of SSTR2 by these neoplasms could be used as a target purpose.

The occurrence of patients with either one or multiple primary lesions in the small bowel gives food for thought. The clinical relevance of this question is debatable, as we did not find any reason that the presence of multifocal disease has a negative impact on survival. The question remains relevant, nevertheless, as such a difference might have a scientific basis. Is extensive multifocal disease a more aggressive type of SB-NEN? Could a clarification for this phenomenon be found in altered genes, similar to for example pancreatic NEN [30]?

One aspect that was not mentioned in this thesis is the health-related quality of life (HRQoL) of patients with SB-NEN. As previously mentioned, GEP-NENs are relatively indolent tumours with most patients living many years after diagnosis. During the lifetime of these patients, NEN-specific symptoms might develop (such as diarrhoea or abdominal pain), which in turn decreases HRQoL [31-33]. We have set-up an observational cohort study to investigate what course HRQoL takes during this time period.

In the past years, artificial intelligence (AI) has made its way into medicine, helping clinicians to predict clinical outcomes [34]. One of the most recent examples is its use to detect and prognosticate patients with coronavirus disease 2019 [35]. A quick search on Pubmed for papers about AI results in 75.431 papers, and indeed shows a very steep increase in the number of papers from 2010 onwards (Search 1). Adding the term “neuroendocrine” to the search, results in (only) 58 papers, a majority of which focuses on pancreatic NEN (Search 2). It is clear that there is a lot to gain in implementing AI projects in the field of NEN.

The common predictor of success of the three sections discussed above is the number of patients included in each study. Scientific success and relevance of studies within this specific field of surgical oncology is highly dependent on the availability of large datasets. This was also one of the main reasons why we decided to set up the International Study Group for Small bowel neuroendocrine neoplasm Surgery. I expect that ISGSS will have significant contributions to the field of NEN research, and above all, be of benefit for patients with SB-NEN.

SUPPLEMENTARY MATERIAL

Search 1 (Pubmed), search date 31-10-2021
Radiomics [tiab] OR deep learning [tiab] OR neural network [tiab] OR artificial intelligence [tiab]

Search 2 (Pubmed), search date 31-10-2021
Neuroendocrine [ti] AND (radiomics [tiab] OR deep learning [tiab] OR neural network [tiab] OR artificial intelligence [tiab])

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APPENDICES

NEDERLANDSE SAMENVATTING

Het algemene doel van dit proefschrift is het evalueren van verschillende aspecten van chirurgische behandeling van patiënten met neuroendocriene neoplasmata (NEN), waaronder de behandeling van levermetastasen, minimaal invasieve resectie van primaire tumoren van de dunne darm (SB-NEN), en de toepassing van fluorescentiegeleide chirurgie. Het eerste deel van dit proefschrift richt zich op de behandeling van neuroendocriene levermetastasen (NELM), epidemiologische en kenmerken van SB-NEN en beschrijft een nieuwe radiologisch en immunologisch verband voor mesenteriale fibrose. Het tweede deel evalueert de toepassing van minimaal invasieve chirurgie voor SB-NEN. Het derde deel onderzocht de toegevoegde waarde van fluorescentiegeleide chirurgie van SB-NEN, NELM en gastroenteropancreatische tumoren (GEP-NEN). Hieronder volgt een samenvatting van deze onderdelen en hun hoofdstukken.

Deel I: Kenmerken van neuroendocriene neoplasma van de dunne darm

Hoofdstuk 1 is een systematische review en meta-analyse en beoordeelt welke behandelingsmodaliteit van NELM in de langste totale overleving (overall survival, OS) resulteert. In totaal werden 712 studies gescreend op geschiktheid, waarvan 11 studies met 1108 patiënten werden geïncludeerd voor analyse. De NELM waren afkomstig van het pancreas, dunne darm of andere locatie in respectievelijk 662 (60%), 164 (15%) en 282 (25%) patiënten. Chirurgische resectie van NELM was geassocieerd met betere overlevingsresultaten in vergelijking met andere behandelingsmodaliteiten (bijv. embolisatie, chemotherapie). Resultaten van deze studie suggereren dat resectie van NELM resulteert in de langste OS voor patiënten met GEP-NEN, om deze reden moet dit per patiënt afgewogen worden.

