Clinical and immunohistochemical studies
of small bowel carcinoid tumours

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Cover illustrations:
Front: Carcinoid with trabecular growth pattern or cord-like appearance.
Back: Carcinoid with insular growth pattern.

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To my family
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This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:


IV. Landerholm K, Shcherbina L, Falkmer SE, Järhult J, Wierup N. Expression of Cocaine- and Amphetamine-Regulated Transcript is Associated with Worse Survival in Small Bowel Carcinoid Tumors. *Submitted manuscript*.

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<table>
<thead>
<tr>
<th>Abbreviation</th>
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<tr>
<td>5-HIAA</td>
<td>5-hydroxyindole acetic acid</td>
</tr>
<tr>
<td>CART</td>
<td>cocaine- and amphetamine-regulated transcript</td>
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<tr>
<td>CgA</td>
<td>chromogranin A</td>
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<tr>
<td>CgB</td>
<td>chromogranin B</td>
</tr>
<tr>
<td>CHD</td>
<td>carcinoid heart disease</td>
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<td>c.i.</td>
<td>confidence interval</td>
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<tr>
<td>CSC</td>
<td>cancer stem cell</td>
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<tr>
<td>EC</td>
<td>enterochromaffin</td>
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<tr>
<td>ENETS</td>
<td>European neuroendocrine tumor society</td>
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<tr>
<td>EPT</td>
<td>endocrine pancreatic tumour</td>
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<tr>
<td>GEP</td>
<td>gastroenteropancreatic</td>
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<tr>
<td>GI</td>
<td>gastrointestinal</td>
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<tr>
<td>HACE</td>
<td>hepatic artery chemoembolization</td>
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<tr>
<td>HAE</td>
<td>hepatic artery embolization</td>
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<tr>
<td>HR</td>
<td>hazard ratio</td>
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<tr>
<td>i.q.r.</td>
<td>interquartile range</td>
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<tr>
<td>IR</td>
<td>immunoreactive</td>
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<tr>
<td>MEN1</td>
<td>multiple endocrine neoplasia type 1</td>
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<tr>
<td>MEN2</td>
<td>multiple endocrine neoplasia type 2</td>
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<tr>
<td>NEC</td>
<td>neuroendocrine carcinoma</td>
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<tr>
<td>NEN</td>
<td>neuroendocrine neoplasia</td>
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<tr>
<td>NET</td>
<td>neuroendocrine tumour</td>
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<tr>
<td>NKA</td>
<td>neurokinin A</td>
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<tr>
<td>NPK</td>
<td>neuropeptide K</td>
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<tr>
<td>NSE</td>
<td>neuron-specific enolase</td>
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<tr>
<td>PET</td>
<td>positron emission tomography</td>
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<tr>
<td>PRRT</td>
<td>peptide receptor radionuclide therapy</td>
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<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>R0</td>
<td>complete resection</td>
</tr>
<tr>
<td>R1</td>
<td>microscopical residual tumour</td>
</tr>
<tr>
<td>R2</td>
<td>macroscopical residual tumour</td>
</tr>
<tr>
<td>RFA</td>
<td>radiofrequency ablation</td>
</tr>
<tr>
<td>SEER</td>
<td>the Surveillance, Epidemiology and End Results Program</td>
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<tr>
<td>SERT</td>
<td>serotonin reuptake transporter</td>
</tr>
<tr>
<td>SPECT</td>
<td>single-photon emission computed tomography</td>
</tr>
<tr>
<td>sstr</td>
<td>somatostatin receptor</td>
</tr>
<tr>
<td>TA</td>
<td>transit-amplifying</td>
</tr>
<tr>
<td>TGF-β1</td>
<td>transforming growth factor beta 1</td>
</tr>
<tr>
<td>TNM</td>
<td>tumour node metastasis</td>
</tr>
<tr>
<td>UICC</td>
<td>union for international cancer control</td>
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INTRODUCTION

A. Morphology and physiology of the small bowel

The small bowel is the site of terminal food digestion, executed by chemicals and enzymes produced in the liver and the pancreas, and by cells in its own mucosa. The digestion is followed by selective absorption of nutrients into blood and lymph capillaries, after which the chyme is transported to the colon. This digestive physiology is orchestrated by enteric nervous and endocrine signals.

MORPHOLOGY OF THE SMALL BOWEL

Estimations of the length of the small bowel vary widely. Hirsch et al. measured the entire small bowel to approximately 270–290 cm in live humans, and pointed out that the intestine gets considerably elongated after death when the normal smooth muscle tone disappears. This may explain that studies on necropsy specimens reported a mean length of the adult human small intestine of 550 cm. Most authors seem to agree that duodenum measures about 20 cm, and that jejunum makes up ⅖ and ileum ⅗ of the remainder. Like the entire gastrointestinal (GI) tract, the small bowel wall is made up by four principal layers: the mucosa, submucosa, muscularis and serosa.

The mucosa of the small bowel is not a smooth surface but instead arranged like a terry cloth, with innumerable villi projecting 0.5–1.5 mm into the lumen. These villi serve to increase the absorptive area of the small bowel tremendously, and the small bowel therefore holds about 90% of the entire mucosal surface of the GI tract. Between the villi are the openings of the intestinal glands, also known as the crypts of Lieberkuhn.

The mucosa of the small bowel contains four major cell types: enterocytes (absorptive cells), goblet cells (mucosecreting cells), Paneth cells and enteroendocrine cells. Enterocytes are tall columnar cells, the most abundant cell type in the small bowel, and mainly dedicated to absorption of nutrients. Goblet cells are mucosecreting and

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INTRODUCTION

become more frequent distally within the small bowel, and are particularly abundant in the colon and the rectum where the stools become increasingly compacted. They secret acid glycoproteins which form a mucus that lubricate and protect the intestinal lining. Paneth cells are exocrine cells secreting antimicrobial agents such as defensins and lysozymes, and are important in the control of the intestinal microbial flora. Recently it has been suggested that the Paneth cells also sustain and regulate the intestinal stem cells. The enteroendocrine cells will be discussed in the next section.

In addition to these four main cell types, there are a few lesser-known cell types in the small bowel mucosa: M (microfold) cells overlie the lymphoid follicles of Peyer’s patches and present luminal antigens to the intestinal immunologic system. Less is known about the origin and function of Brush cells and Cup cells.

ENDOCRINE CELLS OF THE GASTROINTESTINAL TRACT

Endocrine cells are interspersed within the GI mucosa from the stomach to the rectum, making up only approximately 1% of all mucosal cells. Nevertheless, they comprise the largest hormone-producing organ in the body, both in terms of cell number and range of different hormones. The complexity of the GI endocrine system has become evident only in recent decades. For many years, only three gut hormones (secretin, gastrin and cholecystokinin) were known, and it was believed that each hormone was secreted by a separate endocrine cell type in the upper GI tract. Since the 1970s, however, a multitude of different hormones released from at least 15 distinct endocrine cell types in the digestive system (GI tract and pancreas) have been discovered. It has also become clear that hormones are not secreted from separate endocrine cell types of their own, instead various cell types release a mixture of several bioactive substances.

With this increasing understanding of GI endocrinology, it has also become evident that GI hormones are not a matter only for the stomach, bowel and pancreas. On the contrary, they are implicated in the coordination and regulation of many, or perhaps most, physiological functions throughout the body. Of particular current interest are the neurohormonal alterations involved in the decreased calory intake after malabsorptive bariatric surgery. Another fascinating prospect is the possible implications within the psychiatric field.
INTRODUCTION

The diffuse neuroendocrine system

The endocrine cells of the digestive tract are part of the diffuse (or disseminated) neuroendocrine (or endocrine) system, together with similarly interspersed endocrine cells in the skin, thyroid, lung, thymus, and urogenital tract. The diffuse neuroendocrine system stands in contrast to other neuroendocrine cells forming glands, such as the adenohypophysis, the parathyroids, the paragangliae and the adrenal medulla.

Although a heterogenous cell population, the neuroendocrine cells share several characteristic features, including amine and peptide hormone production, and storage of these secretory products in large dense core vesicles (LDCV, diameter 100–400 nm) and small synaptic-like vesicles (SSV, 40–80 nm). The production of neurotransmitters and ultrastructural properties, such as the secretory vesicles, are examples of features that neuroendocrine cells also have in common with neurons. This contributed to the previously held theory that enteroendocrine cells derive from the neural crest, and also to the term "neuroendocrine". It has later been established that enteroendocrine cells derive from the same progenitor cells of endodermal origin as the other cell types of the intestinal mucosa.

The enterochromaffin (EC) cell

The predominating endocrine cell type of the small bowel is the enterochromaffin (EC) cell, which is found in small numbers in the mucosa throughout the GI tract but become increasingly numerous distally in the small bowel. In the terminal ileum, EC cells are by far the most abundant of six different endocrine cell types. The name "enterochromaffin" refers to a deep yellow staining with dichromate, but they are also sometimes referred to as Kulchitsky cells after their discoverer. Although less is known about the EC cells, there is reason to believe that they may be as important in the bowel as G cells are in the stomach, and they are implicated in various pathological conditions, e.g. irritable bowel syndrome.

Like enterocytes and goblet cells, EC cells are found both in the intestinal glands and on the villi. Some EC cells are of the open type with microvilli projecting into the intestinal lumen, others are of the closed type covered by other mucosal cells. EC cells typically have a well developed Golgi apparatus and rough endoplasmatic reticulum, numerous mitochondria, vacuolated granules and many secretory vesicles of various size.
The principal bioactive substance produced by the EC cells is serotonin (5-hydroxytryptamine or 5-HT). Serotonin is a monoamine neurotransmitter present within the central nervous system (CNS) and in the enteric nervous system, but EC cells are responsible for almost 90% of the serotonin production in the body. Serotonin is synthesized from the essential amino acid tryptophan in two enzymatic steps, as illustrated in Figure 1, and stored in LDCVs together with different peptides. Serotonin released from EC cells exerts both paracrine effects on adjacent enterocytes and smooth muscle cells, and systemic endocrine effects. The physiological functions of serotonin within the small bowel include increased mucosal secretion and peristaltic motility, and high levels of serotonin initiates...
nausea, vomiting and diarrhoea. Serotonin may also act as a mitogenic and fibrogenic factor, among several other physiological functions. As illustrated in Figure 2, release of serotonin can be induced by mechanical and chemical luminal stimuli, but is also regulated by paracrine signals as well as cholinergic, adrenergic and noncholinergic/nonadrenergic nervous signals. Most of the serotonin is released into the portal circulation, but smaller amounts are released intraluminally from open EC cells, i.e. with mucosal surface.

Figure 2. Schematic drawing of an enterochromaffin (EC) cell of the open type (with luminal contact), surrounded by absorptive enterocytes. Bioactive substances are released from large dense core vesicles (LDCV) and small synaptic-like vesicles (SSV) at the basal surface membrane, and to a lesser extent at the apical (luminal) surface membrane. These substances exert both paracrine and endocrine effects. Their release is regulated by luminal, neural and hormonal stimuli.
INTRODUCTION

Most of the serotonin content in the blood is found within platelets, which keep plasma levels of serotonin in balance\textsuperscript{23, 30}. Both platelets, neurons and mucosal cells have an active mechanism for serotonin (re)uptake via the serotonin reuptake transporter (SERT) \textsuperscript{23}. Within these cells, as well as in many other cells including hepatocytes, serotonin can be degraded by monoamine oxidase and aldehyde dehydrogenase into 5-hydroxyindole acetic acid (5-HIAA, Figure 1) \textsuperscript{23}.

In addition to serotonin, EC cells synthesize and secrete a group of neuropeptides known as tachykinins because of their contractile effect on the intestine. Another physiological effect of tachykinins is vasodilation. Notable tachykinins secreted by EC cells are substance P, neurokinin A (NKA) and neuropeptide K (NPK) \textsuperscript{22, 25}. Another secretory product, the polypeptide guanylin, is a secretory regulator\textsuperscript{22, 25, 31}.

CELL REPLACEMENT AND DIFFERENTIATION IN THE SMALL BOWEL

The intestinal stem cell

The intestinal mucosa is the most rapidly self-renewing tissue in adults\textsuperscript{7}. New cells are created by mitosis in the base of the intestinal glands, and then continuously migrate upwards through the crypt and onto a villus. Aged cells that have reached the villus tip are constantly being shedded, and the entire mucosa is therefore in a constant move\textsuperscript{9, 18, 32}.

It has long been established that four to six stem cells reside just above the bottom of each crypt, but their identity has been elusive\textsuperscript{9, 32}. There are two separate schools of thought, the first arguing that the so-called +4 cells just above the crypt bottom are the intestinal stem cells\textsuperscript{24}. Advocates of the second school of thought recently provided evidence that the stem cells in fact are the well known crypt base columnar (CBC) cells, found deep within the crypts and hidden between the Paneth cells\textsuperscript{7, 33}. These stem cells are able to sustain its own population (longevity), and they are also able to supply the mucosal tissue with multiple mature progeny cell types (multipotency), both essential features of adult stem cells\textsuperscript{6}. Many stem cells divide only infrequently (quiescence), but the intestinal stem cells are instead very actively proliferating. Each division on average yelds one daughter cell to replace the parent stem cell, and another daughter cell that is rapidly dividing in order to replace the mucosal tissue (asymmetric cell division) \textsuperscript{9, 33}. These transit-amplifying (TA) cells migrate upwards in the crypt and simultaneously proliferate rapidly. At the same time, the TA cells become increasingly committed to a specific cell lineage. When they reach the crypt-villus junction, the proliferation is stopped and the cells irreversibly
Figure 3. Absorptive enterocytes, enteroendocrine cells, goblet cells and Paneth cells all derive from common stem cells located in the crypt base. Transit amplifying (TA) cells proliferate as they migrate towards the intestinal lumen. Math1 expression commits cells into a secretory lineage, and subsequent expression of ngn3 into enteroendocrine cell differentiation. Less is known about the factors that determine the subtypes of endocrine cells, e.g. enterochromaffin (EC) cells.

differentiate into the separate types of mature mucosal cells\textsuperscript{5, 32}. This journey up the crypt takes 48–72 hours, allowing the TA cells time to divide up to six times. This way, the four to six intestinal stem cells in one crypt produce about 300 cells each day\textsuperscript{32}, with six or more crypts surrounding each villus\textsuperscript{9}. The terminally differentiated cells continue to migrate up an adjacent villus until finally reaching the villus tip where they undergo apoptosis and are shedded into the lumen 4–7 days after the initiating stem cell division\textsuperscript{18, 32}.

The Paneth cells as an exception escape the upward stream and instead migrate downwards in the crypt, they also turn over more slowly and survive for 3–6 weeks\textsuperscript{9}. These cells are known to secrete bactericidal products, but recent findings suggest that they are also responsible for creating the necessary stem cell niche that sustains and regulates the stem cell population\textsuperscript{8}.

INTRODUCTION

Lineage determination and differentiation

The intestinal stem cells are pluripotent, giving rise to all four major epithelial cells as well as the other lesser known cell types of the mucosa\textsuperscript{9, 10, 17, 18}. There is only rudimentary knowledge about how the differentiation into the various cell types is directed, schematically illustrated in Figure 3. Lateral inhibitory signalling by the cell surface protein Notch may be a first decisive step. A TA cell that starts to differentiate into secretory lineage (goblet, Paneth or endocrine cells) upregulates Notch ligand δ which binds to Notch on neighbouring TA cells\textsuperscript{10, 18}. Activation of Notch ligand δ increases expression of the gene Math1 which is necessary for secretory differentiation, whereas activation of Notch in the neighbouring cells inhibits Math1 expression and these cells instead begin their maturation into enterocytes\textsuperscript{34}. Next, expression of transcription factor neurogenin3 (ngn3) seems necessary for progenitor cells committed to a secretory lineage to further differentiate into an enteroendocrine cell lineage, at least within the small bowel\textsuperscript{35}. Downstream of ngn3, a number of transcript factors are responsible for segregation into the several different endocrine cells of the intestine, but little is known about their precise nature\textsuperscript{10, 24}:

B. Cancer biology in small bowel carcinoid

TUMOURIGENESIS

Neoplasia develops as the result of genetic defects, more precisely mutations that produce oncogenes with dominant gain of function, and mutations of tumour suppressor genes giving recessive loss of function. Cancer occurs only when several such lesions have accumulated in the cell genome. In two state of the art reviews, Hanahan and Weinberg summarized present knowledge of tumourigenesis, and proposed six to eight capabilities, or hallmarks of cancer, which all need to be acquired by an aspiring cancer cell\textsuperscript{36, 37}.

