Small Intestinal Neuroendocrine Tumor

A Rare Malignancy with Favorable Outcome

OLOV NORLÉN
Abstract

Small intestinal neuroendocrine tumor (SI-NET) is the most common small bowel tumor in Europe and USA, with an annual incidence of around 0.3-1.3/100000 persons. SI-NETs are the most common type of gastroenteropancreatic NETs (GEP-NETs), and they are known for their ability to produce hormones such as tachykinins and serotonin, as well as for their favorable long-term prognosis in comparison to gastrointestinal adenocarcinoma. The overall aim of the thesis was to investigate unknown or unclear aspects of SI-NET disease, in connection with prognosis, treatment and follow-up. Paper I confirmed several known negative prognostic factors and also showed, for the first time, that para-aortal lymph node metastases and peritoneal carcinomatosis were associated with worse survival by multivariable analyses. Locoregional surgery was associated with a low post-operative mortality, and a prolonged long-term survival by multivariable analysis. In Paper II we continued to investigate peritoneal carcinomatosis and found it be a risk factor not only for death, but also for emergency re-surgery. Furthermore, genetic analyses of samples from primary tumors in patients with and without peritoneal carcinomatosis showed a difference in the DNA between these two groups. In Paper III the outcome after liver surgery and/or radiofrequency ablation of liver metastases was investigated. To summarize, no difference in survival was seen in patients treated with surgery/radiofrequency ablation in comparison with matched controls. However, a superior radiological response of liver metastases and lower U-5-HIAA values were seen in patients subjected to liver surgery and/or radiofrequency ablation compared to matched controls. Paper IV compared ultrasonography, computed tomography and 11C-5HTP-PET in the follow-up after radiofrequency ablation of NET liver metastases. The study concluded that 11C-5HTP-PET depicted all residual tumors after RFA and that it, if used, should be combined with computed tomography for easier interpretation, as RFA areas are not clearly distinguishable with 11C-5HTP-PET alone. Paper V studied gallstone complications after somatostatin analog treatment in SI-NET patients, and concluded that there was a rather high risk to be subjected to a cholecystectomy due to biliary colic, cholecystitis, cholangitis or pancreatitis after primary surgery in somatostatin analog treated patients.

Keywords: Neuroendocrine tumor, peritoneal carcinomatosis, single nucleotide polymorphism array, liver metastases, radiofrequency ablation, liver surgery, positron emission tomography, somatostatin analogs, cholecystectomy

Olov Norlén, Uppsala University, Department of Surgical Sciences, Endocrine Surgery, Akademiska sjukhuset ing 70 1 tr, SE-751 85 Uppsala, Sweden.

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“Hard work does not make you tired,
The thing that makes you tired, is sloth”

Freely translated from the, in Swedish,
originally proclaimed:

"Arbete tröttar inte-
Det som tröttar är lättja”

Ulf Haglund, Professor Emeritus of Surgery, Uppsala University

To my family…
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


*Both authors contributed equally to the study. Reprints were made with permission from the respective publishers. Paper I and V: Springer, Paper IV: Elsevier.*
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<th>Definition</th>
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<tbody>
<tr>
<td>PRRT</td>
<td>Peptide receptor radionuclide therapy</td>
</tr>
<tr>
<td>FDG</td>
<td>Fluorodeoxy-glucose</td>
</tr>
<tr>
<td>DOTATOC</td>
<td>DOTA-d-Phe(1)-Tyr(3)-octreotide</td>
</tr>
<tr>
<td>LAR</td>
<td>Long-acting release</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence Interval</td>
</tr>
<tr>
<td>HR</td>
<td>Hazard Ratio</td>
</tr>
<tr>
<td>EHR</td>
<td>Excess Hazard Ratio</td>
</tr>
<tr>
<td>RT</td>
<td>Residual tumor</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleotide acid</td>
</tr>
<tr>
<td>SNP</td>
<td>Single nucleotide polymorphism</td>
</tr>
<tr>
<td>CGH</td>
<td>Comparative genome hybridization</td>
</tr>
<tr>
<td>CNV</td>
<td>Copy number variation</td>
</tr>
<tr>
<td>LOH</td>
<td>Loss of heterozygosity</td>
</tr>
<tr>
<td>CNN-LOH</td>
<td>Copy number neutral loss of heterozygosity</td>
</tr>
<tr>
<td>BAF</td>
<td>B-allele frequency</td>
</tr>
<tr>
<td>LRR</td>
<td>Log R ratio</td>
</tr>
</tbody>
</table>
1 Introduction

This thesis will focus specifically on Small intestinal neuroendocrine tumors, prognostic factors in general, and possible benefits of loco-regional surgery, gallbladder surgery and radiofrequency ablation and/or surgery of liver metastases. Genetic aberrations in tumor samples in patients with different clinical characteristics and imaging before and after radiofrequency ablation of liver metastases will also be studied.
2 Background

2.1 The beginning

In 1907, the German physician Obendorfer described several cases of multiple small tumors in the ileum (1). This was by many contemporaries believed to be the first sighting of small intestinal neuroendocrine tumors (SI-NET). In retrospect, it is clear that these tumors had been described in case reports several decades earlier but nobody had given them a name (1). As Obendorfer recognized these ileal tumors to be histopathologically different from adenocarcinomas, he named them *karzinoide tumoren* (carcinoid tumors or carcinoma-like tumors) (1). Ten years earlier, in 1897, Kulchitsky had discovered the enterochromaffin cell (EC-cell) and in 1914, Masson put two and two together (1). Masson recognized both the EC-cells and the carcinoid tumors to have endocrine features, and he stained them with his silver technique to show their similarity (1). Following this, Friedrich Feyrter’s and Anthony Pearse’s studies mounted the concept of a diffuse endocrine cell system (1). All the tumors originating from this system were named carcinoids and subsequently the ileal tumors firstly described were often called classical carcinoids. The carcinoids are distinguished by the ability to take up amine precursors in similarity with cells in the central nervous system (1). This led to the thought that carcinoids were embryologically derived from the neural crest and the common term neuroendocrine tumor (NET) was proposed, and although that they have later been shown to be of primarily endodermal origin, the term NET has prevailed (1, 2). Thus, tumors originating from the neuroendocrine system in the gastrointestinal tract or pancreas are after the WHO classification 2010 called gastroenteropancreatic neuroendocrine tumors (GEP-NETs) and the term carcinoid will probably become obsolete (2).
2.2 Nomenclature, staging and grading

As symptoms, prognostics, and genetics differ in tumors originating within the gastroenteropancreatic neuroendocrine system, several subdivisions have been attempted for clinical use. A system adopted in 1963 divided the GEP-NETs by embryological origin as foregut (lungs, esophagus, stomach, upper duodenum and pancreas), midgut (lower duodenum, jejunum, ileum and proximal colon) or hindgut (from the distal transverse colon until anus) (3).

The world health organization (WHO) has also made several proposals for subdivision of GEP-NET dependent on their proliferation rate and metastasizing potential, and they have been used clinically with varying degree. The WHO systems are not organ specific and in an effort to make a universal and organ specific TNM-staging system the European Neuroendocrine Tumor Society (ENETS) published a tumor-node-metastasis (TNM) staging classification system of foregut NETs in 2006 and for midgut and hindgut NETs in 2007 (4, 5). This classification system divides tumors depending of organ of origin, and also describes the extent of tumor invasion and dissemination (Table A and B, TNM for SI-NET).

Table A. TNM Clinical Classification of SI- NET (4).

<table>
<thead>
<tr>
<th>T – Primary Tumor*</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>TX</td>
<td>Primary tumor cannot be assessed</td>
</tr>
<tr>
<td>T0</td>
<td>No evidence of primary tumor</td>
</tr>
<tr>
<td>T1</td>
<td>Tumor invades lamina propria or submucosa and is no greater than 1 cm</td>
</tr>
<tr>
<td>T2</td>
<td>Tumor invades muscularis propria or is greater than 1 cm in size</td>
</tr>
<tr>
<td>T3</td>
<td>Tumor invades subserosa</td>
</tr>
<tr>
<td>T4</td>
<td>Tumor perforates visceral peritoneum (serosa) or invades other organs or adjacent structures</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>N – Regional Lymph Nodes</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>NX</td>
<td>Regional lymph nodes cannot be assessed</td>
</tr>
<tr>
<td>N0</td>
<td>No regional lymph node metastasis</td>
</tr>
<tr>
<td>N1</td>
<td>Regional lymph node metastasis</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>M – Distant Metastases</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>M0</td>
<td>No distant metastasis</td>
</tr>
<tr>
<td>M1</td>
<td>Distant metastasis</td>
</tr>
</tbody>
</table>
Table B. TNM Stage of SI-NET (4).

<table>
<thead>
<tr>
<th>Disease stage</th>
<th>T-primary tumor</th>
<th>N-regional nodes*</th>
<th>M-distant metastases**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I-II</td>
<td>T1-3</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>Stage IIIa</td>
<td>T4</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>Stage IIIb</td>
<td>Any T</td>
<td>N1</td>
<td>M0</td>
</tr>
<tr>
<td>Stage IV</td>
<td>Any T</td>
<td>Any N</td>
<td>M1</td>
</tr>
</tbody>
</table>

*Regional nodes = mesenteric lymph nodes; **distant metastases = metastasis at any distant site (including non-regional lymph nodes)

Proliferation is an important prognostic factor in NET and ENETS also proposed a histopathological grading system depending on tumor proliferation (Table C)(4). In the ENETS system grade 1 is denoted \( \leq 2\% \) and grade 2; 3-20\%, a mathematical discrepancy which may confuse users. We have chosen to establish grade 1 as \(<3\%\) throughout this thesis, in accordance with the forthcoming national Swedish NET care program. Patients who exhibit proliferation index above 20\% (grade 3) are rare in NET originating in the small bowel. They are referred to as neuroendocrine carcinomas and generally treated as a separate entity, since prognosis and treatment is fundamentally different from the grade 1-2 tumors.

Table C. ENETS Proliferation Grade of SI-NET (4).

<table>
<thead>
<tr>
<th>Proliferation grade</th>
<th>Ki-67 index (%)*</th>
<th>Mitoses/10HPF**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1</td>
<td>(&lt;3%***)</td>
<td>(&lt;2)mitoses</td>
</tr>
<tr>
<td>Grade 2</td>
<td>3-20% OR</td>
<td>2-20 mitoses</td>
</tr>
<tr>
<td>Grade 3</td>
<td>(&gt;20%) OR</td>
<td>(&gt;20) mitoses</td>
</tr>
</tbody>
</table>

*MIB-1 antibody; % of 2000 tumor cells in areas of highest nuclear labeling.** 10 HPF (High Power Field)=2 mm², at least 40 fields (at 40Å–magnification) evaluated in areas of highest mitotic density.*** In the consensus by Rindi et al it is noted \( \leq 2\% \) instead of \(<3\%\).

2.3 Incidence

SI-NETs are the most common small bowel tumors in the western world, ranging from 27-44\% of all small bowel tumors (6, 7). In the SEER database, regarding incidence, the SI-NETs account for around 19\% of all GI-NETs (8). However, in an autopsy study in a Swedish population 1962-70, the SI-NETs consisted of as many as 73\% of all GI-NETs (9). The same
autopsy study revealed a higher annual frequency of SI-NET, 5.5/100 000, as compared to the clinical incidence of most registry studies, that ranged from 0.1-0.6/100 000 during 1960-75 (7-11). After this, most registry studies report an increase in the incidence of SI-NET. The last decade, registries (SEER, English- and Norwegian- and regional Swedish- cancer registry), report a clinical incidence of 0.3-1.33/100 000 (7, 8, 10, 12). All of these incidence figures are lower than in the above described autopsy study which may be explained by the fact that most of the tumors in the autopsy study had no clinical relevance, as over 90% were discovered post mortem, and only 15% had metastasized (9). In conclusion, the rising incidence of SI-NET might be explained by a combination of improved diagnostic techniques, improved reporting to different registries, and possibly also a true rise in SI-NET incidence. Another imaginable explanation for the increase in clinical incidence might be a greater tendency for metastatic spread during the latter decades in previously dormant SI-NETs.

2.4 Diagnosis and disease progression

SI-NETs originate from the EC-cells in the small intestinal mucosa. The primary tumor is often small but SI-NETs are, irrespective of primary tumor size, nonetheless in a majority of cases spread to mesenteric lymph nodes at time of diagnosis and many patients also display liver metastases (8, 13, 14). The most common symptom at diagnosis is abdominal pain (40-60%), followed by intestinal obstruction (35-50%), diarrhea (20-40%), weight loss (10-35%) and flushing (10-30%).(10) More uncommon presentations include GI-hemorrhage (5-14%), bronchial constriction (2-10%) and carcinoid heart disease (<5%) (10, 15). These symptoms describe the progression of the SI-NET disease very well. Substances secreted by SI-NET cells (among others, Serotonin, tachykinins and/or growth factors) somehow trigger fibrosis around tumor cells and at distant sites although the underlying mechanisms remain unclear (16). The fibrosis commonly occurs in the mesentery, which together with tumor growth often leads to retraction of the mesentery, resulting in kinking of the small bowel (intestinal obstruction, abdominal pain, weight loss) or compression of the mesenteric veins (venous ischemia, abdominal pain, weight loss) (16, 17). In select cases the fibrosis extends to the retroperitoneal space, and occasionally results in compression of the ureter (hydronephrosis) (16).

Metastatic progression of the disease regularly starts with metastases to the mesenteric lymph nodes that subsequently are followed by liver metastasis. Peritoneal carcinomatosis (PC), defined as spread of tumor deposits within the peritoneal cavity, (primary tumor and lymph nodes excluded) is reported in 19-33% of patients with SI-NET at specialized centers (18, 19). When metastases have spread to the liver, retroperitoneal
lymph nodes or ovaries, the first passage metabolism of the liver can no longer dissolve substances produced by the tumor cells (20, 21). The carcinoid syndrome becomes evident and symptoms caused by circulating serotonin and tachykinins such as flush and bronchoconstriction emerges. Also, in cases of an advanced carcinoid syndrome, fibrosis of the myocardium and the tricuspid valve ensues and leads to right-sided heart failure (22). The reason for the left side of the heart to remain unaffected is believed to be clearance of fibrosis-inducing substances in the lung (22, 23). Metastases at extra-abdominal sites such as bones, lungs, mediastinum, lymph nodes, breasts, brain, orbita and skin are also seen throughout disease progression although these patients generally also exhibit liver metastases (19, 24).

