Prognosis, follow-up and quality of life in patients with neuroendocrine tumours

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Contents

Chapter 1

Introduction and aims of the thesis

Introduction

Neuroendocrine tumours (NET) are a heterogeneous group of rare tumours, arising from cells of the neuroendocrine or endocrine system. The cells that belong to this (neuro)endocrine system are dispersed throughout the entire body and capable of secreting several (neuro)endocrine factors; hormones, bioactive amines and polypeptides.

The neuroendocrine cells can be divided into cell types that form localised glands (adenohypophysis, the parathyroids, the paraganglia and the adrenal medulla) and cells that are disseminated throughout the body in other organs: the diffuse neuroendocrine system.[1] Tumours from the adenohypophysis are called adenomas with an indication of the main cell type (for instance corticotroph adenoma), tumours from the parathyroid are mostly adenomas, carcinomas from both are rare. Endocrine tumours originating from chromaffin cells of the adrenal medulla are called phaeochromocytoma. Tumours of the sympathetic and parasympathetic paraganglia are classified as paragangliomas.[2]

The term carcinoid was introduced by Oberndorfer in 1907, to describe a carcinoma-like tumour.[3] Decades later in 1963 Williams and Sandler classified the carcinoid tumours into foregut, midgut and hindgut tumours according to their embryological origin.[4] Foregut endocrine cells give rise to tumours in the respiratory tract, the stomach, the first part of the duodenum and the pancreas. Midgut carcinoid tumours are derived from endocrine cells in the second part of the duodenum, jejunum, ileum and proximal part of the colon. Hindgut carcinoid tumours originate from cell the remaining part of the colon, and rectum.

Recently, in 2000, a new a universal classification from the World Health Organisation (WHO) became available for NETs of the gastrointestinal tract. All gastroenteropancreatic (GEP) NETs are now categorised based on histological features; 1) well differentiated (neuro)endocrine tumours of probable benign behaviour, 2) well differentiated (neuro)endocrine tumours of uncertain behaviour, 3) well differentiated (neuro)endocrine carcinomas and 4) poorly differentiated (neuro)endocrine carcinomas.[5]

The tumour that was originally described by Oberndorfer, the midgut carcinoid tumour is, according to the WHO classification a well-differentiated neuroendocrine carcinoma of the small bowel or proximal colon.

In this thesis the term midgut carcinoid tumour is used to describe this well differentiated NET of the gastrointestinal tract.

Midgut carcinoid tumours

As explained above midgut carcinoid tumours are derived from the enterochromaffin cells in the duodenum, jejunum, ileum and proximal part of the colon.

The incidence rate of midgut carcinoid tumours is about 0.75 per 100.000 per population/year in the United States, whereas the Dutch incidence rate is about 1.95 per 100,000 per population/year for all carcinoid tumours.[6,7]

The enterochromaffin cells are capable of synthesising and secreting serotonin, a biogenic amine. Serotonin is the most important factor secreted by midgut carcinoid tumours and is synthesised from the amino acid tryptophan through 5-hydroxytryptophan (5-HTP) (Figure 1).[8]

Figure 1. Cellular synthesis of serotonin through tryptophan
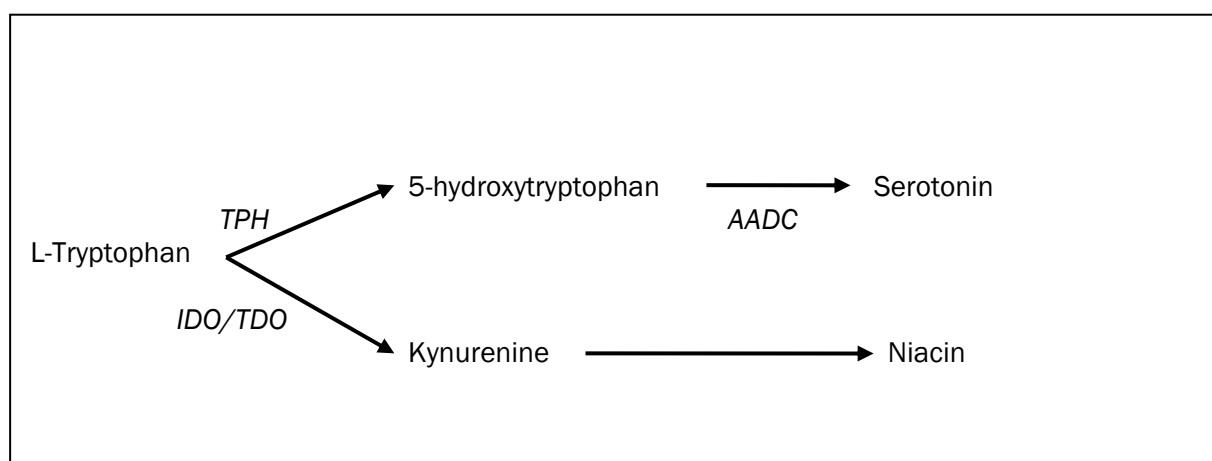
TPH: tryptophan hydroxylase, AADC: aromatic-L-amino acid decarboxylase, IDO: indolamine dioxygenase, TDO: tryptophan dioxygenase.

Next to serotonin, midgut carcinoid tumours are capable of secreting other biogenic amines such as catecholamines and several other factors such as kallikrein, tachykinins, and chromogranin A (CgA).[9-11] When large amounts of serotonin and others factors enter the systemic circulation, usually in the presence of liver metastases, the carcinoid syndrome can develop.

The carcinoid syndrome consists of several symptoms with flushes, diarrhoea and carcinoid heart disease being the most important ones. Identifying the overproduction of serotonin and its major metabolite, 5-hydroxyindolacetic acid (5-HIAA) in the urine assists in the diagnosis and follow-up of midgut carcinoid tumours.[12,13]

Localising the tumour and its metastases is accomplished by ultrasonography, computed tomography (CT), magnetic resonance imaging (MRI), and with nuclear scans. ^{111}In-octreotide scintigraphy (SRS) visualises somatostatin receptors on the cell surface.[14] Metaiodobenzylguanidine (MIBG) is an analogue of norepinephrine taken up by neuroendocrine cells. MIBG can be labelled with [123]iodine(I) or ^{131}I for visualisation.[15] A recent publication shows that 6-[^{18}F]-fluoro-L-dihydroxyphenylalanine (^{18}F-DOPA) positron emission tomography (PET) is the best nuclear scan technique to detect carcinoid tumour lesions by visualising cellular metabolism.[16]

Next to imaging studies to identify tumour localisation, echocardiography is advised for the detection of carcinoid heart disease.[17,18]

The treatment of patients with extensive metastatic disease is, if surgery is no longer possible, palliative and aimed at reducing tumour size, reducing secreted factors by the tumour and improving the quality of life (QoL).[19-22] Somatostatin analogues and interferon (IFN) alpha are both registered agents for this indication and capable to reduce symptoms and lower the urinary 5-HIAA concentrations. Both somatostatin analogues and IFN-alpha have limited effect on tumour growth.[23] Other treatment modalities include radionuclide therapy with radiolabelled somatostatin analogues, radiofrequent ablation and (chemo-) embolisation of liver metastases.[24-27]

Despite the fact that most patients cannot be cured, survival is relatively long. The 5 year survival rate of patients with metastatic carcinoid tumours is about 60% but survival varies greatly between patients and it is extremely difficult to predict the prognosis in an individual patient.[6]

Phaeochromocytomas

Phaeochromocytomas originate from the chromaffin cells in the medulla or paraganglia and secrete biogenic amines: epinephrine, norepinephrine and dopamine. The incidence is about 2.06 per 1,000,000 per population/year.[28] They can occur isolated and in patients with familial syndromes such as multiple endocrine neoplasia (MEN) type 2a and type 2b, von Hippel Lindau disease and in families with succinate dehydrogenase (SDH) mutations. Mutations in one of these genes responsible for the familial syndromes have been found in up to 24% of apparent sporadic cases.[29,30] Symptoms occur when catecholamines are released into the circulation leading to hypertension, palpitations and sweating. Phaeochromocytomas are benign in approximately 90% of the cases, however when left untreated they are fatal because of cardiovascular complications due to

massive release of catecholamines. The diagnosis of a phaeochromocytoma is made by the identification of the metabolites of epinephrine, norepinephrine or dopamine in plasma or in urine.[31] Once the diagnosis is made, localisation is done by CT or MRI and functional scanning with the use of ^{123}I-MIBG scintigraphy. Therapy of a benign phaeochromocytoma consists of resection of the tumour preceded by a preoperatively blockade of the alpha and beta-receptors.[32] After resection lifelong follow-up is needed for the rest of the life since tumour recurrence can occur even after 10 to 20 years.

This thesis

The aim of this thesis is to evaluate the course and follow-up of patients with NETs focussing on the midgut carcinoid tumours and phaeochromocytomas.

In **chapter 2** we present an overview of the current literature concerning symptoms of the carcinoid syndrome, with the emphasis on cardiovascular symptoms.

Known unfavourable factors for survival are age, high urinary 5-HIAA concentrations at first visit, high plasma CgA levels, the presence of liver or lymph node metastases, the presence of carcinoid heart disease, the tumour size, and the histological grade of differentiation. In only one study prognostic factors during follow-up have been identified.[33] Turner and colleagues observed that plasma neurokinin A (NKA) levels during follow-up were related to outcome. For other factors, data were available from only a small number of patients. In **chapter 3.1** we focus on survival and prognostic factors, using a database of midgut carcinoid patients, selecting 76 patients referred between 1992 and 2003. It would be helpful to have a non-invasive cheap marker that indicates prognosis during follow-up in addition to radiological tumour measurements because these are difficult and radiological progression in these patients is generally slow. Especially since numerous targeted drugs are currently under investigation, other prognostic factors may be useful to guide decisions in treatment. Therefore the aim of this study was to determine predictive factors of survival in patients with metastatic midgut carcinoid tumours, with the emphasis on the urinary 5-HIAA concentration. We performed analyses to identify prognostic factors at referral including age, gender, the urinary 5-HIAA concentrations, the presence of liver metastases, the resection of the primary tumour at referral, and liver enzymes at referral. To study the prognostic value of the urinary 5-HIAA concentrations during follow-up, we also performed a multivariate analysis with the urinary 5-HIAA level as a time-dependent covariable.

Chapter 3.2 is our response to a study of Møller and colleagues in which we describe the course of the urinary 5-HIAA concentration during follow-up of patients with the carcinoid syndrome.[34]

In **chapter 4** we study the effect of genetic variations in the metabolism of serotonin on symptoms and survival. Serotonin is taken up by the serotonin transporter (SERT, or 5-HTT) and metabolised to 5-HIAA by monoamine oxidase A (MAO-A). A functional polymorphism in the promoter region of the SERT (*5-HTTLPR*), with a short (S) and a long

(L) allele exists. A functional polymorphic region (upstream variable number of tandem repeats *(uVNTR)*) is also known for the *MAO-A* gene. Given the prominent role of serotonin, we hypothesized that these polymorphisms affect the presence of symptoms of the carcinoid syndrome and survival. A total of 105 patients with metastatic midgut carcinoid tumours were genotyped for *5-HTTLPR* and *uVNTR-MAO-A*. Differences between genotypic groups were tested and correlated with the presence of flushes and diarrhoea. Next we studied the effect of the different genotypes on death within 5 to 10 years after follow-up adjusted for the urinary 5-HIAA concentration at referral, age at referral and the presence of liver metastases.

The new targeted drugs for NETs are mainly directed at angiogenesis, as these tumours are highly vascularised and vascular endothelial growth factors (VEGF) and its receptors are highly present. In **chapter 5** we describe the results of a feasibility study with IFN-alpha (2.5 million U/day subcutaneously), intravenous 5-fluorouracil (5-FU) 750 mg/m^2 and oral leucovorin 180 mg/day as a 2 weekly cycle in patients with metastatic NETs. Because of the suggested synergistic and antiangiogenic effects between 5-FU and IFN-alpha, we determined serial serum VEGF levels to investigate whether serum VEGF levels decrease after treatment.

Next to relieving symptoms and prolonging life, treatment of patients with midgut carcinoid tumours is aimed at but also at improving the QoL. Sexual function is a part of the QoL, but rarely studied. In **chapter 6** we focus on this issue. There are several potential underlying mechanisms that could make patients with midgut carcinoid tumours prone to sexual dysfunction. In the brain serotonin is involved in sexual behaviour. It has been suggested that patients with midgut carcinoid tumours have a depleted serotonin pool in the brain. Furthermore serotonin and other factors have vasoactive properties that could lead to erectile dysfunction in male patients Next to these specific causes, patients can experience pain, fatigue and use medication that may affect the ability and desire to have sexual activity. A total of 43 patients with metastatic midgut carcinoid tumours filled in a validated questionnaire on sexual function. The outcome on different subscales was compared to a population based Dutch reference sample. To study possible underlying mechanisms and create hypotheses for future interventions, we also investigated whether sexual dysfunction was correlated with tumour activity and/or changes in gonadal hormones.

In **chapter 7** we study the role of ^{123}I-MIBG scintigraphy in the work-up of patients with phaeochromocytomas. Recommendations on the use of ^{123}I-MIBG scintigraphy in

localising phaeochromocytomas vary. We determined the accuracy of the ^{123}I-MIBG scintigraphy by evaluating the ^{123}I-MIBG scintigraphy of 30 patients treated in our hospital. We also performed a meta-analysis of previous studies including more than 5 patients with ^{123}I-MIBG scans.

Finally, in **chapter 8** our observations are summarised and future perspectives are addressed.