1. Sustaining proliferative signalling
   Cells normally need mitogenic growth signals from the environment in order to proliferate. Cancer cells overcome this by either producing these signals themselves, or by making surrounding cells produce them, or by overexpressing or structurally altering their cell surface receptors, or alternatively by changing the intracellular signalling downstream of the growth factor receptors.
INTRODUCTION

2. Evading growth suppressors
Normal cells are also under inhibitory control from the environment by antiproliferative signals, something cancer cells need to evade.

3. Resisting cell death
Cells would normally undergo apoptosis under the exceptional stress, including DNA damage, that the developing cancer cell experiences. Cancer cells need to turn off the apoptotic machinery.

4. Enabling replicative immortality
Cell proliferation is not regulated exclusively by external stop and go-signals, but also by an intrinsic, cell-autonomous program that limits proliferation. Shortening of the telomeres which protect the ends of the chromosomes, are most likely involved in this control process. Cancer cells contain greatly increased levels of telomerase, the enzyme which adds telomere segments to the DNA.

5. Inducing angiogenesis
Tissue cells need capillary blood supply within 100 µm for sufficient oxygen and nutrient supply. For tumours to grow any size they therefore need to induce angiogenesis, a process known as angiogenic switch.

6. Activating invasion and metastasis
Epithelial cells are normally anchored to the extracellular matrix by integrins, and to adjacent cells by cell-cell adhesion molecules (CAMs). In a process known as epithelial-mesenchymal transition (EMT), cancer cells gain the ability to invade and migrate in search of greener grass.

(7). Reprogramming Energy Metabolism
The energy metabolism is adjusted in cancer cells, using glycolysis even in the presence of oxygen. This is far less energy-efficient, but serves to supply glycolytic intermediates needed for biosynthesis in the expanding tumour. The markedly increased uptake of glucose is used in 18F-fluorodeoxyglucose (FDG) positron emission tomography (PET).

(8). Evading immune destruction
Cells with genetic and phenotypic alterations would normally be removed by the immune system. Established cancer cells must therefore somehow have managed to escape detection, but the mechanisms largely remain unknown.

Mutations are normally so rare that the chance of a series of genetic lesions leading to cancer in the same cell would be small. One reason is that DNA defects are detected and resolved by a DNA-maintenance machinery (caretakers). Two separate enabling characteristics promote tumourigenesis:

1. Genome instability and mutation
The risk of mutations is increased in cancer cells by an enhanced sensitivity to mutagenic agents and/or by turning off caretaker genes.

2. Tumour-promoting inflammation
Tumours contain inflammatory cells. They have long been considered as the attempt of the immune system to delete the tumour, and this is probably part of the truth. However, inflammation may also sustain tumourigenesis by supplying necessary signalling molecules, enzymes, and mutagenic substances.
INTRODUCTION

Tumours were previously viewed as lumps of cancer cells, but with new insights it has become evident that they function as complex organs. Parallel to the genetic multistep development of cancer cells, a tumour microenvironment is constructed by orchestrating surrounding endothelial cells, inflammatory cells, and fibroblasts.

CANCER STEM CELLS

Emerging evidence suggests that cancer is a disease of stem cells, a theory first proposed by Hamburger and Salmon, who in 1977 discovered that only a fraction of tumour cells were able to form new colonies in vitro. The present apprehension is that cancer stem cells (CSCs) constitute a small subpopulation of tumour cells, responsible for cell renewal and multipotency, much like stem cells in normal tissues.

It is not firmly established whether CSCs arise from mature tissue cells or instead from tissue stem cells or partially differentiated TA cells. In favour of the latter theory is that such cells survive long enough for a sufficient number of mutations to accumulate, and they already have the ability to self-replenish. Their multipotency could also explain that there may be multiple differentiated cell types within a single tumour.

ORIGIN OF SMALL BOWEL CARCINOID

Small bowel carcinoid tumours derive from EC cells, but it is presently not firmly established whether tumourigenesis occurs in the mature cells or in precursors. As already discussed, the endocrine cells of the GI tract are the progeny of tissue stem cells in the intestinal glands. There is increasing evidence that these tissue stem cells are also the source of CSCs in neuroendocrine tumours (NETs). Khan et al. recently found that all examined gastrecteropancreatic (GEP) NETs expressed epithelial cell adhesion molecule (EpCAM), a carcinoma-associated antigen suggesting an epithelial origin. In another fresh study, Gaut et al. demonstrated that a small proportion of NET cells were able to form new spheres in vitro, these cells could be evidence of CSCs also in NETs. It is hypothesized that NETs arising from progenitor cells in early differentiation become poorly differentiated, whereas precursors with a more determined lineage give well differentiated neoplasia. In the case of small bowel carcinoid tumours, the overwhelming majority are well differentiated.
INTRODUCTION

GENETICS OF SMALL BOWEL CARCINOID

Knowledge about the precise genetic and epigenetic alterations leading to induction and progression of NETs within the digestive system remain scarce. Cytogenetic studies have identified structural chromosome alterations occurring with various frequency. The genetic defects identified so far differ significantly between GEP NETs from different sites, which is a further reason not to treat them as a common entity.

The most common genetic aberration in small bowel carcinoid tumours seems to be deletions in chromosome 18. Löllgren et al. found deletions of 18q21–qter in seven out of eight midgut carcinoid tumours. Kytölä et al. could narrow the region to 18q22–qter where losses were identified in 12 out of 18 tumours. Losses were found both in primary tumours and metastases, suggesting that the aberration had occurred early in the tumourigenesis. Other common alterations include losses of 9p and 16q, and gains involving chromosomes 4, 5, 7, 14 and 20.

Kytölä et al. found loss of heterozygosity in five out of eight midgut carcinoid tumours in the succinate-ubiquinone oxidoreductase subunit D (SDHD) gene located at 11q23. Loss of chromosome 11q22–23 was also found in small bowel carcinoid tumours by Andersson et al. Chromosome 11 is also the locus of the MEN1 gene at 11q13. Although small bowel carcinoid is not clinically linked to the multiple endocrine neoplasia type 1 (MEN1 syndrome), genetic aberrations have been suggested in the MEN1 gene in a minority of sporadic small bowel carcinoid tumours, but this was later put into question.

Other genetic syndromes such as multiple endocrine neoplasia type 2 (MEN2 syndrome), tuberous sclerosis complex (TSC), neurofibromatosis type 1 (NF-1), and von Hippel-Lindau syndrome (VHL) do not include GEP NETs. Further, no genetic lesions have been found in the genes associated with these syndromes in sporadic GEP NETs.

Focusing on genetic and epigenetic alterations of tumour suppressor genes and oncogenes known from other neoplasms, some points can be made. The proto-oncogenes β-catenin and cyclin D1 are overexpressed in a majority of GI NETs, whereas tumour-suppressor gene p16INK4a/p14ARF is lost in some. Genetic lesions in oncogene K-RAS or tumour suppressor gene p53 do not seem to be involved in small bowel carcinoid tumourigenesis.
INTRODUCTION

Kidd et al. noted altered response to transforming growth factor beta 1 (TGF-β1) in small bowel carcinoid tumour cells\(^6\). Whereas proliferation was inhibited by TGF-β1 in normal EC cells, carcinoid cells were stimulated and transcription and function of several tumour suppressor genes and oncogenes were regulated in a tumourigenic direction.

In one of few attempts to identify associations between specific genetic lesions and clinical characteristics, Kulke et al. were unable to correlate loss of chromosome 18 with presence of carcinoid syndrome, metastases or survival\(^9\). Gain of chromosome 14 was identified as a predictor of poor survival in one study\(^8\), but this could not be repeated by another research group\(^5\).

In conclusion, a number of genetic lesions have been identified in small bowel carcinoid, but there are most certainly others. Further, the significance of the various lesions are largely unknown.

MULTIPLE PRIMARY TUMOURS

NETs of the digestive tract often present as multiple, discontinuous tumour nodules within the pancreas or within the mucosa and submucosa of the GI tract. It is not clearly established whether this multifocality is the result of intrapancreatic or intraintestinal metastasis or due to the formation of multiple, independent primary tumours. With lymphatic drainage blocked by metastases in the mesentery, the lymph has to drain sideways for some length until the radial drainage is again free. This was suggested to be the cause of metastastic lesions near the intestinal mucosa interpreted as multiple primaries\(^5\). It was also supported by a study investigating clonality by X-chromosome inactivation analysis. A non-random inactivation pattern (monoclonality) was found in all four examined patients with multiple ileal carcinoid tumours, suggesting that multifocal tumours are in fact metastatic lesions\(^6\). In another study, three out of five cases of multifocal small bowel carcinoid similarly showed a nonrandom pattern of X-chromosome inactivation among all coexisting tumours, consistent with a monoclonal origin, whereas two of the five cases seemed to be of oligoclonal origin\(^6\). This study also performed loss of heterozygosity assays with markers for putative tumour suppressor genes in 13 patients with multifocal small bowel carcinoid. Identical loss of heterozygosity pattern was found in all coexisting tumours in three cases, whereas the pattern was different in all coexisting tumours in four cases. In the majority (seven cases), some of the coexisting tumours shared loss of heterozygosity pattern whereas other tumours showed different
patterns, suggesting a mixture of monoclonal (metastatic) and oligoclonal (independent) origin of the multiple tumours. The available results therefore seem to suggest that some patients with multifocal small bowel carcinoid tumours have metastatic lesions, some multiple independent tumours, but in many cases there is a mixture of both.

C. Clinical aspects of small bowel carcinoid

NOMENCLATURE OF NETs

Since NETs were first recognized a century ago, the nomenclature has been under constant evolution. Some historical notes are necessary for understanding.

In the late 19th century, a number of observations were made of tumours in the small bowel that histologically diverged from adenocarcinomas, and the carcinoid syndrome was convincingly described in 1890. In 1907, Oberndorfer described six cases of multiple pea-sized tumours in the ileum. He noticed that their histopathological appearance on the one hand was malignant, but on the other hand different from adenocarcinomas, and called them karzinoide tumoren (carcinoma-like tumours). In 1914, Gosset and Masson recognized the endocrine features of both EC cells and carcinoid tumour cells, and suggested that carcinoid tumours arose from EC cells. They also proposed the existence of a diffuse endocrine system, and subsequently tumours from the diffuse endocrine system were found in locations outside the small bowel. These tumours have often been treated as a common entity, and referred to as carcinoids or more recently neuroendocrine tumours (NETs).

It has, however, become increasingly clear that NETs constitute a heterogeneous group in terms of genetic lesions, morphology, hormone content, and clinical course. Therefore several attempts have been made to subdivide these tumours into useful entities. The first anatomical division was suggested by Williams and Sandler in 1963, dividing carcinoids on the basis of embryonic origin into foregut (lungs, pancreas, stomach, and upper duodenum); midgut (small bowel from mid-duodenum, caecum and colon as far as the mid-transverse colon); and hindgut (the rest of colon and rectum). This division is still frequently used but seems insufficient, as exemplified by the very different prognosis of ileal and appendiceal carcinoids, both midgut.
INTRODUCTION

The first WHO classification of endocrine tumours was published in 1980, but held little of subdivision of the carcinoids. In the second WHO classification from 2000, a three-tier classification with criteria depending on the site of origin was introduced; 1a) Well differentiated neuroendocrine tumour (NET); 1b) Well differentiated neuroendocrine carcinoma (NEC); and 2) Poorly differentiated neuroendocrine carcinoma. The larger group of tumours were now referred to as NETs, the term “carcinoid” was restricted to well differentiated GEP NETs. But the WHO 2000 classification was based on a mixture of clinical and histological characteristics, and did not gain wider acceptance, particularly in the United States.

Next, European Neuroendocrine Tumor Society (ENETS) in 2006 proposed a tumour-node-metastasis (TNM) staging classification of foregut NETs, and in 2007 a similar TNM classification of mid- and hindgut NETs. This classification distinguishes tumours of different origin, and is based on the extent of invasion and dissemination. The ENETS simultaneously proposed a separate histological grading, based on the proliferative activity of the tumour. Both the TNM classification and the histological grading were incorporated in the latest version of the American Joint Committee on Cancer (AJCC) and Union for International Cancer Control (UICC) TNM classification of malignant tumours, with some minor adjustment for endocrine pancreatic tumours (EPTs) and appendiceal NETs. Finally, clinicians and researchers in the field of NETs are endowed with one clinical and one histopathological classification system, as in most other solid tumours.

Table 1. TNM Clinical Classification of jejunoileal carcinoid tumours

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

Note: For any T, add (m) for multiple tumours.

<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>
INTRODUCTION

Table 2. Stage Grouping (Non-appendiceal GI NETs) 69, 70

<table>
<thead>
<tr>
<th>Stage</th>
<th>T</th>
<th>N</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>T1</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>II A</td>
<td>T2</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>II B</td>
<td>T3</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>III A</td>
<td>T4</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>III B</td>
<td>Any T</td>
<td>N1</td>
<td>M0</td>
</tr>
<tr>
<td>IV</td>
<td>Any T</td>
<td>Any N</td>
<td>M1</td>
</tr>
</tbody>
</table>

The new WHO classification from 2010 also adopted the histological grading system proposed by ENETS, retaining the terms NET for G1 and G2 tumours, and NEC for G3 tumours67. The term neuroendocrine neoplasia (NEN) was introduced to cover both NETs and NECs. The TNM staging is also acknowledged in the WHO classification43, 67.

Our research has been focused on well differentiated endocrine tumours of the small bowel, and we have chosen to consistently call them small bowel carcinoids, as opposed to the wider group of NETs from various sites. These tumours may possibly be referred to as NENs in the future, as the new WHO classification suggests.

INCIDENCE

Incidence of NETs

NETs are rather uncommon; bronchopulmonary and gastroenteropancreatic NETs together account for only 0.5 to 1% of all malignant diseases15, 72. As most NETs originate in the digestive system, they comprise a somewhat higher proportion (about 2%) of all GI tract and pancreatic malignant tumours45, 73. However, due to the generally better prognosis the prevalence of GEP NETs is second only to colorectal cancer within the digestive system, more common than for instance esophageal, gastric and pancreatic cancer72.

Table 3. Histopathological Grading69, 70

<table>
<thead>
<tr>
<th>Grade</th>
<th>Mitotic count (per 10 HPF)</th>
<th>Ki67-index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>&lt; 2</td>
<td>≤ 2</td>
</tr>
<tr>
<td>G2</td>
<td>2-20</td>
<td>3-20</td>
</tr>
<tr>
<td>G3</td>
<td>&gt; 20</td>
<td>&gt; 20</td>
</tr>
</tbody>
</table>

*10 HPF: High power field = 2 mm², at least 40 fields (at 40 x magnification) evaluated in areas of highest mitotic density
*Ki-67/MIB1 antibody; % of 2,000 tumour cells in areas of highest nuclear labelling.
INTRODUCTION

The annual incidence of NETs at all sites is reported in the range between 2 and 5 per 100,000 persons and year\textsuperscript{14, 72, 74-77}, and the incidence of GEP NETs between 1 and 4 per 100,000\textsuperscript{14, 72, 73, 75-77}.

In the most recent report from the Surveillance, Epidemiology and End Results (SEER) Program, which currently covers 26% of the US population\textsuperscript{78}, Yao \textit{et al.} demonstrated a steadily increasing incidence of NETs from 1.09 to 5.25 per 100,000 between 1973 and 2004\textsuperscript{72}. A statistically significant increase in incidence was observed also for each separate primary tumour site\textsuperscript{72}. In Norway the incidence of NETs increased from 2.35 per 100,000 in 1993–1997 to 4.06 in 2000–2004\textsuperscript{14}. Most other studies likewise found a dramatically increased incidence of NETs in the West over the last decades\textsuperscript{14, 73, 74, 76, 77}.