2.5 Diagnostic implications of hormone production

Clinical implications of serotonin production include diagnostics and disease monitoring of SI-NET. SI-NETs cells absorb amine precursors and decarboxylate them to produce, among others, catecholamine and serotonin. The uptake of 5-hydroxytryptophan (5-HTP, precursor of serotonin) and L-Dihydroxyphenylalanine (L-DOPA, precursor of dopamine, epinephrine etc.) may be used to depict NET with positron emission tomography (PET), and metabolites of serotonin (Urine 5-hydroxyindoleaceticacid, U-5-HIAA) may be measured in urine samples to facilitate to make the diagnosis and to evaluate treatment effect (25, 26). Other peptides that are possible to measure for diagnosis and evaluation of SI-NET are granins, or more specifically Chromogranin A (CgA). Granins are a family of secretory proteins that are stored in most neuroendocrine cells and plasma CgA levels are elevated in most SI-NET patients. CgA correlates to disease dissemination and can be followed to assess progression or treatment effect (27-29).
2.6 Survival

Survival data for SI-NET mainly originate from registry studies, where the amount of clinical data is low, or from single institutional reports, where the patient count is restricted and survival may be skewed by referral bias. SI-NETs are known for their relatively advantageous survival (6, 8, 12, 30-38). The 5-year overall survival (OS), relative survival (RS) and disease specific survival (DSS) rates in registry studies are shown in Table D (6, 8, 12, 30-38). The corresponding figures, for the single center studies that included over 100 subjects, are noted in Table E (15, 39-41).

Table D. Registry-based studies with over 100 subjects.

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>SI-NET Patients (n)</th>
<th>Overall</th>
<th>Relative</th>
<th>Disease-specific</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilimoria(6)</td>
<td>NCDB, USA 1985-2000</td>
<td>12732</td>
<td>65</td>
<td>79</td>
<td>-</td>
</tr>
<tr>
<td>Modlin(35), Maggard(34)</td>
<td>SEER, USA 1973-1997</td>
<td>≈ 2800</td>
<td>63</td>
<td>55</td>
<td>76</td>
</tr>
<tr>
<td>Zar(36)</td>
<td>Sweden 1960-2000</td>
<td>2437</td>
<td>56</td>
<td>67</td>
<td>87</td>
</tr>
<tr>
<td>Perez(42)</td>
<td>Florida, USA 1981-2000</td>
<td>1428</td>
<td>37</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lepage(33)</td>
<td>UK 1986-1999</td>
<td>1154</td>
<td>-</td>
<td>59</td>
<td>-</td>
</tr>
<tr>
<td>Pashayan(32)</td>
<td>UK 1971-1990</td>
<td>≈ 800</td>
<td>-</td>
<td>46</td>
<td>-</td>
</tr>
<tr>
<td>Hauso(12)</td>
<td>Norway 1993-2004</td>
<td>518</td>
<td>59</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Godwin(31)</td>
<td>ERG, USA 1950-1969</td>
<td>367</td>
<td>-</td>
<td>54</td>
<td>-</td>
</tr>
<tr>
<td>Garcia-Cabanero(37)</td>
<td>Spain 2001-2008</td>
<td>126</td>
<td>83</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Landerholm(13)</td>
<td>Sweden 1960-2005</td>
<td>135</td>
<td>61</td>
<td>-</td>
<td>75</td>
</tr>
<tr>
<td>Lepage(38)</td>
<td>Burgundy, France 1976-2001</td>
<td>102</td>
<td>46</td>
<td>57</td>
<td>-</td>
</tr>
</tbody>
</table>
Table E. Single-center studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>SI-NET Patients (n)</th>
<th>5-year survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Overall</td>
<td>Relative</td>
</tr>
<tr>
<td>Hellman(40)</td>
<td>Uppsala, Sweden 1975-1997</td>
<td>314</td>
<td>67</td>
</tr>
<tr>
<td>Bergestuen(15)</td>
<td>Oslo, Norway 1983-2007</td>
<td>258</td>
<td>72</td>
</tr>
<tr>
<td>Jann(41)</td>
<td>Berlin, Germany 1984-2008</td>
<td>214</td>
<td>-</td>
</tr>
<tr>
<td>Turner(39)</td>
<td>Belfast, UK 1978-2000</td>
<td>139</td>
<td>53</td>
</tr>
</tbody>
</table>

- = not published

A study based on the Swedish cancer registry reports an increased survival over time, with an increase between the first period (1960-70) to the last period (1991-2000) in 5-year OS from 49% to 65%, RS from 59% to 77% and DSS 78% to 88% (36). Conversely, studies from the US, based on the National Cancer Data Base (NCDB) 1985-2000 and SEER 1973-2002 were unable to show any improved survival over time (6, 35).

2.7 Prognostic factors

Advanced dissemination of disease is widely accepted as a poor prognostic factor for survival. The most recent study from the SEER database reports a 5-year OS of 65% for localized disease, 71% for regional disease, and 54% for distant metastasis (8). A Spanish multi-institutional study showed 5-year OS of 100% for localized disease, 74% for regional disease and 82% for distant metastasis (37). A German single-institutional study (n=188) uses the new ENETS staging system (Table A-B) and reports a 5-year DSS of 100% for stage I-II, 97% for stage III and 85% for stage IV(41). A study based on patients from the Swedish cancer registry and local cancer registry of Linköping County, Sweden (n=145) reveals a 5-year OS and DSS of 72% and 100% for localized disease, 68% and 86% for regional disease and 46% and 51% for distant metastasis(13).

The SI-NETs with distant metastases (stage IV) have a favorable survival in comparison to most other stage IV gastrointestinal malignancies. Therefore it is interesting to differentiate patients with stage IV SI-NET into subgroups to more accurately assess prognosis. The liver is the most...
common locale of spread, and the amount of liver metastases (>5 metastases) has been seen to affect prognosis (26). Conflicting reports regarding the prognostic impact of PC in mixed GEP-NET are published, and in total only 37 patients with SI-NET and PC were included in these two studies (18, 24). Patients with NET and ovarian metastases seem to have a similar survival to NET patients with only liver metastases (43). Extra abdominal metastases in NET represent an advanced staged disease and brain metastases are associated with a poor survival (median survival < 10 months) (43). Patients with cardiac metastases also fare worse than patients with liver metastases only (43). The direct prognostic impact of bone metastases or lung metastases is not available in current literature according to a 2010 consensus document (44).

Low tumor proliferation, or more specifically low Ki-67 index, is associated with improved survival by both crude and adjusted analysis in patients with SI-NET (41, 45-47). Other histological prognostic factors include primary tumor size and a solid growth pattern (45, 47).

Carcinoid heart disease is also a major negative prognostic factor for survival in several studies, the largest study reporting a median OS of 2.6 years after diagnosis of carcinoid heart disease (48). Elevated u-5-HIAA, reflecting either a hormonally active tumor, advanced staged disease or non-radical surgery was a negative prognostic factor for survival on multivariable analysis in a report from our institution by Janson et al (26). Ahmed et al reported elevated CgA and elevated u-5-HIAA to be negative prognostic factors on univariable analysis, but not by multivariable analysis (46). Other reports have showed both CgA and u-5-HIAA to be independent prognostic markers for survival on multivariable analyses (15, 49).

Gender was not a prognostic factor for OS or RS in studies from the Swedish cancer registry or by the multicenter UKINETs study, although two other studies (n = 167, and n = 67) found female gender to be a negative prognostic factor for survival by crude and multivariable analysis, respectively (36, 46, 50, 51). Unsurprisingly, studies confirm the inherent factor “age at diagnosis” to be independently prognostic for OS, and also for DSS in a study by Zar et al (13, 26, 36, 46, 48, 52).

2.8 Locoregional surgery

SI-NETs are often discovered during acute surgery due to tumor related intestinal obstruction (13, 19). The standard surgical approach for removal of the primary tumor and regional metastases (locoregional resective surgery) is a distal small bowel resection, often also including right hemicolectomy, (for more occasional jejunal tumors only small-bowel resection), combined with an extensive mesenteric dissection for removal of mesenteric lymph node metastases, with care not to injure the proximal mesenteric vessels (53).
The rationale for dissecting the mesenteric lymph nodes, even in the presence of distant metastases, is to prevent progression of a conglomerate of metastatic and fibrotic tissue encompassing the mesenteric vessels, ultimately causing ileus, cachexia or small bowel ischemia. A growing mesenteric mass has been shown to be a cause of morbidity and mortality in these patients if left untreated (14, 54, 55). Also, locoregional surgery has been shown to improve symptoms cause by intermittent obstruction or incipient ischemia in SI-NET patients (19).

A survival benefit regarding primary tumor surgery of stage IV SI-NET is suggested in a retrospective non-randomized study although the extent of mesenteric dissection was not reported (Table F) (46). This is concordance with a previous report from our center, although in another, somewhat smaller study by Strosberg et al, there was no significant survival advantage of primary tumor surgery (40, 52). A recently published population-based study reports the cancer-survival benefit of radical locoregional resection independent of stage (13).
Table F. Survival after surgical resection of the primary tumor. Only reports with over 100 patients are included.

<table>
<thead>
<tr>
<th>Study population, Author</th>
<th>Type of study, Patients included (n)</th>
<th>5-year survival</th>
<th>Survival difference*</th>
</tr>
</thead>
<tbody>
<tr>
<td>UK 1973-2007 Ahmed (46)</td>
<td>Multi-center, Metastatic SI-NET (360)</td>
<td>74% OS 70% OS</td>
<td>p&lt;0.001 p&lt;0.001</td>
</tr>
<tr>
<td>Sweden, 1975-1997 Hellman (40)</td>
<td>Single-center, All SI-NET (314)</td>
<td>70% OS 58% OS</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>USA 1999-2003 Strosberg (52)</td>
<td>Single-center, Metastatic SI-NET (146)</td>
<td>79% OS 65% OS</td>
<td>NS</td>
</tr>
<tr>
<td>Sweden, 1960-2005 Landerholm (13)</td>
<td>Population based, Surgically treated SI-NET (135)</td>
<td>61% OS* 75% DSS*</td>
<td>- -</td>
</tr>
</tbody>
</table>

*Log rank test, OS = overall survival, DSS disease specific survival. #30-day postoperative mortality excluded.
2.9 Peritoneal carcinomatosis

PC is defined as spread of separate tumor deposits within the peritoneal cavity away from the primary tumor. The spread into the peritoneal cavity can occur in at least two principally different ways. Either the primary tumor (or a metastasis) grows and penetrates the serosal layer of the organ of origin, or surgical trauma releases tumor cells into the peritoneal cavity, either through iatrogenic injury to the organ, lymph or blood vessels (56, 57).

In 2007, the European Neuroendocrine Tumor Society (ENETS) presented a consensus document about PC in GEP-NETs (58). The document concluded that data on PC in GEP-NETs are scarce and it also proposed that one of two principal classifications systems should be used to quantify the extent of PC, namely the PC index (PCI) or the Lyon prognostic index (59, 60). Although the PCI is more advanced and appealing in the prospective setting, the Lyon prognostic index only uses four steps, ranging from small localized nodules, to larger and diffusely spread nodules in the peritoneum and is therefore deemed more feasible than the PCI to use in the case of retrospective studies (Table G)(58, 59). A supplemental system, Global peritoneal score (GPS; Table H), which is used to assess the extent of liver and lymph node metastases, was also suggested by ENETS, as NET patients with PC often are synchronously spread to these organs (58). To date, there are no published studies of SI-NETs that report survival rates according to these classification systems.

**Table G. Lyon prognostic index to classify the extent of peritoneal carcinomatosis.**

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No macroscopic disease</td>
</tr>
<tr>
<td>1</td>
<td>Localized nodules in one part of the abdomen &lt; 5mm in size</td>
</tr>
<tr>
<td>2</td>
<td>Diffuse nodules spread to the whole abdomen &lt;5mm in size</td>
</tr>
<tr>
<td>3</td>
<td>Localized or diffuse nodules 5-20mm in size</td>
</tr>
<tr>
<td>4</td>
<td>Localized or diffuse nodules or masses &gt;20mm in size</td>
</tr>
</tbody>
</table>
Table H. ENETS proposal of abdominal gravity PC score (GPS) grading system based on the association of PC with lymph node and liver metastases.

<table>
<thead>
<tr>
<th>Metastases</th>
<th>0 points</th>
<th>1 point</th>
<th>2 points</th>
<th>3 points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymph node</td>
<td>None or local&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Regional&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Para-aortal or hepatic pedicle</td>
<td>Extra-abdominal</td>
</tr>
<tr>
<td>Metastases</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver metastases</td>
<td>No macroscopic nodules</td>
<td>One lobe less than 5 nodules</td>
<td>Both lobes or 5-10 nodules</td>
<td>Both lobes, more than 10 nodules</td>
</tr>
<tr>
<td>PC</td>
<td>No macroscopic nodules</td>
<td>Lyon I-II resectable</td>
<td>Lyon III-IV resectable</td>
<td>Lyon I-IV unresectable</td>
</tr>
</tbody>
</table>

GPS grade A: 0-3 points, grade B: 4-6 points, grade C 7-9 points

<sup>1</sup>Local: first (adjacent) to the primary tumor relay, Regional: secondary tumor drainage territory relay. Pc = peritoneal carcinomatosis. ENETS = European neuroendocrine tumor society.