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Chapter 2

Complications of midgut carcinoid tumours and carcinoid syndrome

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Abstract

The carcinoid syndrome, associated with carcinoid tumours of the midgut, consists of symptoms such as diarrhoea, flushing, wheezing and cardiovascular symptoms. This review focuses on these symptoms and discusses therapeutic options. The symptoms are caused by the secretion of biogenic amines, polypeptides and other factors of which serotonin is the most prominent. However, diarrhoea is also due to factors such as malabsorption. Besides antitumour therapy, more specific interventions such as serotonin receptor blockers can be useful. The carcinoid heart disease involves the tricuspid and pulmonary valve. In the pathogenesis, serotonin plays a central role. The therapeutic approach is mostly symptomatic. Other cardiovascular complications include bowel ischemia and hypertension. Pellagra and psychiatric symptoms are due to a depletion of tryptophan, which is consumed by the carcinoid tumour for serotonin synthesis. Finally, the follow-up and clinical practice of patients with carcinoid tumours are discussed.

Introduction

Carcinoid tumours are rare slowly growing neuroendocrine tumours, with an incidence of 2/100,000 population per year.[1,2] According to the classification of Williams and Sandler, they are divided into tumours of the fore-, mid- and hindgut.[3] Tumours of the midgut (the classic carcinoid tumours) originate in the enterochromaffin cells of the gut and have the ability to produce various factors.

The carcinoid syndrome, described by Thorson and colleagues in 1954, is associated with carcinoid tumours of the midgut.[4] This syndrome consists of a constellation of symptoms due to the secretion of large amounts of biogenic amines (serotonin, catecholamines and histamine), polypeptides and other factors, of which serotonin is the most prominent factor. Carcinoid tumours of the foregut produce less serotonin compared to midgut tumours, whereas tumours of the hindgut are seldom biochemical active.[5-7] Therefore, the carcinoid syndrome is rarely associated with carcinoid tumours of the fore- and hindgut but typically associated with the carcinoid tumours of the midgut, usually in the presence of liver metastases. Normally serotonin, produced by the enterochromaffin cell or carcinoid tumour cell is transported to the liver through the portal vein and metabolised to 5-hydroxyindoleacetic acid (5-HIAA) by the liver cell (Figure 1).[8] However, when serotonin enters the systemic circulation, as is the case in patients with liver metastases, but also in female patients with metastases in the ovaries, the typical symptoms of the carcinoid syndrome, such as diarrhoea, flushing, and carcinoid heart disease, can occur.

The breakdown product of serotonin, 5-HIAA is secreted into the urine. The urinary 5-HIAA concentration is considered to be a reflection of tumour burden and therefore widely used as a marker for follow-up. Besides symptoms of the carcinoid syndrome, symptoms due to the tumour itself or the metastases can occur. Symptoms and complications of the carcinoid syndrome are becoming more relevant since survival seems to be improving. Five-year survival rates are ranging from 21 to 50% for carcinoid tumours of the small bowel with distant metastases. After the introduction of the somatostatin analogues, survival seemed to improve.[1,2,9]

We will present an overview of symptoms of the carcinoid syndrome, focusing on the pathogenesis and therapeutic options in patients with midgut carcinoid tumours and metastases.

Tryptophan

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                    Tryptophan hydroxylase

      5-hydroxytryptophan              Carcinoid cell

                    Aromatic aminoacid
                    decarboxylase
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Blood

Serotonin → SERT → Platelet

Serotonin transporter (SERT)

Monoamine oxidase-A

5-hydroxyindoleacetaldehyde

Aldehyde dehydrogenase

5-hydroxyindolacetic acid
(5-HIAA)

5-HIAA
(serotonin)

Urine

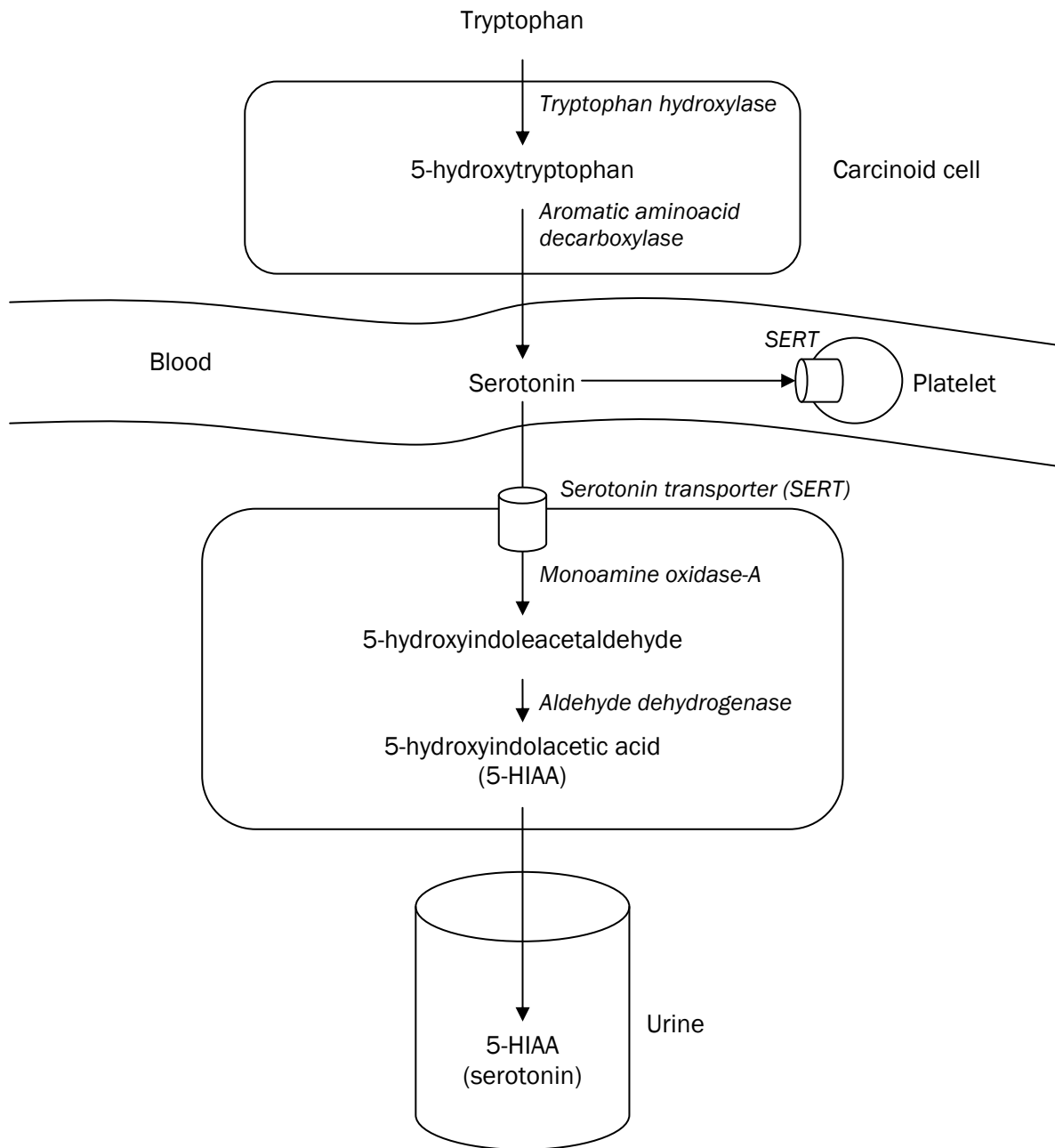**Figure 1.** Synthesis of serotonin from tryptophan and enzymatic steps and major route of metabolism to 5-HIAA (5-hydroxyindolacetic acid)

Diarrhoea

Diarrhoea occurs in about 80% of patients.[10,11] Several underlying mechanisms have been identified, although data on the relative contribution are lacking. Malabsorption of bile acids in the ileum after bowel resection for removal of the primary tumour,

malabsorption of fat and bacterial overgrowth can all contribute to diarrhoea. Serotonin is the most important factor secreted by the tumour and causing incapacitating diarrhoea. Under normal circumstances, serotonin stimulates motility and secretion of the bowel through serotonin receptors.[12] Other secreted factors such as histamine, kallikrein, prostaglandin, substance P and motilin might also play a role in the pathogenesis of diarrhoea.[13,14] Van der Ohe and colleagues studied 16 patients with carcinoid tumours and 16 healthy controls and compared small bowel and colonic transit time.[14] Transit time in the small bowel and colon was increased in the carcinoid patients.

Diarrhoea can be a major burden on normal social life of patients. Therapy is aimed at lowering the secretion of serotonin and other factors by reducing tumour load by surgical intervention or biotherapy. Aggressive surgical treatment of the primary tumour, lymph node metastases and even liver metastases can ameliorate symptoms and improve survival.[15,16] In inoperable patients, biotherapy with somatostatin analogues and interferon (IFN)-alpha are the treatment of choice.[17,18] Blockade of serotonin receptors can also ameliorate diarrhoea.[19,20] Chemotherapy is rarely indicated.

Other medical interventions are aimed at reducing the colonic bile acids (bile acid sequestrants), improving fat absorption (pancreatic enzyme suppletion) and inhibiting peristalsis and decreasing transition time (loperamide).

Flushing

The prevalence of flushing is reported to be about 80%.[10,11] Flushing has been linked to several factors; tachykinins, serotonin, catecholamines, and histamine.[21-23] The exact pathogenesis remains unclear. Flushing usually is most prominent in the malar area of the face, the forehead and the V area of the neck. Certain food and alcohol can provoke it.[24] Therapy consists of lowering the secretion of the various factors by surgical reduction of tumour load, IFN-alpha and/or somatostatin analogues. Some case reports describe the beneficial effect of blocking the histamine 1 and 2 receptor.[25,26]

Wheezing

Although wheezing is often mentioned as a hallmark of the carcinoid syndrome, it is only present in about 10% of the patients.[11] The underlying mechanism is not clear. It often accompanies flushing. It should not be mistaken for asthma since administration of β2 sympathomimetics can provoke a release of biogenic amines.

Carcinoid heart disease and cardiovascular complications

Carcinoid heart disease develops in about 40% of patients.[27,28] It is characterised by so-called 'carcinoid plaques' on the right side with involvement of the tricuspid valve, pulmonary valve and endocardium. The most common manifestations are insufficiency of the tricuspid valve and to a lesser extent stenosis and pulmonary valve stenosis and insufficiency, resulting in right-sided heart failure.[29] The underlying mechanism is considered to be the stimulation of transforming growth factors (TGFβ) by serotonin resulting in an increased collagen synthesis that in turn leads to carcinoid plaques.[30-32] Several studies show that patients with carcinoid heart disease have higher urinary 5-HIAA excretions, compared to patients without carcinoid heart disease, supporting the theory that serotonin plays a major role in the carcinoid heart disease development.[27,29,33,34] Although involvement of the left side of the heart is uncommon, because the pulmonary endothelium, like the liver, can metabolise serotonin to 5-HIAA, it does exist.[35] Prognosis for patients with carcinoid heart disease is worse compared to those without carcinoid heart disease.[29,36] Screening for carcinoid heart disease by performing a transthoracic echocardiography is recommended especially for patients with a urinary 5-HIAA excretion above 100 mg/24 hours.[33] Therapy of carcinoid heart disease is symptomatic with diuretics. It is not clear whether somatostatin analogues inhibit or postpone the development of carcinoid heart disease.[34,37] Surgical valve replacement leads to a decrease of symptoms but has a high perioperative mortality.[35,38]

Other cardiovascular manifestations

Although overproduction of serotonin is the hallmark of the carcinoid syndrome and carcinoid heart disease, other biogenic amines are also important. Catecholamines are produced and secreted in about 40% of patients.[39,40] Heart rate variability, a sign of autonomic dysfunction, is impaired in an early stage of carcinoid heart disease and related to an increased urinary secretion of catecholamines and its metabolites.[41-43] Catecholamines can also lead to hypertension; however, data on the prevalence of hypertension are sparse.

Elastic vascular sclerosis is a condition of the mesenteric blood vessel, consisting of a proliferation of the adventitial and intimal elastic fibers. The exact mechanism has to be elucidated, but it has been linked to serotonin, histamine and bradykinin.[44] It can lead to ischemic intestinal changes in about 33% of the patients.[44,45] These ischemic intestinal changes however can also result from entrapment of vessels in the fibrotic changes of

the mesentery.[46] Another cardiovascular manifestation that has been described in some case reports is peripheral vasospasm.[47]

Fibrotic complications

Although not completely elucidated, serotonin is also held responsible for the fibrotic changes elsewhere throughout the body besides the heart. Extensive fibrotic changes may occur in the mesentery leading to obstruction and abdominal pain.[46] Several patients with hydronephrosis due to retroperitoneal fibrosis have been reported.[48,49]

Pellagra

Pellagra is characterised by the triad dermatitis, diarrhoea and dementia. It is a result of a deficiency of niacin. Under normal circumstances, niacin can be obtained directly from the diet or it can be synthesised from tryptophan.[50] Carcinoid tumours can consume about 60% of the body tryptophan as it is a precursor of serotonin. This leads to an impaired endogen niacin production. The prevalence of pellagra in patients with carcinoid tumours is about 5%.[10] Treatment consists of the administration of niacin.

Neuropsychological symptoms

A depletion of body tryptophan can also result in an intracerebral deficiency of serotonin. The brain has its own ability to synthesise serotonin from tryptophan, since serotonin, in contrast to tryptophan, is not able to cross the blood-brain barrier. Intracerebral produced serotonin is involved in the regulation of sleep, sexuality, mood and eating. Neuropsychological investigation in patients with carcinoid syndrome has indicated that patients have an impaired sustained visual attention and an impulse control disorder.[51,52] Treating depression in patients with carcinoid syndrome can be a challenge for the treating physician, since the commonly used selective serotonin reuptake inhibitors might interfere with peripheral serotonin metabolism and therefore worsen symptoms of the carcinoid syndrome.