Hoofdstuk 2 is een retrospectieve cohortstudie die epidemiologische, behandelings- en overlevingsresultaten analyseert van patiënten met graad 1 en 2 SB-NEN in Nederland tussen 2005-2015. De gegevens zijn geëxtraheerd uit de Nederlandse Kankerregistratie (NCR) en het Landelijk Netwerk en Registratie Histo- en Cytopathologie in Nederland (PALGA). In totaal werden 1132 patiënten geïncludeerd in de epidemiologische analyses, die een toename in incidentie lieten zien van 0.52 in 2005 tot 0.81 per 100.000 patiënten per jaar. Tweeëntachtig procent van de patiënten had een graad 1 tumor en 17% had graad 2. De meerderheid van de patiënten onderging een chirurgische resectie (86%), gevolgd door het gebruik van somatostatine-analogen (30% in totaal, 50% van de patiënten in stadium IV). Gegevens over overlevingsresultaten waren beschikbaar voor 975/1132 (86%) van de patiënten. De 5-jaars overleving was 75% voor stadium I-II, 75% voor stadium III en 57% voor stadium IV-ziekte. Uit dit onderzoek blijkt dat de incidentie van SB-NEN (zoals verwacht) stijgt, dat chirurgische resectie een hoeksteen is in de behandelstrategie en dat de overlevingskansen relatief hoog zijn.

In het cohort wat in **Hoofdstuk 2** wordt gepresenteerd had 62% van de patiënten lymfekliermetastasen. Een van de gevolgen van lymfekliermetastasen van SB-NEN is het ontstaan van mesenteriale fibrose. Aanwezigheid van (uitgebreide) mesenteriale fibrose resulteert in een moeilijkere chirurgische resectie, en dan met name als deze wordt uitgevoerd middels een minimaal invasieve benadering. **Hoofdstuk 3** is een exploratieve onderzoek

naar de associatie tussen IgG4-expressie in mesenteriale tumorafzettingen en de mate van mesenteriale fibrose die wordt gezien op preoperatieve beeldvorming. Om dit verband te kunnen onderzoeken hebben we een nieuw scoresysteem ontwikkeld om de omvang van mesenteriale fibrose te kwantificeren. IgG/IgG4-kleuringen werden uitgevoerd in formaline gefixeerd en in paraffine ingebed weefsel, en handmatig gescoord door de IgG/IgG4-verhouding te berekenen in het gebied met de meeste IgG4-positieve cellen. In totaal werden 14 patiënten geïncludeerd. Zoals verondersteld was de IgG/IgG4-ratio hoger in de groep met uitgebreidere mesenteriale fibrose. Ook werd een hogere IgG/IgG4-verhouding gezien bij graad 2 tumoren en bij stadium IV-ziekte. Deze bevindingen kunnen mogelijk leiden tot een nieuwe indicatie voor preoperatieve toediening van corticosteroïden (bijv. prednison) of de ontwikkeling en toepassing van (nieuwe) immunomodulerende therapieën.

Deel II: Minimaal invasieve chirurgie voor neuroendocriene neoplasma van de dunne darm

Een van de beperkingen van **Hoofdstuk 2** was de afwezigheid van gegevens over chirurgische uitkomsten van SB-NEN. Als gevolg van deze beperking hebben we in **Hoofdstuk 4** een systematische review en meta-analyse uitgevoerd om de postoperatieve morbiditeit en mortaliteit na chirurgische resectie van SB-NEN te beoordelen. Na screening van 2416 artikelen zijn er 13 in de meta-analyse geïncludeerd. Dit resulteerde in een onderzoekspopulatie van 1087 patiënten, waarvan 62% stadium IV-ziekte had en 76% een segmentale dunne darm resectie onderging. Gepoolde ernstige morbiditeit (Clavien-Dindo graad III-IV) was 7% (95% BI 4-13%, I2 = 71%), gepoolde 30 dagen mortaliteit was 2% (95% BI 1-3%, I2 = 0 %), de gepoolde mortaliteit na 90 dagen was 2% (95% BI 2-4%, I2 = 0%) en de gepoolde mortaliteit in het ziekenhuis was 1% (95% BI 0-2%, I2 = 0%). Ernstige morbiditeit (Clavien-Dindo graad III-IV) was lager in ziekenhuizen met een jaarlijks volume > 9 resecties, terwijl de 90-dagen mortaliteit juist hoger was. Toekomstig onderzoek zou zich moeten richten op het effect dat ziekenhuis- en chirurgvolume hebben op postoperatieve morbiditeit en mortaliteit.