\textit{Incidence of small bowel carcinoid}

Malignant tumours in the small bowel consist mainly of adenocarcinomas, carcinoids, lymphomas and sarcomas. Adenocarcinomas occur most frequently in the duodenum whereas carcinoids and lymphomas are more common in the jejunum and the ileum. Sarcomas are evenly distributed throughout the small bowel\textsuperscript{79-81}.

The incidence of malignant tumours in the small bowel has increased\textsuperscript{79-82}, in particular carcinoid tumours which now is the most common histologic subtype of small bowel malignancies\textsuperscript{80, 81}. Within the SEER database, the annual incidence of small bowel malignancy increased from 1.18 per 100,000 in 1973 to 2.27 in 2004. This development was mainly explained by a more than four-fold increase of the incidence of carcinoid tumours from 0.21 to 0.93 per 100,000, whereas the incidence of adenocarcinomas changed only moderately from 0.57 to 0.73 per 100,000\textsuperscript{81}. A similar development, with carcinoids surpassing adenocarcinomas as the most common malignant small bowel tumours in the United States, was observed in the National Cancer Data Base (NCDB). In 2005 44.3\% of small bowel cancers within the NCDB were carcinoid tumours, the proportion of adenocarcinomas was 32.6\%, lymphomas 14.8\%, gastrointestinal stromal tumours (GIST) 7.1\%, and other sarcomas 1.2\%\textsuperscript{81}. In England, carcinoid tumours comprised 26.6\% of malignant small bowel tumours between 2000 and 2006\textsuperscript{75}.

Several other reports from the SEER database have demonstrated an increasing incidence of small bowel carcinoid tumours\textsuperscript{72, 74, 77}, the most recent study by Yao \textit{et al.} found a statistically significant increase in incidence of jejunal and ileal carcinoid tumours between 1973 and 2004, reaching 0.67 per 100,000 between 2000 and
2004. Ellis et al. reported the incidence of small bowel carcinoid tumours in England to have increased from 0.12 in men and 0.11 in women per 100,000 in 1971–1978 to 0.46 and 0.32, respectively, in 2000–2006. The incidence in Norway increased from 0.60 per 100,000 in 1993–1997 to 1.01 in 2000–2004. A study based on the Digestive Cancer Registry of Burgundy found an incidence of small bowel endocrine malignancies as low as 0.07 per 100,000 between 1976 and 1988, rising slightly to 0.25 in men and 0.17 in women between 1989 and 2001. An incidence of approximately 0.3 per 100,000 was reported from the Netherlands between 1989 and 1997, and about 0.4 per 100,000 between 1983 and 2003 in western Norway.

The account of small bowel tumour incidence is valid foremost for developed Western countries. In fact, there is reason to believe that small bowel carcinoid tumours are far less common in other parts of the world where lymphoma is the predominating histological subtype.

**Why are small bowel tumours so rare?**

The small bowel represents 90% of the absorptive surface area of the entire GI tract. Why then do less than 3% of all digestive system malignancies occur in the small bowel? By comparison, colon cancer comprises 42.6% of all digestive system cancer in Sweden, and 36.5% in the United States (50.9% colorectal cancer).

A long list of mechanisms responsible for the much lower tumour frequency in the small bowel has been put forward. The discussion again refers to small bowel malignancies in general, not only carcinoid tumours, and concerns on the one hand a less hostile environment, and on the other hand better protective properties:

**Environment**

1. The liquid chyme of the small bowel causes less mechanical trauma than the more solid fecal contents of the colon.
2. The rapid transit of the bowel contents through the small bowel reduces the exposure to carcinogens.
3. The considerably lower bacterial load in the small bowel leads to less formation of potential carcinogens from bile acid breakdown.
4. The alkalinity of the small bowel contents leads to less formation of potentially carcinogenic nitrosamines.
5. The levels of endogenous reactive oxidative species (ROS) are lower in the small bowel than in the colon.
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Protection

1. There are relatively fewer stem cells in the small bowel, and they are located deeper within the crypts, and therefore better protected from carcinogens than stem cells at other sites. 
2. The well-developed local IgA-mediated immune system.
3. The removal of cells with genetic defects is prompter in the small bowel than in the colon.
4. The mucosal cells in the small bowel turn over fast, which some argues decreases the likelihood of tumourigenesis and others that it is increased.
5. The lower levels of activating enzymes of precarcinogens and also presence of other enzymes converting carcinogens into less toxic metabolites, e.g. benzopyrene conversion.

ASSOCIATION WITH OTHER DISEASES

Association with cancer at other sites

In a case-control study, Hassan et al. found an increased risk for small bowel carcinoid tumours in individuals with a family history of any cancer. However, no such association was found in a large registry study from Sweden. Another case-control study did not find an increased incidence of cancer among first-degree relatives of patients with GI NETs.

Some studies have pointed out an association with colorectal cancer. There is a geographical correlation between the incidence of small bowel malignancy (adenocarcinoma, carcinoid and sarcoma) and the incidence of colon cancer. This may primarily pertain to small bowel adenocarcinomas since a bidirectional association was found between adenocarcinomas in the small bowel and in the colon or rectum, i.e. having either means an increased risk of later having the other. Hassan et al. found that a family history of colorectal cancer was associated with an increased risk of developing small bowel carcinoid. However, other studies did not find an association between small bowel carcinoid and colorectal cancer. Patients with colorectal cancer was not at increased risk of later developing small bowel carcinoid tumours themselves, or vice versa. Neither did individuals with a family history of colorectal cancer have an increased risk of small bowel carcinoid, or vice versa.

Two studies found an increased risk of prostate cancer in patients who previously had small bowel carcinoid, and vice versa. Another study similarly found an increased risk of prostate cancer in patients who previously had any type of NET.
INTRODUCTION

Hassan et al. again found that a family history of prostate cancer was associated with an increased risk of developing small bowel carcinoid\(^9\) whereas other studies did not\(^9, 95, 98\).

Several other neoplasms have been proposed as potentially associated with small bowel carcinoid: cancers of breast, kidney, nervous system, and endocrine glands (in particular the thyroid), as well as squamous cell skin cancer and melanoma\(^7, 98\).

**Association with coeliac disease**

It has long been argued that patients with coeliac disease are at increased risk of developing certain tumours in the small bowel, in particular lymphomas but also adenoscarcinomas\(^99-102\). Some cases probably result from raised vigilance, and accordingly West et al. found that the risk of small bowel malignancies other than lymphoma was not increased beyond the first year after the coeliac disease was diagnosed\(^103\). Other studies found no increased risk at all for either small bowel lymphoma or adenocarcinoma\(^104, 105\). There is likewise no evidence of an association between coeliac disease and small bowel carcinoid tumours\(^100, 101, 104, 105\), although cases with both diseases have been reported\(^99, 106\). Interesting in this context is that the EC cell population increases in coeliac disease\(^107, 108\). Of further interest, more than 90% of patients with coeliac disease possess the HLA class II gene HLA-DQ2, and an ongoing project of ours shows that HLA-DQ2 is overrepresented also in small bowel carcinoid patients\(^109\).

**Association with genetic syndromes**

Patients with the MEN1 syndrome are commonly affected by EPTs or other foregut NETs\(^55, 110\). By contrast, small bowel carcinoid tumours do not occur as part of any established genetic syndrome.

HEREDITY

Small bowel carcinoid tumours are generally considered sporadic, but a number of case reports have described families with two or three affected members\(^111-114\), suggesting the existence of an inherited variant. There are also epidemiological studies showing an increased risk of developing GI NETs\(^95\) or small bowel carcinoid tumours\(^94, 98\) in individuals with a parental history of GI NETs or small bowel carcinoid tumours, respectively. Cunningham et al. recently described nine Swedish
INTRODUCTION

families with 23 individuals affected by small bowel carcinoid. The pattern of inheritance was suggestive of an autosomal dominant two-hit inherited susceptibility. Clinical features, histopathological characteristics and genetic aberrations were similar between the familial tumours and sporadic small bowel carcinoid tumours.

RISK FACTORS

Intrinsic risk factors

Most studies found a higher incidence of NETs within the digestive system in men than in women, and also a higher incidence of small bowel carcinoid in men. The other three main histological subtypes of small bowel cancer were also more common in men than women.

Studies from the United States comparing incidence between races demonstrated a markedly higher incidence in African Americans than in Caucasians, both of NETs in general and of small bowel carcinoid.

Two separate studies based on the SEER registry found the median age at diagnosis of jejunoileal carcinoid to be 66 years. As might be expected, the average age was lower in studies from referral centres; between 57.5 and 62 years.

Lifestyle risk factors

Owing to its rarity compared to other digestive cancers, relatively little attention has been paid to risk factors of small bowel malignancies. The results from the available studies are conflicting, and there are therefore no established lifestyle risk factors for small bowel carcinoid. For instance, two case-control studies found an association between tobacco smoking and increased risk of small bowel carcinoid, whereas a larger study did not. Similarly, alcohol intake was associated with an increased risk in one study but not in two others. In a case control study not distinguishing the histological subtypes, neither smoking habits nor alcohol intake was associated with an increased risk of small bowel malignancy. The same study suggested an association between frequent consumption of red meat and risk of small bowel carcinoid, whereas Cross et al. found no such association but instead between higher intake of saturated fat and risk of small bowel carcinoid. The results of a major prospective cohort study indicated that a high dietary fibre intake might be protective against small bowel neoplasia, in particular against carcinoid tumours. This finding and the arguable association with colorectal cancer discussed above, has
led some to believe that small bowel malignancies share risk factors with colorectal cancer.

A multicentre study of occupational risk factors found an increased risk of developing small bowel carcinoid after certain exposures, but the study included too few cases to allow any reliable interpretation.\textsuperscript{128}

**SYMPTOMS**

Symptoms of small bowel carcinoid tumours vary with the extent of the disease. Localized tumours may attract attention by causing symptoms such as bowel obstruction or haemorrhage, but they are commonly asymptomatic and discovered only incidentally. In fact, most localized small bowel carcinoid tumours are not diagnosed at all, as demonstrated in a classical autopsy study.\textsuperscript{129} When metastases occur within the mesentery, the liver or elsewhere, patients experience progressive symptoms. Many patients suffer from recurrent episodes of abdominal distension and colicky pain due to mesenteric fibrosis and partial bowel obstruction. These symptoms were previously often misinterpreted for several years before the diagnosis was reached, but this has probably changed with the introduction of computed tomography (CT) and other imaging techniques. Other patients suffer from the carcinoid syndrome with cutaneous flushing, diarrhoea and other symptoms. Some patients present with more general malignant symptoms such as weight loss or a palpable mass, typically in the right lower quadrant of the abdomen. Symptoms may rarely result from metastases at other sites.

A large proportion of patients with small bowel carcinoid report no symptoms at all before the disease presents as an abdominal emergency, predominantly bowel obstruction. The frequency of different symptoms at diagnosis from previous studies is summarized in Table 4. The symptoms are described in more detail below.

<table>
<thead>
<tr>
<th>Symptom</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal pain</td>
<td>40–60</td>
</tr>
<tr>
<td>Bowel obstruction</td>
<td>35–50</td>
</tr>
<tr>
<td>Weight loss</td>
<td>10–35</td>
</tr>
<tr>
<td>GI haemorrhage</td>
<td>5–10</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>20–40</td>
</tr>
<tr>
<td>Flushing</td>
<td>10–30</td>
</tr>
<tr>
<td>Bronchial constriction</td>
<td>5–10</td>
</tr>
<tr>
<td>Carcinoid heart disease</td>
<td>&lt; 5</td>
</tr>
</tbody>
</table>
INTRODUCTION

General symptoms

Like other gastrointestinal tumours, small bowel carcinoid tumours may cause diffuse abdominal pain, haemorrhage, malaise and weight loss. As already discussed, the primary tumours are generally small, and rarely lead to partial or complete bowel obstruction in themselves.

Carcinoid syndrome

The classical sign of small bowel carcinoid is the carcinoid syndrome including hormone-related symptoms such as cutaneous flushing (80–90%), diarrhoea (70%), and bronchial constriction (15%). Most authors also include long-term symptoms such as the carcinoid heart disease (CHD) (30–40%) in the carcinoid syndrome. Other manifestations include telangiectasia (25%) and pellagra-like skin lesions (5%) \(^{133, 134}\).

Diarrhoea may occur with any GI tract malignancy but is much more common and prominent in patients with small bowel carcinoid. This tendency towards diarrhoea is mainly due to the paracrine effects of serotonin which increases secretion of fluid and causes hypermotility of the bowel\(^{26, 135, 136}\), but tachykinins and other bioactive substances may also contribute\(^{137}\). Less often, diarrhoea may result from bowel ischaemia which is discussed below\(^{135}\).

When liver metastases occur, tumour-derived substances may reach the systemic circulation and give rise to distant symptoms, in particular flushing and bronchoconstriction. These symptoms are paroxysmal and occur either seemingly spontaneously, or triggered by alcohol, physical exercise, mental stress and tyramine-containing foods such as chocolates, walnuts and bananas\(^{138}\).

Flushing appears as pink or red colour of the face, neck and upper chest, typically lasting for a few minutes\(^{132, 138}\). Bronchial constriction, also known as wheezing, is far less frequent. The exact mechanisms and the proportional contribution of individual substances such as serotonin, tachykinins and histamine remain unknown\(^{15, 26, 135-137}\).

Fibrosis

Fibrosis is another distinctive mark of small bowel carcinoid tumours\(^{139, 140}\). This fibrosis progresses slowly and steadily as a result of longstanding disease, and occurs both locally and at distant sites.
INTRODUCTION

Locally, mesenteric fibrosis is often extensive already at the time of diagnosis due to the otherwise often inconspicuous symptoms\textsuperscript{23, 141}. This mesenteric fibrosis, or desmoplastic response, causes kinking of the bowel which in many patients leads to the typical longstanding episodic and colicky abdominal pain from incomplete or intermittent bowel obstruction, as well as weight loss. Eventually the fibrosis may become severe enough to cause a complete bowel obstruction by fibrotic luminal obstruction or by kinking of adherent bowel loops. At emergency surgery for intestinal obstruction, marked mesenterial fibrosis is encountered in approximately $\frac{2}{3}$ of the patients\textsuperscript{117, 142}. The mesenteric fibrosis may also engage the mesenteric vessels and cause bowel ischaemia, and retroperitoneal fibrosis occasionally results in stenosis of the ureters.

Another characteristic form of fibrosis in small bowel carcinoid patients is the carcinoid heart disease, involving the mural and valvular endocardium of the right side of the heart\textsuperscript{26, 140, 141}. The carcinoid plaques consist of smooth muscle cells, myofibroblasts and matrix-rich connective tissue deposited on the endocardial surface, leaving the underlying tricuspid and pulmonary valves intact. The ensuing valvular insufficiency and pulmonary regurgitation leads to right sided cardiac failure, which is claimed to account for up to $\frac{1}{3}$ of all deaths in patients with the carcinoid syndrome\textsuperscript{140, 143}. CHD as a rule affects only patients with hepatic metastases, and primarily the right side of the heart because apart from the liver, also the lungs clear the fibrogenous agents from the circulation. Exceptions are seen only in the rare circumstances of ovarian metastases or patent foramen ovale\textsuperscript{26, 140}. CHD is a late symptom, uncommon at diagnosis\textsuperscript{144} but estimated to occur in $40\%$ of patients with the carcinoid syndrome\textsuperscript{140}.

Fibrosis also seems to occur in the pleura without causing any apparent symptoms\textsuperscript{145}, and infrequently in the skin\textsuperscript{26, 141}.