Clinical practice and follow-up of patients with carcinoid syndrome

The above-mentioned signs and symptoms are, like one would expect, variable in expression. It seems difficult to predict which symptoms will develop in an individual patient. However, despite the slow-growing nature of this malignancy and the relatively long survival of patients, a carcinoid tumour has a great impact on a patient's life and the

quality of life can be affected.[53] It is not clear whether somatostatin analogues or IFN-alpha improve survival, although increasing evidence for this hypothesis exists.[2,9] Data about the optimal initial work-up and follow-up of a patient are lacking. After the diagnosis has been made, further diagnostics usually compromise an ^{111}In-octreotide scintigraphy (somatostatin receptor scintigraphy: SRS) for the localisation of metastases and primary tumour. A computed tomography (CT) scan of the abdomen is often performed to evaluate liver metastases. Both diagnostics are helpful in the consideration of surgical interventions. A promising new technique is visualisation of the tumour and its metastases by 6-[^{18}F]-fluoro-L-dihydroxyphenylalanine (^{18}F-DOPA) positron emission tomography (PET). Biochemical evaluation includes chromogranin A and urinary excretion of 5-HIAA, markers for the effect of therapy and tumour progression.[54] By regular measurement of these markers, e.g. every 3 months, one can evaluate the rate of progression of the tumour in individuals. Serotonin in platelets is the most sensitive indole marker in the primary biochemical diagnosis but not useful in follow-up.[55] It is not clear whether measuring tryptophan every 3 months can predict symptoms of pellagra. Questions remain about performing SRS or CT scans regularly. It does not seem necessary for predicting symptoms of the carcinoid syndrome since an increment in the urinary excretion of 5-HIAA often seems to precede these symptoms. Evaluating carcinoid heart disease is recommended initially and for patients who are at risk, i.e. patients with an urinary 5-HIAA excretion above 100 mg/24 hours.[33] Regular evaluation is important since one can anticipate right-sided heart failure and perhaps identify patients who will benefit from surgical valve replacement.[35,38] It seems justified to perform other diagnostics only when an individual patient complains of certain mentioned symptoms, e.g. examination of 24-hour stools for fat malabsorption.

Conclusion

The carcinoid syndrome consists of a spectrum of symptoms. Treatment of these symptoms should not only consist of antitumour therapy but should also be based on the underlying pathogenesis, i.e. blocking a specific receptor. The optimal follow-up of this group of patients is not yet totally clear. Large databases will likely give more insight into this subject in the near future.

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Chapter 3.1

Persistent low urinary excretion of 5-HIAA is a marker for favourable survival during follow-up in patients with metastatic midgut carcinoid tumours

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Abstract

Introduction

Survival of patients with metastatic midgut carcinoid tumours varies. We investigated which factors predict survival at referral and during follow-up, with the emphasis on urinary 5-hydroxyindolacetic acid (5-HIAA) levels.

Patients and methods

Between 1992 and 2003, 76 patients were studied; urine was prospectively collected over a 24 hours period every 3 months in order to measure urinary 5-HIAA levels. Uni- and multivariate analyses were performed.

Results

Median follow-up was 55 months with a median survival of 54 months. Prognostic factors for poor survival were high age, high gamma-glutamyltransferase levels and greatly increased urinary 5-HIAA levels (> 20 mmol/mol creatinine) The Hazard Ratio (HR) of a greatly increased urinary 5-HIAA level was 3.33 (95% confidence interval (CI) 1.66-6.66, P=0.001).

In a multivariate survival analysis with the urinary 5-HIAA level as time dependent variable the HR for the 5-HIAA level was 1.007 (95% CI 1.004-1.010, P=0.000).

Conclusion

In conclusion, patients with persistent low urinary 5-HIAA levels have favourable outcome.

Introduction

Midgut carcinoid tumours are rare, slowly growing neuroendocrine tumours (NET) originating in the small intestine and proximal colon. They are derived from enterochromaffin cells and capable of secreting serotonin.[1] The World Health Organisation nowadays classifies these tumours as well differentiated neuroendocrine carcinomas of the small bowel.[2] However, the term midgut carcinoid tumour is still being used for those tumours originating from the enterochromaffin cells, with serotonin secretion and associated with the carcinoid syndrome. In patients with metastatic midgut carcinoid tumours an increased urinary 5-hydroxyindolacetic acid (5-HIAA) level, the metabolite of serotonin, is found.[3] The urinary 5-HIAA level is used as a diagnostic test and is used in the evaluation of disease progression and treatment.[4,5] Treatment of patients with metastatic midgut carcinoid tumours is primarily palliative and aimed at ameliorating symptoms, improving quality of life and prolonging survival. This can be achieved by somatostatin analogues, interferon (IFN) alpha, targeted radionuclide therapy and in selective patients by surgery.[6-9] Treatment leads, in the majority of patients, to a reduction of the urinary 5-HIAA levels but rarely to objective tumour responses. Recently however several targeted drugs such as sunitinib, bevacizumab and mTOR inhibitors showed signs of antitumour activity in these tumours.[10-14] This raised the interest in the course of the disease in order to decide, in the future, when during this protracted disease to start which drug.

Survival varies greatly between these patients and it is difficult to predict the course of an individual patient. In a large population based study 5 year survival rate was 44.1% for patients with metastatic midgut carcinoid tumours.[15] In several studies prognostic factors were studied (Table 1).[9,15-37] Unfavourable factors for survival are high urinary 5-HIAA levels at first visit in referral centres, high plasma Chromogranin A (CgA) levels, the presence of liver or lymph node metastases, carcinoid heart disease, tumour size, histological grade of differentiation and high age (Table 1).

With the exception of the study by Turner and colleagues no studies are available that focus on biochemical parameters during follow-up to predict survival.[34] Therefore, the aim of the present study was to determine factors that predict survival of patients with metastatic midgut carcinoid tumours at referral and during follow-up with the emphasis on the urinary 5-HIAA level.

Table 1. Previous studies identifying prognostic factor at baseline for survival for patients with midgut carcinoid tumours

Author	Nº	Tumour	5-year survival	5-year survival rate subgroups	Prognostic factors univariate	Prognostic factors, multivariate
Gatta[17]	7693	GEP-NET[P]	47%			age, time period, histological grade of differentiation
Janson[19]	256	midgut[R]	63%		liver metastases, 5-HIAA (>300 μmol/24 hour), high CgA, NPK, age	age
Wangberg[35]	64	midgut[R]	69%			
Hellman[9]	314	midgut[R]	57%	liver metastases median 4.9 year	resection primary and/or lymph nodes, liver metastases, 5-HIAA (>250 μmol/24 hour), biotherapy	
Maggard[23]	2778	small bowel[P]	55%	distant metastases 32%		tumour size
Makridis[24]	121	midgut[R]			age	
Modlin[15]	3458	small bowel[P]	61%	midgut, distant metastases 44%		
Pape[26]	254	GEP-NET[R]	57%	ileojejunum 67%	tumour size (>2 cm), grade of differentiation	
Agranovich[6]	87	GEP-NET[R]			grade of differentiation, 5-HIAA	
Shebani[30]	150	GI carcinoid[R]		ileojejunum 68%	tumour site, male gender, age	age, liver/lymph node metastases, male sex
Turner[34]	139	midgut[R]	53%		age, 5-HIAA, NKA, liver metastases, resection primary tumour	age
Söreide[33]	154	GI carcinoid[R]	69%		age, tumour site, tumour size, depth of invasion, liver/lymph node metastases	age, liver metastases
Soga[32]	748	CS[P]	76%	digestive 67% extra digestive 89%		
Westberg[36]	64	CS[R]	CHD + 30% CHD - 75%		age, tricuspid abnormalities	
Zar[37]	2526	small bowel[P]		duodenal 60% ileojejunum 56%	age, period of diagnosis	age, period of diagnosis

Table 1. continued

Author	No	Tumour	5-year survival	5-year survival rate subgroups	Prognostic factors univariate	Prognostic factors, multivariate
Quaedvlieg[28]	2391	carcinoid[P]	72%	GI tumour, distant metastases 21%		age, stage, gender, tumour site, year of diagnosis
Kirshbom[20]	434	carcinoid[R]	50%*	midgut, distant metastases 10 year survival 28%		
McDermott[25]	188	GEP-NET[R]		small bowel 66%	male sex, T4 tumour, tumour size, liver/lymph node metastases	male sex, metastases
Greenberg[18]	270	carcinoid[P]		small bowel 66%	tumour site, stage	age, tumour site, stage
Lepage[21]	229	GEP-NET[P]	43%	small bowel 48%	age, male sex, stage, curative resection	age, tumour site, stage
Lepage[22]	3233	GEP-NET[P]	57%	small bowel 59%	age, male sex, tumour site	
Sjoblom[31]	48	small bowel[R]		liver metastases 54%		
Saha[29]	112	GEP-NET[R]		ileojejunum 30%		
Pellika[27]	132	CS[R]	CHD + 20% CHD - 50%		CHD	

GEP-NET: gastroenteropancreatic neuroendocrine tumour, GI: gastrointestinal, CS: carcinoid syndrome, CHD: carcinoid heart disease,

5-HIAA: 5-hydroxyindolacetic acid, NKA: neurokinin A, NPK: neuropeptide K, R: referral based, P: population based, CgA: Chromogranin A. * Midgut 10 year survival.

Patients and methods

Patients

Between January 1992 and December 2003 all patients with a metastatic midgut carcinoid tumour who were referred for treatment at the Department of Medical Oncology, University Medical Centre Groningen, were studied. The diagnosis of a metastatic midgut carcinoid tumour was based on the review of operation reports, the pathology specimens, thoracoabdominal computed tomography (CT), ^{111}In-octreotide scintigraphy and increased levels of 5-HIAA in a 24 hours urine collection (upper reference limit 3.8 mmol/mol creatinine) and/or in increased level of serotonin in platelets (upper reference limit 5.4 nmol /10^9 platelets). Data on gender, age at diagnosis, race, metastases at presentation, resection of the primary tumour, the presence of flushes and or diarrhoea, the use of somatostatin analogues and/or IFN-alpha and survival were derived from the carcinoid database of the Department of Medical Oncology. This database contains medical information from the first appointment at the outpatient clinic and during follow-up of all patients with a midgut carcinoid tumour referred since 1987. Patients are regularly seen during follow-up every 3 to 6 months by the medical oncologist. An echocardiography is performed at referral and thereafter when patients are suspected of having carcinoid heart disease, i.e. complaints of dyspnoea and/or oedema, new findings on cardiac auscultation or physical signs of heart failure. In this database the patient is anonymous using a unique patient code, only known to one dedicated data manager. The database can only be checked through this data manager. Last moment of follow-up was February 2007.

Laboratory and biochemical markers

Serotonin in platelet-rich plasma was determined by high performance liquid chromatography (HPLC) with fluorometric detection, expressed in nmol/10^9 platelets.[38] The upper reference limit of alkaline phosphatase (ALP) was 120 U/l and the upper reference limit of gamma-glutamyltransferase (GT) was 50 U/l. The urinary 5-HIAA was determined by HPLC with fluorometric detection, in a 24 hours urine collection and expressed as mmol/mol creatinine.[39]

These data were prospectively collected every 3 to 6 months and put in the carcinoid database.

Statistical analysis

Differences in survival between groups were analysed using Kaplan Meier survival curves and tested with the log rank test. We used the following parameters: gender, the presence of liver metastases at referral, resection of the primary tumour prior to referral, plasma ALP and plasma GT levels (above or beneath the reference limit) and the urinary 5-HIAA levels at referral. Patients were grouped according to their urinary 5-HIAA levels with a cut-off level of 20 mmol/mol creatinine according to previous reported prognostic levels.[9,19] Hazard ratios (HR) were calculated using a univariate Cox regression analysis with plasma ALP, plasma GT, age and urinary 5-HIAA levels as a continuous variable. Since laboratory investigations were determined at presentation, "survival since referral" instead of "survival since diagnosis" was used in the survival analyses. To study the effect of several prognostic factors at presentation we performed a multivariate Cox regression analysis including factors related to outcome in the univariate analysis (P<0.1). All variables were included in this analysis as continuous variables, the urinary 5-HIAA level was also included as a grouping variable. Next to survival after referral we also studied the prognostic value of the urinary 5-HIAA levels across time using a Cox Regression model with the urinary 5-HIAA level as a time-dependent variable, also including factors mentioned above. The urinary 5-HIAA level was evaluated as a 3 monthly measurement. If data on the urinary 5-HIAA level was missing (203 (23%) out of 666 measurements), we interpolated using the level 3 months prior and 3 months after the missing date. The urinary 5-HIAA level 6 months prior to the last moment of follow-up was used to predict survival. If the difference between the actual follow-up since referral and the last moment of an urine collection was more than 6 months, then the last urinary 5-HIAA level was extrapolated. For patients with missing data on urinary 5-HIAA levels for more than 12 months before the last moment of follow-up, the last follow-up date was determined as 6 months after the last urine collection. As a consequence these patients were alive at this moment of follow-up.

To determine the effect of the cumulative exposure to serotonin we also used the cumulative 5-HIAA level at every moment of follow-up as a time-dependent variable. The cumulative 5-HIAA level was calculated as the sum of the separate urinary 5-HIAA levels measured every 3 months. All tests were performed two-sided and a P-value of <0.05 was considered statistically significant. All statistical analyses were performed using SPSS version 14.0.

Results

Patients

A total of 76 patients, 33 male and 43 female patients, all Caucasian, were included. Table 2 shows the characteristics of patients at referral.

Table 2. Characteristics of patients at referral

Characteristic	
Mean±SD age at diagnosis – years	59.4±9.6
Mean±SD age at referral – years	60.7±9.3
Median duration of complaints before referral – months (range)	20 (1-577)
Primary tumour site – *n* (%)	
Ileum	43 (56.6)
Small bowel	4 (5.3)
Colon	7 (9.2)
Unknown	22 (28.9)
Presence of liver metastases – *n* (%)	61 (80.3)
Complaints – *n* (%)	
Diarrhoea	49 (64.5)
Flushes	51 (69.9)
Treatment – *n* (%)	
Somatostatin analogues	11 (14.5)
Interferon-alpha	1 (1.3)
Laboratory values	
Median alkaline phosphatase - U/l (range)	93 (35-401)
Median gamma-glutamyltransferase – U/l (range)	41 (12-505)
Mean±SD serotonin in platelets – nmol/10^9 platelets	26.2±10.5
Median urinary 5-HIAA level – mmol/mol creatinine (range)	21.0 (1.3-418.4)

5-HIAA: 5-hydroxyindolacetic acid.