Hoofdstuk 5 is een internationale enquête onder chirurgen die patiënten met SB-NEN behandelen. Het doel was om de internationale praktijk met betrekking tot minimaal invasieve chirurgie voor SB-NEN, de huidige mening en toekomstperspectieven ten aanzien van minimaal invasieve chirurgie voor SB-NEN in kaart te brengen, en om een internationale studiegroep op te zetten. Een anonieme enquête werd verspreid via internationale colorectale en (neuro-) endocriene tumor verenigingen. In totaal zijn 58 respondenten uit 20 landen geïncludeerd. Negenenzestig procent van de respondenten gaf aan (wel eens) minimaal invasieve chirurgie uit te voeren voor SB-NEN. Over het algemeen werd de voorkeur gegeven aan een minimaal invasieve benadering vanwege de peri-operatieve voordelen op korte termijn, terwijl een open benadering de voorkeur had i.v.m. een betere lymfadenectomie en tastzin. Ongeacht eerdere ervaring met geavanceerde minimaal invasieve chirurgie, gaf 52% van de respondenten aan mogelijk baat te hebben bij aanvullende training voor deze techniek. Naar aanleiding van deze studieresultaten hebben wij de *International Study Group for Small bowel neuroendocrine neoplasm Surgery* (www.ISGSS.org) opgericht om internationaal multicenter onderzoek te kunnen doen in grotere onderzoekspopulaties.

Hoofdstuk 6 is een retrospectieve cohortstudie die een institutionele verandering evalueerde van open naar laparoscopische resectie van SB-NEN, onafhankelijk van de locatie van mesenteriale lymfekliermetastasen. Patiënten die tussen 2003 en 2019 een chirurgische resectie van SB-NEN ondergingen werden gescreend op inclusie. Vierendertig patiënten werden geïncludeerd, van wie 11 (32%) een open resectie ondergingen en 23 (68%) een laparoscopische resectie. Er waren geen significante baseline- of pathologische verschillen. De primaire tumor werd pre-operatief geïdentificeerd bij 95% van de patiënten in de laparoscopische groep en 36% van de open groep (P <0.001). De mediane duur van het ziekenhuisverblijf was 4 dagen in de laparoscopie groep en 8 dagen in de open groep (P = 0.036). Er waren geen verschillen in postoperatieve morbiditeit of mortaliteit. Het lijkt er dus op dat laparoscopische resectie van SB-NEN, zoals uitgevoerd in ons tertiair verwijzingscentrum, resulteert in vergelijkbare pathologische uitkomsten en een kortere ziekenhuisopname. Toekomstig onderzoek zou zich moeten richten op de lange termijn oncologische uitkomsten van laparoscopische resectie, en hoe het ziekenhuis- of chirurgvolume dit beïnvloedt.

In **Hoofdstuk 6** konden alleen peri-operatieve korte termijn uitkomsten worden vergeleken tussen laparoscopische en open resectie van SB-NEN. Gegevens over de overleving op lange termijn ontbraken echter in deze studie. Om deze reden hebben we in **Hoofdstuk 7** een retrospectieve cohortstudie uitgevoerd om de overlevingsresultaten op lange termijn te beoordelen na laparoscopische resectie van SB-NEN. Gegevens van patiënten die tussen 2005 en 2015 een chirurgische resectie hebben ondergaan, zijn verzameld uit de NKR en PALGA database. In totaal werden 482 patiënten geïncludeerd, van wie 342 (71%) een open resectie en 140 (29%) een laparoscopische resectie ondergingen. Histopathologisch onderzoek toonde aan dat bij patiënten in de open resectiegroep significant meer multifocale tumoren waren verwijderd, meer pN2-lymfeklieren en stadium IV-ziekte aanwezig was. Onafhankelijke voorspellers van kortere OS waren leeftijd boven de 60 jaar en stadium IV ziekte, terwijl een laparoscopische resectie een langere OS voorspelde. Tumorgraad, resectiemarges en aanwezigheid van multifocale tumoren hadden geen invloed op de OS. Toekomstige studies met meer gedetailleerde peri-operatieve gegevens zijn nodig om de oncologische veiligheid van een laparoscopische SB-NEN resectie te bevestigen.