Although the mesenteric\textsuperscript{131} and cardiac\textsuperscript{146} fibrosis have been recognized for decades, the underlying mechanisms remain obscure. Tumour infiltration may contribute locally, but one or several substances secreted by tumour cells into the circulation seems necessary to explain fibrosis at distant sites. Serotonin was proposed long ago\textsuperscript{147} and remains one of the main candidates\textsuperscript{26, 140}. However, it seems that other agents may be more important and the correlation between serotonin and fibrosis may rather be an epiphenomenon\textsuperscript{141}. Tachykinins have been proposed, but more evidence suggest that growth factors including connective tissue growth factor (CTGF) and TGF-β1 are involved\textsuperscript{26, 28, 139, 141, 148, 149}. 
INTRODUCTION

DIAGNOSIS

Up to half of the patients with small bowel carcinoid tumours present with bowel obstruction or otherwise acute abdomen and undergo emergency surgery\(^{117,150}\). Some initial imaging may have been undertaken, but the carcinoid diagnosis is in many cases not established before the operation. Numerous other (11–34%) small bowel carcinoid tumours are detected incidentally during surgery or investigations for other conditions\(^{119,120,131}\). The remainder of patients seek their physician for symptoms which may be very general or, alternatively, typical for metastatic small bowel carcinoid. The symptoms along with other circumstances will obviously determine which investigations are planned, but CT or abdominal ultrasonography will be usually undertaken at an early stage. Suspected small bowel carcinoid should be confirmed by biochemical markers, whereas hepatic or mesenteric metastases of unknown origin often are biopsied percutaneously for histological analysis. When the diagnosis has been established, single-photon emission computed tomography (SPECT) or positron emission tomography (PET) should be considered for more precise localization of primary tumours and metastases.

Imaging

The introduction of new imaging techniques has revolutionized the diagnosis of metastatic carcinoid. A few decades ago investigators were restricted to variants of conventional X-ray such as small bowel follow-through or double-contrast technique (enteroclysis). Although enteroclysis may be highly sensitive\(^{89}\), it has largely been superseded by other modalities. Follow-through still has a role in the emergency setting with suspected bowel obstruction.

In practice, examinations with CT or abdominal ultrasonography will in many cases be a first choice to investigate pain or a palpable mass today. Ultrasonography may detect mesenteric and hepatic metastases. CT may be highly indicative of small bowel carcinoid based on characteristic signs: the mesenteric fibrosis typically appears with calcification and radiating strands resembling a spoke-wheel\(^{151,152}\). Magnetic resonance imaging (MRI) better identifies bone metastases, but otherwise adds little additional information over CT\(^{89,153}\).

Endoscopic examination of the entire small bowel is possible but rather complicated and not widely used. Capsule endoscopy can be useful particularly when investigating GI haemorrhage or when searching for unknown primary tumours after mesenteric NET metastases have been found\(^{154}\).
Nuclear medicine techniques exploit the presence of somatostatin receptors on NET cells. Krenning et al. first reported that NETs could be visualized with $\gamma$-camera after infusion of radiolabelled somatostatin analogue octreotide. Various agents were developed but the most commonly used for somatostatin receptor scintigraphy has been $^{[111\text{In-DTPA]}}$octreotide, commercially known as OctreoScan™. $^{68\text{Ga}}$ has proved a better radionuclide and is expected to supersede $^{111\text{In}}$. SPECT allows 3-dimensional information and gives superior anatomical localization of detected lesions.

Still better resolution is gained with PET when combined with CT (PET-CT). The most widely used PET radioligand $^{18\text{F}}$-fluorodeoxyglucose (FDG) accumulates in tumour tissue due to the higher glucose uptake of most tumour cells, but it is not suitable for the generally slow-growing NETs. A number of different other radionuclide-coupled ligands have been introduced for NETs, some of them with affinity for the somatostatin receptors or precursors used for synthesis of e.g. serotonin. Skeletal metastases may be detected by bone scintigraphy.

Upon diagnosis of a metastasized small bowel carcinoid tumour, echocardiography should be performed to evaluate presence of carcinoid heart disease. Valvulopathy with thickening and retraction of the tricuspid and pulmonary valve leaflets resulting in both regurgitation and stenosis is pathognomonic.

### Biochemical markers

Chromogranin A (CgA) and the other members of the granin family are secretory proteins stored in secretory LDCVs in virtually all cells in the neuroendocrine system. They appear to contribute in the formation of secretory granules and in secretory protein sorting and activation. The plasma levels of granins, in particular CgA, are elevated in the vast majority of NETs and can be used as a general diagnostic NET biomarker. The highest CgA levels are found in small bowel carcinoid and the CgA concentration correlates to tumour burden. CgA can also be used to monitor tumour growth or treatment response, as well as for detection of recurrent disease.

CgA levels may be “falsely” elevated in patients with some other cancers as well, most commonly prostate cancer which often contain neuroendocrine cells. Furthermore, CgA concentrations increase in the hypergastrinaemia seen with both proton pump inhibitor (PPI) treatment and in autoimmune chronic atrophic gastritis. Another pitfall is renal failure in which CgA increases in proportion to the degree of
insufficiency. Chromogranin B (CgB) levels are far less affected by PPI treatment and renal failure and has been suggested as a complement to CgA in these situations.

Production of serotonin is characteristic of midgut carcinoid tumours, although small amounts may be produced also by foregut NETs. As already described, plasma levels of serotonin is regulated by platelet uptake via SERT. For this reason, and because concentrations vary with the time of day, plasma serotonin levels are not measured in clinical practice. Platelet serotonin has been proposed as a sensitive marker, but is not in widespread use. Instead, measurement of the breakdown product 5-HIAA in 24-hour urine collection is an established, albeit somewhat inconvenient, method for diagnosis and follow-up of midgut carcinoid. The sensitivity of 5-HIAA is considerably lower than that of CgA, but the specificity is high and, when positive, 5-HIAA discerns carcinoid tumours of midgut origin.

Serum levels of neuron-specific enolase (NSE) may be measured as a general neuroendocrine biomarker, and levels of the tachykinins NKA, NPK and substance P as biomarkers for midgut carcinoid.

Histopathology

Tumour specimens can be retrieved either by surgical resections or by biopsies, preferentially percutaneously. In the microscope, several morphological growth patterns can be discerned, as described by Soga and Tazawa. Most commonly small bowel carcinoid tumours appear as rounded nests of closely packed tumour cells which often form a typical peripheral palisading, this pattern was named insular by Soga and Tazawa. Although areas with insular pattern are present in the majority of carcinoid tumours, some contain other areas with trabecular or glandular (acinar, rosette) growth patterns. The growth pattern is useful for descriptive purposes, but has previously not been accredited with any prognostic relevance. Recently, Cunningham et al. introduced two new growth patterns: small nest and solid. The latter was proposed to correlate with shorter survival.

Carcinoid tumour cells show little pleomorphism, i.e. the cells and their nuclei are uniform in size and shape. The mitotic activity is measured as the percentage of cells expressing Ki-67. This proliferation index is typically low in small bowel carcinoid, most often below 2%. Normally, a high proliferation index in NETs is equivalent to a low degree of differentiation, and vice versa. According to the WHO classification of tumours of the digestive system, high grade/poorly differentiated
INTRODUCTION

NETs (or NECs) do not occur within the small bowel\(^{19}\). However rare cases have been reported\(^{171, 172}\).

The neuroendocrine origin of NETs is verified by positive Grimelius silver nitrate stain for argyrophilia, as well as by strong immunoreactivity for CgA, CgB and synaptophysin\(^{43, 173, 174}\). Less reliable neuroendocrine markers are cytosolic NSE and neural cell adhesion molecule (NCAM or CD56) in the plasma membrane\(^{12}\). The specific jejunoileal EC cell origin is verified by positivity for the Masson-Fontana argentaffin stain\(^{173, 174}\), and immunoreactivity for serotonin and substance P\(^{43, 167}\).

An international and multidisciplinary NETs expert group recently drafted a statement of the minimum information in pathology reports on NETs\(^{175}\). Size, histological grade (i.e. proliferation index), pTNM and presence of vascular invasion, perineural invasion or tumour necrosis are data considered crucial. Although not mandatory according to this statement, immunohistochemical staining for general (e.g. CgA and synaptophysin) and specific (e.g. serotonin) neuroendocrine tissue markers is considered standard in NETs pathology\(^{160}\).

Future markers

Presently used biochemical and histopathological markers for NETs are diagnostic, and to some extent prognostic. However, useful markers for prediction of treatment response is lacking. It has been argued that traditional methods have been exhausted\(^{12}\), others hope that studies using proteomics and tissue arrays will be able to identify new biochemical and histopathological markers\(^{15}\).

Increasing knowledge about tumourigenesis in small bowel carcinoid may provide genetic markers or marker constellations that can be used for prognostic and predictive purposes\(^{12, 64, 45}\). Drozdov et al. studied expression of a panel of nine genes known or suspected to be involved in NET tumourigenesis, and could accurately predict metastasis\(^{176}\). Andersson et al. found that small bowel carcinoid tumours with gain of chromosome 14 were associated with worse survival\(^{164}\).

STAGE AT DIAGNOSIS

Metastases are present in the majority of small bowel carcinoid patients at diagnosis, primarily regional in the mesentery and/or distant in the liver. Distant metastases may less often occur at other sites including peritoneal carcinomatosis\(^{177}\), lung\(^{178}\),
INTRODUCTION

bone, brain, heart, ovaries and eyes. The most recent update from the SEER database (1973–2004) reported that 29% of jejunoileal carcinoid tumours were localized at diagnosis, 41% had regional metastases, and 30% distant metastases. Quadflieg et al. found locoregional disease in 56% of small bowel carcinoid patients in the Netherlands, 26% had distant metastases and 18% were unstaged. Lepage et al. observed that 41% of small bowel carcinoid patients in Burgundy had distant metastases at diagnosis.

Due to selection of patients, single-centre studies reported still higher proportions with metastases. Bergstuen et al. found that 7% of small bowel carcinoid tumours were localized, 27% had regional metastases, and 66% distant metastases. Onaitis et al. reported proportions to be 23% localized, 20% with regional metastases and 57% with distant metastases. In the series described by Thompson et al., 11% of tumours were localized, 70% had regional metastases and 20% distant metastases. Jann et al. used the new TNM stage classification and found 1% of tumours to be stage I and 3% stage II (i.e. 4% localized tumours), 20% were stage III (with mesenteric involvement) and 77% were stage IV (with distant metastases).

The study by Hellman et al. included 91% of patients with mesenteric metastases and 81% with hepatic metastases, and that of Shebani et al. 70% with mesenteric and/or distant metastases.

TREATMENT

Surgery is the only curative treatment and therefore first-line strategy in patients with small bowel carcinoid. The extent of the disease determines what other treatments are necessary and feasible. Patients with localized disease are generally cured by a limited surgical procedure. As already described, most patients present with metastatic disease, but at least in locoregional disease there is still a chance for cure by a complete resection. Nevertheless, due to the initially often discrete symptoms, many patients have overly advanced disease already at diagnosis. A multitude of invasive and pharmacological palliative treatments are available in this situation.

Surgery

Primary small bowel carcinoid tumours are characteristically small, flat, and often multiple, and can therefore at times be difficult to localize during surgery. The pathognomonic mesenteric fibrosis seen with regional metastases poses a more severe
INTRODUCTION

challenge for the surgeon, with shrinkage and fixation of the mesentery and its root to the retroperitoneum. Profuse tumour growth and fibrosis may encircle the mesenteric blood vessels which may be at risk of stenosis or occlusion. The fibrosis thus complicates the resection and increases the risk of accidentally compromising vascular supply to the remaining bowel. Hepatic metastases are frequent already at presentation and necessitates difficult strategical decisions.

Technical aspects of surgery

To meet these challenges, some specific surgical techniques have been developed. The primary tumours and mesenteric metastases are preferentially resected en bloc with a wedge resection of the small bowel, or often as an ileocaecal resection or right-sided hemicolectomy. Öhrvall et al. proposed that the operation starts with manual exploration of the entire small bowel, from the ligament of Treitz to the ileocaecal valve, in order to localize primaries, mesenteric metastases and to estimate the required length of bowel resection\textsuperscript{150}. Next, the mesentery of the right colon and distal small bowel is mobilized from the retroperitoneum up to the level of the horizontal duodenum, allowing both anterior and posterior access to the mesenteric root. The mesenteric peritoneum is incised and unwrapped ventrally from the mesenteric root. Now the course of the major vessels and the extent of the mesenteric metastases may better be determined, and the resection is performed carefully to preserve the vascular supply and to limit the intestinal resection. Also, great care has to be taken in order not to injure the duodenum, the pancreas or the inferior caval vein. Tumours growing retroperitoneally, behind or above the pancreas, or encasing the proximal superior mesenteric artery are considered unresectable\textsuperscript{150}. When all mesenteric tumour growth cannot be safely removed without risk of compromising the vascular supply, the resection sometimes has to be carried out through tumour tissue\textsuperscript{159}. Stenting of the superior mesenteric vein was recently proposed in cases of unresectable mesenteric metastases obstructing the superior mesenteric vein with ensuing venous stasis\textsuperscript{186}.

An alternative approach to determine the mesenteric resection margins by injection of blue dye around the primary tumours, was put forward by Wang et al\textsuperscript{59}. The authors argue that the normally radial lymphatic drainage is blocked by mesenteric metastasis, and instead becomes longitudinal to the bowel lumen for a distance until the radial drainage is again free. This technique could theoretically decrease the risk of local recurrence.
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Handheld γ-detecting probes have also been tried to identify small tumours intraoperatively, but have not proved very helpful 153, 187.

Removal of liver metastases can be accomplished by combinations of lobectomy, segmental or atypical resection, or enucleation 187-189. Liver metastases may be removed with curative intent defined as no gross residual tumour in 20% of patients or more, provided that there are no other distant metastases or limiting comorbidity 187, 188, 190. Even when a macroscopically complete resection cannot be done, debulking resections of liver metastases can be considered for symptomatic patients if at least 90% of the hepatic metastases can be removed 189.

Strategical approach to surgery

Even with unresectable mesenteric or hepatic metastases, the general consensus is that maximal debulking resections should be performed. This serves to relieve already present symptoms, as well as to prevent future chronic symptoms or acute complications 117, 190-194. Especially with modern palliative treatments available, many patients survive for long periods with decent symptom control. Eventually most of them will suffer from symptoms caused by mesenteric metastasis and fibrosis if left in situ 195. Further, cytoreductive surgery increases the efficacy of these other treatments 185.

An active surgical approach is also supported by studies reporting improved survival 81, 117, 190, 195, 196. Wängberg et al. described improved survival in patients with disseminated midgut carcinoid after aggressive multimodal treatment including maximum resections 190. Chen et al. found that complete resection of liver metastases from various NETs resulted in better survival compared to patients with unresectable metastases 198. Hellman et al. found that complete resection of mesenteric metastases was associated with better survival, even in the presence of unresectable liver metastases 117. Givi et al. claimed that resection of the primary tumour was associated with improved survival 197. However, it is difficult to deduce with certainty whether or not this rather could be ascribed to clearance of mesenteric metastases. Hellman et al. also found better survival after resection of the primary tumour, but this difference was not seen with remaining mesenteric or hepatic metastases 117.

In multivariable analysis of 11,095 small bowel carcinoid patients, Bilimoria et al. found that complete resection of all primary tumours and metastases was associated with significantly better survival, irrespective of disease stage 41.
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Since many patients undergo emergency operations for acute abdominal symptoms with no prior knowledge about the carcinoid, adequate resections are not always performed. These patients should generally be planned for a second, measured debulking procedure.

Liver-directed surgery and other invasive treatments

Hepatic metastases are common at diagnosis of small bowel carcinoid. As described above, curative and palliative resections of liver metastases may yield symptomatic relief and prolonged survival. The reduction of biochemical markers after surgery is predictive of symptomatic relief and disease control. Unfortunately, most tumours with hepatic metastases recur after some time, 50–60% within 5 years.

Liver transplantation in NET patients is controversial but may be considered in highly selected patients. This may achieve significant palliative results but long-term cure is exceptionally rare. Other liver-directed treatment options may be used as alternatives or supplements to liver surgery. Radiofrequency ablation (RFA) is the preferred locally ablative technique, causing heat and necrosis of metastases up to a size of 3–4 cm in moderate numbers. RFA can be employed percutaneously with ultrasonographic guidance, at laparoscopy or during open surgery. There is limited data on the efficacy of RFA, but some of the larger series indicate good symptomatic relief and tumour control.