Patients were referred to our hospital after a median duration of disease of 2 months (range 0-184 months). The median duration of complaints before referral for patients with an urinary 5-HIAA level of ≤ 20 mmol/mol creatinine was 13 months (range 1-577 months) compared to 26 months (range 1-247 months) for patients with an urinary 5-HIAA level > 20 mmol/mol creatinine (P=0.229).

Nine patients (11.8%) out of 76 patients developed carcinoid heart disease during the follow-up. Patients with carcinoid heart disease tended to have higher urinary 5-HIAA levels at referral, 52.8 mmol/mol creatinine (range 8.5-252.0 mmol/mol creatinine) compared to patients without carcinoid heart disease, 18.6 mmol/mol creatinine (range 1.3-418.4 mmol/mol creatinine) (P=0.085).

Sixty-one (80.3%) out of 76 patients had liver metastases at referral. Patients with liver metastases tended to have higher levels GT at referral, 42 U/l (range 12-505 U/l) versus 32 U/l (range 13-118 U/l) (P=0.072) respectively. Plasma ALP levels were 99 U/l (range 35-401 U/L) and 74 U/l (range 50-177 U/l) (P=0.316) in patients with and without liver metastases respectively.

The median follow-up of all patients was 55 months (range 0.5-143 months). Figure 1 shows the median urinary 5-HIAA levels during follow-up.
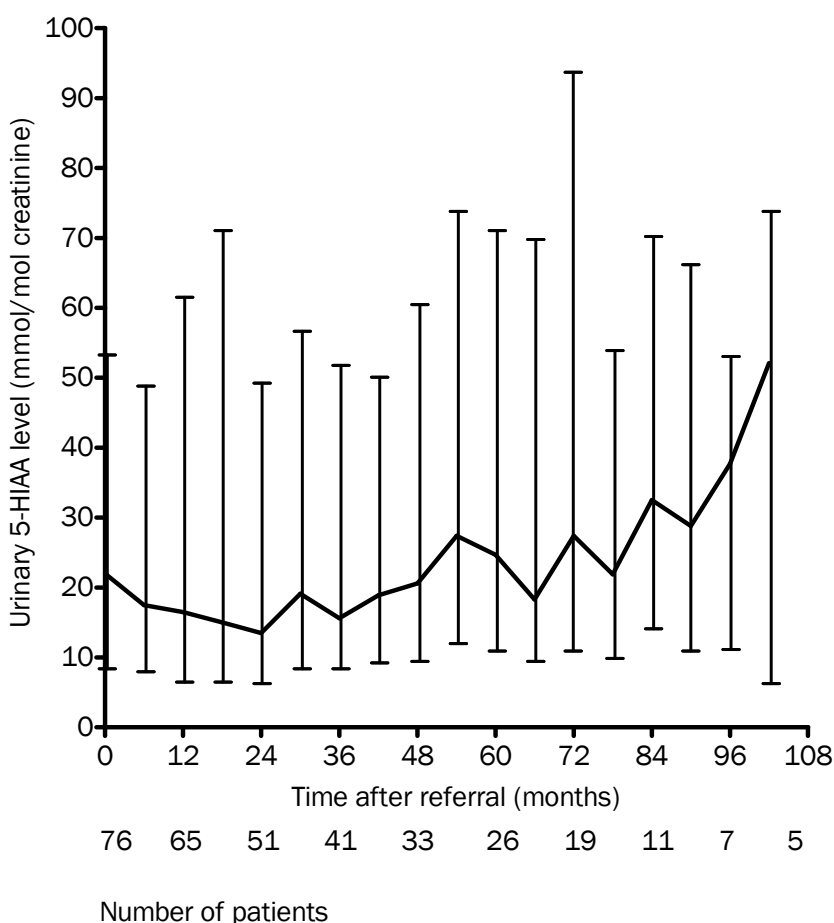
Figure 1. Median urinary 5-hydroxyindolacetic acid (5-HIAA) levels (mmol/mol creatinine) during follow-up (months) with interquartile ranges

Survival, univariate testing

Overall median survival since diagnosis and since referral was 75 months (95% confidence interval (CI) 61-88 months) and 54 months (95% CI 42-67 months) respectively, with a 5 year survival rate of respectively 56.8% and 48.5% (Figure 2). Age as a continuous variable was a prognostic factor with a HR of 1.043 (95% CI 1.014-1.073, P=0.003). Next to age, GT (HR 1.009; 95% CI 1.004-1.013, P=0.000), ALP (HR 1.006; 95% CI 1.003-1.010, P=0.001) and urinary 5-HIAA levels at referral (HR 1.004; 95% CI 1.001-1.006, P=0.003) were prognostic factors for poor survival. Table 3 shows the univariate analysis of different characteristics at referral.

Lowering or raising the cut-off level of the urinary 5-HIAA level did not increase the difference in survival between the groups when compared to the difference of 57 months for patients with a urinary level of ≤ 20 mmol/mol creatinine and > 20 mmol/mol creatinine (Figure 3).
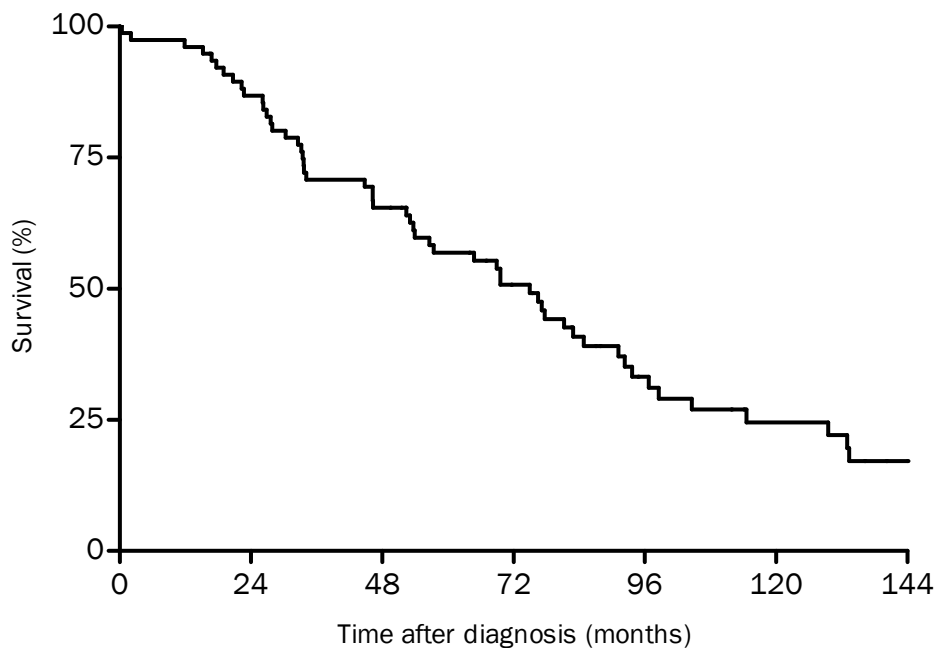
Figure 2. Survival after diagnosis (months)

Table 3. Univariate analysis with respect to survival after referral

Factor	Nº	HR	95% CI	P	Median Survival (months)	95% CI	5 year survival (%)	P
Age (years)	76	1.043	1.014-1.073	0.003				
Gender								
Male	33	0.786	0.462-1.336	0.372	65	54-77	60	0.670
Female	43				51	41-61	40	
Liver metastases								
Present	61	1.790	0.845-3.789	0.104	52	37-69	45	0.123
Not present	15				64	31-97	64	
Resection primary tumour								
Yes	27	0.606	0.341-1.076	0.087	75	44-107	57	0.084
No	49				52	37-68	44	
Gammaglutamyltransferase (U/l)		1.009	1.004-1.013	0.000				
Normal	47				66	53-79	60	0.026
Raised	29				43	23-64	28	
Alkaline phosphatase (U/l)		1.006	1.003-1.010	0.001				
Normal	59				64	51-77	55	0.063
Raised	17				29	23-35	25	
Urinary 5-HIAA level (mmol/mol creatinine)		1.004	1.001-1.006	0.003				
≤ 20	37				90	69-111	72	0.000
> 20	39				33	29-36	26	

HR: hazard ratio, CI: confidence interval, 5-HIAA: 5 hydroxyindolacetic acid.
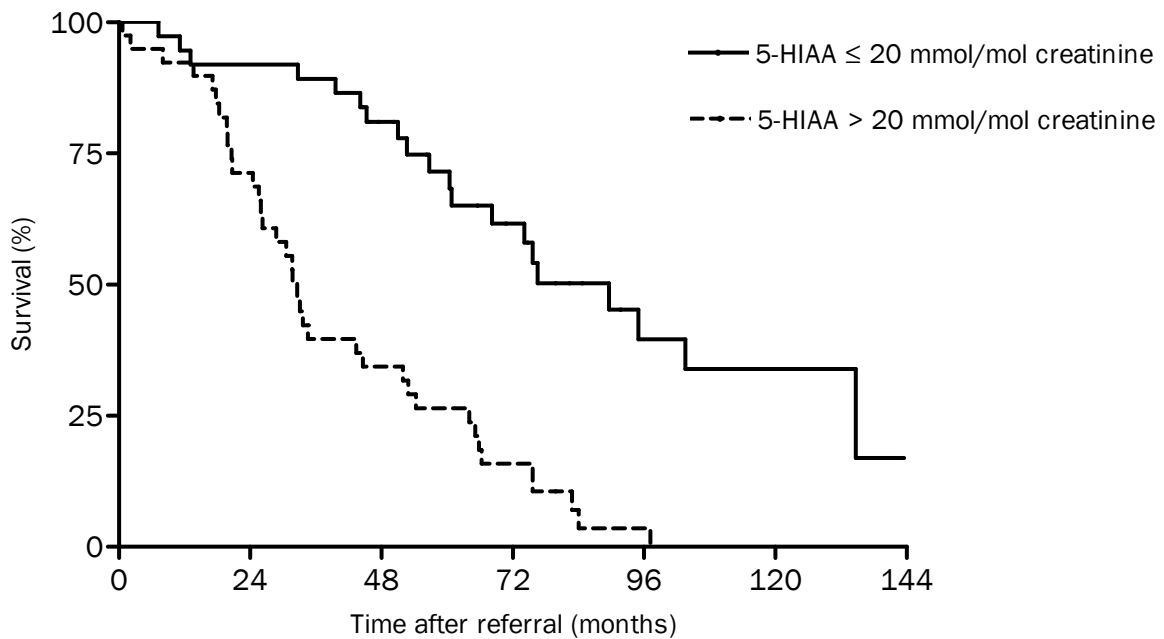
Figure 3. Survival after referral, difference between patients with urinary 5-hydroxyindolacetic acid (5-HIAA) ≤ 20 mmol/mol creatinine and > 20 mmol/mol creatinine

Survival, multivariate testing

In the multivariate model, age, a greatly increased urinary 5-HIAA level (> 20 mmol/mol creatinine) and a raised GT level at referral were independent prognostic factors for poor survival. The multivariate analysis is presented in Table 4. Patients with an urinary 5-HIAA level of > 20 mmol/mol creatinine at referral had a worse survival compared to patients with a urinary 5-HIAA level of ≤ 20 mmol/mol creatinine with a HR of 3.33 (95% CI 1.66-6.66, P=0.001).

Table 4. Multivariate analysis with respect to survival

Factor	HR	95% CI	P
Age	1.052	1.02-1.084	0.001
Resection primary tumour	0.581	0.306-1.104	0.097
Gamma-glutamyltransferase	1.009	1.003-1.015	0.002
Alkaline phosphatase	1.002	0.997-1.008	0.400
Urinary 5-HIAA level (mmol/mol creatinine)	1.003	1.000-1.006	0.033

HR: hazard ratio, CI: confidence interval, 5-HIAA: 5-hydroxyindolacetic acid.

Urinary 5-HIAA level as a time dependent variable

Eleven patients (14%) did not collect urine for measurement of 5-HIAA levels for > 12 months prior to the last follow-up date. Median difference between the actual follow-up and the follow-up until the last urine collection in these patients was 38 months (range 14-80 months). Five (45.5%) out of 11 patients had a change in vital status; deceased at the last follow-up moment of February 2007, but still alive 6 months after the last moment of urine collection.

In the extended multivariate Cox regression analysis the HR of the urinary 5-HIAA level as a time dependent variable was 1.007 (95% CI 1.004-1.010, P=0.000). The cumulative urinary 5-HIAA level as a time dependent variable had an HR of 1.001 (95% CI 1.000-1.001, P=0.000).