Deel III: Fluorescentiegeleide chirurgie van neuroendocriene neoplasmata

Hoofdstuk 6 en 7 hebben laten zien dat een laparoscopische resectie van SB-NEN technisch haalbaar is en geen aanleiding geeft tot bezorgdheid over oncologische veiligheid. Zoals eerder beschreven in **Hoofdstuk 2 en 3**, maakt de aanwezigheid van mesenteriale metastasen (en daaraan gerelateerde fibrose) een chirurgische resectie moeilijker. Daarom hebben we in **Hoofdstuk 8** een exploratieve studie uitgevoerd om de potentiële waarde van intra-operatieve fluorescentie angiografie met behulp van indocyanine groen (ICG) tijdens chirurgische resectie van SB-NEN te evalueren. We veronderstelden dat het gebruik van ICG van toegevoegde waarde zou zijn, vanwege de nabijheid van mesenteriale metastasen tot mesenteriale vaten. Vanwege het exploratieve karakter van de studie hebben we pragmatisch 10 patiënten geïncludeerd. Fluorescentie angiografie werd uitgevoerd na mobilisatie van

de dunne darm en markering van het transsectieniveau door de chirurg. Hierna werd een bolusinfusie van ICG met een dosis van 0.1 mg/kg toegediend. Verandering in management werd gedefinieerd als het uitvoeren van de transsectie op een ander niveau dan eerder door de chirurg was geselecteerd. Het gebruik van fluorescentie angiografie leidde bij acht patiënten tot verandering in het management. Vier patiënten ondergingen een uitgebreidere resectie (3 tot 25 cm), en bij vier patiënten (5 tot 35 cm) kon een gedeelte van dunne darm worden gespaard. De mediane postoperatieve opnameduur was 4 dagen, zonder ernstige complicaties (Clavien-Dindo graad 3 of hoger). Hoewel deze resultaten veelbelovend zijn, moet toekomstig onderzoek met grotere cohorten dit bevestigen, met een bijzondere focus op de effectiviteit van een spaarzame resectie.

Zoals eerder beschreven in **Hoofdstuk 1**, resulteert chirurgische resectie van NELM in de langste OS, en volledige verwijdering van NELM draagt bij aan gunstige oncologische uitkomsten. **Hoofdstuk 9** is een proof-of-concept studie die de haalbaarheid van fluorescentiegeleide chirurgie voor NELM met behulp van ICG evalueerde. Onze hypothese was dat het van toegevoegde waarde zou zijn vanwege de hypervasculaire eigenschappen van NELM. Patiënten kregen ongeveer 24 uur voor de operatie een bolusinfusie van ICG met een dosis van 10 mg. Om het ICG-signaal te vergelijken, werden patiënten met colorectale levermetastasen (CRLM) geïncludeerd. Tijdens de onderzoeksperiode (2019-2021) werden drie patiënten met NELM geïncludeerd, waarvan er één uiteindelijk geen resectie kreeg vanwege onvoorziene uitgebreide bilobaire metastasen. Drie patiënten met CRLM werden geïncludeerd om het ICG-signaal te vergelijken. Opname van ICG was aanwezig bij alle patiënten met NELM en liet ofwel een randvormig of homogene opname zien. Er waren geen ernstige complicaties (Clavien-Dindo graad 3 of hoger) gerelateerd aan het gebruik van ICG voor dit doel. Toekomstige studies, waaronder grote cohorten, zijn nodig om de effectiviteit van ICG-geleide resectie van NELM te beoordelen, vooral in termen van het bereiken van radicale resectiemarges.

De vorige hoofdstukken hebben aangetoond dat chirurgische resectie van NEN belangrijk is om goede overlevingsresultaten te bewerkstelligen (**Hoofdstuk 1, Hoofdstuk 2 en Hoofdstuk 7**), terwijl er enkele technische uitdagingen zijn: vanwege de grootte of nabijheid van mesenteriale metastasen (**Hoofdstuk 6**), aanwezigheid van mesenteriale fibrose (**Hoofdstuk 3**), of door een minimaal invasieve benadering te gebruiken (**Hoofdstuk 5**). Nauwkeurige identificatie van tumorweefsel tijdens de operatie zou dus van pas komen tijdens de operatie. **Hoofdstuk 10** is het onderzoeksprotocol van de PHOTON trial, waarin we een fluorescente tracer ontwikkelen voor intra-operatieve identificatie van GEP-NEN. De PHOTON-studie omvat een preklinische veiligheidsbeoordeling van PHT001, een nieuwe, op somatostatine receptor type 2 gerichte fluorescente tracer, en een klinische studie om de eerste gegevens bij mensen te verkrijgen. Preklinische veiligheidsbeoordeling zal worden uitgevoerd conform ICH M3(R2), middels een microdosing studie (totale dosis ≤ 100 microgram). Dit omvat een uitgebreide studie met enkelvoudige doses in muizen, en een klinische fase 0 studie bestaande uit 3-5 patiënten. Dit zal nieuwe data opleveren waarmee we fase I/II studies kunnen uitvoeren.