Hepatic artery embolization (HAE) takes advantage of the dual blood supply of the liver, with metastases mainly dependent on arterial blood whereas the normal parenchyma primarily is supplied by the portal venous system. Historically, the common hepatic artery was ligated, a method which was associated with high mortality and low efficacy because of collateral blood supply. In HAE, selective catheterization is instead undertaken and ischemia achieved by injection of embolization material, a procedure which can be repeated. Hepatic arterial chemoembolization (HACE) involves addition of a chemotherapeutic agent to the embolization material, resulting in selective delivery and less washout of the drug. HAE/HACE is particularly suitable for treating multiple, diffuse liver metastases. Embolization leads to complete or partial tumour response in approximately half of patients and symptom control in still more, with no difference demonstrated between HAE and HACE. Prolonged survival has not been firmly demonstrated, but Swärd et al. found better survival in 107 patients with unresectable liver metastases from midgut carcinoid tumours treated with HAE compared to historical data.
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Prophylactic cholecystectomy

Treatment with somatostatin analogues increases the risk of gallstone formation, and gallbladder-related complications may result from RFA and HAE of hepatic metastases. It is therefore recommended that an accompanying prophylactic cholecystectomy is performed during laparotomy if any of these treatments may later be an option. However if a laparotomy is not otherwise needed, the treatments can be given with the gallbladder in situ as the risk for complications is limited.

Biotherapy

Somatostatin is produced in the brain, by δ-cells in the pancreatic islets, by D-cells in the GI tract, in various endocrine organs and by immune cells. It occurs naturally as somatostatin-14 and somatostatin-28, depending on the peptide length. Both peptides bind with similar affinity to five distinct G-protein-coupled receptor subtypes (sstr1-5). Among other effects, somatostatin exerts inhibition of hormone and growth factor secretion, as well as inhibition of proliferation of normal and tumour cells – useful features in the treatment of neuroendocrine tumours.

There are two commercially available somatostatin analogues, both in common clinical use: octreotide (Sandostatin®) and lanreotide (Somatuline®). They have a biological half-life of 90–120 minutes, compared to the less stable natural somatostatin with a half-life of less than three minutes. Both are also available in a slow-release form which is injected every 3–6 weeks. More than 80% of GEP NETs express somatostatin receptors on the cell surface. Better differentiated tumours express higher levels and more of the five receptor subtypes. The most frequent subtype in GI NETs is sstr2, and both octreotide and lanreotide preferentially bind to sstr2. A novel synthetic analogue, pasireotide (SOM230), has high affinity for four of the receptor subtypes (sstr1, sstr3, sstr5) and appears to be effective for some tumours refractory to octreotide and lanreotide. The level of uptake on somatostatin scintigraphy may give some indication about how effective treatment with somatostatin analogues will be.

The efficacy of octreotide and lanreotide is similar, and involves symptomatic improvement in 40–80% of patients, reduced biochemical markers in 40–60%, but partial or complete response in less than 10%. Octreotide was compared to placebo in a double-blind, randomized study of 85 patients with metastatic midgut carcinoid. Stable disease was observed in 9/5 of patients in the treatment group after 6 months, significantly better than in the placebo group. Treatment with somatostatin analogues is usually well tolerated with few and minor side effects.
Currently, somatostatin analogues are recommended as first-line medical treatment in patients with hormonal symptoms. Whether it should be used in the absence of the carcinoid syndrome is not clear. Because of more pronounced side effects, interferon is only recommended as a second-line treatment. The combination of somatostatin analogue and interferon-α was studied in three randomized controlled trials. Faiss et al. found no significant difference in time to tumour progression between patients treated with lanreotide, interferon-α or the combination. Köbly et al. found that patients treated with octreotide and interferon-α had a reduced risk of progressive disease compared to patients receiving only octreotide, but there was no significant difference in survival. Arnold et al. found no significant difference between octreotide alone or combined with interferon-α in terms of progression-free or overall survival. However, criticism has been raised that both studies of long-term survival were underpowered. Thus, there is no compelling evidence that somatostatin analogues and/or interferon-α improves survival in small bowel carcinoid.

**Chemotherapy**

The presently available chemotherapeutic agents have been tested as single agents and in combinations with poor results in patients with small bowel carcinoid tumours, and they are not to be recommended other than in poorly differentiated neuroendocrine carcinomas.

However, some agents introduced in recent years for treatment of other malignancies show promise in patients with advanced GEP NETs. Bevacizumab (Avastin®) is a monoclonal antibody targeting vascular endothelial growth factor A (VEGF-A), and an angiogenesis inhibitor. Another angiogenesis inhibitor, sunitinib (Sutent®), is a tyrosine kinase inhibitor targeting several different VEGF receptors and platelet-derived growth factor (PDGF) receptors. Everolimus (Afinitor®), is an inhibitor of the mammalian target of rapamycin (mTOR), a central regulator of protein synthesis and involved in proliferation, angiogenesis, cell metabolism and apoptosis. The best results have been observed for patients with advanced EPTs, but response has been noted also in advanced GI NETs. There are ongoing randomized controlled trials of bevacizumab and everolimus in advanced GI NETs.
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Peptide receptor radionuclide therapy

In analogy to somatostatin scintigraphy, peptide receptor radionuclide therapy (PRRT) targets the somatostatin receptors that are overexpressed on the cell surfaces of most carcinoid tumours. Binding to the receptors is followed by endocytosis of the radionuclides which are retained in the tumour cell for a long time, exerting selective radiation therapy. PRRT is recommended in symptomatic, somatostatin scintigraphy-positive tumours refractory to biotherapy. 90Y and 177Lu are the currently recommended radionuclides.

There is no randomized phase III-study of PRRT, but in the largest reported series of 310 GEP NET patients treated with 177Lu-octreotate, Kwekkeboom et al. found that both progression-free survival and overall survival compared favourably with historical data. Complete or partial tumour response was reached in 23% of the 188 included GI NETs. In a number of smaller studies, complete or partial response was reported in 4–37% of patients, and stable disease in 51–88%. A recent study suggested that PRRT might be used as a neoadjuvant therapy to downstage primary unresectable NETs. A possible role for PRRT as an adjuvant treatment in NETs remains to be evaluated.

Treatment of carcinoid heart disease

Initial symptoms of right-sided heart failure may be relieved by water restriction, compression stockings and diuretics. Specific treatment of CHD involves cardiac valve surgery with either mechanical or bioprosthetic valves.

The prognosis for patients with CHD appears to have improved after introduction of valve replacement surgery, but the coinciding introduction of biotherapy might also have contributed. However, already established CHD lesions do not respond to decreased hormonal levels.

Perioperative care

In its extreme form, the carcinoid syndrome may amount to a carcinoid crisis. This medical emergency is caused by massive release of tumour-derived substances, occurring either spontaneously or provoked by endoscopy, induction of anesthesia, surgery, invasive treatment of hepatic metastases, chemotherapy or PRRT. Symptoms involve hyperthermia, profound flushing, diarrhoea, bronchial constriction, unstable blood pressure, cardiac arrhythmias, confusion and stupor, and is a life-threatening condition. To avoid a carcinoid crisis, prophylactic
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Treatment with somatostatin analogues should be given pre- and perioperatively\textsuperscript{231, 232}. If a crisis occurs despite prophylaxis, higher doses of somatostatin analogues should be given and the handling of the tumour should be halted. Adrenergic drugs should be avoided as they may worsen the situation by increased hormone release\textsuperscript{233}.

SURVIVAL

Small bowel carcinoid tumours are characterized by a high frequency of metastatic disease at diagnosis on the one hand, but usually slow progression on the other hand. There are studies indicating that survival in small bowel carcinoid has improved over the last few decades, but this conclusion is controversial.

Survival for patients with small bowel carcinoid has been studied principally by two methods; population-based registry studies and observations from single institutions. The strength of the registry studies is the inclusion of many patients, the disadvantages are that the included information about the patients and their tumours is limited and that the data may be incorrectly reported. By contrast, while studies based on material from single centres often are detailed, they are commonly subject to selection bias as patients usually have been referred to a centre of excellence. Unfortunately, many studies did not analyse survival for small bowel carcinoid alone, but as part of wider entities like GI NETs, GEP NETs or NETs. Furthermore, comparisons between various studies are hindered by the alternating use of overall, relative and disease-specific survival. The following account of survival in small bowel carcinoid includes prominent registry studies and selected single-centre studies. Results are summarized in Table 5.

Registry studies

Godwin reported from the End Results Group (ERG) of the National Cancer Institute (NCI), based on three state registries and over 100 hospitals in the United States\textsuperscript{233}. A relative 5-year survival of 54% was found in patients diagnosed with small bowel carcinoid between 1950 and 1969; 75% with localized tumour, 59% with regional metastases, and 19% with distant metastases. The ERG was succeeded by the SEER Program in 1973\textsuperscript{78}, and several reports on survival in small bowel carcinoid have since appeared from the SEER database\textsuperscript{72, 74, 77, 115, 234}. In the most recent report, including 9,266 midgut carcinoid patients diagnosed 1988–2004, Yao \textit{et al.} found an overall 5-year survival in jejunoileal carcinoid of 65% with localized tumour, 71% with regional metastases and 54% with distant metastases\textsuperscript{72}. 

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Bilimoria et al. identified 11,095 patients who had undergone surgical resection between 1985 and 2000 from the National Cancer Data Base (NCDB) covering 70% of all hospitals in the United States, and found an overall 5-year survival of 64.6% and a relative 5-year survival of 79.1%.

Another study was based on the Florida Cancer Data System, which covers 6% of the US population. From the start of the registry in 1981 and until 2000, some 1,428 patients were diagnosed with small bowel carcinoid with an overall 5-year survival as low as 36.8%.

Data from the Digestive Cancer Registry of Burgundy included 102 cases of “endocrine small bowel cancer” diagnosed between 1976 and 2001. The 5-year overall survival was 45.7% and the relative 5-year survival 56.8%.

Based on the National Cancer Registry of England and Wales, Pashyan et al. reported a relative 5-year survival of 46% for over 800 patients diagnosed with small bowel carcinoid between 1971 and 1990. A more recent study from the same registry found 1,154 patients with well differentiated small bowel carcinoid tumours diagnosed 1986–1999 with a relative 5-year survival of 58.7%.

A study from the Norwegian Registry of Cancer showed an overall 5-year survival of 59% for patients diagnosed between 1993 and 2004 with small bowel NETs, including less differentiated neuroendocrine carcinomas.

In a study based on the Swedish National Cancer Registry, Zar et al. described long-term survival in jejunoileal carcinoid. There was no data on disease stage available, but all three measures of survival were given; the 5-year overall survival was 56%, the 5-year relative survival was 67% and the 5-year disease-specific survival was 87%.

Lepage et al. demonstrated that there are significant geographical differences in survival of GEP NETs with an increased hazard ratio (HR) in Eastern Europe and decreased HR in Western and Northern Europe compared to the United Kingdom.

Single-centre studies

In an early series from the Mayo Clinic, Moertel et al. reported that 5-year overall survival in 50 patients with small bowel carcinoid diagnosed at surgery 1938–1954 was 64% for those with localized disease, 71% for those with resected metastases and 27% for those with unresectable metastases. In a later study from the Mayo Clinic,
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Table 5. Summary of previous reports on overall, relative and disease-specific 5-year survival in small bowel carcinoid

<table>
<thead>
<tr>
<th>Reference</th>
<th>Location</th>
<th>Period</th>
<th>Tumour site</th>
<th>n</th>
<th>Loc</th>
<th>Reg</th>
<th>Dist</th>
<th>All stages</th>
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<td>Registry studies</td>
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<tr>
<td>Zar\textsuperscript{237}</td>
<td>Sweden</td>
<td>1960–2000</td>
<td>JI</td>
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<td>Modlin\textsuperscript{234}</td>
<td>USA SEER</td>
<td>1973–1991</td>
<td>D, JI</td>
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<td>Yao\textsuperscript{72}</td>
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<td>Thompson\textsuperscript{133}</td>
<td>Roch., MN, USA</td>
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<td>66</td>
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<td>Shebani\textsuperscript{134}</td>
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<td>JI</td>
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<td>1971–1990</td>
<td>D, JI</td>
<td>&gt; 800</td>
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<td>258</td>
<td></td>
<td></td>
<td></td>
<td>78</td>
</tr>
<tr>
<td><strong>Disease-specific survival</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Registry studies</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Zar\textsuperscript{237}</td>
<td>Sweden</td>
<td>1960–2000</td>
<td>JI</td>
<td>2,437</td>
<td></td>
<td></td>
<td></td>
<td>87</td>
</tr>
<tr>
<td>Maggard\textsuperscript{17}</td>
<td>USA SEER</td>
<td>1973–1997</td>
<td>D, JI</td>
<td>2,778</td>
<td>94.5</td>
<td>84.4</td>
<td>51.2</td>
<td>78.1</td>
</tr>
<tr>
<td><strong>Single-centre studies</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jann\textsuperscript{17}</td>
<td>Berlin, Germany</td>
<td>1984–2008</td>
<td>JI</td>
<td>214</td>
<td>100</td>
<td>97.1</td>
<td>84.8</td>
<td>88.9</td>
</tr>
</tbody>
</table>
| \* Including patients after surgical resection.

Values of 5-year survival are percentages.

D = duodenum, JI = jejunum/ileum, IC = ileocecal valve.
INTRODUCTION

Thompson et al. found an overall 5-year survival of 62% in 66 patients with ileal carcinoid tumours.

Jansson et al. included 256 midgut carcinoid patients between 1978 and 1993, of note was that as many as 76% of the patients had liver metastases at diagnosis. Nevertheless, the 5-year overall survival was 63%.

Another Uppsala study by Hellman et al., seemingly including the majority of patients from Janson et al., described 314 patients with midgut carcinoid diagnosed between 1975 and 1997. Despite liver metastases in 81% of patients at diagnosis, the 5-year overall survival was 67%.

A study from a tertiary referral centre in Belfast identified 139 patients with midgut carcinoid diagnosed 1978–2000, 80.4% of whom had metastases, and found an overall 5-year survival of 53%.

In a study including all 258 small bowel carcinoid patients seen at a Norwegian referral centre between 1983 and 2007, Bergstuen et al. found 78% relative survival after 5 years, and 53% after 10 years.

Nykjær et al. described 56 midgut carcinoid patients with an overall 5-year survival of 72% from a Danish referral centre.

A recent study by Jann et al. described 214 jejunoileal carcinoid tumours diagnosed 1984–2008 at one German and one French referral centre. As an exception, this study included disease-specific survival reported at 88.9% after 5 years and 69.2% after 10 years.

PROGNOSTIC FACTORS

Many studies include a mixture of NETs or GEP NETs from various sites in the analysis of prognostic factors. The following account focuses as far as possible on studies limited to small bowel carcinoid.

Age

There is universal agreement that patient age at diagnosis is a prognostic factor – older patients having a worse survival. However, only Zar et al. found a steadily increasing hazard ratio with advancing age in multivariable analysis of disease-specific survival. The other studies used overall survival, which is
problematic when analyzing age as a risk factor. This was exemplified by a study reporting age as a prognostic factor for overall survival, but not for disease-specific survival.  

Gender  
Five studies reported no significant difference in survival between men and women with small bowel carcinoid. In two studies based on the SEER database, Maggard et al. and Bilimoria et al. found a significantly worse overall survival in men.  

Race  
American studies as a rule include race in the analysis of prognostic factors. The results are conflicting; Maggard et al. and Bilimoria et al. found that black patients have worse overall survival in multivariable analysis, whereas Landry et al. found that black patients have better survival in univariable analysis, with no significant difference in multivariable analysis.  

Location of primary tumour  
The location of the primary carcinoid tumour within the small bowel as a risk factor was examined in two different studies. Landry et al. found an increased hazard ratio for primary location in the jejunum compared to the duodenum and ileum, but neither this study nor that by Bilimoria et al. found any difference in multivariable models.  

Disease stage  
That the extent of dissemination at diagnosis is associated with survival is uncontroversial, although the classifications of disease stage have changed over the years. The SEER database contains information of disease stage defined as localized, regional or distant disease. Several studies based on this registry have found that more advanced disease stage is associated with worse survival. However, studies including significance testing between each of the three stages have not found significantly worse survival associated with regional metastases compared to localized tumours, neither in registry studies nor in single-centre studies. In fact, in multivariable analysis, Bilimoria et al. counterintuitively found a significantly better survival in patients with regional metastases. Jann et al. applied the recently
INTRODUCTION

introduced TNM stage classification, and found that stage I and II (localized) jejunoileal carcinoid tumours were associated with significantly better disease-specific survival than stage III (regional) and IV (distant) \(^{171}\). The result was the same comparing stage I–IIIa with stage IIIb-IV, but no significant difference was found between stages I–II and III.