Discussion

To our knowledge, this is the first study to demonstrate that the urinary 5-HIAA level is an independent prognostic factor both at referral and during follow-up for patients with a metastatic midgut carcinoid tumour. Patients with a greatly increased urinary 5-HIAA level at referral (> 20 mmol/mol creatinine) had a median survival of 33 months compared to 90 months for patients with a moderately increased level (≤ 20 mmol/mol creatinine). Next to the urinary 5-HIAA level we found that high age and a high GT level at referral were independent prognostic factors for a poor survival. An extended multivariate analysis identified the urinary 5-HIAA level as an independent predictor for poor survival with an HR of 1.007 for each increment of the urinary 5-HIAA level (mmol/mol creatinine). The data obtained with a 5 year survival rate of 56.8% are comparable to the survival found in previous studies (Table 1) and therefore allows generalisation of our findings. Several studies did observe urinary 5-HIAA levels at presentation to be related with outcome in univariate analysis but after correction for other factors such as age it did not stand out.[9,19,34] Our study with a relatively high number of measurements per patient, validates the use of the urinary 5-HIAA level during follow-up not only for monitoring the effect of therapy but also for predicting survival in an individual patient at any moment during follow-up.[3,5] The HR of the cumulative 5-HIAA level, as a time dependent variable was 1.001. This suggests that the absolute urinary 5-HIAA level, and thus the absolute

serotonin production is important as previously described by Denney and colleagues for the development of carcinoid heart disease.[40] A cumulative 5-HIAA level does not seem to give additive information in predicting survival. At presentation there seems to be a threshold for the urinary 5-HIAA level of 20 mmol/mol creatinine for predicting survival, since raising the cut-off level (to the level of 40 mmol/mol creatinine) did not influence the difference in survival of 57 months between the groups (data not shown).[9,19] Serotonin has its harmful effects through seven different serotonin receptors, saturation of these receptors, could potentially well explain this threshold.

We observed that GT levels predicted survival, probably as a reflection of the presence, or the extent of liver metastases, since GT and ALP rise in the presence of liver metastases.[41,42] Clancy and colleagues also observed that ALP predicted survival in patients with metastatic neuroendocrine tumours.[43] Their analysis did not include GT levels. A recent study of Formica and colleagues also showed that raised liver enzymes were associated with a worse prognosis, including GT but not ALP.[44] The HR of the presence of liver metastases did not reach statistical significance in our analysis; however, we did not collect information on the extent of the liver metastases.

Our findings might have a number of implications for the clinic. First, since urinary 5-HIAA levels are prognostic at every moment during follow-up it seems justified to initiate treatment not only for symptoms but also because of greatly increased urinary 5-HIAA levels.[6,7] In 2 previous studies it was already suggested that somatostatin analogues, by lowering serotonin secretion, prolong survival in patients with midgut carcinoid tumour.[28,34] Secondly when a patient first presents it is often difficult to predict prognosis and justify starting antitumour treatment with e.g. the newer tumour agents. In clinical trials currently often clear radiological tumour progression is required. However based on our observations patients with a high urinary 5-HIAA level might be eligible without proof of recent anatomical tumour progression.[45]

A limitation has to be mentioned, although this is also true for other prognostic studies. Patients with midgut carcinoid tumours also with metastatic disease often present following a long patient and doctors delay.[5] As a consequence these analyses are based on values from the period after referral and not after disease onset.

In summary our study shows that next to high age and a high GT level at referral, the urinary 5-HIAA level is an independent prognostic factor of survival of patients with midgut carcinoid tumours both at referral and during follow-up.

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Chapter 3.2

Carcinoid heart disease

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To the editor

Møller and colleagues (March 13 issue) addressed the topic of progression of carcinoid heart disease.[1] Serial echocardiography was available in 71 of 273 referred patients. The median baseline urinary 5-hydroxindolacetic acid (5-HIAA) level was in patients with carcinoid heart disease 209 mg/24 hour, and without carcinoid heart disease 110 mg/24 hour, with a relatively short median duration of the syndrome of 1.0 and 1.8 years, respectively.

Remarkably, literature data on 5-HIAA levels during the course of this disease are hardly available. We therefore analysed patients with carcinoid syndrome referred between 1985-2002 of whom at least two 5-HIAA levels were measured with a 1 year interval (*n*=73). The median urinary 5-HIAA level gradually increased during follow-up, reaching 110 mg/24 hour only after 7 years (Figure 1).
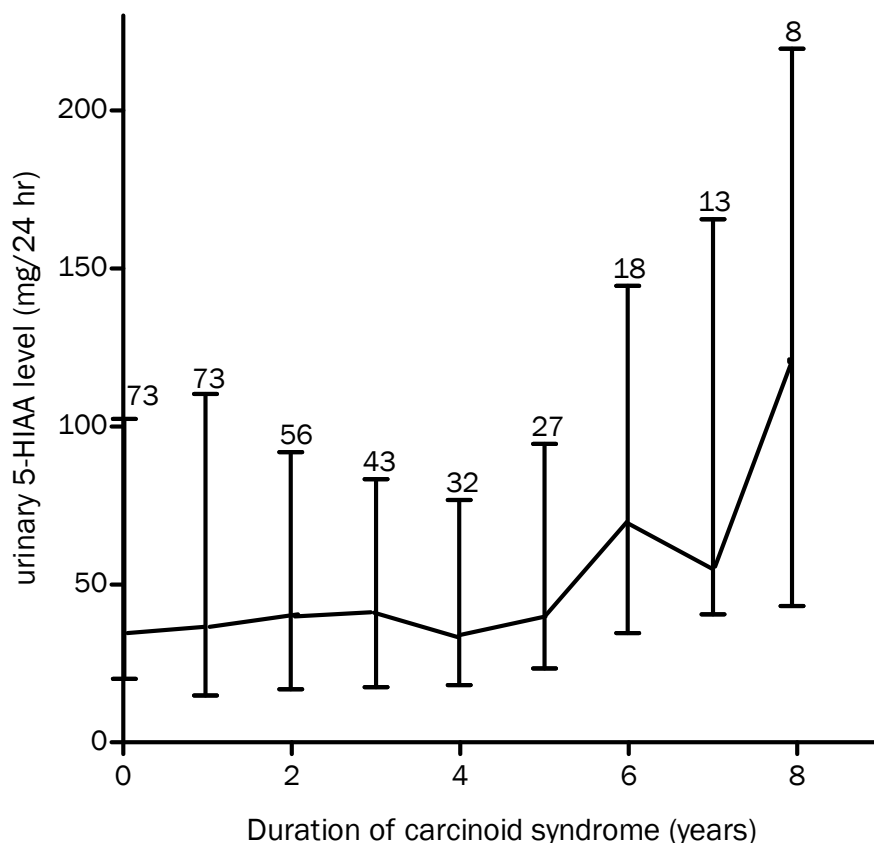
Figure 1. Course of urinary 5-hydroxyindolacetic acid (5-HIAA) levels in time, median values with interquartile range. Numbers in figure denote number of patients.

The extreme urinary 5-HIAA levels and the relatively high incidence of carcinoid heart disease compared to another recent study suggests that Møller and colleagues have studied a highly selected group.[2] This underscores that although somatostatin analogues did not prevent carcinoid heart disease in their population, effects are not precluded in less advanced disease like in most patients referred to us.

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Chapter 4

Serotonin transporter promoter polymorphism predicts survival in patients with metastatic midgut carcinoid tumours

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Abstract

Introduction

In midgut carcinoid tumour patients, serotonin secretion induces carcinoid syndrome and affects survival. Serotonin is taken up by cells by the serotonin transporter (SERT) and metabolised to 5-hydroxyindolacetic acid (5-HIAA) by monoamine oxidase A (MAO-A). The *SERT* promoter region has a functional polymorphism (*5-HTTLPR*), with a short (S, functional less active) and long (L) allele. A functional polymorphic region, upstream variable number of tandem repeats (*uVNTR*) is present in *MAO-A*. We studied whether these polymorphisms affect symptoms and overall survival in carcinoid patients.

Patients and methods

105 patients with metastatic midgut carcinoid tumours and elevated serotonin production were genotyped for *5-HTTLPR* and *MAOA-uVNTR*. Endpoints were presence of flushes, diarrhoea and survival. In addition effects of genotypes, age, urinary 5-HIAA and liver metastases at first visit on death within 5, 6, 7, 8, 9 and 10 years after referral were determined with multiple logistic regression analysis.

Results

Patients with S'S' genotype of *5-HTTLPR* (*n*=29) had a shorter median survival (69.6 months, 95% confidence interval (CI) 20.8-118.3 months) compared to patients with L'S' (*n*=49, 114.6 months, 95% CI 49.1-180.1 months) or L'L' genotypes (*n*=26, 91.2 months, 95% CI 41.1-141.2 months) (P=0.01). Survival was not influenced by *MAOA-uVNTR*. Patients with the S'S' genotype of *5-HTTLPR* had a greater risk to die within 7 to 10 years after referral, independent of high age, high urinary 5-HIAA concentrations and liver metastases. The different genotypes were not related to the presence of flushes and diarrhoea.

Conclusions

The S'S' genotype of *5-HTTLPR* is associated with a worse survival in patients with a metastatic midgut carcinoid tumour.

Introduction

Midgut carcinoid tumours are rare well differentiated neuroendocrine tumours originating in the small intestine and proximal colon. Midgut carcinoid tumours secrete serotonin and other vasoactive amines. When serotonin enters the systemic circulation in high concentrations, the carcinoid syndrome develops.[1,2] This syndrome is characterised by various symptoms including flushes, diarrhoea, and carcinoid heart disease, with diverse presentation between patients.[3-5] High concentrations of free circulating serotonin are harmful and cleared from the plasma by cellular uptake in liver, lung and platelets by the serotonin transporter (SERT or 5-HTT). The liver and lung deaminate serotonin by the mitochondrial enzyme monoamine oxidase A (MAO-A) and thereafter it is oxidised to 5-hydroxyindoleacetic acid (5-HIAA) and secreted in the urine (Figure 1).[6]

Survival varies greatly between patients with metastatic midgut carcinoid tumours and 5-year survival rates range from 21%-63%.[7-9] The urinary 5-HIAA concentration at presentation is a known prognostic factor for survival and lowering serotonin secretion appears to improve survival, suggesting that serotonin is partly responsible for the mortality in these patients.[7,9-11]

Differences in plasma clearance of free serotonin due to genetic variation might explain why some patients with minimal tumour burden have symptoms whereas others do not despite extensive metastatic disease and why there is a broad range in survival duration in these patients. Variation in the expression of the SERT protein is associated with various mood disorders since SERT removes serotonin from the synaptic cleft thereby terminating its action at the receptor.[12] Increased uptake of serotonin by SERT is known to stimulate hyperplasia in pulmonary artery smooth muscle cells in patients with primary pulmonary hypertension.[13]

The promoter region of the *SERT* gene contains a polymorphic region, a 44-base pairs (bp) deletion/insertion (*5-HTTLPR*).[14,15] The *5-HTTLPR* consists of a short (S, functional less active) and a long variant (L, functional active). In the S variant the transcriptional activity of the promoter is reduced which coincided with about 2 times decreased SERT protein expression and serotonin uptake in lymphoblast cell lines.[14,15] Recently an A-G substitution in the L allele was described.[16] The L_G allele decreases transcriptional activity of *SERT* in lymphoblast cell lines, in the same manner as the S allele does. Thus, *5-HTTLPR* can be regarded as a triallelic locus with alleles designated as L_G, L_A, and S.[16]
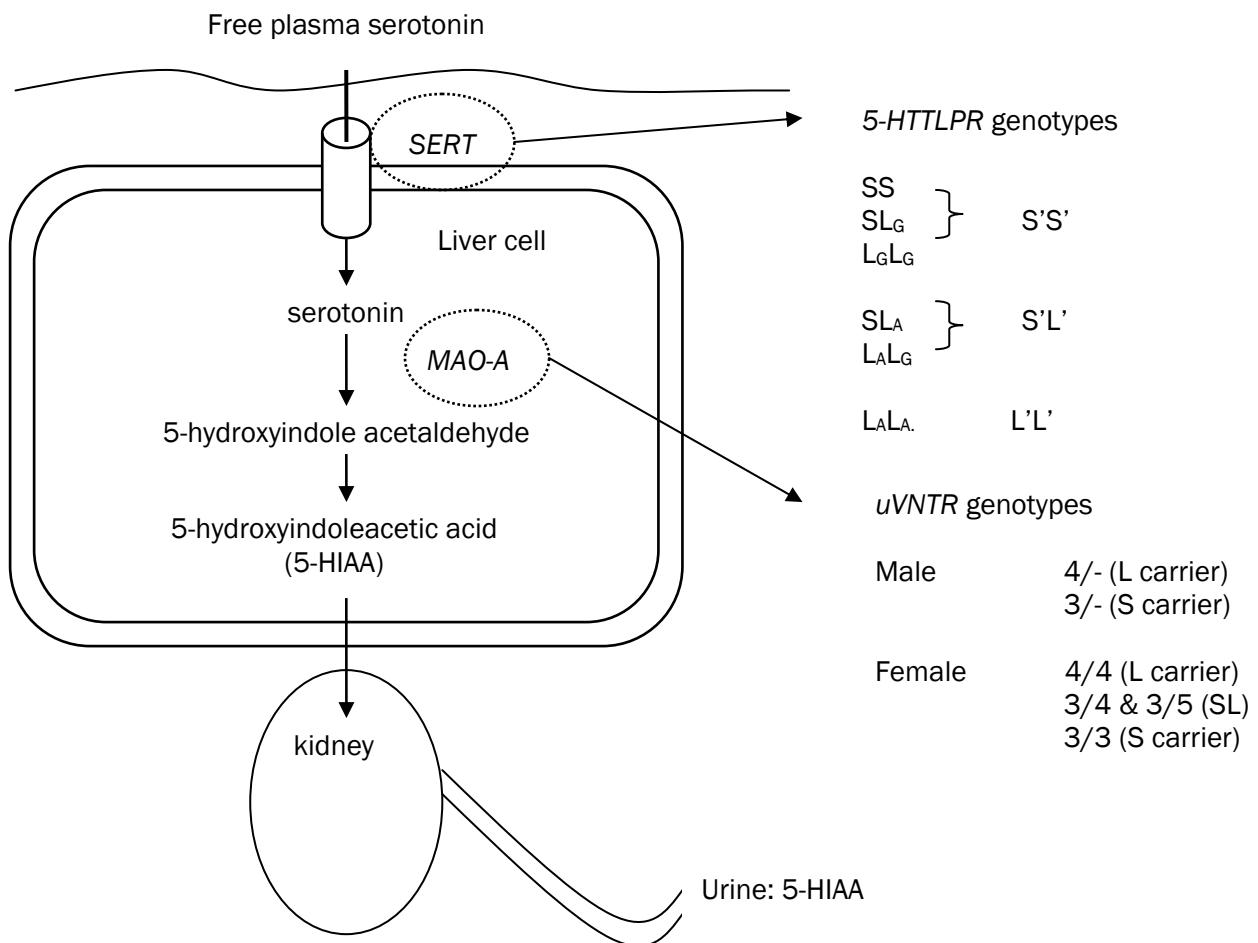
Figure 1. Clearance of free serotonin by liver cell, with sites of the serotonin transporter (SERT) and monoamine oxidase A (MAO-A), and different genotypic variants (L: long, S: short)

MAO-A is located on the X chromosome and has a polymorphic region consisting of an upstream variable number of tandem repeats (*uVNTR-MAO-A*). This polymorphism consists of 4 variants each with 3 or more repeats of 30 bp. Alleles containing 3.5 or 4 or 5 repeats (long (L)) are functionally more active in transcriptional activity of the promoter, compared to the alleles with 3 (short (S)) repeats.[17,18]

These 2 frequent polymorphisms might well affect the clearance of free serotonin and thus influence the important, harmful role of free serotonin and affect the morbidity and mortality of patients with metastatic midgut carcinoid tumours. Currently there are no data available on the role of these polymorphisms in carcinoid patients. We therefore

examined the relationship among the polymorphisms of *SERT* and *MAO-A*, the presence of symptoms of the carcinoid syndrome and survival.