During the last decades, cytoreductive surgery (CRS) with the aim to remove all visible tumor, in combination with heated intra-peritoneal chemotherapy (HIPEC), has shown favorable survival in patients with pseudomyxoma peritonei, mesothelioma and colorectal cancer (61-63). In light of this, some institutions currently perform CRS with HIPEC also for SI-NET tumors. However, there is only one published study that focuses on CRS with intra-peritoneal chemotherapy in NET patients (n=17), which reports a 5-year overall survival of 66,2 %, although most patients recurred during follow-up. One fear regarding the use of CRS and HIPEC in NET patients is the lack of knowledge concerning effective chemotherapy for NET with low proliferation.
2.10 Treatment of liver metastases

Around a third of SI-NET patients present with liver metastases and the many of the rest develop liver metastases during follow-up (8, 13). Treatment of liver metastases is performed to palliate symptoms from the carcinoid syndrome, and in an effort to improve survival. Most reports comprise of SI-NET, other GI-NET and P-NET and the following is therefore a survey of treatment regarding liver metastases of mixed GEP-NET populations. First of all, it should be noted that no randomized trials have been undertaken to study whether surgery, current best medical treatment, ablative procedures, embolization procedures or peptide receptor therapy is the most effective treatment in patients with metastatic NET (64). With that said, liver surgery has shown excellent results regarding symptom palliation and favorable five-year OS rates (Table I) (65-78).

Table I. Outcome after resection of NET liver metastases.

<table>
<thead>
<tr>
<th>First Author</th>
<th>Patients (n)</th>
<th>NET</th>
<th>SI-NET</th>
<th>Period</th>
<th>Mortality (%)</th>
<th>Symptom control (%)</th>
<th>OS (median, 5-year)</th>
<th>PFS (median)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mayo(75)</td>
<td>339&lt;sup&gt;a&lt;/sup&gt; 83</td>
<td>1985-2010</td>
<td>&lt;1</td>
<td>123, 74%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Glazer(77)</td>
<td>172&lt;sup&gt;b&lt;/sup&gt; 65</td>
<td>1978-2009</td>
<td>0</td>
<td>116, 77%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Sarmiento(71)</td>
<td>170 85</td>
<td>1977-1998</td>
<td>1</td>
<td>81, 61%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Saxena(78)</td>
<td>74 32</td>
<td>1992-2009</td>
<td>4</td>
<td>95, 63%</td>
<td>23</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Osborne(76)</td>
<td>61 36</td>
<td>2000-2004</td>
<td>0</td>
<td>93</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Ahmed(46)</td>
<td>50 50</td>
<td>1973-2007</td>
<td>-</td>
<td>135,</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Elias(73)</td>
<td>47 14</td>
<td>1985-2000</td>
<td>5</td>
<td>62, 71%</td>
<td>48</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Eriksson(79)</td>
<td>42&lt;sup&gt;c&lt;/sup&gt; 23</td>
<td>1998-2006</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>McEntee(74)</td>
<td>37 20</td>
<td>1970-1989</td>
<td>-</td>
<td>68</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Chamberlain(68)</td>
<td>34 -</td>
<td>1992-1998</td>
<td>6</td>
<td>90, 76%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Nave(70)</td>
<td>31 17</td>
<td>1983-1995</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>47%</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Mazzaferro(80)</td>
<td>36 -</td>
<td>1987-2006</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>89%</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Only reports with more than 30 subjects are included, and only the most recent report if there are several from the same center. Median overall survival (OS) and progression free survival (PFS) displayed in months. - = Not published. <sup>a</sup>, <sup>b</sup>, <sup>c</sup> = in conjunction with RFA of liver metastases in 66<sup>a</sup>, 41<sup>b</sup>, and 17<sup>c</sup> cases.

Morbidity is around 14% and operative mortality is reported to be between and 0-4% in five reports that include more than 50 patients each (71, 75-78). NET liver metastases commonly recur after perceived radical liver surgery...
and the procedure should probably be considered as palliative although anecdotal reports of long-term disease free survivors exist (71, 81). This is supported by a study from Elias et al that shows that more than half of all NET liver metastases are undetectable by our standard imaging procedures rendering a high probability for undiagnosed disease to be left after liver surgery (82). However, cancer reductive surgery removing at least 90% of the depicted liver tumor mass is also considered to alleviate symptoms although symptomatic recurrence rates are higher than for patients that undergo apparent complete resection (71, 81). Around 30% of the liver parenchyma should be left after surgery to avoid hepatic failure (83). In selected cases, preoperative portal vein embolization can be performed to induce liver regeneration to expand the number of patients eligible for liver surgery (83).

It is not uncommon for the spread of liver metastases to be both extensive and bilobar and not all patients with NET are eligible for liver surgery. For non-resectable liver metastases, radiofrequency ablation (RFA) and microwave ablation (MWA) has been proven safe and to offer local tumor control and palliation of the carcinoid syndrome (72, 79, 83-92). Both techniques can be performed either through an ultrasound (US)/ computed tomography (CT)/ magnetic resonance imaging (MRI) guided percutaneous route, or via laparotomy or laparoscopy, at times also in combination with liver resections (75, 77, 79, 87, 93-95).

In case of RFA, a needle is inserted into the liver metastasis, the power of the generator is turned on and a high frequency alternating current generates heat that destroys the tissue around the tip of the needle (87). Generators are commonly regulated by the tissue impedance, which increases in association with carbonization around the tip of the needle, implying that there are no remaining viable cells in the area when impedance reaches maximum (87). The aim of RFA is to achieve an ablation zone with a margin of at least 10mm around each metastasis.

More than a dozen lesions can be treated in a single patient and many patients tolerate repeat ablations when they experience recurrence (96). Tumors up to 12 cm in diameter have been treated with radiofrequency ablation, but survival and recurrence rates are worse for patients with larger metastases and most centers refrain from ablating tumors larger than 3 to 5 cm (84, 89, 97, 98). Incomplete ablations are frequent close to large vessels due to a “heat-sink effect” from the cooling blood flow (98). Factors that improve ablation results include general anesthesia, absence of extra-hepatic metastases, operative approach, vascular occlusion, at least 1 cm-ablative margin and a well-experienced physician (91, 98-100). Local recurrence rates ranging from 2%-60% are seen, and many patients also develop new metastases in the liver, provided that the follow-up is long enough (75, 79, 91, 96, 98). Akyildiz et al and Karabulut et al report survival rates in the
lower range compared with those seen after liver surgery (Table J) (72, 91). Symptom control is also readily achieved with RFA (Table J) (72, 79, 91).

**Table J. Outcome after RFA of NET liver metastases.**

<table>
<thead>
<tr>
<th>First Author</th>
<th>Patients (n)</th>
<th>NET SI-NET</th>
<th>Period</th>
<th>Mortality (%)</th>
<th>Follow-up (median)</th>
<th>Symptom control (%)</th>
<th>OS (median, 5-year)</th>
<th>Recurrence* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Akyildiz(91)</td>
<td>89</td>
<td>55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1996-2009</td>
<td>1</td>
<td>30</td>
<td>97</td>
<td>- , 57%</td>
<td>60</td>
</tr>
<tr>
<td>Karabulut(72)</td>
<td>69</td>
<td>44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1996-2010</td>
<td>-</td>
<td>22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>91</td>
<td>73&lt;sup&gt;c&lt;/sup&gt;, -</td>
<td>22</td>
</tr>
<tr>
<td>Eriksson(79)</td>
<td>50&lt;sup&gt;d&lt;/sup&gt;</td>
<td>22</td>
<td>1998-2006</td>
<td>0</td>
<td>-</td>
<td>70</td>
<td>-</td>
<td>82&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mayo(75)</td>
<td>76&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-</td>
<td>1985-2010</td>
<td>0</td>
<td>43</td>
<td>-</td>
<td>-</td>
<td>47</td>
</tr>
<tr>
<td>Glazer(77)</td>
<td>41&lt;sup&gt;f&lt;/sup&gt;</td>
<td>-</td>
<td>1978-2009</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Only reports with more than 30 subjects are included, and only the most recent report if there are several from the same center. * Recurrence within the liver - = not published, <sup>a</sup> = GI-NET, <sup>b</sup> = mean, <sup>c</sup> = patients with shorter follow-up than 24 months excluded. <sup>d</sup>,<sup>e</sup>,<sup>f</sup> = in conjunction with resection of liver metastases in 17/50<sup>d</sup>, 66/76<sup>e</sup> and 23/41<sup>f</sup> cases.

MWA uses a similar technique as RFA, although the heat is generated through microwaves instead of electric current. The microwave technique has been shown to have a deeper penetration into tissues and less “heat-sink effect”- and has therefore been recommended for larger tumors, or tumors close to large vessels (101, 102). No comparative studies have yet showed any difference in local recurrence between these two ablative procedures (90, 102).

Percutaneous alcohol injection (PAI) is another technique that has been used as a local therapy for NET liver metastases, but a recent meta-analysis has showed PAI monotherapy to be inferior to RFA in hepatocellular cancer (HCC) and one may speculate that this holds true also for NET (103). However, the combination of PAI-RFA is more effective than RFA alone in HCC (104). PAI is also not vulnerable to the “heat sink effect” and small liver lesions close to large vessels or bile ducts may be treated with adequate results (83).

For extensive NET liver tumor load, hepatic artery embolization/ chemoembolization (HAE/HACE) emerged as an alternative treatment during the 1990s. HAEs/HACEs can be accomplished with various agents such as gelfoam (Pharmacia and Upjohn Co, Kalamazoo, MI), Polyvinyl alcohol particles (Ivalon, Fabco Inc, Altoona, PA), or trisacryl gelatin microspheres (Embospheres; Biosphere Medical Inc, Rockland, MA) (105). One of these agents is injected via a catheter into the left or right hepatic artery, with or without supplementary chemotherapy and complete or partial
response is achieved in 33%-73% of all patients (Table K) (105, 106)(100)(106-109). The right and left liver lobe is treated at separate sessions and never simultaneously. Side-effects include nausea, fever, upper right quadrant abdominal pain and elevated liver enzymes (105). Adverse events include ileus, hepatic abscesses, portal-vein thrombosis, ischemic cholecystitis, ischemic enteritis, spleen infarctions and liver failure (110, 111). Relative contra-indications for HAE/HACE include portal-vein thrombosis, liver dysfunction and aberrant arterial vascular anatomy (i.e. superior mesenteric artery originating from hepatic artery). Furthermore HAE has shown inferior results in comparison to reductive liver surgery regarding survival and symptomatic recurrence in a non-randomized study, however the study was of course limited by “confounding by indication” (76).

Table K. Outcome after HAE/HACE of NET liver metastases.

<table>
<thead>
<tr>
<th>First Author</th>
<th>Patients (n)</th>
<th>NET</th>
<th>SI-NET</th>
<th>Modality</th>
<th>Period</th>
<th>Mortality (%)</th>
<th>Symptom response (%)</th>
<th>OS (median, 5-year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mayo (75)</td>
<td>441</td>
<td>119</td>
<td></td>
<td>HAE/HACE*</td>
<td>1985-2010</td>
<td>2.4</td>
<td>-</td>
<td>33.5, 30%</td>
</tr>
<tr>
<td>Dong (112)</td>
<td>123</td>
<td>21</td>
<td></td>
<td>HACE</td>
<td>1990-2005</td>
<td>0</td>
<td>-</td>
<td>-, 36%</td>
</tr>
<tr>
<td>Christante (107)</td>
<td>77</td>
<td>37</td>
<td></td>
<td>HACE</td>
<td>1994-2007</td>
<td>7</td>
<td>-</td>
<td>51, 30%</td>
</tr>
<tr>
<td>Wangberg (106)</td>
<td>40a</td>
<td>40</td>
<td></td>
<td>HAE</td>
<td>1987-1995</td>
<td>3</td>
<td>-</td>
<td>108b, 63%</td>
</tr>
<tr>
<td>Eriksson (113)</td>
<td>42</td>
<td>21</td>
<td></td>
<td>HAE</td>
<td>1981-1995</td>
<td>5</td>
<td>91c</td>
<td>80c, 60%*</td>
</tr>
<tr>
<td>Osborne (76)</td>
<td>69</td>
<td>-</td>
<td></td>
<td>HAE</td>
<td>2000-2004</td>
<td>-</td>
<td>91</td>
<td>-</td>
</tr>
<tr>
<td>Gupta (108)</td>
<td>81</td>
<td>43</td>
<td></td>
<td>HAE/HACE</td>
<td>1992-2000</td>
<td>1</td>
<td>63</td>
<td>31, 24%</td>
</tr>
<tr>
<td>Moertel (109)</td>
<td>65a</td>
<td>-</td>
<td></td>
<td>HAE/HACE</td>
<td>1982-1991</td>
<td>5</td>
<td>98</td>
<td>27c/49d, 39%c/30%c</td>
</tr>
</tbody>
</table>

Only reports with more than 30 subjects are included, and only the most recent report if there are several from the same center. * Including also RAE, - = not published, a = GI-NET, b = Value is approximate, c = HAE, d = HACE e = For SI-NET patients

A different method to treat hepatic metastases is radioembolization (RAE) with 90Y embedded in either a resin microsphere or a glass microsphere (105, 114). This technique enables direct delivery of a radionuclide with a long-range tissue penetration to hepatic metastases (105). Contrary to HAE/HACE, total circulatory arrest in tumor feeding arteries is not necessary or even wanted, and therefore side effects appear to be milder (105, 114). Also, patients with contraindications for HAE due to liver dysfunction may be able to undergo RAE (114). Adverse effects of RAE include ischemic enteritis or pneumonitis due to erroneous infusion or shunting of embolization material into other arteries (105). Radiological
response rate in one multicenter study (n=148) was 63%, while another study showed a response rate of 51% (115, 116).