Tumour size

Two separate studies based on the SEER database found that larger primary tumours were associated with worse overall survival in multivariable analysis\(^{81, 116}\). Two single-centre studies did not find an association between tumour size and overall survival in univariable\(^{120}\) or multivariable\(^{243}\) analyses, respectively.

Histopathology

Jann et al. did not find any statistically significant difference in disease-specific survival between G1 (Ki-67 index \(\leq 2\%\)) and G2 (3–20%) jejunoileal carcinoid tumours\(^{171}\). However, survival was significantly worse in a few G3 (> 20%) carcinoid tumours. Cunningham et al. observed that Ki67 > 1% was associated with worse disease-specific survival in ileocaecal carcinoid\(^{170}\). Panzuto et al. reached similar results on overall survival using the established cut-off at Ki67 > 2%, and Bergestuen et al. with Ki67 \(\geq 5\%\)\(^{120, 244}\). Other studies mixed NETs of different origins\(^{245, 246}\).

Cunningham et al. first described the solid growth pattern in ileocecal carcinoid tumours, and found it to be associated with worse survival\(^{170}\).

Biochemistry

Urinary 5-HIAA levels\(^{120, 239, 247}\) as well as plasma CgA levels\(^{120, 239}\) at presentation have been identified as independent prognostic factors in multivariable analyses. Plasma levels of NKA was the only independent prognostic biomarker in one study\(^{168}\).

Period of diagnosis

In multivariable analysis, Zar et al. found that disease-specific survival in small bowel carcinoid improved steadily over the years – the hazard ratio for patients diagnosed 1990–2000 was 0.2 (95% confidence interval (c.i.) 0.1 to 0.3) compared to those diagnosed 1960–1970\(^{137}\). In a multivariable analysis of relative survival, Lepage et al. noted a trend towards better survival for patients diagnosed more recently, although
INTRODUCTION

it did not reach statistical significance\textsuperscript{386}. By contrast, Bilimoria \textit{et al.} did not observe any improvement in survival from 1985 to 2000 in a multivariable analysis\textsuperscript{311}. Neither could Modlin \textit{et al.} detect an improved overall survival between 1973 and 1997 in univariable analysis\textsuperscript{74}. An unaltered prognosis is surprising in view of the new treatment modalities that have appeared during the last decades, but also given the improved diagnostic possibilities which would presumably lead to earlier detection and thereby to lead time bias.

After a review of the available data on prognostic factors, Modlin \textit{et al.} recently constructed a nomogram to predict survival for individual small bowel carcinoid patients\textsuperscript{248}. Points are assigned for each of 15 weighted prognostic variables, with Ki-67 index by far considered the most decisive. The sum of points is compared with the nomogram to read the the probability to survive for 5 and 10 years.

D. Cocaine- and amphetamine-regulated transcript (CART)

DISTRIBUTION AND PHYSIOLOGICAL FUNCTION OF CART

\textit{Cocaine- and amphetamine-regulated transcript} (CART) was discovered by Douglass \textit{et al.} in 1995 as an mRNA that was upregulated in the rat brain in response to psychostimulant administration\textsuperscript{249}. CART peptides have since been found in the central, peripheral and enteric nervous systems, and also in endocrine cells in the pancreatic islets\textsuperscript{250-252}, the GI tract mucosa\textsuperscript{253, 254}, the thyroid\textsuperscript{255}, and the adrenal medulla\textsuperscript{256}.

It thus appears that CART peptide is another brain-gut peptide, acting both as a neurotransmitter and as a hormone. Within the CNS, the spatial distribution of CART peptides together with various experimental studies suggest a role of CART peptides in regulating food intake and body weight\textsuperscript{257, 258} with an overall anorexigenic effect via largely unknown mechanisms\textsuperscript{257, 259}. This is also supported by observations that Cart null mice and humans carrying a mutated Cart gene develop obesity and signs of type 2 diabetes\textsuperscript{260-262}. In addition, there is evidence of CART involvement in other brain processes such as mechanisms of reward and stress response\textsuperscript{257, 258, 263}. 41
INTRODUCTION

Hormonal expression of CART in pancreatic islets occurs mainly in somatostatin-producing δ-cells, and the produced CART peptides participate in the regulation of insulin, glucagon, and somatostatin secretion. Indeed, CART is necessary for normal islet function, since Cart null mice have diminished insulin secretion and reduced glucose elimination. Interestingly, there is recent evidence that CART is crucial for pancreatic islet β-cell viability, both by reducing apoptosis and by increasing proliferation via several different pathways.

Within the GI tract mucosa, CART expression has been identified mainly in gastrin-producing G-cells but also in EC cells of the small bowel. The physiological function of CART in the enteric neurons and GI endocrine cells remains poorly elucidated.

In view of the importance of CART in the regulation of body weight, addiction and insulin secretion, the possibility of developing drugs that target CART receptors attracts a lot of attention. Evidence suggests that some of the effects of CART are mediated via a G-protein-coupled receptor, but the nature of this CART receptor has not yet been identified.

CART SYNTHESIS

The human Cart gene has been mapped to chromosome 5q13–q14. Transcription of the gene in rats and mice yields two different propeptide isoforms due to alternative splicing: one longer proCART 1–102 and one shorter proCART 1–89. In humans, only the short proCART 1–89 has been found. The proCART peptides are then subject to prohormone convertase-mediated processing at several different dibasic cleavage sites, resulting in at least two biologically active CART peptides. From the long proCART derives CART 55–102 and CART 62–102, and from the short proCART derives CART 42–89 and CART 49–89. CART 55–102 is identical to CART 42–89 (CART I), as is CART 62–102 to CART 49–89 (CART II). A high level of sequence homology between Cart genes and CART peptides of different species indicates an evolutionary conserved function.

CART IN TUMOURS

CART expression in neoplastic tissue was first demonstrated in rat pancreatic islet tumours by Jensen et al. Next, Wierup and Sundler found CART to co-localize
with insulin in human insulin-producing EPTs. Interestingly, it seemed that CART immunoreactivity (IR) was more abundant in well differentiated tumours than in less differentiated tumours.

We recently undertook a study to explore the presence of CART in human NETs originating in the stomach, ileum, rectum, pancreas, and thyroid. Expression of CART was found in a similar proportion of tumours regardless of the tumour site of origin. The frequency of CART IR cells varied from none in some tumours to a majority of the tumour cells in others. Commonly the pattern of CART IR was heterogeneous even within the same tumour. In small bowel carcinoid tumours, CART was consistently co-expressed with serotonin and CgA within the same tumour cell, and sometimes also with the tachykinin NPK.

Bech et al. found that circulating levels of CART were raised in patients with a wide range of various NETs, although there was some uncertainty regarding the molecular identity of this circulating CART. Interestingly, patients with progressive disease had higher CART levels than those with stable disease.
AIMS OF THE STUDY

Small bowel carcinoid is a rare disease and therefore difficult to study. We found that previous studies either described selected patients at referral centres, or were based on limited data from large registries. The main objective of this thesis was to investigate small bowel carcinoid patients from a geographically defined cohort with no selection bias.

The specific aims were:

- To investigate the incidence, histopathological characteristics, stage at diagnosis, symptomatology, surgical treatment, prognostic factors and survival of small bowel carcinoid.

- To describe the occurrence of hereditary small bowel carcinoid in three consecutive generations.

- To investigate whether content of CART in small bowel carcinoid tumours is associated with tumour characteristics, symptoms and survival.
PATIENTS AND STATISTICAL METHODS

PATIENTS

Papers I and II

All patients resident in Jönköping County when diagnosed with small bowel carcinoid between 1960 and 2005 were included, i.e. also patients who were diagnosed at hospitals elsewhere in Sweden. Initially 167 patients were identified as eligible for inclusion through the Swedish Cancer Registry and the local cancer registry, 22 of them were excluded for various reasons. Seven patients had incorrectly been included in the registries. Ten patients were excluded as the carcinoid diagnosis was found to be incorrect after our renewed histopathological examination. Five patients were excluded because the medical records could not be found. Eventually, 145 patients (79 men and 66 women) with small bowel carcinoid diagnosed ante mortem were included in the study, all of them ethnic Swedes.

Paper III

We encountered a family with three members in consecutive generations affected by metastasizing small bowel carcinoid.

Case 1 was a 57-year-old man admitted to the local district hospital in 1958 for an elective stomach resection on suspicion of a duodenal ulcer, after episodes of profuse gastrointestinal haemorrhage. At operation, however, no lesion was found in the stomach or duodenum, and the surgeon therefore went on to examine the intestines. In the distal part of the jejunum, a tumour measuring 2 cm was found and removed by a 10-cm small bowel resection. There were no signs of any metastases in the mesentery or the liver. The carcinoid tumour was classified as pT3N0M0 (stage IIB) \(^{69,70}\). Following the operation, the patient was well for 18 years before he was acutely admitted due to acute cholecystitis. At the ensuing elective cholecystectomy, the surgeon incidentally found a 6-cm-large carcinoid recurrence centrally located in the mesentery. The tumour was resected together with 50 cm of compromised ileum, and histopathological examination confirmed a lymph node metastasis from the previous tumour. The patient was followed for 5 years and had no complaints other
than occasional diarrhoea. Urinary 5-HIAA was normal and no further treatment was given. In 1992, at the age of 91, the patient was admitted to the surgical department twice due to malaise and colicky pain. Investigations including gastroscopy, plain abdominal X-ray and ultrasonography of the liver could not provide any explanation to his symptoms. He returned home and died a couple of months later. Autopsy was not performed.

Case 2 was the eldest son of Case 1. He was 77 years old when diagnosed with a small bowel tumour and enlarged mesenteric lymph nodes on CT in 2003, after 6−7 years of intermittent colicky abdominal pain. At surgical exploration at his local district hospital, the tumours were considered unresectable and a palliating ileo-ileal shunt was performed. A different opinion was held at the central district hospital where his abdomen was re-explored 3 months later, and the ileal tumour and mesenteric metastases were removed. The surgeon found the liver macroscopically free from metastases, confirming the CT results. The postoperative course was complicated by anastomotic insufficiency which resolved only after two additional abdominal procedures. The histopathological examination of the specimens revealed a large (4 cm) metastasizing ileal carcinoid tumour with a small (0.5 cm) satellite lesion nearby, and the tumours were classified as pT4(m)N1M0 (stage IIIB). At follow-up, his urinary 5-HIAA levels increased 2½ years postoperatively, and liver metastases appeared on CT. The patient was given increasing doses of somatostatin analogue, but his general health deteriorated and he died peacefully in his home 4½ years after the initial diagnosis.

Case 3 was the youngest daughter of Case 2. She was 53 years old when she visited her general practitioner in 2007 with complaints of fatigue, diffuse abdominal pain, and flushing for the last 3 years. Laboratory investigations revealed raised levels of serum CgA and urinary 5-HIAA, and CT displayed multiple small bowel tumours and a 10-cm-large metastatic lesion in the liver. The patient was operated with resection of 40 cm of the proximal ileum along with adjacent lymph nodes up to the mesenteric root. Also, segments 5−8 of the liver were resected at the primary operation. The histopathological examination showed nine separate small carcinoid tumours in the ileum (the largest measuring 8 mm) and multiple metastatic lesions both in mesenteric lymph nodes and the liver. The tumours were classified as pT2(m)N1M1 (stage IV). During follow-up investigations, new hepatic metastases emerged in early 2010, and the patient was subjected to renewed liver resection.
PATIENTS AND STATISTICAL METHODS

Paper IV

The 145 patients included in papers I and II were all eligible for inclusion in paper IV, provided that paraffin-embedded tumour material could be retrieved for analysis. Eventually, 97 patients with adequate specimens were included. Patients with distant metastases were somewhat underrepresented among the included patients compared to the eligible (28% versus 36%) due to the fact that some patients with distant metastases never underwent surgical resection.

The investigations were approved by the Regional ethical review board at Linköping University.

STATISTICAL METHODS

Non-normally distributed data, in particular duration of time, was presented as median with interquartile range (i.q.r.). Differences in proportions were tested using the $\chi^2$ test, or Fisher’s exact test when the expected number in any cell was less than five. Differences in proportions of recurrence by disease-stage (ordinal scale) were tested using $\chi^2$ test for linear trend. Non-normally distributed variables were compared between groups using the Mann-Whitney U test. The strength of relationship between variables on ordinal scales was estimated using Kendall’s rank correlation coefficient ($\tau$, tau). The differences in mean cell viability in mediums containing control, CART or glucagon-like peptide-1 (GLP-1) were tested using one-way analysis of variance, followed by Dunnet’s multiple comparisons test.

Incidences were age-standardized to the Swedish population in 2005 using the direct method. Change in incidence over time was assessed using Mantel-Haenszel test for trend.

Overall, disease-specific, and recurrence-free survival was estimated according to the Kaplan-Meier method, and the logrank test was used to test the difference between survival curves. Relative survival was calculated as the ratio between overall survival in the study cohort and the expected survival in the reference population. Calculations were performed by the Ederer II method, whereby the matched individuals are considered to be at risk until the corresponding patient with carcinoid dies or is censored. Expected survival estimates were calculated from information obtained from Statistics Sweden, regarding the number of deaths and mid-year population for each sex, using intervals of one year for age and calendar year.
PATIENTS AND STATISTICAL METHODS

Univariable and multivariable Cox proportional hazards regression models were used to calculate the hazard ratio for prognostic factors. Co-variables were selected by step-up regression in paper II and by forced-entry in paper IV. In order to describe long-term survival, patients who died within 30 days of diagnosis were excluded from survival analyses in paper II. This could not be done in paper IV because it resulted in too few uncensored cases left in one group.

All tests were two-tailed and \( P \)-values < 0.05 were considered statistically significant.
RESULTS AND DISCUSSION

PAPERS I AND II

Incidence

The age-adjusted incidence was 1.12 per 100,000 persons and year (95% c.i. 0.95 to 1.31) during the entire study period 1960–2005 (Table 6). The incidence was found to increase ($P < 0.001$), and reached 1.33 (95% c.i. 1.03 to 1.70) in the last third of the study period (1991–2005).

The incidence in our material compares high to previous reports which mostly range between 0.3 and 0.7 per 100,000. $^{14, 72-75, 84}$ The highest previously reported incidence was 1.01 per 100,000 between 2000 and 2004 in Norway. $^{14}$ The observed increase in incidence was in accordance with previous studies $^{14, 72-74, 83}$, two of these studies included significance tests $^{72, 74}$. It seems likely that improved diagnostic techniques have contributed to this increase. Changes in the reporting of carcinoid to registries may also account for some of the increase, but a “true” change in epidemiology can not be ruled out either. There were more men than women in our material, but no significant difference in incidence between the genders ($P = 0.239$). Previous epidemiological studies have consistently reported more men than women with small bowel carcinoid $^{14, 72-74, 83}$. This difference was statistically significant in the study by Yao et al. $^{72}$, but not in that by Modlin et al. $^{74}$.

Table 6. Age-adjusted incidence of small bowel carcinoid in Jönköping county 1960–2005

<table>
<thead>
<tr>
<th>Time period</th>
<th>Male and Female</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>1960–1975</td>
<td>0.58 (0.38–0.84)$^a$</td>
<td>0.43 (0.20–0.78)</td>
<td>0.76 (0.46–1.22)</td>
</tr>
<tr>
<td>1976–1990</td>
<td>1.28 (0.97–1.66)$^a$</td>
<td>1.63 (1.15–2.26)</td>
<td>0.97 (0.60–1.45)</td>
</tr>
<tr>
<td>1991–2005</td>
<td>1.33 (1.03–1.70)$^a$</td>
<td>1.47 (1.02–2.05)</td>
<td>1.19 (0.80–1.72)</td>
</tr>
<tr>
<td>1960–2005</td>
<td>1.12 (0.95–1.31)</td>
<td>1.20 (0.96–1.49)$^b$</td>
<td>1.00 (0.77–1.26)$^b$</td>
</tr>
</tbody>
</table>

$^a$Annual age-adjusted incidence per 100,000 persons with 95% confidence interval in parentheses. Age standard is the Swedish population 2005.