Samenvatting van onderzoeksvragen en hoofdbevindingen

Chapter	Research questions
1	Welke behandeling resulteert in de langste totale overleving voor patiënten met neuroendocriene levermetastasen? *Chirurgische resectie van neuroendocriene levermetastasen resulteert in de langste totale overleving, in vergelijking tot geen resectie, chemotherapie, embolisatie en levertransplantatie.*
2	Wat zijn de epidemiologische, behandel en overlevings karakteristieken van graad 1 en 2 dunne darm neuroendocriene tumoren? *De incidentie van dunne darm neuroendocriene tumoren rees tot 0.81 per 100.000 personen per jaar in 2015, de behandeling geschied vaak middels een chirurgie resectie (in 86% van de patiënten). Vijf-jaar totale overleving voor stadium I-II en stadium III ziekte is 75%, en 57% voor stadium IV ziekte.*
3	Wat is de relatie tussen immunoglobuline G4 expressie en de mate van mesenteriale fibrose in dunne darm neuroendocriene tumoren? *Het IgG/IgG4 is hoger in patiënten met uitgebreidere mesenteriale fibrose, er is echter geen statistisch significante relatie gevonden.*
4	Wat is de morbiditeit en mortaliteit na chirurgische resectie van dunne darm neuroendocriene tumoren, en wat is het effect van het ziekenhuis volume hierop? *Ernstige morbiditeit en 30-/90-dagen mortaliteit na chirurgische resectie van dunne darm neuroendocriene tumoren is respectievelijk 7% en 2%. Ernstige morbiditeit kwam minder vaak voor in hoog-volume ziekenhuizen. Het omgekeerde werd gezien voor mortaliteit.*
5	Wat zijn op dit moment de internationale gebruiken en meningen over minimaal invasieve resectie van dunne darm neuroendocriene tumoren? *In het onderzochte cohort gaf 69% van de respondenten aan minimaal invasieve resectie van dunne darm neuroendocriene tumoren uit te voeren. De voorkeur voor deze benadering had te maken met peri-operatieve voordelen, terwijl een open benadering de voorkeur had in verband met zorgen over de oncologische veiligheid (van de minimaal invasieve benadering), en om de gehele dunne darm te kunnen palperen.*
6	Wat zijn de peri-operatieve verschillen tussen patiënten die een laparoscopische of open resectie van een dunne darm neuroendocriene tumor ondergaan? *Patiënten die een laparoscopische of open resectie ondergingen hadden vergelijkbare baseline karakteristieken. Ziekenhuis opname was korter in de laparoscopie groep. Post-operatieve morbiditeit en pathologische uitkomsten waren vergelijkbaar.*

7 Wat is de meest voorkomende benadering voor chirurgische resectie van dunne darm neuroendocriene tumoren in Nederland?
In dit cohort werd in 71% van de patiënten een open resectie uitgevoerd, en in 29% van de patiënten een laparoscopische resectie. Patiënten die een open resectie ondergingen hadden vaker multifocale tumoren, pN2 lymfeklieren en stadium IV ziekte.

8 Wat is de waarde van fluorescentie angiografie middels indocyanine groen gedurende chirurgische resectie van dunne darm neuroendocriene tumoren?
Fluorescentie angiografie middels indocyanine groen kan verschillen in vascularisatie na mobilisatie van de dunne darm zichtbaar maken. Dit resulteert in verandering van het beleid, waardoor er meer of minder darm gereseceerd kan worden.