Liver transplantation in NET has been performed in highly selected patients with bilobar extensive disease, but it should probably be considered as a palliative procedure because most patients experience recurrence. Nevertheless, one center reports a low postoperative mortality and a 5-year survival of around 90% after liver transplantation in NET (117). Other studies report postoperative mortality rates are as high as 10-20% (105). In the two large meta-analyses (n=150 and n=103), the 5-year survival rates after liver transplantation for NETs were 49% and 47% respectively, and only 27% were disease free in the latter study (118, 119). Patients over 55-years, and patients with the need for concurrent resection of extra-hepatic disease fare worse on multivariable analysis (83). The survival benefits of liver transplantation in metastatic NETs remain uncertain, and should be performed only after careful consideration in select cases.

2.11 Radiology

Radiological imaging in patients with SI-NET is mainly used for the following purposes: To diagnose the patient, to assess disease dissemination (staging), to evaluate treatment effect and to investigate complications due to treatment or from the disease itself. Several different imaging techniques can be used and the following is a survey of the most common imaging modalities with focus on evaluation of disease dissemination and treatment effect (mainly RFA). Most studies regarding liver metastases are of mixed NET populations. Imaging of locoregional tumor, extra-hepatic metastases, liver metastases and finally imaging after RFA will be discussed.

The method of choice to evaluate primary tumor and locoregional disease is CT. CT is fast, widely available and acceptably accurate (120). Also, many SI-NET patients present with abdominal pain or ileus and CT with subsequent follow-through is commonly used to assess bowel obstruction. Moreover, CT may reveal a pathognomonic radiating mesenteric mass “a spoke wheel sign” (120). CT-enteroclysis to detect the primary tumor has in one study of mixed small-bowel tumors (19/219 were SI-NET) shown sensitivity 85% and specificity 97% and in another study of SI-NET (n=8) the sensitivity and specificity 50% and 25% respectively (121, 122). Localization of the primary tumor is, however, rarely needed since these patients are generally subjected to laparotomy where primary tumor is easily palpated. CT can depict the involvement of tumor and fibrosis around the mesenteric root and thus be used to assess the possibility of radical resection (120). US can be used to detect a mesenteric mass and to guide biopsy, but it is not well studied in comparison with CT or MRI in evaluation of locoregional disease (120). MRI is not well studied in imaging of
locoregional SI-NET disease in comparison to CT and it is more time consuming and less available (120).

CT can also be used to detect extra-hepatic abdominal soft tissue metastases with 63-100% sensitivity and 98-100% specificity (12-15). Likewise, MRI was excellent for detecting extra-hepatic soft tissue metastases in a study by Cwikla et al (123). MRI is also considered superior to CT in detection of bone and brain metastases of NET (120).

The most common site of distant SI-NET metastases is the liver. Contrast enhanced CT (CECT) of the liver should always be performed as a “triple-phase-CT” i.e. before and during contrast-enhancement in the late arterial (or portal-venous inflow) phase and in the venous (or portal-venous) contrast-enhancement phase to ensure a high detection rate for the generally well-vascularized NETs (120, 124). The detection rate for NET liver metastases with clinical data as the gold standard is 80-81% for CT and 80-100% for MRI on a lesion basis (123, 125-131). However, detection rate on a lesion basis was as low as 38% for CECT, 38% for CEUS and 49% for MRI when thin-slice pathology was used as gold standard (82). In conclusion, MRI seems to be more sensitive than CT when screening for liver metastases and might be the optimal method of choice, but drawbacks of MRI include, as noted above, an increased cost and time consumption and the technique is generally less available than CT(83, 120). Although contrast enhanced US (CEUS) has a high specificity to characterize single liver metastases, it is unreliable to assess tumor load in extensive disease (120, 132). CEUS is rarely used in clinical studies as it does not reproduce images that are easy to reevaluate and because it is very operator dependent. A benefit of US and MRI imaging in comparison to CT is the lack of radiation exposure which make them suitable for young patients with a long life-expectancy (120).

2.12 Nuclear medicine imaging

Nuclear medicine imaging is founded upon the knowledge that certain isotopes of selected elements exert radiation and that this radiation is possible to measure. Planar, two-dimensional (2-D), single-photon imaging or its 3-D counterpart, single photon emission computed tomography (SPECT), uses the single photon (i.e. gamma ray) emitting properties of certain radioactive isotopes. These isotopes may be used to label biological molecules (tracers) that are injected into a patient, upon where the gamma camera registers radiation from where the tracer has accumulated, such as in tumors. SPECT collects multiple planar images and then uses tomographic reconstruction to recalculate these images into a 3-D dataset that are displayed as transverse, coronal and sagittal images. The most commonly used isotopes for single-photon imaging is $^{99m}$Tc (Half-life = $t_{1/2} = 6h$) and $^{111}$Indium ($t_{1/2} = 67h$). $^{99m}$Tc is readily available from a generator in the
nuclear medicine department whereas $^{111}$Indium is usually produced in a cyclotron at a vendor and then transported to the nuclear medicine department. Both isotopes are fairly cheap since an on site cyclotron is not needed.

Single photon nuclear imaging in SI-NET utilizes the abundance of somatostatin receptors (SSTr) expressed on most SI-NET cells. There are a variety of somatostatin analogues that bind to SSTr, and the most used somatostatin analogue in somatostatin receptor scintigraphy (SRS) is Octreotide (25). Octreotide is commonly labeled with $^{111}$In, using the chelator diethyle-triamine-pentaaceticacid (DTPA) to produce $^{111}$In-DTPA-octreotide, also known as the commercial product Octreoscan® (25). Other somatostatin analogues, chelators and isotopes have also been tested in SRS (25). Several Studies have shown improved sensitivity of SRS when SPECT of the abdomen or thorax is performed in addition to hole-body planar imaging planar imaging (25). SRS has reported a sensitivity of 86-95% in detecting GI-NET tumors on a patient basis (25). Other authors have noticed that small tumors may remain undetected, and this fact is supported by the lesion based 28% sensitivity with SRS regarding NET liver metastases with thin slice pathology as the gold standard (82, 133). Besides detection of tumors, SRS is used to investigate the extent of SSTr positivity prior to treatment with somatostatin analogues and peptide receptor radionuclide therapy (PRRT) (134).

Positron emission tomography (PET) relies on a specific type of isotopes that emit positrons. Within approximately a millimeter of emission these positrons will have interacted with an electron and annihilated, giving rise to a pair of photons that are emitted in opposite (180 degrees) directions. The PET-camera can detect both these gamma rays simultaneously, registering the isotope to be somewhere on this line of decay. This co-incidence imaging makes PET more effective than single photon imaging and offers a higher spatial resolution, approximately 0,5cm compared to the 1,5cm spatial offered by SPECT. Unfortunately, the short half-life of the most commonly used positron emitting nuclides $^{18}$F ($t_{1/2}=110$min), $^{11}$C ($t_{1/2}=20$min) and $^{15}$O($t_{1/2}=2$min) require a very expensive on-site production in a cyclotron, although $^{18}$F-tracers can be transported from a nearby production site (25). A recent trend in PET imaging is the increased use of positron emitter $^{68}$Ga, that does not need an on-site cyclotron for synthesis and like $^{99m}$Tc can be eluted from a generator and therefore is much cheaper (135). A recent study showed, in a clinical setting, that PET with $^{68}$Ga-labelled octreotide was cheaper than SRS (Octreoscan®)(136).

During the last years a combination of PET and CT (PET/CT) has become clinical routine and hybrid MRI/PET cameras are emerging into several institutions at present. This development has lead to faster PET examinations and an excellent anatomical map for correlation of functional findings. (25)
Several different tracers for PET are used for cancer imaging; the most common is $^{18}$F-labelled Fluorodeoxy-glucose (FDG). FDG is accumulated in highly proliferative tissues with elevated metabolism such as most cancers or inflammation (25). However, SI-NETs are typically low proliferative and therefore FDG has low sensitivity for these tumors (25). In a recent study in patients with a variety of NETs, the combination of FDG-PET and SRS, however, was superior to each imaging technique alone (137). The few SI-NET that can be identified by 18-FDG PET have a high proliferation rate and a poor prognosis (137). Interestingly, the uptake of FDG in NET has shown to be an independent predictor of progression free survival (137).

Since most NETs have low proliferation, specific tracers have been developed for NETs. The most common type of PET tracers for NET imaging use two typical features of SI-NET cells: The expression of somatostatin receptors (SSTr), and the uptake of amine precursors such as L-Dihydroxyphenylalanine (L-DOPA) or 5-hydroxytryptophan (5-HTP) (25). Several different somatostatin analogues have been tested for PET. The most commonly used are $^{68}$Ga-labelled octreotide and octreotate, $^{68}$Ga-DOTA-TOC and $^{68}$Ga-DOTA-TATE (25, 138).

$^{68}$Ga-DOTA-TOC-PET has in GI-NET patients shown to have better sensitivity than SRS (139). Similarly, in another study $^{68}$Ga-DOTA-TOC-PET showed to be especially advantageous in the detection of small tumors with low tracer uptake (140). Furthermore, Frilling et al showed that $^{68}$Ga-DOTA-TOC-PET/CT detected additional hepatic or extra-hepatic metastases in 66% of all patients with liver metastases (n=33) in comparison to CT or MRI and also changed the treatment decision in more than every second patient (138).

As mentioned, other PET tracers used include $^{18}$F-L-DOPA and $^{11}$C-5-HP. No comparative studies have been performed between these and $^{68}$Ga-DOTA-TOC (25). In a comparative study by Koopmans et al $^{18}$F-L-DOPA-PET/CT was superior to $^{11}$C-5-HTP-PET/CT in patients with GI-NET although the reverse was true for patients with P-NET (141). On a lesion basis, sensitivities in GI-NET were with $^{18}$F-L-DOPA-PET (87%) the hybrid exam $^{18}$F-L-DOPA-PET/CT (98%), $^{11}$C-5-HTP-PET (78%), $^{11}$C-5-HTP-PET/CT (89%), SRS (49%), SRS/CT (73%) and CT alone (63%) (141). Other studies have also shown a superior detection rate of metastases for $^{18}$F-L-DOPA-PET and $^{11}$C-5-HTP-PET in comparison to SRS, CT and US but no studies have focused exclusively on liver metastases (142-145).

2.13 Imaging after radiofrequency ablation

Imaging after RFA is performed to assess complete ablation and to screen for new metastases. At our institution we perform routine post-RFA imaging on Day 1 by means of contrast-enhanced US (CEUS) and later follow up
with CEUS and contrast-enhanced CT (CECT) for at least 12 months. The sensitivity and specificity of the morphological imaging methods CECT, CEUS, and MRI are imperfect for assessing the liver for residual tumor (RT) following RFA (146-149). Moreover, the contrast-enhancement in an early local inflammatory response may sometimes be difficult to differentiate from RT (149). Functional imaging with FDG-PET in combination with CT has demonstrated promising results in depicting RT after RFA of colorectal metastases and hepatocellular carcinoma (150-152).

As described above, other tracers than FDG are used for NET and our center employs imaging with $^{11}$C-5-HP while several other centers have employed $^{68}$Ga-DOTA-TOC or $^{18}$F-L-DOPA, possibly due to the more complicated radiosynthesis of $^{11}$C-5-HP.

2.14 Drugs and radionuclide therapy

Somatostatin receptors (SSTRs) are expressed in most SI-NET tumors. SSTRs consist out of five different subtypes, all expressed on the NET cells. The two currently clinically used somatostatin analogues (SSA) are octreotide and lanreotide. Both have high affinity to receptor subtype 2 and moderate affinity to subtype 5 (153). They are available in long-acting release (LAR) forms and are both well-tolerated treatments, although adverse effects such as steatorrhea, nausea, bloating and formation of gallstones occur (105). A novel SSA, pasireotide, with affinity for 4 out of the 5 SSTR subtypes, has been tested with promising results in patients refractory to octreotide treatment (154). Treatment with long-acting SSA is considered to offer marked improvement of life-quality in patients with symptoms of the carcinoid syndrome, and may possibly prevent development and/or progression of the carcinoid heart disease (105, 155). SSA therapy is therefore recommended as first-line medical treatment in symptomatic tumors (156). One prospective randomized trial has shown prolonged PFS in patients allocated to long-acting SSA (Octreotide LAR). Median time to progression was 14,3 months in the treatment arm as compared to 6 months in the placebo arm. Treatment with SSA is therefore indicated also in asymptomatic non-radically operated patients (157).

Interferon therapy is recommended as second-line treatment of functioning NETs of the jejunum-ileum with a low proliferation rate (156). Response rates for interferon in NET ranges from 0-27% in various trials, and one randomized trial has shown comparable response rates for interferon, SSA, and a combination of the two, although clinically significant differences might have gone undetected due to small treatment groups (158, 159). Drawbacks of interferon include adverse effects such as hyperthyroidism, immunosuppression, and depression. Contraindications for interferon include severe heart disease, kidney failure and liver failure (158).
Cytotoxic chemotherapy such as 5-Fluorouracil, streptozocin and doxorubicin has meager or no positive effect on low-proliferative SI-NETs and adverse effects can be considerable (160-162). However, mTOR Inhibitor Everolimus has in a randomized phase III trial demonstrated longer PFS in GI-NET, 16.4 months in the treatment arm versus 11.3 months in the placebo arm (p=0.026) (163). In SI-NET there was a non-significant superior PFS in the treatment arm, measuring 18.6 months in comparison to 14 months in the placebo arm (163). Angiogenesis inhibitors have a theoretically promising use in NET as these tumors in general are highly vascularized(164). Moreover NETs frequently over-express the vascular endothelial growth factor (VEGF) receptor and ligand (165). Bevazizumab is a monoclonal antibody to circulating VEGF and this drug has shown a positive effect on PFS in randomized study of mixed GI-NET (n=44) (165). SI-NET comprised of 54.5% of all patients, but results where not reported separately.