Patients and methods

Patients

All patients with a metastatic midgut carcinoid tumour and elevated serotonin production who were referred for treatment and follow-up to the Department of Medical Oncology of the University Medical Centre Groningen between January 1987 and July 2004 were eligible for inclusion. The diagnosis was based on the review of surgery reports, pathology specimens, thoracoabdominal computed tomography, ^{111}In-octreotide scintigraphy and increased 5-HIAA concentrations in 24 hour urine and/or an increased serotonin in platelets. The study was approved by the Medical Ethics Committee. All patients gave written informed consent. Patients who were deceased were included according to local regulations by obtaining DNA from an anonymous serum bank. Data on gender, age at diagnosis, race, metastases at presentation, resection of the primary tumour, the use of somatostatin analogues or interferon (IFN)-alpha and the presence of carcinoid heart disease were derived from the carcinoid database of the Department of Medical Oncology. This database contains medical information and information on treatment from the first appointment at the outpatient clinic and during follow-up of all patients with a midgut carcinoid tumour referred between 1987 and 2004. Patients visit the outpatient clinic during follow-up every 3 to 6 months. Treatment consists of removal of the primary tumour if possible. Somatostatin analogues are started in patients with complaints of the carcinoid syndrome. If tumour progresses or complaints worsen IFN-alpha is administered. Carcinoid heart disease is diagnosed by echocardiography, which is routinely performed in patients at referral. During follow-up echocardiography is performed in case of suspicion of carcinoid heart disease (complaints of dyspnoea and/or oedema, new findings on cardiac auscultation or physical signs of heart failure). In the database the identity of the patient is anonymously used with an unique patient code, only known to one dedicated data manager. The database can only be checked through this data manager. Last moment of follow-up was January 2007.

Genotyping

DNA was isolated from freshly taken peripheral EDTA anti-coagulated blood samples (alive patients) and from frozen serum and plasma samples, stored at -20 ^{0}C (deceased patients). Genomic DNA was isolated from whole blood and serum using the QIAamp DNA Mini Kit (Qiagen, Venlo, The Netherlands).

Genotyping 5-HTTLPR, including S, L_A and L_G

5-HTTLPR genotypes were determined using the HTTp2a and HTTp2B primer set to amplify 406 (S) and 450 (L) bp fragments with the polymerase chain reaction (PCR).[19] The L_A, L_G and S alleles were measured by incubation of the PCR product with the restriction enzyme Msp I (New England Biolabs, Westburg, Leusden, The Netherlands) for at least 3 hours at 37° C. Msp I cuts the GGCC sequence, resulting in fragments of 329, 62, and 59 (L_A), 174, 155, 62 and 59 bp (L_G), and 285, 62 and 59 bp (S) respectively. The resulting restriction fragments were separated using a 2% agarose gel and visualized using GelStar (SYBR-green; Cambrex Bio Science, Rockland, ME, USA). The triallelic genotypes were reclassified into a biallelic model by their level of expression as follows: L_GS, L_GL_G, and SS were reclassified as S'S', L_AS and L_AL_G were reclassified as L'S', and L_AL_A was reclassified as L'L' (Figure 1).[16]

Genotyping uVNTR region of the MAO-A gene

MAO-A genotyping was essentially performed as described by Sabol and colleagues.[18] The resulting PCR fragments were separated on a Spreadex EL 800 mini gel (Elchrom Scientific AG, Cham, Switzerland), using the SEA 2000 Electrophoresis Apparatus (Elchrom) and visualized using GelStar (SYBR-green).

Since the MAO-A is X-linked we classified the different genotypes as L carrier, LS carrier and S carrier (Figure 1).

Symptoms of carcinoid syndrome

Information regarding the presence of diarrhoea and flushing at first visit was collected from the carcinoid database of the Department of Medical Oncology.

Biochemical markers

Serotonin in platelet-rich plasma (upper reference limit 5.4 nmol /10^9 platelets) and urinary 5-HIAA in a 24 hour urine collection (upper reference limit 3.8 mmol/mol

creatinine) were determined by high performance liquid chromatography with fluorometric detection as previously described.[20]

Power analysis

To determine sample size, we performed a power-analysis based on the primary endpoint diarrhoea and *5-HTTLPR* as a biallelic allele. We assumed that 50% of patients with the LL genotype for *5-HTTLPR* had complaints of diarrhoea compared to 80% of patients with the LS and 80% of patients with the SS genotype. Among Caucasian individuals the frequency of the L allele is 60% and the frequency of the S allele is 40%.[15] Group sample sizes of 32 were required to detect a 30% difference with a power of 80% between 2 groups. Therefore, we aimed at collecting 32 patients for each genotype of the three *5-HTTLPR* genotypes, resulting in a total of at least 96 patients.

We report here the results of the *5-HTTLPR* as a triallelic locus as it became clear in 2006, 2 years after the start of this study that the *5-HTTLPR* had to be regarded as a triallelic locus.[16]

Statistical analysis

Differences between groups were tested using one way Anova or Kruskall-Wallis tests for continuous variables, and the Chi-square test was performed to test differences for categorical variables. Differences in overall survival according to genotypes were analysed using Kaplan Meier survival curves and formally tested with the log rank test. To study the independent effect of genotypes on overall survival we performed multiple logistic regression analyses with the outcome death within 5, 6, 7, 8, 9 respectively 10 years after referral. We adjusted for the urinary 5-HIAA concentration at referral (as a continuous variable), age at referral (as a continuous variable) and the presence of liver metastases. Since the urinary 5-HIAA concentrations, age and the presence of liver metastases were determined at referral, the 'death within 5, 6, 7, 8, 9 respectively 10 years after referral' instead of ' death within 5 to 10 years after diagnosis' was used in these survival analyses. All tests were performed two-sided and a P value of <0.05 was considered statistically significant. All statistical analyses were performed using SPSS version 14.0.

Results

Patients

A total of 105 (96.3%) out of 109 eligible patients, all Caucasian, were included. In 87 (82.9%) patients pathology reports showed tumours with low grade malignancies. The 18 (17.1%) patients in whom no pathology was available had a median survival of 129.5 months and are thus also regarded as low grade malignances.

Four patients could not be genotyped for the *uVNTR-MAO-A* and 5 patients not for *5-HTTLPR* due to limited availability of DNA. Median follow-up of all patients since diagnosis was 55.7 month (range 0.4-281.1 months).

Patient characteristics and allele frequencies for the different genetic variants of the *5-HTTLPR* and the *uVNTR-MAO-A* are presentated in Table 1. All polymorphisms were in Hardy-Weinberg equilibrium.

There were no statistically significant differences between different genetic variants with respect to patient characteristics at referral. Patients with the S'S' variant of the *5-HTTLPR* tended to have lower urinary 5-HIAA concentrations at referral (22.0 mmol/mol creatinine, range 1.3-166.2 mmol/mol creatinine) compared tot patients with the L'S' (30.6 mmol/mol creatinine, range 2.5-418.4 mmol/mol creatinine) and the L'L' variant (23.5 mmol/mol creatinine, range 3.2-271.6 mmol/mol creatinine) (P=0.1).

Ten patients (9.5%) were using somatostatin analogues and 2 patients (1.9%) IFN-alpha at referral.

Symptoms of carcinoid syndrome

Flushes and diarrhoea were present in respectively 79 (75.2%) and 66 (62.9%) out of 105 patients at referral. There were no differences in the presence of flushes or diarrhoea between the different genotypes of the *5-HTTLPR* or the *uVNTR-MAOA* (Table 2). Symptomatic carcinoid heart disease, confirmed with echocardiography, developed in 15 (14.3%) out of 105 patients during follow-up. Seven (26.9%) out of 26 patients with the L'L' genotype developed carcinoid heart disease compared to 4 (8.2%) out of 49 patients in L'S' and 4 (13.8%) out of 29 patients in the S'S' genotypic group (P=0.09).

Survival

Median overall survival since diagnosis was 77.7 months (95% confidence interval (CI) 59.2-96.2 months). Five and 10 year survival rates were 58.8% and 32.9% respectively. A

total of 77 patients (73.3%) died during follow-up. The median overall survival of the S'S' genotype was 69.6 months (95% CI 20.8-118.3 months) compared to a median overall survival of the L'S' and the L'L' genotype of 114.6 months (95% CI 49.1-180.1 months) and 91.2 months (95% CI 41.4-141.2 months) respectively (P=0.01) (Figure 2). Ten year survival rates were 17.1% for patients with the S'S' genotype, 49.6% for patients with the L'S' genotype, and 28.2% for patients with the L'L' genotype.
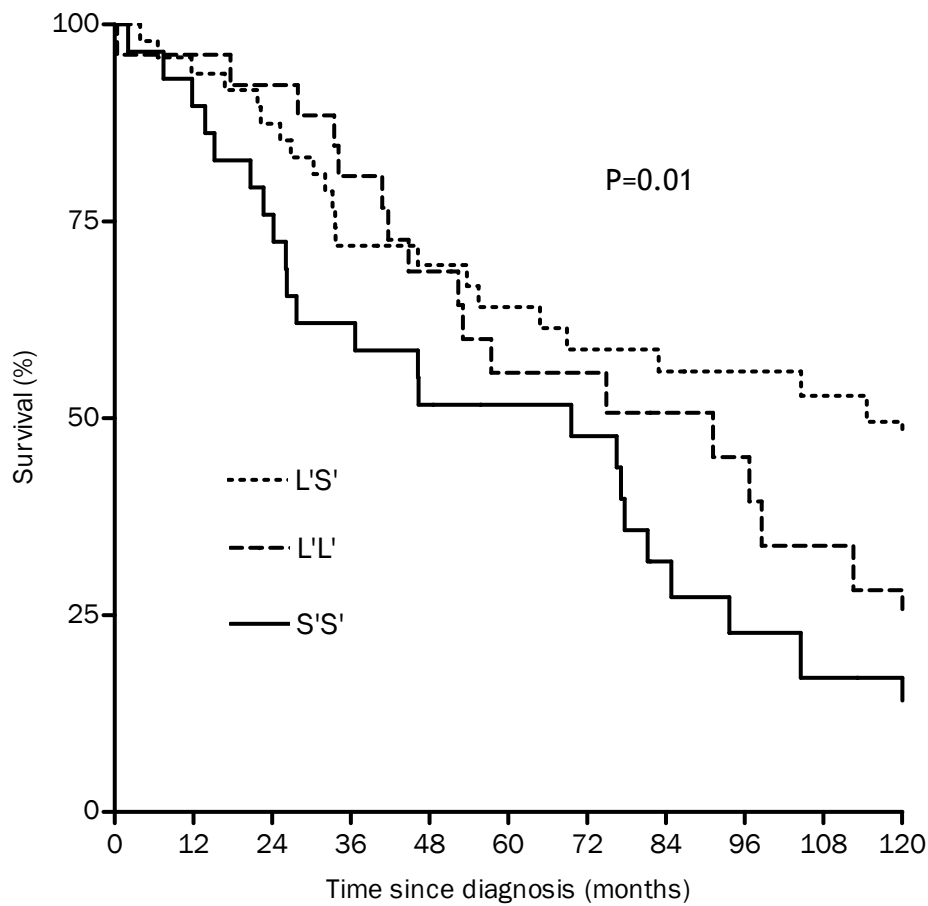
L'L'	25	24	21	17	13	12	9	8	6	5
L'S'	45	41	31	27	24	21	20	18	17	15
S'S'	26	22	18	15	13	12	7	4	3	2

Number of patients

Figure 2. Kaplan-Meier survival curve from time of diagnosis for the different genotypic variants of the serotonin transporter gene–linked polymorphic region (*5-HTTLPR)*

Table 1. Basic characteristics at referral of patients for the different genotypic variants of the *5-HTTLPR* and the *uVNTR* region of the *MAO-A* gene

	5-HTTLPR				uVNTR			
	L'L'	L'S'	S'S'	P	L carrier	S carrier	LS	P
Number of patients	26 (25.0)	49 (47.1)	29 (27.9)		50 (47.6)	31 (29.5)	24 (22.9)	
Sex male	11 (42.3)	23 (46.9)	10 (34.5)	0.60	22 (44.0)	22 (71.0)	0 (0.0)	<0.00
Mean±SD age at diagnosis – years	57.2±9.4	57.7±11.7	61.7±9.7	0.18	59.3±9.9	56.5±12.9	60.1±9.2	0.39
Tumour resection	13 (50.0)	17 (34.7)	12 (41.4)	0.43	22 (44.0)	10 (32.2)	12 (50.0)	0.38
Liver metastases	19 (73.1)	39 (79.6)	25 (86.2)	0.48	41 (82.0)	27 (87.1)	15 (62.5)	0.07
Median urinary 5-HIAA – mmol/mol creatinine (range)	23.5 (3.2-271.6)	30.6 (2.5-418.4)	22.0 (1.3-166.2)	0.10	19.6 (2.8-398.0)	27.5 (1.3-418.4)	25.7 (2.3-175.5)	0.15

Data are numbers (%) unless otherwise indicated. *5-HTTLPR*: serotonin transporter gene–linked polymorphic region, *uVNTR*: upstream variable number of tandem repeats, MAO-A: monoamine oxidase-A, 5-HIAA: 5-hydroxyindolacetic acid, CI: confidence interval.