9 Wat is de waarde van fluorescentie geleide resectie van neuroendocriene levermetastasen middels indocyanine?
Indocyanine groen accumuleert in neuroendocriene levermetastases, waardoor het helpt om tumorweefsel van gezond leverweefsel te onderscheiden. Opname door neuroendocriene levermetastasen ziet er ofwel ringvormig uit of als homogene opname.

10 Is PHT001 veilig voor gebruik in mensen, en kan het tumorgrenzen goed onderscheiden van gezond weefsel?
Op dit moment hebben we nog geen antwoord op deze onderzoeksvraag. We verwachten dat gebruik veilig zal zijn en goed zal presenteren in de klinische studies.

PhD PORTFOLIO

Name PhD student: Enes Kaçmaz
PhD period: November 2019 – March 2022
Promotores: prof. dr. E.J.M. Nieveen van Dijkum, prof. dr. P.J. Tanis
Copromotor: dr. A.F. Engelsman, dr. H.J. Klümpen

PhD training	Year	ECTS
Courses		
BROK	2021	1.5
Advanced topics in biostatistics	2021	2.1
Data visualization	2021	0.1
FameLab	2020	1.0
Writing e learning	2020	1.5
Didactical skills	2020	0.4
MRI cursus	2020	1.0
Practical biostatistics	2019	1.4
Vascular Surgery, Geneva University Hospitals	2019	0.5
Seminars, worshops and master classes		
Weekly department research meetings	2019-2021	2.0
Monthly department journal club	2019-2021	1.0
Colorectal surgery research meetings	2019-2021	1.0
Endocrine surgery research meetings	2020-2021	1.0
Presentations		
European Society of Endocrine Sugery, Athens,	2022	0,5
European Neuroendocrine Tumor Society, Barcelona	2022	0.5
International Hepato-Pancreato Biliary Association, New York	2022	0.5
European Society of Surgical Oncology, Lisbon	2021	0.5
Wetenschapsdag Chirurgie Regio 1&2, Amsterdam	2021	0.5
Neuroendocrine Tumor Research Foundation, virtual	2021	0.5
Dutch Digestive Days, virtual	2021	0.5
Neuroendocrine Tumor Research Foundation, virtual	2020	0.5
European Neuroendocrine Tumor Society, virtual	2020	0.5
European Neuroendocrine Tumor Society, Barcelona	2019	0.5
European Society of Surgical Research, Geneva	2019	0.5
Science Exchange Day, Amsterdam	2019	0.5
Symposium Experimenteel Onderzoek Heelkundige Specialismen, Amsterdam	2019	0.5

PhD training	Year	ECTS
Parameters of esteem		
Cancer Center Amsterdam research grant, €160k	2020	-
Amsterdam Gastroenterology Endocrinology Metabolism grant, €15k	2020	-
MD/PhD Scholarship, Amsterdam UMC, University of Amsterdam, €108k	2019	-
Travel grant, Amsterdam University Fund	2019	-
Travel grant, Collegium Chirurgicum Neerlandicum	2019	-
Teaching and supervising		
Journal club for bachelor students	2020	1.0
Devano Boesewinkel, bachelor thesis	2020	1.0
Yara Riethoven, bachelor thesis	2020	1.0
Astrid Boodt, master thesis	2021	2.0
Felix Hers, master thesis	2021	2.0

LIST OF PUBLICATIONS

THIS THESIS

1. **Kaçmaz E**, Azhdarinia A, Tanis PJ *et al.* Tumour-specific fluorescence-guided surgery for gastroenteropancreatic neuroendocrine neoplasms using PHT001: a phase 0, open-label, single-arm, microdosing study – PHOTON-trial
   Submitted

2. **Kaçmaz E**, Slooter MD, Tanis PJ *et al.* Indocyanine green fluorescence guided resection of neuroendocrine liver metastases: a proof-of-concept study
   Submitted

3. **Kaçmaz E**, Engelsman AF, Koppes JCC *et al.* IgG4 expression in small bowel neuroendocrine neoplasms with radiological signs of mesenteric fibrosis and the introduction of the mesenteric fibrosis score; a new tool for quantifying mesenteric fibrosis
   Submitted

4. **Kaçmaz E**, Engelsman AF, Bemelman WA *et al.* International survey on opinions and use of minimally invasive surgery in small bowel neuroendocrine neoplasms
   European Journal of Surgical Oncology 2021

5. **Kaçmaz E**, Chen JW, Tanis PJ *et al.* Post-operative morbidity and mortality after surgical resection of small bowel neuroendocrine neoplasms: a systematic review and meta-analysis
   Journal of Neuroendocrinology 2021