PRRT is a treatment option for metastatic SI-NET that exhibit equal or higher uptake than the normal liver parenchyma with SRS (134). PRRT is recommended in symptomatic patients refractory to medical treatment with inoperable disease (156). PRRT with $^{177}$Lutetium-DOTA-Tyr3-octreotate compare favorably to other possible treatments and patients that have undergone such PRRT have an OS that compares favorably to historical controls (134). Complete and partial response rates for mixed GI-NET total 23%, and only 20% of all patients have progressive disease after 3 months according to Kwekkeboom et al (134). Side effects of PRRT include nausea, vomiting and abdominal pain. Adverse events such as sub acute hematologic toxicity, renal insufficiency, liver dysfunction and myelodysplastic syndrome are rare (134). The limiting factor for continued treatment with PRRT is often renal impairment due to a high-absorbed dose of $^{177}$Lutetium-DOTA-Tyr3-octreotate in the kidneys (166).

2.15 Gallbladders and Gallstones in SI-NET

Long-acting SSAs have become a cornerstone for the treatment of SI-NET patients. However, the treatment may have adverse effects such as increased formation of cholesterol-rich crystals in the bile and suppression of cholecystokinin, which may impair gallbladder emptying and inhibit relaxation of sphincter Oddi, leading to increased gallstone formation (167-172). The prevalence of gallstones ranges from 35-47% in SSA treated patients with acromegaly and the prevalence was 53% in the only SSA treated SI-NET population studied (n=55) (173-175). The corresponding prevalence of gallstones in the Swedish population varies between 11% and 35% in different screening studies depending on the age and gender (176-179). In the study of SI-NET, there was 6.8% risk to suffer a complication
that necessitated cholecystectomy during a mean follow-up of 44 months that may be compared with the 5-year risk of around 1.3% to suffer a gallstone complication in a general population with age >65 (175, 180). Reports of ischemic cholecystitis as a complication of liver embolization and gallbladder injury caused by RFA have also been considered to strengthen the indication for prophylactic cholecystectomy in NET patients although these complications are rare (81, 85, 111).

The above-mentioned facts has lead to a consensus statement recommending prophylactic cholecystectomy during laparotomy for SI-NET disease in 2008 (156). However no large studies that investigate the clinical impact of a remaining gallbladder in SI-NET are published and our previous clinical strategy has been, in contrast to the 2008 consensus statement, to leave the gallbladder in-situ during laparotomy in most SI-NET patients.
2.16 Genetics in SI-NET

The human genome is built out of pairs of complimentary DNA strands, one inherited from our mother, and one from our father. These two different strands are called alleles.

One example of genetic aberrations frequently seen in the DNA is single nucleotide polymorphisms (SNPs), which are defined as common variations of the individual bases (A-adenine, G-guanine, C-cytosine, T-thymine). They occur in a certain frequency of the population, and most of them do not cause disease. In a normal cell some SNP are homozygous for the two alleles (i.e. A-A, T-T) and some are heterozygous (i.e. A-G, C-T).

Other examples of structural variation of the genome include copy number variations (CNV), which originally were defined as gains or losses of DNA segments that are substantially larger than SNPs, often >1000 bases in size. A genome wide description of CNVs may be determined with several different methods, for example with comparative genome hybridization (CGH) arrays or single nucleotide polymorphism (SNP) arrays (181). A SNP array investigates if parts of one allele is lost or duplicated (i.e. a CNV) by the pattern of the signal intensities of each SNP call. A rise in signal intensity of several adjacent SNPs indicates a gain, and a decrease in signal intensity indicates a loss. This may be visualized in a log R ratio (LRR) plot (Fig 1A-D). Analysis of SNP arrays also takes advantage of the fact that many SNPs are heterozygous. Loss of heterozygosity (LOH) means that there are only homozygous SNP calls in that part of the genome, which in turn is a sign of a deletion of that part of one allele. Loss of heterozygosity is visualized in the B-allele frequency (BAF) (Fig 1C-D). By combining the data on signal intensities and the absence of heterozygous SNP calls, copy number neutral LOH may be detected, which is defined as loss of one allele and duplication of the remaining allele (Fig 1C). CNV gains will, as described above, increase the signal intensity of SNP calls, but also alter the B-allele Frequency due to imbalance of heterozygous SNPs (Fig 1B).

In contrast to the NETs included in the multiple endocrine neoplasia 1 (MEN-1) syndrome, which are caused by a mutation in the Menin gene located at chromosome 11, the genetics of SI-NETs are poorly understood. To date, no mutations in specific genes have been discovered in putative proto-oncogenes or tumor-suppressor genes. However, CGH-arrays and SNP-arrays show CNV loss and/or LOH at chromosome 18 in many primary tumors indicating that genes located on this chromosome may be involved in early tumorigenesis (181-185). A study by Cunningham et al showed a correlation between gains at chromosome 7 and a solid growth pattern at pathological examination, which in turn has been linked to worse prognosis (181). Gains at chromosome 14 have in another study been associated with worse survival, while the study by Cunningham et al could not verify this finding (181, 183).
Figure 1. Examples of findings in log R ratio and B-allele frequencies.

(A) Normal DNA. The log R Ratio (LRR) shows normal signal intensity (calls in LRR centered on 0) and the B-allele frequency (BAF) is heterozygous, (AA calls around 0, AB calls centered on 0.5 and BB calls around 1).

(B) Example of copy number (CN) gain. The LRR is elevated, and there is a splitting of heterozygous SNP calls in the BAF.

(C) Example of copy number neutral loss of heterozygosity (CNN-LOH) marked by yellow field in the BAF. The log R ratio is normal (calls centered around 0) but there is LOH (no AB calls).

(D) Example of loss of CN (marked by red in the LRR) and LOH (marked by yellow in the BAF). The LRR is lowered and there are no heterozygous SNPs (AB calls) in the BAF.

Figures 1A-D are created in software Nexus 6.1 (Biodiscovery, Hawthorne, USA)
3 The rational for the thesis

Paper I
- Prognostic factors of SI-NET are not well studied in any large cohorts. Locoregional surgery of SI-NET in stage IV patients is controversial and there are conflicting results in previously published cohorts.

Paper II
- There are only a few reports of peritoneal carcinomatosis in SI-NET patients reported within the literature, and also conflicting data regarding the prognostic impact of this manifestation. Furthermore, no studies have primarily focused on genetic aberrations of SI-NETs with and without peritoneal carcinomatosis.

Paper III
- Results after RFA and liver surgery are only reported as case series and it is unknown if liver surgery and/or RFA prolong life and hinder disease progression in SI-NET.

Paper IV
- There are limitations in CEUS, MRI and CECT in evaluation after RFA of NET liver metastases. $^{115}$HTP-PET is an excellent tool for diagnosing and depicting NET lesions, however, no studies have investigated the use of $^{11}$C-5-HTP-PET in follow-up after local ablative procedures, such as RFA.

Paper V
- The incidence and impact of gallstone complications and effect of a remaining gallbladder in SI-NET has previously not been studied in a large population.
4 Aims of the thesis

The overall aim of the thesis was to investigate unknown or unclear aspects of SI-NET disease, in connection with prognosis, treatment and follow-up.

Specific aims:

**Paper I**
- To assess survival and clinical prognostic factors in patients with SI-NETs, and to explore a possible association between locoregional surgery and survival.

**Paper II**
- To assess the prognostic impact of peritoneal carcinomatosis in patients with SI-NETs, and to explore possible genetic aberrations in tumor samples from patients with and without PC.

**Paper III**
- To assess survival, progression and U-5-HIAA values in patients with SI-NETs subjected to RFA/liver surgery in comparison to a matched control-group.

**Paper IV**
- To assess the feasibility of $^{11}$H-TP-PET in the follow-up imaging after radiofrequency ablation of NET liver metastases.

**Paper V**
- To explore the risk of gallstone formation and gallstone complications in patients with SI-NETs treated with SSA.
5 Patients & Methods

5.1 Ethical considerations
The local ethics committee gave written approval for all studies.
5.2 Patients and methods

Common patients and methods paper I-III:
We included 603 consecutive patients that were diagnosed and admitted between 1985 and 2010 to the Departments of Surgery or Endocrine Oncology at Uppsala University Hospital (paper II and III: 672 patients and until 2012). A database search was performed to identify all patients with the (ICD-9/10) diagnostic codes for small intestinal tumor (152 C/C17.0-9) and/or Carcinoid syndrome (259 C/E34.0). We included patients with a histopathological diagnosis of SI-NET made by microscopy and immunohistochemical staining of either liver metastasis biopsy material or surgical tumor specimens. Specific staining for CgA, synaptophysin, and serotonin confirmed the SI-NET diagnosis. Proliferation rate was estimated by Ki-67 index. Patients with a known proliferation rate equal to or higher than a Ki-67 index of 20% at baseline (Neuro-endocrine carcinomas) and non-Swedish residents were excluded (due to follow-up issues). The patients were followed until death or their last follow-up at the Departments of Surgery or Endocrine Oncology (until December 2010 (paper I) or 2011 (Paper II and III)).

Patient charts were reviewed for; Age, calendar year, gender, symptoms such as flush, diarrhea and abdominal pain at baseline (baseline was in some cases different for paper I, II and III), diagnosis of carcinoid heart disease by echocardiography, lymph node metastases, peritoneal carcinomatosis, liver metastases, distant metastases, proliferation rate of primary tumor and metastases, abdominal surgery, liver debulking treatments, PRRT (i.e. 177Lutetium-DOTA-Tyr3-octreotate), and medical treatment, and u-5-HIAA values (co-morbidity also included for III). During follow-up we noted subsequent treatment, complications thereof, progression of disease and death. Causes of death were established through consensus between medical records and the Swedish death registry (paper II and III).
Additional methods paper II:
All patients in this study with peritoneal carcinomatosis (PC) (n=73) had a diagnosis of peritoneal carcinomatosis confirmed by histopathology. Patients were staged according to the Lyon prognostic index and the GPS (Table F, G). Tumor samples from 15 patients (with PC, n=8) were investigated with a single nucleotide polymorphism array.

Sample preparation
To ensure high tumor cell content, five µm sections were taken both before and after the collection of tumor tissue for DNA extraction and were stained with haematoxylin-eosin to verify tumor content. The tumor samples were, when necessary, macrodissected with the aim to ensure at least 70% tumor cell content. In total 10 sections á 20 µm were used for the DNA extraction.

Immunohistochemistry
Immunohistochemistry was performed on acetone-fixed frozen tissue sections of 5 µm. The tissue sections were incubated with 0.3 % hydrogen peroxide to block endogenous peroxidase activity. Avidin/Biotin blocking kit (Vector Laboratories Inc, Burlingame, CA) and normal horse serum was added to the sections. Monoclonal mouse anti-human Ki-67 antibody (M7240, DakoCytomation) was used and the antibody was diluted in phosphate-buffered saline with 2 % bovine serum albumin (Sigma-Aldrich Inc, Stockholm, Sweden) and incubated at room temperature for 1 hour. A biotinylated secondary mouse antibody was added to the sections, followed by Vectastain Elite ABC (Vector Laboratories). The reaction product was revealed using 3-amino-9-ethylcarbazole (Sigma-Aldrich, Inc) as chromagen, and sections were counterstained with Mayer’s hematoxylin. Negative control included omission of antibody.

Single nucleotide polymorphism array
DNA extraction was performed with the QIAGEN genomic-tip according to manufacturer’s instructions (Qiagen Nordic, Sollentuna, Sweden). The DNA samples were genotyped with the Infinium assay using the HumanOmini2.5-beadchip (Illumina Inc, San Diego, USA) at the SNP&SEQ Technology Platform in Uppsala, Sweden (www.genotyping.se) (186, 187).

The Illumina HumanOmini array investigates a fixed set of 2.4 million SNPs with the Infinium II assay (186, 187). First a whole-genome amplification step is used to increase the amount of DNA and then the DNA is fragmented (186). A probe sequence, 75 bases long, whereof 25 bases are designed to attach to a bead and 50 bases are designed to hybridize adjacent to the SNP query site is used (186). After target hybridization to the BeadArray, the SNP locus–specific primers are extended in the presence of hapten-labeled dideoxynucleotides. Biotin-labeled ddCTP and ddGTP, and
2,4-dinitrophenol (DNP)-labeled ddATP and ddUTP are the different bases that may be incorporated at the SNP-locus (186). A subsequent two-color antibody-based staining and signal amplification is used to detect a SNP. Visually, three different genotypes are discernable: red (homozygous AA), yellow (heterozygous AB) or green (homozygous BB)(186). Polar transformation of these data provides normalized intensity values and allelic intensity ratios (188). These values are used to calculate two metrics (LRR and BAF) for each SNP marker in a sample relative to those expected from a standard cluster position, which are used to determine SNP genotypes and copy number estimates (188).

In our study, the standard cluster positions were retrieved from samples from individual 13 and 01 in CEPH/Utah Pedigree 1362. Unsupervised hierarchical clustering based on CNV and LOH data and analysis of LOH was performed with Nexus 6.1 software (BioDiscovery, Hawthorne, USA). To confirm our results of LOH at chromosome 18 in another software and cluster file, Genome studio (Illumina, Inc) was used, and for this analysis the standard Illumina cluster file (based on DNA samples from the HAP-MAP) was used for computation of LRR and BAF.

**Patients and methods paper IV:**
Six consecutive patients with a histopathological diagnosis of NET liver metastases deemed suitable to undergo RFA at the Department of Surgery (Uppsala University Hospital, Uppsala, Sweden) were included. Three patients had SI-NET and three had P-NET. The aims and indications for RFA were to achieve full remission of liver metastases, to reduce symptoms of hormone release or to decrease morbidity. After inclusion into the study, RFA was performed in a prospective part of the study on 1-4 metastases per patient, and on 11 lesions (9 metastases and 2 RTs) in total. Five patients had also previously undergone RFA of NET liver metastases (n=19), and the outcomes of these treatments were included in a retrospective part of the present study. The RFAs for all six patients were performed through an ultrasound-guided percutaneous approach. All patients underwent CECT, CEUS and $^{11}$C-5-HTP-PET (baseline examinations) up to 8 months before RFA. After RFA, the scheduled radiological follow-up included CEUS and CECT on Day 1, and CEUS, CECT as well as $^{11}$C-5-HTP-PET at 1-2 and 6-11 months. The patients were also subjected to an extended follow-up to corroborate the image findings during the scheduled follow-up. This included evaluation of CECT up to 43-77 months and MRI up to 6-11 months performed as part of clinical routine. During scheduled follow-up, complete ablation or RT adjacent to the RFA areas were noted, as well as new hepatic metastases in other locations. Because biopsies of RT and new metastases for histopathological examination could not be performed for ethical reasons, findings from US, CT, $^{11}$C-5-HTP-PET and MRI were used.
as the gold standard. Tumor lesions visualized by at least two imaging modalities (CEUS, CECT, $^{11}$C-5-HTP-PET, MRI) during “scheduled follow-up” or “extended follow-up” were defined as definite tumor.