$^b$ $P < 0.001$ (Mantel-Haenszel test for trend)

$^b$ $P = 0.239$ (Fisher’s exact test)
RESULTS AND DISCUSSION

Age

Median age at diagnosis was 69 years (i.q.r. 59 to 77), 66 years in men and 73 years in women ($P = 0.027$). Yao et al. reported a median age of 66 years at diagnosis of jejunoileal carcinoid from the SEER database. By contrast, probably due to selection bias, studies from referral centres reported average age at diagnosis in the range 57.5 to 62 years.

Symptoms

One hundred and twenty-two patients were diagnosed as a result of symptoms from the carcinoid tumour. The duration of symptoms before diagnosis was in median 5 (i.q.r. 1 to 15, range 0 to 180) months. Symptoms are outlined in Table 7, notably flushing was seen in only 16 patients at the time of diagnosis. Instead, the most common presenting symptom was abdominal pain, either chronic or acute. Other common symptoms were complete or partial bowel obstruction, diarrhoea and weight loss. No less than 17 patients presented with overt GI haemorrhage, in eleven patients as the only symptom.

Overall, our results describe that presenting symptoms of small bowel carcinoid are commonly uncharacteristic, in accordance with many previous studies. Hormonal symptoms, on the other hand, are relatively infrequent at diagnosis, flushing being reported in 2–29% of patients in the same studies. Gastrointestinal haemorrhage is generally not considered a common feature of small bowel carcinoid, but has been noted in 5–9% of patients in a few previous studies.

<table>
<thead>
<tr>
<th>Symptom</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal pain</td>
<td>61 (50)</td>
</tr>
<tr>
<td>Intestinal obstruction</td>
<td>43 (35)</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>32 (26)</td>
</tr>
<tr>
<td>Weight loss</td>
<td>29 (24)</td>
</tr>
<tr>
<td>Gastrointestinal haemorrhage</td>
<td>17 (14)</td>
</tr>
<tr>
<td>Flushing</td>
<td>16 (13)</td>
</tr>
<tr>
<td>Bronchial constriction</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Right-sided heart failure</td>
<td>0</td>
</tr>
</tbody>
</table>

Values in parentheses are percentages.

*More than one symptom possible for each patient.
RESULTS AND DISCUSSION

Diagnosis

The diagnosis of small bowel carcinoid was confirmed by means of preoperative investigations in 28 patients (19%), by elective surgery in 40 (28%) and by emergency surgery in 54 patients (37%). By contrast, the carcinoid tumour was incidentally found in 23 patients (16%) by the surgeon or pathologist in connection with surgery for other conditions. Thus, due to the initially inconspicuous symptoms, a substantial proportion of small bowel carcinoid tumours are diagnosed on an emergency basis or incidentally. Similar to our finding, emergency operations accounted for 46% of operations in a study by Hellman et al.117, and incidental detection of jejunoileal carcinoid has been reported in 6–34% in some previous studies118-120,131.

TNM stage classification

Paper I was written before the TNM classification and histopathological grading systems were introduced, and we were urged by the reviewers to include the WHO classification of 2000. In paper II we re-examined all available tumour specimens and introduced the new classification systems, separate for clinical stage and histological grade67, 69, 70. Adequate tumour specimens from 110 patients (75.9%) were available for histopathological re-examination. The remaining tumours were classified from the original pathology report. According to the TNM stage classification69, 70, local invasiveness of the primary tumour was classified as T1 in three patients; T2 in 17; T3 in 83; and T4 in 24 patients (Table 1). Tumour invasiveness could not be decided (TX) in 18 patients272, most often because no resection of the primary tumour was performed. Localized tumours were stage I in three, IIA in 11, and IIB in 12 patients (Table 2). The T category could not be established for one localized tumour which was therefore classified as TX N0 M0. Regional tumours invaded the mesentery in two patients (stage IIIA), and appeared as lymph node metastases in 64 patients (stage IIIB). Distant metastases were present in 52 patients at diagnosis (stage IV). As expected, the risk of metastases was significantly lower for incidentally detected tumours (P = 0.041); still 65% had metastases (Table 8). The sites of distant metastases at diagnosis and cumulatively are detailed in Table 9.

Table 8. Dissemination of small bowel carcinoid tumours at diagnosis in 145 patients.

<table>
<thead>
<tr>
<th>Mode of detection</th>
<th>Localized</th>
<th>Regional</th>
<th>Distant</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical symptomatic (n = 122)</td>
<td>19 (16)</td>
<td>54 (44)</td>
<td>49 (40)</td>
<td></td>
</tr>
<tr>
<td>Incidental (n = 23)</td>
<td>8 (35)</td>
<td>12 (52)</td>
<td>3 (13)</td>
<td>0.041</td>
</tr>
<tr>
<td>All (n = 145)</td>
<td>27 (19)</td>
<td>66 (46)</td>
<td>52 (36)</td>
<td></td>
</tr>
</tbody>
</table>

Values in parentheses are percentages.

*Localized versus regional and distant metastases, compared to clinical symptomatic (Fisher’s exact test).
RESULTS AND DISCUSSION

Table 9. Sites of distant metastasis in 145 small bowel carcinoid patients at the time of diagnosis and cumulatively

<table>
<thead>
<tr>
<th>Site of metastases</th>
<th>At diagnosis</th>
<th>Cumulatively</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>45 (31)</td>
<td>65 (45)</td>
</tr>
<tr>
<td>Peritoneum</td>
<td>13 (9)</td>
<td>15 (10)</td>
</tr>
<tr>
<td>Bone</td>
<td>3 (2)</td>
<td>12 (8)</td>
</tr>
<tr>
<td>Lung</td>
<td>1 (1)</td>
<td>8 (6)</td>
</tr>
<tr>
<td>Ovaries*</td>
<td>3 (5)</td>
<td>5 (8)</td>
</tr>
<tr>
<td>Myocardium</td>
<td>1 (1)</td>
<td>3 (2)</td>
</tr>
<tr>
<td>Pancreas</td>
<td>2 (1)</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Kidney</td>
<td>0</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Eye</td>
<td>0</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Testicle*</td>
<td>0</td>
<td>1 (1)</td>
</tr>
</tbody>
</table>

Values in parentheses are percentages.
*Per cent of male or female patients, respectively.

Moertel et al. described that 9% of incidentally detected small bowel carcinoid tumours were metastasized, as opposed to 93% of symptomatic tumours. Two other studies reported lower risk of dissemination in incidentally encountered GI NETs.

Histopathological grade

The Ki-67 proliferation index could be established in primary tumours from 79 patients. Median Ki-67 index was 1% (range 0 to 10%). According to the WHO 2010 classification (Table 3), there were 74 G1 and five G2 tumours.

Jann et al. reported that 63% of 153 examined jejunoileal NETs were G1; 35% G2; and 3% G3. Bergstuen et al. found Ki67 index < 5% in 78% of 130 small bowel carcinoid tumours, and ≥ 5% in 22%.

Primary surgery

One-hundred and thirty-five patients underwent surgery within 10 weeks of diagnosis. Eight of them were subjected to a second elective operation within 6 months because the first operation only involved exploration, sometimes with biopsies or intestinal bypass. Small bowel resection was performed in 80 patients and right-sided hemicolecction in 38 patients.
RESULTS AND DISCUSSION

Residual tumour after surgery was classified according to UICC, considering the local site as well as regional and distant sites\textsuperscript{70, 273}. R0 implies complete resection with no residual tumour evident macroscopically, on histology, on imaging or in laboratory investigations. R1 indicates histologically verified residual tumour and R2 macroscopically visible residual tumour. RX is used when it cannot be established whether the resection was complete or not. The resection of all local, regional and distant tumours was considered complete (R0) in 74 patients (54.8\%) (Table 10). Tumours with regional metastases were considered R0 in 47 patients (71\%). All but two localized tumours were R0, whereas only two tumours with distant metastases were completely resected. Recurrent disease was observed in 23 patients (31\%) with R0 resection, after a median of 85 (i.q.r. 48 to 160) months. The risk of recurrence increased with the stage at diagnosis ($P < 0.001$). Recurrences most often occurred in the mesentery (14 patients) and in the liver (17 patients).

Other treatments

Adjuvant and palliative treatments were used infrequently in our investigation as few options were available during the first part of the study. Thirty-nine patients were treated with somatostatin analogues for a median of 50 (i.q.r. 13 to 91) months and 25 patients with interferon for 32 (i.q.r. 15 to 94) months. Chemotherapy was given to seven patients and external radiotherapy to four. Liver metastases were treated in 15 patients with one or more of the available techniques: surgical resection, radiofrequency ablation and ischaemic treatment by hepatic artery ligation or embolization. No patient received PRRT. In addition, 16 patients underwent later elective debulking surgery and 15 required surgery to resolve bowel obstruction.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Patients</th>
<th>Operated</th>
<th>Completeness of resection</th>
<th>Recurrence</th>
<th>after R0</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>R0</td>
<td>R1</td>
<td>R2</td>
</tr>
<tr>
<td>Localized</td>
<td>27 (18.6)</td>
<td>27</td>
<td>25 (93)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Regional</td>
<td>66 (45.5)</td>
<td>66</td>
<td>47 (71)</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>Distant</td>
<td>52 (35.9)</td>
<td>42</td>
<td>2 (4)</td>
<td>0</td>
<td>40</td>
</tr>
<tr>
<td>Total</td>
<td>145 (100)</td>
<td>135</td>
<td>74 (54.8)</td>
<td>2</td>
<td>52</td>
</tr>
</tbody>
</table>

Values in parentheses are percentages.
* $P < 0.001$ ($\chi^2$ test for linear trend)
RESULTS AND DISCUSSION

Survival

Patients who survived the first 30 days after diagnosis were included in the survival analyses, ten patients who died in the postoperative period were excluded. This method, with its pros and cons, was chosen in order to describe long-term survival and associated prognostic factors. For the remaining 135 patients, median overall survival time was 7.2 (i.q.r. 2.9 to 12.8) years and median disease-specific survival time 12.3 (i.q.r. 4.7 - never reached 3rd quartile) years. The overall survival at 5, 10 and 15 years was 60.7, 40.6 and 22.1%, respectively. The relative survival was 73.9, 60.5 and 40.4% of the expected survival in the general population at 5, 10 and 15 years of follow-up, respectively. Disease-specific survival rates were similar to relative survival rates: 75.0, 63.5 and 47.7% after 5, 10 and 15 years, respectively.

Relative survival is suitable for describing excess mortality in larger populations provided that the studied disease is not correlated with some other condition that affects mortality. Disease-specific survival is the alternative measure when cause of death can be established. However, determining the cause of death inherently involves some degree of subjectivity, and further distinguishes those who died from the disease and those who did not, disregarding that the disease may have contributed to death, but not be the sole reason for it. The relative and disease-specific survival rates were very similar in our investigation, our interpretation is that both are accurate measures of excess mortality.

Overall, relative, and disease-specific survival in our material compared similar to most previous studies.

Prognostic factors

In univariable analyses, disease-specific survival was associated with disease stage (Figure 4), size of primary tumour, T category and whether resection was complete or not (Table 11). We were particularly interested in the patients with mesenteric involvement but no distant metastases, and found that R0 in this group was associated with better survival (P = 0.005) (Figure 5).

In multivariable Cox proportional hazards regression, R stage remained an independent prognostic factor whereas tumour size did not. T3–T4 tumours were associated with worse survival, but this did not reach statistical significance (HR 3.80, 95% c.i. 0.87 to 16.63; P = 0.076). The majority of TX tumours were never resected, because they were considered unresectable or the patient unfit for surgery, and were therefore associated with an increased HR. Distant metastases were
associated with worse survival (HR 14.44, 95% c.i. 1.59 to 131.36; \( P = 0.018 \)). Regional metastases were also associated with an increased risk of carcinoid-related death, but this did not reach statistical significance (HR 2.66, 95% c.i. 0.32 to 22.20; \( P = 0.367 \)). Multivariable analysis identified worse survival for older patients and indicated better survival for patients diagnosed during the latter half of the study period. Histological grade was not included in the multivariable analysis due to many missing values.

### Table 11. Uni- and multivariable Cox proportional hazards regression of disease-specific survival in 135 patients with small bowel carcinoid tumour.

<table>
<thead>
<tr>
<th></th>
<th>Univariable analysis</th>
<th>Multivariable analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hazard ratio</td>
<td>( P )</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male ((n = 74))</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Female ((n = 61))</td>
<td>0.84 (0.49, 1.45)</td>
<td>0.534</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\leq 60 ) ((n = 44))</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>61–74 ((n = 48))</td>
<td>1.81 (0.96, 3.40)</td>
<td>0.065</td>
</tr>
<tr>
<td>(\geq 75 ) ((n = 43))</td>
<td>2.02 (0.98, 4.17)</td>
<td>0.056</td>
</tr>
<tr>
<td>Period</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1960–1982 ((n = 37))</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>1983–2005 ((n = 98))</td>
<td>1.05 (0.59, 1.90)</td>
<td>0.860</td>
</tr>
<tr>
<td>No. of primary tumours</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solitary ((n = 87))</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Multiple ((n = 35))</td>
<td>0.98 (0.50, 1.90)</td>
<td>0.946</td>
</tr>
<tr>
<td>Unknown ((n = 13))</td>
<td>6.98 (3.27, 14.88)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Size of primary tumour (mm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\leq 10 ) ((n = 29))</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>10–100 ((n = 83))</td>
<td>3.88 (1.36, 11.04)</td>
<td>0.011</td>
</tr>
<tr>
<td>Unknown ((n = 23))</td>
<td>14.43 (4.69, 44.41)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>T category (primary tumour)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1–T2 ((n = 19))</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>T3–T4 ((n = 101))</td>
<td>4.65 (1.12, 19.36)</td>
<td>0.034</td>
</tr>
<tr>
<td>TX ((n = 15))</td>
<td>16.47 (3.62, 74.81)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Localized tumour ((n = 25))</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Regional met ((n = 62))</td>
<td>6.01 (0.79, 45.48)</td>
<td>0.083</td>
</tr>
<tr>
<td>Distant met ((n = 48))</td>
<td>32.90 (4.51, 239.98)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Resection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R0 ((n = 68))</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>R1, R2 or RX ((n = 67))</td>
<td>6.45 (3.31, 12.55)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Values in parentheses are 95% confidence intervals.
RESULTS AND DISCUSSION

We found that complete resection of all tumours was associated with better survival, as previously demonstrated by Bilimoria et al.\textsuperscript{81}. We concluded that this pertained foremost to patients with mesenteric (but not distant) metastases, some of whom seemed cured by surgery. Similar findings were previously reported by Hellman et al.\textsuperscript{117}. Whether resections were complete or not could hypothetically be due to either the skill of the individual surgeon or the extent of regional disease (number; size and location of metastases; vascular encasement; overgrowth on adjacent organs). Only two patients with hepatic metastases were considered tumour free after R0 resection, both suffered recurrences but may still have gained from surgery as indicated by previous results\textsuperscript{190, 196}.

Regional or distant metastases at diagnosis were associated with worse disease-specific survival, although this did not reach statistical significance for regional disease. In previous multivariable analyses of prognostic factors for overall survival, Bergstuen et al. found similar results, whereas Bilimoria et al. found the hazard ratio to be even lower for patients with mesenteric metastases\textsuperscript{81, 120}.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{disease_specific_survival.png}
\caption{Disease-specific survival for 135 patients with small bowel carcinoids with localized tumours, tumours with regional metastases and tumours with distant metastases. $P < 0.001$ (logrank test)}
\end{figure}
RESULTS AND DISCUSSION

Figure 5. Disease-specific survival for 56 patients with small bowel carcinoid and regional metastases after complete resection (R0) and incomplete resection (R1-R2). $P = 0.005$ (logrank test)

Age was an independent prognostic factor for disease-specific survival, as previously found by Zar et al.\(^{237}\).

In multivariable analysis, patients diagnosed 1983–2005 had significantly better survival than those diagnosed 1960–1982. Zar et al. described this development between 1960 and 2000\(^{237}\), whereas Bilimoria et al. did not find any improved prognosis during the shorter time span 1985–2000\(^{81}\). The reasons for this possibly improved survival is difficult to discern; it coincides with the introduction of new treatment options on the one hand, and with the emergence of CT and other diagnostic techniques on the other hand. The latter may lead to detection of more tumours and at an earlier stage (lead time bias).