Table 2. Presence of flushes and diarrhoea at referral and overall survival since diagnosis between the different genotypic variants of the *5-HTTLPR* and the *uVNTR* region of the *MAO-A* gene

Outcome	5-HTTLPR				uVNTR			
	L'L'	L'S'	S'S'	P	L carrier	S carrier	LS	P
Flushes	19 (73.1)	37 (75.5)	21 (72.4)	0.98	35 (70.0)	26 (83.9)	17 (70.8)	0.48
Diarrhoea	16 (61.5)	31 (63.3)	17 (58.6)	0.73	26 (52.0)	19 (61.3)	19 (79.2)	0.12
Median survival – months (95% CI)	91.2 (41.4-141.2)	114.6 (49.1-180.1)	69.6 (20.8-118.3)	0.01	77.7 (60.0-95.4)	98.6 (56.2-140.9)	53.7 (20.7-86.6)	0.28

Data are numbers (%) unless otherwise indicated. *5-HTTLPR*: serotonin transporter gene–linked polymorphic region, *uVNTR*: upstream variable number of tandem repeats, MAO-A: monoamine oxidase-A, CI: confidence interval.

In the multiple logistic regression analysis with the outcome death within 5 years after referral, high age was a prognostic factor for dying. In the multiple logistic regression analysis with the outcome death within 6 to 10 year after referral, the S'S' genotype of the *5-HTTLPR*, high age and high urinary 5-HIAA concentrations were independent predictors when the outcome was dying within 7, 8, 9 and 10 years after referral. Patients with the L'S' genotype had a better overall survival compared to patients with the S'S' genotype. Patients with the S'S' genotype had an increased risk to die within 10 years after referral (Odds Ratio (OR)=4.36 (95% CI 1.33-14.30, P=0.02) compared to patients with the L'S' genotype. The OR for patients with the S'S' genotype compared to patients with the L'L' genotype was 1.64 (95% CI 0.43-6.23, P=0.47). The OR for dying within 6, 7, 8 and 9 years within referral of patient with the S'S' genotype compared to patients with the L'S' genotype was 1.86 (95% CI 0.64-5.42, P=0.26), 3.26 (95% CI 1.04-10.26, *P*=0.04), 3.06 (95% CI 1.00-9.30, P=0.05) and 4.65 (95% CI 1.43-15.18, P=0.01) respectively. Including the *uVNTR-MAO-A* into the model did not affect these results.

Median overall survival of the different genotypes of the *uVNTR-MAO-A* is presented in Table 2. There were no significant differences with regard to survival between the different genotypes.

Discussion

This study shows that patients with a metastatic midgut carcinoid tumour and the S'S' genotype of *5-HTTLPR* have a worse overall survival compared to the patients with the L'L' and L'S' genotypes. No association was observed between the different genotypic variants of the *5-HTTLPR* and symptoms of flushes or diarrhoea at the moment of referral.

The association between the genotypes of *5-HTTLPR* and death after referral from 7 years onwards is independent of other known risk factors for a worse survival, such as high age, high urinary 5-HIAA concentrations and the presence of liver metastases.[7,9] It is also independent of the *uVNTR* polymorphism of *MAO-A*.

The median survival of our population is comparable with the survival rates reported in previous studies with patients with midgut carcinoid tumours.[7,8,21] To our knowledge this is the first report on the relation between *5-HTTLPR* and survival in patients with

metastatic midgut carcinoid tumours. No interest in these genes contrasts with the research performed in other diseases such as several psychiatric disorders. A body of evidence exists on the relation between the *5-HTTLPR* polymorphism and a disturbance in the neurotransmission of serotonin.[15,16,22] Outside the central nervous system *5-HTTLPR* polymorphism is associated with the development of primary pulmonary hypertension and it has been suggested as a potential gene involved in the irritable bowel syndrome.[13,23,24]

The relation between *5-HTTLPR* polymorphism and survival in patients with a metastatic midgut carcinoid tumour can be explained by several mechanisms. The S and L_g alleles have lower transcriptional activity, leading to a lower expression of SERT in cells with the S'S' variant.[15,16] Hence patients with the S'S' genotype are likely to be exposed to free circulating serotonin for a longer period of time. This is of clinical importance since patients with metastatic carcinoid tumours have high free serotonin levels.[25]

Free serotonin has several harmful acute and chronic cardiovascular effects.[26] The prominent cardiovascular effect of serotonin in patients with metastatic midgut carcinoid tumours is the fibrous thickening of the heart valves, also known as carcinoid heart disease, leading to right sided heart failure and early death.[27-29] There is clear evidence for the causal role for serotonin in the development of carcinoid heart disease. Rats who received serotonin injections for 3 months developed lesions comparable to those seen in carcinoid heart disease.[30] Patients using fenfluramine, a sympathomimetic amine that activates serotonergic pathways also formed such fibrous thickening of the heart valves.[31] In addition a recent study showed that mice deprived of their SERT (*SERT-* knock out), were prone to develop fibrosis and functional alteration of the heart.[32] We found a slightly higher prevalence of clinical carcinoid heart disease in patients with the L'L' genotype, this did however not reach statistical significance. This may be due to too low number of patients with carcinoid heart disease in our study and needs to be evaluated in a larger population.

In clinical practice if our results are confirmed in other series, genotyping patients with a metastatic midgut carcinoid tumour for the *5-HTTLPR* may be of value to identify patients at risk for poor survival. This could lead to a more tailored approach in patients with the S'S' genotype of the *5-HTTLPR* e.g. by using early somatostatin analogues, or newer targeted drugs such vascular endothelial growth factor inhibitors.[33-35]

In conclusion the S'S' genotype of the *5-HTTLPR* is associated with a shorter survival in patients with metastatic carcinoid midgut carcinoid tumours.

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Chapter 5

Effect of interferon and 5-fluorouracil on serum VEGF levels in neuroendocrine tumours

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Established systemic treatment choices for metastatic neuroendocrine tumours (NETs) are somatostatin analogues and alpha interferons (IFN-alpha) both reducing the secretory activity of the tumour. IFN-alpha also has an anti-proliferative effect, inhibiting angiogenesis, blocking the cell cycle, and stimulating apoptosis.[1] Chemotherapy has limited value, used in NETs with a high-proliferative index.

Angiogenesis has raised interest as a new target in NETs. This interest is fuelled by the fact that NETs are highly vascularised and vascular endothelial growth factor (VEGF) and its receptors are present.[2] We performed a study with IFN-alpha, 5-fluorouracil (5-FU) and leucovorin in patients with metastatic NETs because of the suggested synergistic effects between 5-FU and IFN-alpha, and promising results of this combination obtained by others.[3] In this feasibility study, approved by the Medical Ethical Committee, patients were treated with IFN-alpha-2b (2.5 million U/day subcutaneously), and after 2 weeks, intravenous 5-FU 750 mg/m^2 (day 2) and oral leucovorin 180 mg/day (day 1 and 2) were added as a 2 weekly cycle.

The primary endpoint was toxicity, the secondary endpoints included radiological and biochemical response. In addition, serum was prospectively stored for serum VEGF level measurements.

This study was prematurely closed after 9 patients because of side effects. After 1 cycle, 3 patients had to stop, because of grade 3 fatigue (n=2) and heart failure (n=1). Three additional patients discontinued after respectively 2, 3 and 4 cycles because of arthritis and polyneuropathy (n=1) and grade 3-4 diarrhoea and fatigue (n=2). The remaining 3 patients completed 10, 19 and 20 cycles. Median survival was 22 months, 2 patients are still alive after 144 months. All 3 radiological evaluable patients experienced stable disease lasting 5 - 84+ months.

Serum VEGF levels (R&D Systems, Minneapolis, USA) decreased in 7 out of 8 evaluable patients, from a median level of 352 pg/ml (range 41-1276 pg/ml) to 219 pg/ml (range 18-938 pg/ml), a median reduction of 40% in 88 days (median) (range 34-462 days). This suggests an antiangiogenic effect of the combination IFN-alpha and 5-FU. One study showed that IFN-alpha alone did not reduce serum VEGF levels in patients with carcinoid tumours, although it did in another study (median reduction 33%) also decreasing VEGF mRNA expression in liver metastases.[1,4]

In the present study toxicity was considerable. Next to the synergistic effect of this regime, IFN-alpha also can potentiate the toxicity of 5-FU. Four different dosages of 5-FU

and INF-alpha were previously studied in patients with NETs, only the regimen in a study of Andreyev and colleagues was well tolerated in which 5-FU was given continuously.[3]

Bevacizumab, a monoclonal antibody against VEGF was found to prolong progression free survival compared to IFN-alpha in carcinoids while sunitinib, a tyrosine kinase inhibitor, induced stable disease in NETs.[5,6] Recently Zhang and colleagues showed that bevacizumab inhibited tumour growth in carcinoid xenograft, but at the same time up regulation of VEGF transcription occurred as a possible resistance mechanism.[2] During sunitinib therapy elevated baseline serum VEGF also increased, possibly due to resistance.[7]

We conclude that this combination of 5-FU and IFN-alpha is not feasible due to side effects. However taken our observation of decreased VEGF levels, less toxic modifications, e.g. 5-FU given continuously or IFN-alpha combined with capecitabine, an oral fluoropyrimidine prodrug, in combination with one of the new antiangiogenic drugs can be envisioned. Such a regime might potentiate the effect of angiogenesis inhibitors.

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Chapter 6

Sexual function in patients with carcinoid tumours

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Abstract

Introduction

Sexual dysfunction is a poorly studied aspect of Quality of Life (QoL) in patients with midgut carcinoid tumours. We investigated whether carcinoid patients experience sexual problems.

Patients and methods

Patients with metastatic midgut carcinoid tumours filled in a validated questionnaire for screening of sexual dysfunction (QSD). The prevalence of sexual dysfunction on the subscales arousal, erection, lubrication, orgasm and dyspareunia, was compared to a Dutch reference population. Plasma concentrations of gonadal hormones, tryptophan and urinary 5-hydroxyindolacetic acid (5-HIAA) concentrations (µmol/24 hours) were measured.

Results

Forty three patients were studied, 27 men, mean±SD age 61.8±8.3 years and 16 women, mean±SD age 58.5±10.2 years. Sexual dysfunction was present in 29.6% of men (*n*=8) and in 6.3% of the women (*n*=1). The prevalence of sexual dysfunction on the different subscales did not differ from the reference population. Male patients with a sexual dysfunction had, compared to those without a sexual dysfunction, a longer duration of disease, namely 95.3 months (range 5.4-314.5 months) versus 18.6 months (range 0.6-167.9 months) (P=0.024), lower plasma tryptophan concentrations (±SD) of respectively 31.5±16.1 µmol/L and 48.9±14.5 µmol/L (P=0.031), and used more frequently interferon-alpha, respectively 4 out of 8 patients (50%) and 2 out of 19 (10.5%) (P=0.044).

Conclusion

Patients with metastatic midgut carcinoid tumours do not experience sexual problems more often than a reference population. Male patients with sexual dysfunction are characterised by a more longstanding disease and lower tryptophan concentrations.

Introduction

Sexual dysfunction is a common problem in patients with cancer, that can affect their quality of life (QoL).[1] Few studies evaluated the QoL of patients with midgut carcinoid tumours.[2-4] Only one study in the early seventies specifically studied sexual dysfunction.[5] In this study in 16 carcinoid patients, including 10 male patients with the carcinoid syndrome and elevated urinary 5-hydroxyindolacetic acid (5-HIAA) concentrations, a decrease in libido after the onset of the disease was observed.[5] In another study, investigating the QoL in carcinoid patients, distress about possible changed sexual function was present in 13 (36%) out of 37 patients, although this was evaluated with just one single question.[2]

Several factors could potentially play a role in a changed sexual function in patients with a midgut carcinoid tumour. First, serotonin changes in the brain have been reported to interfere with sexual desire, arousal and orgasm.[6] Previous data suggest that patients with midgut carcinoid tumours have a serotonergic dysfunction due to peripheral consumption by the tumour of the precursor tryptophan.[7] Second, serotonin and other factors have vasoactive properties which can lead to endothelial dysfunction and can interfere with the erectile function.[8,9] Furthermore, patients can suffer from pain and fatigue and often medication is used that may affect the ability to and the desire for sexual activity.[1,8]

In this study we investigated the sexual function of patients with metastatic midgut carcinoid tumours. To gain more insight in the pathogenesis of sexual dysfunction in this population and for hypotheses generation for possible future treatment strategies, we also evaluated the biochemical activity of the tumour and gonadal hormone concentrations in relation to sexual function.

Patients and methods

Patients

Patients with metastatic midgut carcinoid tumours were recruited in 4 Dutch hospitals: the University Medical Centre Groningen (UMCG) in Groningen, the Erasmus Medical Centre (Erasmus MC) in Rotterdam, the Medical Spectrum Twente in Enschede, and the

West Friesch Gasthuis in Hoorn. Inclusion criteria were: (1) Diagnosis of a midgut metastatic carcinoid tumour based on the review of surgery reports, histology specimens, thoracoabdominal computed tomography, ^{111}In-octreotide scintigraphy and increased concentrations of 5-HIAA in a 24 hour urine collection and/or in increased level of serotonin in platelets, (2) life expectancy of more than 3 months, and (3) age 18 years or older. All eligible patients were informed about the purpose and the character of the study. Participating patients received the questionnaires, which they returned by mail. Information on the duration of the carcinoid tumour, the use of somatostatin analogues and/or interferon (IFN)-alpha were derived from the medical charts.