**Patients and methods paper V:**
The study included 235 patients (130 men and 105 women) who were admitted to the Department of Oncologic Endocrinology, Uppsala University Hospital, with SI-NET diagnosis between January 1980 and December 2001. The median age at diagnosis was 60 years (range 22-84). Patient charts were analyzed retrospectively, and the following data were registered: gender and age at diagnosis, if and when cholecystectomy was performed; if and when locoregional surgery was performed; prevalence of lymph and liver metastases at the time of locoregional surgery; SSA treatment; prevalence of gallstones; rate of gallstone complications (cholangitis, pancreatitis or acute cholecystitis); type of treatment for liver metastases including radiofrequency ablation treatment and liver embolization, and possible gallbladder-related complications.
5.3 Statistics

**Basic Statistics**
Continuous variables were given as mean (±SD) or median (range). For comparisons between groups different statistical tests were used for unpaired, paired, parametric/non-parametric continuous or categorical data as deemed appropriate. A kappa statistic was used to assess the inter-rater agreement regarding the Lyon prognostic score (Paper II). For all statistical tests, p<0.05 was considered statistically significant. A sample size calculation for the primary outcome (OS between matched groups) using a power of 80% and an alpha value of 0.05 was performed in Paper III.

**Survival analyses**
Kaplan Meier curves were drawn to illustrate survival and cumulative incidence. Crude and multivariable survival analysis for overall, disease specific and progression free survival as well as cumulative incidence were performed with a log-rank test, or with Cox-proportional hazards models. Stratified versions of the log-rank test and the Cox proportional hazards model were used for paired data (paper III). All Cox-regression models were truncated at 10 years follow-up with the aim to preserve proportional hazards. Relative survival was calculated as the ratio of the observed survival in the study population to the expected survival of the background population (189). The expected mortality rates were assessed from gender-, age- and period-specific life tables for Sweden. RS models calculating the excess mortality rate, i.e. the difference between the observed number of deaths and the expected number of deaths per person-year, were built in the Poisson regression model suggested by *Dickman et al* and modeled up to 10 years follow-up (190).
Propensity score matching
A propensity score was calculated by logistic regression with the baseline characteristics for each person as specified by Rosenbaum and Rubin (191). Propensity score matching between the two groups was performed on the logit of the propensity score, using a 1:1 matching procedure with no replacement, with a caliper width of 0.2 standard deviations of the propensity score logit, as proposed by Austin et al (192). Multilevel categorical values were recoded into dummy binary values and balance in baseline characteristics between the two matched groups was controlled by standardized differences for both continuous and binary variables as suggested by Austin et al (193). There is no agreement on firm cut-off levels for proper balance, although, for binary values, standardized differences <10% can most often be interpreted as negligible imbalance, and values <20% may also indicate limited imbalance (193). For continuous variables, a standardized difference of 10% has been shown to equal 7.7% non-overlap between groups (193).
6. Results

6.1 Paper I: Long-Term Results of Surgery for Small intestinal Neuroendocrine Tumors at a Tertiary Referral Center.

Out of 603 patients, 325 (53.9%) were male. Age at diagnosis was 63.1±11.3 years. Mean duration of follow-up from diagnosis was 6.9±5.2 years and 351 patients died during follow-up. Median OS was 8.4 years, 5-year OS was 67% (64-71%) and 5-year RS was 74% (70-79%).

Patients were staged according to the current ENETS criteria 2010, (4) (Table A-B). A more advanced stage was associated with worse OS and RS on crude analysis. The proliferation marker Ki-67 was analyzed in 299 patients, thus allowing grading according to ENETS proposal (Table C - Only Ki67 used for grading) and the OS and RS was significantly higher for grade 1 than for grade 2 (log rank test, p<0.001 and Poisson regression, p<0.001) (4).

Four different multivariable models for OS and RS were built. In the first model, we investigated prognostic factors that were readily available in all patients; gender, age at diagnosis, symptoms, carcinoid heart disease, metastatic distant abdominal lymph nodes, liver tumor load, and extra-abdominal metastases, and we included all patients with complete data (n = 562; Table L).


### Table L. Prognostic factors by multivariable regression models

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<th>p-value</th>
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<th>95% CI</th>
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<td>0.82</td>
<td>(0.59,1.12)</td>
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Cox regression (overall survival, OS) and Poisson regression model (relative survival, RS) for all patients with complete data. HR= Hazards ratio, EHR= excess hazards ratio, CI= confidence interval

In the second model (table not shown), we aimed for testing also for WHO grade, but as this parameter was only available from the late 1990 s, we only included patients diagnosed from 1998 to 2009 (n = 308). Complete data for all prognostic factors used in the first model, as well as ENETS grade, were available in 260 patients. In the third model (table not shown) we wanted to perform an adjusted analysis targeting peritoneal carcinomatosis and mesenteric lymph node status, which were findings at operation. Thus, this model included the same parameters as the first model but excluded all patients that were not operated on (patients with complete data, n = 481). Significant negative prognostic factors for OS and RS on multivariable analysis in all models included old age at diagnosis, carcinoid heart disease, and liver tumor load. High ENETS grade (grade 2–3 versus grade 1, HR [± 95% CI]; 1.83 [1.09–3.08]), peritoneal carcinomatosis (HR [± 95% CI]; 1.76 [1.26–2.45]), and pathological mesenteric lymph nodes (HR [± 95% CI];
3.92 [1.44–10.7]) were also shown to be negative prognostic factors for OS and RS in the second and third multivariable models, respectively.

Finally we used a fourth multivariable model to target possible survival benefits of locoregional resective surgery (table not shown). In this model we included the same parameters as in our first multivariable model for prognostic factors but also added the parameter “locoregional resective surgery” (n = 562). Locoregional resective surgery was associated with a decreased HR (95% CI) 0.46 (0.33; 0.65, p < 0.001), and a decreased EHR 0.41 (0.27; 0.62, p < 0.001) when patients “not operated on” were used as the standard of reference.

6.2 Paper II: Peritoneal carcinomatosis in small intestinal neuroendocrine tumors: A more aggressive disease?

Median OS and DSS for the PC group were 5.1 and 5.8 years, and 5-year OS and DSS rates were 50% and 57%. In comparison, patients without signs of PC (control population) had a favorable median OS and DSS of 11.1 and 12.6 years respectively and 5-year OS and DSS rates of 78% and 83% (both p<0.001).

After a median (range) of 2.6 years (0-11), 19 (26%) patients in the PC-group had undergone an emergency re-operative procedure. The control population had a similar median time to emergency re-surgery, 2.9 (0-14) years, but the proportion of patients (n=40, 8.5%) subjected to an emergency re-operative procedure was much lower (p<0.001).

The preoperative Lyon prognostic index could not separate patients with different extent of PC according to survival. The median OS rates were for stage 1; 8.0 years, stage 2; 6.3 years, stage 3; 6.8 years and stage 4; 6.1 years (p=0.322). On the contrary, the post-operative Lyon prognostic index was an excellent indicator of prognosis and median OS rates were for stage 0; 11.5 years, stage 1; 8.5 years, stage 2; 6.3 years, stage 3; 5.6 years and stage 4; 4.0 years (p=0.007). Correspondingly, the median OS for GPS were: Grade A; 8.7 years, grade B; 7.0 years, grade C; 5.2 years (p=0.058). A Cox-regression analysis (endpoint=OS) including age and co-morbidity was performed to examine the three different contributors within the GPS system (lymph node metastases, liver metastases and PC). It showed that the only significant prognostic factors were patient age and the post-operative quantity of PC (Non-resected PC Lyon stage 4 versus resected PC, hazard ratio (95% CI) 4.1 (1.05-16.1) and p=0.042)(Table not shown).

Unconditional hierarchical clustering based on CNV and LOH data from the SNP array from the primary tumors revealed two apparent clusters, A and B (Fig 2). Cluster A contained a higher proportion of primary tumors
from patients with PC (86% n=6/7) than cluster B (25%, n=2/8) (Fisher’s exact test p=0.042). The obvious difference between the groups on a chromosomal level was that cluster A (with 86% PC) showed LOH at the entire or major part of chromosome 18, whereas cluster B (with 25% PC) only showed limited LOH (n=7) or no LOH (n=1, ID 5) at chromosome 18.

**Figure 2. Unsupervised hierarchical clustering of all primary tumors**

ID 1-15 corresponds to primary tumors from patients in Table 4 of the manuscript. PC= peritoneal carcinomatosis at baseline or follow-up, no PC = without Peritoneal carcinomatosis at diagnosis nor follow-up. Fisher’s exact test confirms a higher proportion of primary tumors from patients with PC in Cluster A (86%, n=6/7) compared with Cluster B (25%, n=2/8)(p=0.042).
6.3 Paper III: Outcome after surgery and radiofrequency ablation of liver metastases in small-intestinal neuroendocrine tumors.

**Unmatched RFA/liver surgery group**
Liver surgery and/or RFA were performed in a total of 107 patients; however, due to missing data, four (3.7%) of these patients were not evaluated further (no information on size and/or amount of liver metastases). Liver surgery was performed alone in 31 patients and in combination with RFA in another 31 patients. A total of 192 metastases were resected, in median two (range 1-25) per procedure and in median two (range 1-25) per patient. RFA without resection was performed in another 41 patients, and consequently the total number of patients subjected to RFA was 72. In total 165 RFA sessions were performed, 1-7 times per patient, and a total of 382 metastases were treated by RFA. Macroscopic complete resection/ablation of liver metastases after the first procedure (RFA and/or liver surgery) was achieved in 44 (43%) cases. Most of these patients (n=28 (64%)) recurred during follow-up, and 5-year disease free survival was 25%.

**Unmatched Control group**
An unmatched control group of 273 (145 male) patients with liver metastases not treated by liver surgery nor RFA was identified within our cohort.

**Matching the “unmatched RFA/surgery group” to the “unmatched control group”**
The baseline characteristics showed that the patients in the surgery/RFA group were younger, had less advanced disease, and less co-morbidity. A propensity score match that counterbalanced for these disparities at baseline was performed, and this provided us with a matched-control study that included two groups of 72 patients each with similar baseline appearances, which in turn enabled us to evaluate outcome in a less biased manner.

**Comparison of outcome between the “matched surgery/RFA group” and the “matched control group”**
After matching, the OS and DSS of the matched control group were not different from the matched RFA/surgery group (Fig 3, only OS shown). Furthermore, there was no difference in progression to extra-abdominal sites, and 5-year PFS was 71% in the matched control group versus 67% in the matched RFA/surgery group (pairs with extra-abdominal disease at baseline excluded).
Stratified log-rank test showed no difference in survival (p=0.866). No. at risk = number at risk.
As expected, the matched RFA/surgery group displayed more response and stable disease within the liver than the matched control group after two and five years of follow-up (McNemar Bowkers’s test, p<0.001 and p<0.001) (patients with shorter follow-up than two and five years excluded, respectively)(Fig 4). However, the patients denoted as stable disease in the matched RFA/surgery group actually exhibited recurrence of disease that equaled the amount of tumor load at baseline, and they could therefore be considered to have progressive disease. Accounting for this, the proportion of patients with progressive disease within the liver was not different between the two groups after two (35% vs. 32%) or five years (44% vs. 44%)(Fig 4). This probably reflects similar tumor biology between the groups, which was not altered by RFA and/or liver surgery.

Figure 4. Treatment results of liver metastases at 2 and 5-years follow-up versus baseline.

Response = Decrease ≥30% of total liver tumor diameter. Progress= Increase by ≥20% of total liver tumor diameter. Stable = no progression or regression versus baseline. Pairs of patients with shorter follow-up than 2 and 5-years were excluded, respectively.
The matched RFA/surgery group U-5-HIAA values were lower than in the matched control group after both two and five years (Wilcoxon signed ranks test, p=0.019 and p=0.005, respectively)(Fig 5).

**Figure 5. U-5-HIAA value at baseline, 2 and 5-year follow-up for both groups.**

P-values test the null hypothesis of equivalence between the treatment groups by Wilcoxon signed ranks test. Dotted line represents reference value (<50 μmol/dl/24 hours). Pairs of patients with shorter follow-up than 2 and 5-years excluded, respectively.
6.4 Paper IV: $^{11}$C-5-Hydroxytryptophan Positron Emission Tomography after Radiofrequency Ablation of Neuroendocrine Tumor Liver Metastases

Retrospective analysis

Five patients had undergone RFA before inclusion in the study. At baseline there were 19 previously established RFA areas (1-7 areas per patient) and 5 of these areas displayed RT, none of which had been identified by earlier routine therapy monitoring by CECT and CEUS. The baseline examinations demonstrated sensitivities for $^{11}$C-5-HTP-PET (100%), CEUS (100%) and CT (100%), and specificities for $^{11}$C-5-HTP-PET (100%), CEUS (82%) and CT (78%) concerning complete ablation using both scheduled and extended follow-up as the gold standard.

Prospective study

Three out of the 11 evaluated RFA areas were depicted with RT, two of which were visualized by CEUS and one by CECT 1-2 days after RFA. All 3 lesions were subsequently confirmed by means of CEUS, CECT, and $^{11}$C-5-HTP-PET examinations in the scheduled follow-up after 1-2 months (Fig 6). When combining the results from the retro- and prospective parts of the study the sensitivities and specificities for establishing complete ablation at the first post RFA imaging were 100% and 100% for $^{11}$C-5-HTP-PET and 100% and 88% for both CECT and CEUS.
Figure 6. Residual tumors depicted by $^{11}$C-5-HTP-PET and CECT during follow-up imaging.