6. **Kaçmaz E**, Farina Sarasqueta A, van Eeden S *et al.* Update on incidence, prevalence, treatment and survival of patients with small bowel neuroendocrine neoplasms in the Netherlands.
   World Journal of Surgery 2021

7. **Kaçmaz E**, Klümpen HJ, Bemelman WA *et al.* Evaluating nationwide application of minimally invasive surgery for small bowel neuroendocrine neoplasms and the impact on survival.
   World Journal of Surgery 2021

8. **Kaçmaz E**, Slooter MD, Nieveen van Dijkum EJM *et al.* Fluorescence angiography guided resection of small bowel neuroendocrine neoplasms with mesenteric lymph node metastases.
   European Journal of Surgical Oncology 2020

9. **Kaçmaz E**, van Eeden S, Koppes JCC *et al.* Value of laparoscopy for resection of small bowel neuroendocrine neoplasms including central mesenteric lymphadenectomy.
   Diseases of the Colon & Rectum 2020

10. **Kaçmaz E**, Heidsma CM, Besselink MG *et al.* Treatment of liver metastases from midgut neuroendocrine tumours: a systematic review and meta-analysis.
    Journal of Clinical Medicine 2019

OTHER PUBLICATIONS

1. **Kaçmaz E**, de Betue CTI, Slooter MD *et al.* Full laparoscopic D3 lymphadenectomy for central mesenteric lymph node metastases from a small bowel neuroendocrine neoplasm – a video vignette.
   Colorectal Disease 2020

2. **Kaçmaz E**, Zwart MJW, Engelsman AF *et al.* Robotic enucleation of an insulinoma in the pancreatic head: a video vignette.
   Journal of Visual Experiments 2020

3. Slooter MD, Blok RD, **Kaçmaz E** *et al.* Fluorescence angiography of a pedicled omentoplasty for pelvic filling - a video vignette.
   Colorectal Disease 2019

4. Blok RD, **Kaçmaz E**, Hompes R *et al.* Gluteal turnover flap for perineal reconstruction following abdominoperineal resection for rectal cancer - a video vignette.
   Colorectal Disease 2019

5. **Kaçmaz E**, Slooter MD, Nieveen van Dijkum EJM *et al.* Laparoscopic assisted central mesenteric lymph node dissection with bowel sparing resection of small bowel neuroendocrine tumours using fluorescence angiography – a video vignette.
   Colorectal Disease 2019

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Lieve **mam** en **pap**, ben bildiğimden itibaren ablamın ve benim okuyup, güzel bir iş sahibi olmamızı istediniz. Bizim okul hayatımıza verdiğiniz önem için çok çok teşekkür ederim. Şu an elde ettiğimiz başarıları sizin gayretler ve desteğinize borçluyuz. Bu yaşımda dilini, kültürünü ve geleneklerini bilmediğim bir yabancı ülkeye gidip, orda bir hayat kurmanın ne kadar zorlayıcı olduğunu tahmin bile edemiyorum. Bunu böylesine başardığınız için sizinle sonsuz gurur duyuruyorum. Sevgi dolu bir yuva kurdunuz ve sayenizde ikimizde güzel bir iş sahibi olduk :) Bu yazdığım tezi size armağan ediyorum.

ABOUT THE AUTHOR

Enes Kaçmaz was born in Amsterdam on the 3rd of June 1997. After finishing pre-university education, he studied applied mathematics at the Delft University of Technology for a couple of months. Hereafter, he started studying medicine at the University of Amsterdam in 2016.

He began as a student researcher at the Department of Surgery in 2017 under supervision of prof. dr. Els J.M. Nieveen van Dijkum and dr. Anton F. Engelsman. In 2019, Enes received an MD/PhD Scholarship from the AMC/UvA, which enabled him to conduct research and write the current PhD thesis. After obtaining his PhD degree, Enes will continue as a postdoctoral researcher at the Department of Surgery, with a special focus on neuroendocrine neoplasms. He recently started with clinical internships and expects to obtain his medical degree at the end of 2023.

Besides his academic and medical ambitions, he aspires to obtain his pilot license and set-up a charity to provide medical care and equipment in developing countries. In his spare time, he enjoys oil painting and sports, but above all, spending time with family and friends.