In conclusion, the population-based cohort studied in papers I and II had a higher incidence of small bowel carcinoid than previous studies, and the incidence increased during the study period. Disease-specific survival after 5 years was 75\% and after 10 years 63\%, independent prognostic factors were higher age at diagnosis, more advanced disease-stage at diagnosis and incomplete tumour resection.
RESULTS AND DISCUSSION

PAPER III

Histopathology

Apart from the similarities in their clinical appearance, the tumours showed close resemblance on most histopathological examinations. An insular growth pattern was predominating in primary tumours and metastases from all three family members. All tumour specimens were positive for argyrophilia using the Grimelius silver nitrate procedure, as well as immunoreactive to antibodies raised against CgA, synaptophysin and CD56 – all confirming a neuroendocrine origin12, 43, 173, 174. Further, all tumour specimens were positive for the Masson-Fontana technique for argentaffinity, as well as immunoreactive to antibodies raised against serotonin and substance P – all confirming EC cell origin43, 167, 173, 174. All specimens were negative for immunohistochemistry using antibodies against the peptide hormone ghrelin, which is secreted by neuroendocrine cells in the stomach and the pancreas275.

Two interesting differences were apparent between Case 1 on the one hand, and Cases 2 and 3 on the other. Ki-67 proliferation index was 1–2% (G1) in Case 1, 2–5% (G2) in Case 2 and 0–2% but with hot spots of 10% (G2) in Case 3. Furthermore, CART IR was present in some tumour cells of Cases 2 and 3, but absent in Case 1. Histological grade is an established prognostic factor in small bowel carcinoid, and expression of CART is proposed as a negative prognostic factor in paper IV. These findings fit well with the much less aggressive clinical course in Case 1.

Genetics and heredity

Foregut NETs are occasionally associated with mutations in the MEN1 gene even in the absence of a hereditary MEN1 syndrome45, 276. Small bowel carcinoid is not a part of any genetic syndrome such as MEN1 or MEN2, but genetic lesions in the MEN1 gene have been proposed in some sporadic cases46. We found no evidence of abnormalities of the MEN1 gene in the tumours from the three family members, although a common mutation could not be completely excluded. There are several other described genetic lesions that occur in small bowel carcinoid tumours45, and the three tumours are presently being further genetically examined.

There are a number of previous case reports that describe small bowel carcinoid in two first-degree relatives111–114. This could be indicative of a familiar predisposition, but it could also be the result of chance277. However, epidemiological studies show an
increased risk of developing GI NETs or small bowel carcinoid tumours in individuals with a parental history of the disease.

Our article was the first to describe metastasizing ileal carcinoid tumours in three consecutive generations, which is unlikely to occur by chance in view of the low incidence. We therefore consider this familial occurrence strongly suggestive of a hereditary disease form.

**Association with coeliac disease**

Interestingly, three close relatives of the carcinoid patients had coeliac disease. A markedly increased number of EC cells has previously been found in the intestinal wall of patients with coeliac disease. Furthermore, patients affected by both coeliac disease and small bowel carcinoid tumours have been described. The majority of coeliac patients are HLA DQ2-positive, and an ongoing study shows that HLA-DQ2 is overrepresented also in patients with midgut carcinoid, indicating a shared hereditary risk for the two conditions. None of the three Cases in this family had clinical signs of coeliac disease, and analyses of IgA antibodies against transglutaminase were negative in Cases 2 and 3. Case 3 was found to be HLA DQ2-positive, this was not examined in Cases 1 or 2.

**PAPER IV**

A first brief notice about CART in human NETs was made by Wierup and Sundler who observed CART in insulin-secreting EPTs. We have subsequently demonstrated CART-expression in tumour cells in several types of NETs, including small bowel carcinoid tumours. Bech et al. found raised plasma levels of CART in a similarly wide range of NET types. In paper IV we investigated whether CART expression was associated with tumour characteristics, symptoms and survival.

Based on the proportion of CART IR cells, we introduced a CART score with three tiers: *No CART*, *Low CART* and *High CART*. One-hundred and thirty-one specimens from the 97 patients were analyzed; 79 of them were from primary tumours at the time of diagnosis. The CART score of the primary tumour was used for analyses when available. We found a good correlation between the CART score in primary tumour and metastases when both were available (\( \tau \) coefficient 0.453 and \( P = 0.013 \)), therefore the CART score in metastases was used in 15 patients where no primary tumour specimens were available.
Some level of CART IR was detected in tumours from 81 of the 97 patients (84%). We tested whether CART expression was associated with the prognostic factors age, disease stage and histological grade and found an association between the presence of CART (CART 0/+ and grade \( P = 0.011 \)), but not between CART 0/+ and age or disease stage. Neither was there any significant correlation between CART score and age, stage, or grade. We further addressed whether CART expression correlated with clinical symptoms. Weight loss and hormonal symptoms, such as flushing and diarrhoea, were of particular interest since CART normally regulates hormone secretion and downregulates body weight. However, there were no tendencies for any associations between CART expression and any hormonal or other symptom.

A main finding of the study was that tumour expression of CART \( (P = 0.011) \) and increasing CART score \( (P = 0.033) \) were associated with worse disease-specific survival (Figure 6). Adjusting for age, disease stage and tumour grade in multivariable analysis, CART expression was still associated with worse survival (Low CART HR 5.47, 95% c.i. 0.71 to 42.46; and High CART HR 9.44, 95% c.i. 1.14 to 78.14) (Table 12). The effect of CART on cell viability was assessed \textit{in vitro} using an enteroendocrine cell line (GLUTag) \textsuperscript{27b}. Supporting our clinical data, we found that CART promoted tumour cell viability \textit{in vitro} \( (P < 0.01) \). This is in agreement with
RESULTS AND DISCUSSION

our previous observations that CART is crucial for regulation of pancreatic islet β-cell viability, both by reducing apoptosis and by increasing proliferation\textsuperscript{265, 266}. Interesting in this context is that Bech\textit{et al.} found higher levels of circulating CART in patients with progressive NET disease\textsuperscript{271}.

These findings suggest CART as a possible prognostic biomarker, although our results have to be repeated and validated for clinical use\textsuperscript{279, 280}. CART also emerges as a potential anti-tumour treatment target.

<table>
<thead>
<tr>
<th>Table 12. Uni- and multivariable Cox proportional hazards regression of disease-specific survival in 97 patients with small bowel carcinoid</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Univariable analysis</strong></td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td><strong>Hazard ratio</strong></td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
</tr>
<tr>
<td>≤ 60 (n = 29)</td>
</tr>
<tr>
<td>61–75 (n = 36)</td>
</tr>
<tr>
<td>&gt; 75 (n = 32)</td>
</tr>
<tr>
<td><strong>Stage</strong></td>
</tr>
<tr>
<td>Localized (n = 18)</td>
</tr>
<tr>
<td>Regional met (n = 52)</td>
</tr>
<tr>
<td>Distant met (n = 27)</td>
</tr>
<tr>
<td><strong>Grade</strong></td>
</tr>
<tr>
<td>G1 (n = 84)</td>
</tr>
<tr>
<td>G2 (n = 13)</td>
</tr>
<tr>
<td><strong>CART</strong></td>
</tr>
<tr>
<td>0 (n = 16)</td>
</tr>
<tr>
<td>+ (n = 81)</td>
</tr>
<tr>
<td>No CART (n = 16)</td>
</tr>
<tr>
<td>Low CART (n = 61)</td>
</tr>
<tr>
<td>High CART (n = 20)</td>
</tr>
</tbody>
</table>

Values in parentheses are 95% confidence intervals.
CONCLUSIONS

• A higher incidence of small bowel carcinoid than previously described was found. The incidence has increased during the study period.

• Symptoms were most often uncharacteristic. Many small bowel carcinoid tumours present as surgical emergencies without preceding symptoms.

• The majority of small bowel carcinoid tumours have metastasized to the mesentery or the liver at diagnosis.

• Disease-specific survival after 5 years was 75.0% and after 10 years 63.5%. Median disease-specific survival was 12.3 years.

• Independent prognostic factors for worse disease-specific survival were higher age at diagnosis, more advanced disease stage at diagnosis and incomplete tumour resection. Completeness of resection pertained in particular to tumours with regional metastases.

• The occurrence of metastasized small bowel carcinoid in three consecutive family members was described for the first time. This was highly indicative of a hereditary form of small bowel carcinoid.

• Presence of CART IR tumour cells in small bowel carcinoid tumours was associated with histological grade, but not with stage or patient age.

• CART expression in small bowel carcinoid tumours was not associated with clinical symptoms.

• Increasing levels of CART IR in small bowel carcinoid tumour cells was associated with worse disease-specific survival, suggesting that CART could be used as a prognostic biomarker and that CART is a potential anti-tumour treatment target.
Tunntarmscarcinoider uppstår från så de kallade enterokromaffina cellerna (EC-cellerna) i tarmslemhinnan. EC-cellerna är en av de omkring femton olika typerna av endokrina (hormonproducerande) celltyper som finns i matsmältningssystemet. EC-cellerna producerar en rad ämnen som på givna signaler utsöndras i syfte att reglera funktioner både lokalt i tarmen och i övriga kroppen. Störst betydelse av dessa ämnen har serotonin som bland annat bidrar till ökad utsöndring av vätska från tarmslemhinnan och till ökade tarmrörelser. Liksom vid andra så kallade neuroendokrina tumörer (NETs) kan förhöjd utsöndring av hormoner leda till symptom hos patienter med tunntarmscarcinoid. Det för tunntarmscarcinoider karaktäristiska carcinoidsyndromet uppstår i regel först när dottertumörer uppträder i levern. Carcinoidsyndromet innebär att patienten lider av diarréer och episodiska anfall av flusher (kraftigt uppblossande hudrödning och hjärtklappning) och astmaliknande andningsbesvär. På längre sikt leder de förhöjda hormonnivåerna till utveckling av fibros (överdriven bindvävsbyggning) dels i tarmkäxet och dels i hjärtat. Fibrosutvecklingen i tarmkäxet kan vara mycket kraftig och leder typiskt till att patienten får periodiska knipsmärtor och inte sällan tarmvred. Vad gäller hjärtat drabbas i första hand den högra sidans klaffar vilket leder till högersidig hjärtsvikt.

Tumörer i tunntarmen är anmärkningsvärt ovanliga – trots att tunntarmen utgör 90% av ytan i magtarmkanalen så uppstår där mindre än 3% av all cancer i matsmältningssystemet. Insjuknandet i carcinoid har ökat betydligt under de senaste decennierna och carcinoider är numera den vanligaste tumörtypen i tunntarmen. Tidigare undersökningar har antytt att vissa tunntarmscarcinoider kan ha en ärftlig bakgrund.

Eftersom moder tumörerna ofta är små, och sällan i sig själva ger symptom, finns oftast dottertumörer när diagnosen ställs. Det enda sättet att bli botad från tunntarmscarcinoid är genom kirurgi, men det är inte alltid möjligt vid spridd sjukdom. Operationer av tunntarmscarcinoider innebär särskilda utmaningar, framför allt försvårar en botande operation av den kraftiga bindvävsomvandlingen i tarmkäxet, vilken även kan inbegripa tarmens kärlförsörjning. I de fall man inte lyckas få bort all tumörvävnad, eller om tumören återkommer, finns en lång rad av
SVENSK SAMMANFATTNING


Den långsamma tillväxten medför också att överlevnaden vid spridd tunntarmscarcinoid är förhållandevis lång jämfört med vanlig tarmcancer – även med levermetastaser kan överlevnaden vara många år. Man vet att spridningsgraden och tumörcellernas delningshastighet är av betydelse för sjukdomens förlopp. Det finns också en rad andra kända faktorer som påverkar utgången vid tunntarmscarcinoid, men resultaten från olika studier är motsägelsefulla. Därför pågår forskning i syfte att hitta bättre metoder att kunna förutsöja sjukdomens förlopp och också avgöra vilka behandlingar som kan vara lämpliga i det enskilda fallet.

DELARBETE I OCH II


Vi fann 145 fall av tunntarmscarcinoid under perioden, sedan obduktionsfall exkluderats. Nyinsjuknandet var 1,12 per 100 000 personer och år, vilket är en högre siffra än tidigare studier har visat. Nyinsjuknandet ökade efter hand under den studerade tidsperioden. Sjukdomen var lika vanlig hos män som hos kvinnor. Genomsnittsaldern vid diagnos var 69 år vilket är högre än vad tidigare studier har visat, särskilt jämfört med studier från högspecialiserade kliniker. Vid diagnostillfället hade 81% dottertumörer i tarmkäxet, levern eller andra organ. Trots det var carcinoidsyndromet inte så vanligt: 13% hade flusher, endast ett par patienter astma.
och ingen typisk hjärtsvikt. Vanligast var buksmärtor (50%), tarmvred (35%), diaré (26%) och kraftig viktnedgång (24%). Dessa symptom är vanliga vid alla typer av tarmcancer, men kan också påverkas av överproduktion av serotonin.

Genom kännedom om dödsorsaken hos avlidna patienter kunde vi räkna ut den sjukdoms-specifika överlevnaden, det vill säga utan hänsyn till dödsfall av andra orsaker. För hela gruppen var den sjukdoms-specifika överlevnaden i genomsnitt 12,3 år. Ett annat sätt att beskriva sjukdomens betydelse är genom relativ överlevnad, det vill säga hur stor andel av den drabbade gruppen som lever efter en viss tid jämfört med den förväntade överlevnaden i motsvarande befolkning. Den relativna överlevnaden vid tunntarmscarcinoid var efter 5 år 73,9% och efter 10 år 60,5%. Faktorer av betydelse för den sjukdoms-specifika överlevnaden var ålder, tumörstadium och huruvida operationen varit till synes botande. Vid carcinoid-sjukdom begränsad till tarmen blir tumören i regel helt borttagen, detta blir sällan fallet när det finns dottertumörer i levern eller andra organ. Vid förekomst av dottertumörer enbart i tarmkäxet kunde all tumörvävnad avlägsnas hos 71% av patienterna, och mer än hälften av dessa förblev tumörfria under resten av observationstiden.

DELARBETE III

Det tredje delarbetet beskriver för första gången en familj, där tre medlemmar i rakt nedstigande led drabbats av spridd tunntarmscarcinoid. Undersökningar av tumörerna visade att de i hög grad liknade varandra vad gäller tumörens mikroskopiska utseende och tumörcellernas innehåll av olika ämnen. Fynden talar mycket starkt för att det finns en ärftlig variant av tunntarmscarcinoid.

DELARBETE IV

Vi visade nyligen att peptidhormonet CART finns i flera olika typer av neuroendokrina tumörer, bland dem tunntarmscarcinoider. I delarbete IV gick vi vidare och undersökte alla tillgängliga tumöreeparat från delarbetena I och II med avseende på CART-förekomst. Intressant nog visade det sig att ju mer CART som fanns i tumören, desto sämre var den sjukdoms-specifika överlevnaden för patienten. Skillnaden kvarstod även sedan man i analysen justerat för ålder, spridningsgrad och tumörcellernas tillväxthastighet. Vi fann också att CART ökade överlevnaden av odlade tumörceller. Fynden behöver särskiljas genom ytterligare studier men talar
SVENSK SAMMANFATTNING

för att CART kan användas som markör för förväntat sjukdomsförlopp vid tunntarmscarcinoid. Mer hypotetiskt skulle CART och dess signalvägar kunna vara en möjlig angreppspunkt för framtida tumörbehandlingar.
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REFERENCES

1. Eroschenko VP. *di Fiore's atlas of histology with functional correlations (8th ed.)* Williams & Wilkins: Media, PA, USA, 1996.


REFERENCES


REFERENCES

REFERENCES


REFERENCES


78. Web page of the Surveillance Epidemiology and End Results (SEER) Program of the National Cancer Institute: http://seer.cancer.gov/about/.


REFERENCES


REFERENCES


REFERENCES


133. Öberg K, Astrup L, Eriksson B, Falkmer SE, Falkmer UG, Gustafsen J et al. Guidelines for the management of gastroenteropancreatic neuroendocrine tumours (including
REFERENCES


REFERENCES


REFERENCES


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