RT marked by white arrow or black arrow. $^{11}$C-5-HTP-PET images visualize the corresponding lesion to the CT next to it. The dotted white line represents the liver contour, and the unbroken white line the contour of the spleen in the PET images.
Sensitivity for detecting tumor lesions by different imaging modalities

By using a combination of $^{11}$C-5-HP-PET, CEUS, CECT (and also MRI in 6 cases) and extended follow-up (CECT) as the gold standard, we calculated the sensitivity for $^{11}$C-5-HP-PET, CEUS and CECT in detecting metastases and RT. On a lesion-by-lesion basis, the sensitivity for $^{11}$C-5-HP-PET, CEUS and CECT upon inclusion was 92%, 44%, 16%; at 1 month 95%, 32% and 63%, and at 6 months 78%, 35% and 74%, respectively. When comparing the sensitivity for detecting tumor lesions on a patient-by-patient basis, $^{11}$C-5-HP-PET was superior to CECT in three patients, equal in one and inferior in two patients. In comparison with CEUS, $^{11}$C-5-HP-PET was superior to CEUS in four patients, and equal in two patients.

6.5 Paper V: Prophylactic Cholecystectomy in Midgut Carcinoid (SI-NET) patients.

The 187 patients with a remaining gallbladder at diagnosis were treated according to clinical routine at the time. Forty-three of these patients were not treated with SSA when they still had a remaining gallbladder, and this group was followed on average 82±101 months after diagnosis. None of these 43 patients developed cholangitis, pancreatitis or acute cholecystitis during follow-up.

SSA treatment was given to 144 out of the 187 patients during the time these patients had a remaining gallbladder. The observation times were 89±73 months after diagnosis and 55±57 months after start-up of SSA treatment. Thirty-two out of these 144 patients (22%) underwent cholecystectomy (n=31) or drainage (n=1) during follow-up. Cholecystectomy was performed in conjunction with primary carcinoid surgery in 5 cases and after primary surgery in 26 cases.
Figure 7. Cumulative incidence of cholecystectomy due to cholangitis, pancreatitis, cholecystitis, or gallstone-related pain in somatostatin analog-treated (SSA+) and non-treated (SSA-) patients.

The 5-year cumulative risk (from diagnosis) to undergo cholecystectomy due to gallstone-related complications or pain was 2.3% (95% CI, 0–6.9) for untreated patients and 19% (95% CI, 10–28) for somatostatin analog treated patients (from treatment startup), respectively. Log-rank test for test of difference between the two groups was however not statistically significant (Fig 7). The absolute risk of undergoing cholecystectomy with gallbladder related complications (cholangitis, cholecystitis, and pancreatitis) as indication was 7.2% was in the SSA treated group with a remaining gallbladder after primary surgery (10/139). The absolute risk for undergoing cholecystectomy (all indications) after primary surgery in SSA treated patients with a remaining gallbladder was 19% (26/139).
7 General Discussion

7.1 Survival, prognostics and locoregional surgery

A majority of patients displayed tumor spread to both lymph nodes (88%) and/or liver (61%) at diagnosis. Despite this, fairly favorable 5-year OS and RS rates of 68% and 75% were encountered in our series, consistent with previous results from the Swedish cancer registry of patients diagnosed 1991–2000, showing 5-year OS of 65% and RS 77%. Other registry studies report a 5-year OS and RS ranging from 37-65% and 46-79% respectively (6, 8, 12, 30-34). Relative survival is in general an interesting measure of cancer survival, and may be considered as the gold standard for measuring cancer-mortality in a population based setting, however in a single center study such as ours, relative survival rates may be skewed due to referral bias.

Metastatic distant abdominal lymph nodes (i.e. paraaortal), PC, and extra-abdominal metastases were shown to be independent prognostic factors for survival, and these are to our knowledge new findings. It should, however, be noted that the parameters “distant abdominal lymph nodes” and “extra-abdominal metastases” were non-significant in our multivariable model where proliferation grade was included, although this may be due to fewer patients and shorter follow-up diminishing the power of the model. Also the number of statistical tests performed in the current study should be noted. We pondered if correction for multiple comparisons should have been done, but it was considered unnecessary, as all tests performed are published. A correction has the benefits of decreasing the risk of a type I error, but instead increases the risk of a type II error. If not done, the reader may still easily do corrections at their own discretion, for example by multiplying the p-values by the number of tests performed (Bonferroni).

Interestingly, none of the patients subjected to locoregional surgery with apparent stage I–II (n = 18) in our study had a recurrence during follow-up, which rendered a 100% OS indicating possible curative resection in these patients. Three other studies similarly noted 100% survival (one study OS, two studies DSS) in their patients with stage I–II SI-NET (13, 37, 41). A previous study from our own institution of patients radically operated with localized disease diagnosed 1980-1990 showed recurrence in 2 out of 8 patients (19). Also, Landerholm et al report a low rate (4%) of recurrence after surgery in localized disease (13). It is therefore not certain that these patients need to be followed after surgery. Regional disease has a much
higher rate of recurrence after perceived radical surgery and it was 40% in a population-based study and 82% in a previous study from our institution, and these patients should be followed to assess and treat recurrence (13, 19).

Conflicting results regarding locoregional resection of the primary and mesenteric tumor in metastatic SI-NET have been published, and uncertainties as to what type of surgical procedures have been performed complicate the picture (46, 52, 194). A multivariable analysis in Paper I showed that surgery of the primary tumor was associated with a superior survival. However, a retrospective cohort study, such as ours, always has the disadvantage of “confounding by indication” when studying effects of treatment. In retrospect we could of course have made an attempt to control also for co-morbidity, as we did in paper III, and this shortcoming hampers interpretation of our results. Also, multivariable analyses needs at least some patients with similar baseline characteristics to avoid heroic modeling assumptions, and most of our patients eligible for locoregional surgery were also subjected to such surgery which makes the comparison between locoregional surgery and no locoregional surgery difficult. Prospective randomized controlled trials are needed to formulate traditional evidence-based criteria regarding survival after surgical treatment of SI-NETs. However, such trials are most likely impossible to conduct, due to the scarcity of patients and the indolent course of the disease and difficulty to include patients in such a study. The primary conclusion to draw from the surgical part of the study is that locoregional surgery can be performed with a low risk for complications, and that there is no evidence pointing to a negative effect on long-term survival.
7.2 Peritoneal carcinomatosis in SI-NET

PC is a negative prognostic factor for survival, in this study reflected by a median survival of only 5.1 years in the patients with PC. In an earlier study from our group a multivariable Cox-regression model also showed that PC was a poor prognostic factor (Hazard ratio [95%] CI) 1.76 [1.26-2.45]).

The fact that patients without PC at baseline had less than half the risk of undergoing emergency re-surgery in comparison to patients with PC points to the fact that PC may be harmful. The patients subjected to radical resection of PC had a very favorable median survival (11.5 years), which resembles the median survival of patients without PC (11.1 years). Of course, it must be noted that the patients subjected to radical resection of PC within our cohort generally had very confined PC. Therefore, it is still unknown if surgical resection of PC grants increased survival and decreased risk of re-surgery. In a multivariable model, we also showed that the only significant prognostic factor within the GPS was the post-operative Lyon score. We therefore conclude that one of the most import clinical prognostic factors in SI-NET patients is the amount of PC post laparotomy, more important than both the amount of liver metastases and metastatic lymph nodes in these patients.

From the clinical data we hypothesized that patients with PC may harbor genetic aberrances different to the patients without PC that may be driving the more aggressive phenotype. In this patient cohort we report that primary tumors from patients with PC in general clustered differently from primary tumors in patients without PC based on profiling from CNV and LOH data. The main finding at the chromosomal level was a difference in the quantity of LOH at chromosome 18, where tumors in cluster A (86% with PC) showed LOH at all or most of chromosome 18, whereas tumors in cluster B (25% with PC) only showed small stretches of LOH. Aberrations at chromosome 18 are the most frequently seen in SI-NET patients ranging from 43-100% (181-185). This is in correspondence to our study, as all but one of the primary tumor samples showed LOH at chromosome 18. However, our results contrast to two other studies that have not found the same differences in SI-NET patients with and without PC (182, 183).

Varying definition of peritoneal carcinomatosis (in conjunction with the primary tumor or distant from the primary tumor, macroscopic/ microscopic etc.), variable patient inclusion criteria, contamination with non-tumorous tissue and diverse array methods (CGH vs. 100k SNP versus 2.4million SNP) may explain differences between studies. Also, it is unclear if any of the patients without PC at diagnosis developed PC during follow-up in those studies (182, 183).

Our study is limited by size and it remains to verify whether patients with PC present a distinct sub-group of SI-NETs that follow a specific genetic
pathway. The findings will have to be addressed further in a larger cohort of patients.

7.3 Long term effect of RFA/liver surgery on tumor load and U-5-HIAA values

Although a multicenter study of a GEP-NET population used propensity scores to describe attributes in patients that were subjected to either liver surgery or HAE/HACE (75), our study is the first to date regarding treatment of SI-NET liver metastases that uses propensity score matching between a surgery/RFA group and controls, and also displays balance in baseline characteristics between the two groups.

The patients in the matched RFA/surgery group did not have superior OS or DSS rates (both 5-year 74%) when compared with the matched controls in whom the OS and DSS rates were at least equally beneficiary (5-year 74% and DSS 78%). Progression-free survival was also no different between the matched RFA/surgery group and the matched control group, indicating that RFA and/or surgery of liver metastases do not inhibit spread to extra-abdominal sites. There are several possible explanations for the findings above. One is that RFA and/or liver surgery does not affect progression to extra abdominal sites or survival in SI-NET, and previously observed advantageous survival rates in RFA/surgery cohorts are mainly due to selection bias of healthier patients for surgery. Furthermore, RFA/liver surgery was not shown stop progression of liver metastases, as a fairly equal proportion of patients in both matched groups displayed progression or recurrence of tumor after two and five years than at baseline. On the other hand, more patients in the matched RFA/liver surgery than in the matched control group displayed stable disease, partial or complete response of liver metastases after two and five years compared to baseline. This indicates a feasible local long-term result and chance to slow development of extensive tumor load within the liver. Also, U-5-HIAA values were lower in the matched RFA/surgery group both after two and five years, indicating treatment effect. Finally, the frequency of given PRRT and/or RAE was slightly higher in the matched control group and this might have affected outcome in favor of the control group.

Limitations of Paper III include a long accrual time leading to differences in radiology exams and treatment, although we did adjust for calendar year at baseline to counterbalance this. Furthermore, although we tried to control extensively for presumed confounders in our analysis, we cannot be entirely certain that we did not oversee some critical ones. Theoretically, imaginable remnant confounders may inflict bias, making the matched RFA/surgery group and matched control group non-comparable. The sample size of our
study may also lack power to ascertain a difference in survival and progression of extra-abdominal disease that may still be clinically important. However, when examining the HR and 95% CI for OS and progression to extra abdominal disease, there are no trends that points to a positive, but non-significant effect of RFA and/or liver surgery in these aspects. Difference in radiology exams performed during follow-up may also skew results, although most patients were examined at least annually with abdominal CT. The changes in U-5-HIAA values should also be interpreted with some caution, since supplemental treatments such as somatostatin analogues, which also affect U-5-HIAA values, were initiated during the same period of time in a number of patients. Moreover, our study is not population-based and may therefore be skewed by referral bias. Nevertheless, it is the largest study to date of surgical treatment of SI-NET liver metastases and we believe that it displays new important insights of the outcome after RFA and liver surgery.

We believe that RFA/liver surgery is indicated in symptomatic disease when symptoms are refractory to medical treatment. For asymptomatic patients, evidence does not unambiguously encourage widespread use of RFA and/or liver surgery in SI-NET, although our data show less progression of metastases within the liver and lower U-5-HIAA values during follow-up in the RFA/surgery group than in a matched control group. A randomized controlled trial to evaluate survival, progression, symptoms and quality of life is needed, although most likely impossible to perform, due to the scarcity of the disease and difficulties to include patients in such a study.

7.4 Imaging before and after RFA

In our study, $^{11}$C-5-HTP-PET detected RT earlier than CECT or CEUS. Furthermore, the sensitivity of $^{11}$C-5-HTP-PET exceeded that of CECT and CEUS for early visualization of liver lesions before and after RFA.

As mentioned above, $^{11}$C-5-HTP-PET offered superior detection of RT, as has been shown with FDG-PET after RFA of liver metastases from tumor entities other than NETs (150-152, 196). It is well known that FDG accumulates in inflammatory lesions, and false positive FDG-PET findings have been encountered in the immediate post-RFA follow-up. $^{11}$C-5-HTP has not previously been shown to accumulate in inflammatory and infectious foci, and no false positive uptake of $^{11}$C-5-HTP was observed in the current study, using radiological follow-up as the gold standard. In an earlier study, $^{11}$C5-HTP-PET positivity has shown a specificity of 100% for neuroendocrine tumor using histopathological diagnosis (surgical biopsies) as the golden standard (143). When using $^{11}$C-5-HTP-PET, additional tumor lesions were detected as compared with CEUS and CECT. CEUS
detected fewer metastases than CECT and $^{11}$C-5-HTP-PET. In the retrospective setting, it was possible to compare the image findings from CECT and $^{11}$C-5-HTP-PET with those from previous examinations, which was not the case with CEUS, and although this may have facilitated the detection of metastases by CECT and $^{11}$C-5-HTP-PET, as compared to CEUS, the difference in the diagnostic yield is still notable. Operator dependency for CEUS must also be taken into account in this comparison. However, both radiologists who performed all but two examinations in this series are highly experienced with CEUS and no significant diagnostic differences were noted between these two operators.

One reason for the false negative CECT results may be related to the contrast enhancement technique. Poor timing of the CECT examination in relation to the contrast medium injection can also make comparisons between examinations difficult and especially when the administered volume and concentrations of iodine in the contrast medium differ between examinations (124).

Nevertheless, CECT detected 5 lesions in two different patients that were not diagnosed by $^{11}$C-5-HTP-PET during follow-up. Earlier studies have demonstrated FDG-Pet’s poor sensitivity for small hepatic lesions, not only due to its low spatial resolution but also because of blurring of tracer uptake due to liver movements during breathing (197). This drawback with PET might be resolved with respiratory-gated imaging, although this technique was not implemented in our institution at the time of the present study. All lesions that were detected during follow-up by CECT, but not by $^{11}$C-5-HTP-PET, were smaller than 0.8 cm, and these false negative results from PET may partly be explained by this phenomenon. The only $^{11}$C-5-HTP-PET negative lesion in one of our two patients with PET-negative lesions was situated adjacent to a lesion with a high tracer uptake that might have concealed presence of the former metastasis. Theoretically, dedifferentiation of tumors may result in lower uptake of $^{11}$C-5-HTP, which may explain the findings in the other patient, who died 7 months after RFA with widespread metastatic disease. Differences in $^{11}$C-5-HTP uptakes after different medical treatments are also possible, although no patients changed medication during the follow-up of our trial. We could not correlate any differences in sensitivity of $^{11}$C5-HTP-PET to difference in medical treatment between subjects.

The advantage of $^{11}$C-5-HTP-PET in screening may be underestimated in our study since one of the inclusion criteria was that patients have at least one liver metastasis detectable by CEUS or CECT, thus leading to a selection bias. Conversely, the sensitivity of $^{11}$C-5-HTP-PET might be overestimated later during follow-up as only lesions seen by morphologic imaging modalities were ablated during this study, omitting lesions depicted exclusively by PET. The small number of patients in combination with the lack of a histopathological gold standard for the image findings also makes it
hard to draw firm conclusions from our data. A recent report on NET liver metastases using thin slice pathology as the gold standard did nevertheless demonstrate similarly poor results for morphological imaging in detecting hepatic metastases: only 38% sensitivity was reported for CECT and CEUS and 49% for MRI (82). $^{11}$C-5-HTP-PET has also been found superior to CT, US and SRS for the detection of NET metastases in other settings (142-145). PET imaging is generally a whole body scan and therefore also has an advantage over CEUS and CECT in the search for extra-hepatic disease.

The results from previous reports on screening for NET metastases by $^{11}$C-5-HTP-PET combined with those in this small series provide grounds for further $^{11}$C-5-HTP-PET/ CT studies on therapy monitoring following RFA and screening for metastatic disease. However, the drawbacks of $^{11}$C-5-HTP-PET/ CT imaging include high cost, limited availability and an increase of the radiation dose to the patient in comparison to CEUS and CECT.

7.5 Impact of a remaining gallbladder in patients with midgut carcinoids (SI-NET)

In population studies the prevalence of gallbladder stones ranges from 10–20% and gallstones are almost twice as common in women as in men (172). The prevalence of gallbladder stones increases with increasing age, and in some studies it comprises of more than 50% in individuals older than aged 75 years (198). It may be argued that the high prevalence of gallbladder stones in our study material is due to selection bias of patients undergoing radiological examinations, but the minimum prevalence could still be calculated to 40% (58/144). Moreover, it may be difficult to state whether gallbladder stones existed before SSA treatment was initiated. However, our findings correlate well with one study of patients treated with SSA for acromegaly, which showed increased prevalence of gallstones from 26% to 47% (174). Another study of patients with acromegaly showed 35% prevalence of gallbladder stones in the SSA treated group compared with 8.6% in patients not subjected to SSA therapy (173). In one study of NET patients (n= 44) who received SSA treatment, the gallbladder stone prevalence increased from 2.2% to 53% during a mean 44 (range, 18–84) months of follow-up (175). However, most gallstones remain asymptomatic, and in a general population (older than 65 years of age), the 5-year incidence of cholecystitis, cholangitis, and gallstone-related pancreatitis is approximately 1.3%, with cholecystitis accounting for 75–85% of all complications (180, 199, 200). The 5-year incidence of cholecystitis, pancreatitis, and cholangitis and gallstone-related pain leading to cholecystectomy in a general population (older than 65 years of age) is approximately 3% compared to the cumulative 5- year incidence of 19%
(95% CI, 10–28) in our SSA treated patients (180). However, our calculated 5-year cumulative incidence is skewed by the competing risk of cancer-related death that increases the number of censored cases (and hence also increases the cumulative risk) in our Kaplan-Meier analysis. To make a less biased comparison, another method of censoring (ignore death) or a cumulative incidence competing risks method to assess for competing risks could have been used, which would find the adjusted 5-year cumulative incidence to be lower when accounting for death (201). Another way to easier compare our results to the general population would have been to calculate standardized incidence ratios using the incidence ratios of cholecystectomies in age, calendar year and gender matched controls, as in a study by Plecka-Ostlund et al (202). The absolute risk (which is not influenced by competing risks) in patients treated with SSA of undergoing cholecystectomy or drainage with a remaining gallbladder due to gallstone related pain or complications after diagnosis was 15% (95%CI [9-21%]) (n=21/144) in our study.

A Swedish population based study (1987-2008) showed 8.2% risk of undergoing cholecystectomy (all indications) and a 3.2% absolute risk concerning the need for an imperative cholecystectomy after obesity surgery, which seems to be lower than in our cohort (19% and 7.2%, respectively)(202). However, absolute risks are dependent on length of follow-up and therefore difficult to compare straightforwardly.

The actual origin of the common symptom of abdominal pain in SI-NET may be difficult to establish in patients with a remaining gallbladder. Furthermore the risk of injury to the gallbladder during RFA or HAE should be considered. However, reports of ischemic cholecystitis as a complication of liver embolization or gallbladder injury caused by RFA are rare in the literature and in our study there was a mere 4.8% (2/43) bile-duct-related complication rate after liver embolization, and no gallbladder-related complications were noted after any of the 16 RFA sessions performed (81, 85, 111).

Most authors recommend conservative management by observation alone for asymptomatic gallbladder stones in normal individuals (203-209). However, for SI-NET patients treated with SSA the risk to suffer a complication that necessitates cholecystectomy is rather high and therefore concomitant prophylactic cholecystectomy during laparotomy may be recommended in patients with SI-NET. The indication for cholecystectomy becomes stronger if the patient has liver metastases and is planned to undergo hepatic artery embolization. We believe that there is no indication for prophylactic cholecystectomy if the patient is not planned for laparotomy.
Conclusions

- Age, carcinoid heart disease, proliferation grade, metastatic mesenteric lymph nodes, distant abdominal lymph nodes, liver metastases, peritoneal carcinomatosis, and extra-abdominal metastases, were demonstrated to be independent prognostic factors for survival with multivariable analysis.
- Surgical removal of the primary tumor was a positive prognostic factor for survival by both crude and multivariable analysis.
- The amount of peritoneal carcinomatosis after surgery was a significant prognostic marker for survival.
- Primary tumors in SI-NET patients with and without peritoneal carcinomatosis clustered differently according to analysis of CNV and LOH from SNP array data.
- Surgery and/or RFA of liver metastases did not show an association with improved survival or less progression to extra abdominal sites in comparison to a matched control group.
- Surgery and/or RFA of liver metastases were associated with less tumor load within the liver during follow-up than at baseline and lower U-5HIAA values after two and five years compared to a matched control group.
- $^{11}$C-5-HTP-PET can be used in the post-RFA follow-up for the purpose of detecting residual tumor, should be combined with CT for easier interpretation, and may provide additional information to morphologic radiology when screening for hepatic metastases.
- Somatostatin analog treated SI-NET patients with a remaining gallbladder after primary locoregional surgery have a substantial risk to suffer symptoms that necessitate cholecystectomy.
9 Future perspectives

Based on our findings, additional genetic and clinical studies on SI-NET patients with peritoneal carcinomatosis are warranted. Also, randomized studies regarding locoregional surgery, liver surgery and radiofrequency ablation are needed, however these studies are extremely difficult to perform due to the scarcity and indolent course of the disease as well as difficulties to include patients in such studies. A registry-based approach to evaluate these treatments might be more practical, and the emerging Swedish liver registry and the anticipated Swedish NET registry are proper initiatives that possibly will help us to draw some future conclusions. Imaging of NET tumors with different PET-tracers, CT, US and also MRI needs further analysis, although we also need to determine how to treat metastatic disease of varying extent, as we most likely will detect more small metastases. Is all visible tumor tissue supposed to be removed surgically, or are somatostatin analogs and watchful waiting in asymptomatic patients the proper way? Cancer is a heterogeneous disease and maybe some metastases are more dangerous than others? In this case we need a reliable way to identify which metastases to treat. Some surgeons indicate that individualized treatment for all patients and maybe even for specific metastases is the only way to go, although this approach is far from optimal for future patients, as no firm conclusions will then be drawn regarding current treatments. Hopefully, at least a few prospective randomized multicenter studies are forged in the near future that evaluate some of the principal questions above; otherwise we may be stuck at the current level of evidence regarding surgery and RFA within the foreseeable future.
10 Summary of the thesis in Swedish

Populärvetenskaplig sammanfattning

Neuroendokrina tunntarmstumörer, tidigare benämnda midgut carcinoider, har en årlig incidens av 0,3-1,3 per 100 000 invånare. En direkt följd av att de är så ovanliga är att de studier av neuroendokrina tunntarmstumörer som beskriver prognostiska faktorer och effekt av behandlingsmetoder, såsom kirurgi, är få, och begränsade till antalet patienter. Uppsala har under de senaste årtiondena varit ett stort specialistcenter för patienter med dessa tumörer.

I delarbete 1, 2 och 3 har vi studerat patienter med neuroendokrin tunntarmstumör som diagnosticerats och vårdats på Akademiska sjukhuset mellan 1985-2010 (-2012 för delarbete 2 och 3). Totalt inkluderade vi 672 patienter, vilket torde utgöra ungefär en fjärdedel av alla patienter diagnosticerade i hela Sverige under samma tidsperiod. Vi studerade sedan prognostiska faktorer för överlevnad. Vi kunde visa att medianöverlevnad för patienter med neuroendokrin tunntarmstumör var 8,4 år och att metastaser i lymfkörtlar bredvid aorta, tumörspridning till bukhinnan, levermetastaser, och tumörväxt utanför buken alla var oberoende negativa prognostiska faktorer för överlevnad. Vi kunde också visa att de patienter som opererats bort sin primärtumör (och ev. lymfkörtelmetastaser) överlevde längre än de som inte opererades, justerat för de nämnda prognostiska faktorerna. Hos patienter med neuroendokrin tunntarmstumör är spredning av cancer till bukhinnan (peritoneal carcinos) den tredje vanligaste lokalen för spredning, näst lever och lymfkörtelmetastaser. Det finns bara enstaka publicerade arbeten omfattande mindre än 20 patienter, som fokuserar på peritoneal carcinosis hos patienter med neuroendokrina tunntarmstumörer. Då patienter med peritoneal carcinosis föreföll ha en annorlunda, kliniskt mer aggressiv sjukdom så var vår hypotes att det fanns bakomliggande genetiska skillnader hos de tumörer som sprid sig till bukhålan jämfört med de som inte gjorde det.

I delarbete 2 inkluderades alla patienter som genomgått laparotomi och vårdats på Akademiska Sjukhuset 1985-2012. Vi identifierade 73 patienter med peritoneal carcinosis bekräftad med mikroskopi och speciell färgning av tumör celler. Utbredningen av denna bedömdes genom ett validerat prognostiskt index och beskrev sedan naturalförloppet vad gäller överlevnad och reoperationer på grund av peritoneal carcinosis. Vi kunde visa att patienter
med kvarvarande peritoneal carcinos efter operation hade kortare överlevnad och högre risk för reoperation. I en andra del av detta arbete påvisades skillnader i DNA från tumörprover på patienter med, respektive utan, peritoneal carcinos när detta kartlades med en högupplöst så kallad "single nucleotide polymorphism array".


I delarbete 4 studerade vi olika typer av radiologisk uppföljning efter RFA; för tillfället används i klinisk rutin ofta en kombination av ultraljud (UL) och datortomografi (CT). Flera studier har dock pekat på begränsningar i såväl sensitivitet som specificitet för dessa undersökningar när det gäller...
bedömning av eventuella lokalrecidiv. \(^{18}\)Flor-deoxyglukos positron emissions tomografi (FDG-PET) som uppföljning efter RFA av levermetastaser från tjocktarmscancer och primär levercancer har visat lovande resultat, dessvärre är FDG-PET undermålig på att visualisera tumörer med låg tillväxttakt, såsom neuroendokrina tumörer ofta har. Däremot har PET med \(^{11}\)C-5-hydroxytryptophan (HTP) visats ha en god förmåga att avbilda neuroendokrina tumörer. Vi utförde en studie på sex konsekutivt insamlade patienter med NET och levermetastaser, som alla var planerade att genomgå RF. HTP–PET, UL med kontrast, CT i trefas med kontrastförstärkning utfördes alla innan, ca 1 månad samt ca 6 månader efter RF behandling. Vårt mål med studien var att undersöka möjligheterna till detektion av lokalrecidiv efter RF samt nya levermetastaser för HTP-PET i jämförelse med våra traditionella röntgenundersökningar (UL, CT). PET avbildade samtliga lokalrecidiv enligt vår ”golden standard” emedan UL och CT var något mindre sensitivt. Vi upptäckte också fler nyttillkomna levermetastaser med PET jämfört med CT och UL. Vår konklusion blev att HTP-PET kan användas för detektion av nya levermetastaser samt övervakning efter RF hos patienter med NET, och att PET ger ytterligare information om tumörspridning inom levern.

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Most abbreviations within this thesis are written on the front page. If you manage to find out which ones that are missing- send me an email: olov.norlen@me.com
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