Patient's data on medical history with emphasis on co-morbidity, smoking and medical therapy, were obtained through a questionnaire. All patients gave written informed consent. The questionnaires were only marked by a study number and entered in a database by a data manager not involved in routine patient care. The study was approved by the Medical Ethics Committees of all hospitals.

Questionnaire on sexual dysfunction

The short version of the Questionnaire for screening Sexual Dysfunction (QSD) was used to measure sexual function.[10] This questionnaire assesses the frequency and experienced distress of sexual problems on different subscales, e.g. orgasm, erection, arousal, pain. Different versions for men and women, relationship status and sexual orientation are available. Research on the validity of this instrument is available.[10] Frequencies of sexual problems are rated on a five point ordinal scale, ranging from almost never (score 1) to always (score 5). Experienced distress accompanying these problems is also rated on a five point scale from no distress (score 1) to severely stressful (score 5). We defined a sexual problem as clinically relevant or as a dysfunction when scores on the frequency or experienced distress scale were 3 or higher. This means that a sexual problem is experienced 'regularly' to 'always' and the distress with this problem is scored as 'stressful' to 'severely stressful'. We selected the subscales concerning problems with sexual arousal, erection, orgasm and ejaculation for male patients and the subscales concerning problems with sexual arousal, lubrication, orgasm and dyspareunia for female patients. Satisfaction is scored on a five point scale ranging from very dissatisfied (score 1) to very satisfied (score 5). To compare the results of patients with midgut carcinoid tumours to the general population we used data from the Rutger Nisso Foundation, who used the QSD in a random Dutch population.[11]

Health related QoL

Health related QoL was assessed using the Dutch version of the European Organization of Research and Treatment of Cancer (EORTC) QoL-Core 30 Questionnaire (QLQ-C30).[12] This questionnaire has five functional scales: physical, role, cognitive, emotional and social functioning and three symptom scales: fatigue, pain and nausea and vomiting. This questionnaire has also six single-item questions about dyspnoea, insomnia, loss of appetite, constipation, diarrhoea, and financial difficulties; and two questions about global health and QoL. Answers on the questions range from not at all (score 1) to very much (score 4). Answers on the items for the global health/quality of life scale range from very poor (score 1) to excellent (score 7). Scores were transferred to a score from 0 to 100, according to the EORTC QLQ-C30 scoring manual.[13] A high score for the symptom/item scales represents a high level of symptoms/problems, whereas a high score for the functioning scales and the global health or general QoL scales represents a high level of functioning, global health or general QoL.

Laboratory investigations

Urine and blood samples were taken within 2 months after the questionnaires were returned.

Urinary 5-HIAA was determined with HPLC with fluorometric detection.[14,15] All urinary 5-HIAA concentrations were expressed in µmol/24 hours (normal range 14.7-97.2 µmol/24 hours). Plasma tryptophan was measured as previously described.[16] Serum testosterone was measured by a Radio-immuno-assay (lower reference value 12 nmol/L). Luteinizing hormone (LH) and follicle stimulating hormone (FSH) were determined by an Enzyme Immuno Assay. Free testosterone was calculated from serum total testosterone using sex hormone-binding globulin (SHBG) and the albumin serum concentration (reference value in male patients 300-650 pmol/L).[17]

Statistical analysis

The prevalence of sexual dysfunction on the different subscales is descriptive.

In 2006, the prevalence of sexual dysfunction using the same QSD in the general Dutch population (reference population), age up to 69 years, became available.[11] However in this reference population not all questions for the different subscales were included. From this reference population we selected men and women in the same age range as

our patients with midgut carcinoid tumours. For comparison between prevalences of sexual dysfunction on different subscales we used a Chi-square test or Fisher exact test as appropriate.

Since not all questions belonging to a subscale were asked in this reference population, we recalculated the prevalence of dysfunction in our patients using only those questions that were asked in the population study.

To compare patients with and without sexual dysfunction, we used t-tests for normally distributed variables, the non-parametric Mann-Whitney U test for variables not normally distributed and a Fisher's exact test for differences for categorical variables. P values <0.05 were considered to be significant. All data analysis were performed using SPSS (version 14.0).

Results

Patients

A total of 43 (78.2%) out of 55 eligible patients returned the questionnaire, 27 men, 26 with a female partner and 1 man without a partner and 16 women, 9 with a male partner and 7 without a partner. Baseline demographic and clinical characteristics are presented in Table 1. Mean±SD age was 61.8±8.3 years in male patients and 58.5±10.2 years in female patients.

Questionnaire for sexual dysfunction

The prevalence of sexual dysfunction in male and female patients with carcinoid tumours is shown in Table 2. The prevalence of sexual dysfunction in male patients regarding sexual arousal, orgasm and erection in our study population was 22.2% (n=6), 14.8% (n=4) and 25.9% (n=7) respectively. Nineteen (70.4%) out of 27 men did not have a sexual dysfunction on any of the selected subscales. None of the male patients reported ejaculation problems. Only one (6.3%) woman reported an orgasm dysfunction, no other problems were reported by the female patients in this study.

Table 1. Basic characteristics of participating patients with metastatic midgut carcinoid tumours

Variable	
Patients	43
Male	27 (62.8)
Male patients with female partner	26 (96.3)
Female patients with male partner	9 (56.3)
Mean±SD age – years	60.6±8.8
Median time since diagnosis – months (range)	27 (1-314)
Liver metastases	37 (90.2)
Medical therapy	
Somatostatin analogues	31 (77.5)
Interferon-alpha	7 (17.5)
Symptoms of carcinoid syndrome	
Diarrhoea	21 (53.8)
Flushes	20 (50.0)
Median urinary 5-HIAA concentration – μmol/24 hour (range)	599 (24-5102)
Medical history	
Diabetes	5 (11.6)
Surgery pelvic region	9 (20.9)
Intoxication	
Smoking	4 (9.3)
Use of medication	
Beta blocker	12 (27.9)
ACE inhibitor and/or AT II antagonist	11 (25.6)
Benzodiazepines	2 (4.7)
Calcium channel blocker	3 (7.0)
Spironolactone	3 (7.0)
Tricyclic antidepressant	1 (2.3)
Progesterone	1 (2.3)

Data are numbers (%) unless otherwise indicated. 5-HIAA: 5-hydroxyindolacetic acid, ACE: angiotensin converting enzyme, AT: angiotensin.

Table 2. Prevalence of sexual dysfunction of different subscales in patients with metastatic midgut carcinoid tumours

	Male patients	Female patients
Patients	27	16
Sexual arousal	6 (22.2)	0 (0.0)
Orgasm	4 (14.8)	1 (6.3)
Erection	7 (25.9)	
Ejaculation	0 (0.0)	
Lubrication		0 (0.0)
Pain		0 (0.0)
Mean±SD satisfaction score	2.92±1.03	3.60±0.95

Data are numbers (%) unless otherwise indicated.

Comparison with reference population

The age range in the reference population was 52-69 years in men (*n*=583, weighted for age) and 44-69 years for women (*n*=1032, weighted for age). The prevalence of sexual dysfunction in male patients using only those questions that were asked in the reference population, was 3.9% (*n*=1) regarding arousal, 25.9% (*n*=7) regarding erection and 11.1% (*n*=3) regarding the orgasm. Table 3 shows the prevalence of sexual dysfunction in the reference population compared to patients with metastatic carcinoid tumours. No statistical significant differences were found between male or female patients and the reference population.

Table 3. Frequencies of sexual dysfunction in different subscales in patients with metastatic midgut carcinoid tumours compared to the reported prevalence (%) in a Dutch population (reference population)

	Male	References	P	Female	References	P
Patients	27	583		16	1032	
Sexual arousal	1 (3.7)	10 (1.7)	0.38	0 (0.0)	36 (3.5)	1.00
Orgasm	3 (11.1)	29 (5.0)	0.15	1 (6.3)	87 (8.4)	1.00
Erection	7 (25.9)	96 (16.5)	0.18			
Lubrication				0 (0.0)	88 (8.5)	0.39
Pain				0 (0.0)	45 (4.4)	1.00

Data are numbers (%).

Patients with and without sexual dysfunction

Only one woman reported orgasm problems, therefore a comparison between female patients with and without sexual dysfunction was not made. Eight male patients (29.6%) were defined to have a sexual dysfunction and were compared to the 19 male patients who experienced no problems on the selected subscales (Table 4).

Table 4. Male patients with metastatic midgut carcinoid tumours, comparison of patients with and without sexual dysfunction

	Sexual dysfunction	No sexual dysfunction	P
Patients	8 (29.6)	19 (70.4)	
Mean±SD age – years	60.3±5.4	62.4±9.2	0.54
Median duration disease – months (range)	95.3 (5.4-314.5)	18.6 (0.6-167.9)	0.024
Median Ur 5-HIAA – µmol/24 hour (range)	872.5 (55.1-3806.0)	259.0 (24.3-2376.2)	0.163
Mean±SD tryptophan – µmol/L	31.5±16.1	48.9±14.5	0.031
Medical therapy			
Somatostatin analogues	7 (87.5)	15 (78.9)	1.00
Interferon	4 (50.0)	2 (10.5)	0.044
Beta blocker	1 (12.5)	3 (15.8)	1.00
Mean±SD total testosterone – nmol/L	14.0±5.3	15.4±5.4	0.53
Median LH –U/L (range)	4.80 (2.43-21.30)	5.22 (2.35-18.7)	0.90
Median FSH – U/L (range)	8.23 (1.66-28.7)	6.61 (2.4-28.90)	0.69

Data are numbers (%) unless otherwise indicated, 5-HIAA: 5-hydroxyindolacetic acid; ur: urinary; LH: luteinizing hormone; FSH: follicle stimulating hormone

In male patients who reported a sexual dysfunction, the disease duration was significantly longer 95.3 months (range 5.4-314.5 months) compared to patients who did not reported sexual dysfunction, 18.6 months (range 0.6-167.9 months) (P=0.024). Male patients with sexual dysfunction also had lower plasma tryptophan concentrations (±SD) compared to patients without sexual dysfunction, respectively 31.5 (±16.1) µmol/L and 48.9 (±14.5) µmol/L (P=0.031). Plasma concentrations of total testosterone and gonadotrophins did not differ between patients with and without a sexual dysfunction. Mean total testosterone (±SD) concentration in the all 27 male patients was 15.9 (±5.9) nmol/L. Seven (25.9%) out of 27 men had a total testosterone concentration below the reference level of 12 nmol/l. In contrast to other laboratory investigations, free plasma testosterone concentrations were only available in 21 (77.8%) out of 27 men. Nine

(42.9%) out of 21 male patients had a free testosterone concentration below the reference level of 300 pmol/L.

Quality of life

The summary of the EORTC QLQ-C30 is presented in Table 5. The Global QoL score tended to be lower in male patients with sexual dysfunction compared to male patients without sexual dysfunction, respectively 52.1±18.2 versus 62.7±19.3 (P=0.20).

Table 5. Summary of EORTC-C30 in patients with metastatic midgut carcinoid tumours

Domain	Score
Functioning scales	
Physical	72.7±19.9
Role	63.2±31.8
Emotional	83.0±19.1
Cognitive	83.3±23.3
Social	76.4±30.7
Global QoL	61.8±21.8
Symptom scales	
Fatigue	33.9±26.0
Nausea/vomiting	5.8±14.0
Pain	19.4±25.4
Single item scales	
Dyspnoea	19.4±26.5
Insomnia	31.0±35.9
Anorexia	7.8±20.3
Constipation	6.2±18.2
Diarrhoea	39.5±34.3
Financial impact	10.1±23.6

Data are mean±SD. QoL: Quality of Life.

Note. Scores range from 0 to 100. A high score for the symptom/item scales represents a high level of symptoms/problems, a high score for the functioning scales and the global health or general QoL scales represents a high level of functioning or general QoL.

Discussion

In our study 29.6% of male patients with a metastatic midgut carcinoid tumour and elevated serotonin production reported a clinical relevant sexual problem concerning arousal, erection or orgasm. The prevalence of sexual dysfunction on the different subscales was however for all carcinoid patients studied similar to a Dutch reference population.[11] Only one woman in our population reported sexual dysfunction. This low prevalence could be an underestimation, because 7 out of 16 women were not involved in a relationship. According to the QSD, a person not sexually active is defined as having not a sexual dysfunction. In addition in women, feelings of intimacy are often more important than feelings of sexual arousal, in particular when dealing with illness. In men sexual dysfunction is more visible and thus classified by men as a problem.[8]

In 1974 Feldman et al were the first to study the sexual function of patients with a carcinoid tumour.[5] We can not compare our data properly with Feldman's as they did not used a specific questionnaire. However 5 (50%) out of 10 male patients in their study with a carcinoid syndrome had almost a complete impotence. The urinary 5-HIAA concentration in the patients studied by Feldman was higher (mean 835 μmol/24 hour) so maybe they studied patients with more extensive disease, explaining the higher prevalence of sexual dysfunction.

In accordance with the definition of sexual dysfunction by the DSM IV, the QSD, which was used in our study, explicitly takes into account the experienced burden or distress by patients.[18] This is necessary for a proper judgment of sexual dysfunctions. Data from population studies are also difficult to compare. They use different questionnaires and do not always take into account this experienced distress.[19,20] This underscores the necessity to unify the tools for investigation of sexual dysfunction.

Mean plasma tryptophan concentrations were lower and median urinary 5-HIAA concentrations tended to be higher in 8 male patients with sexual dysfunction. This could be explained by the fact that patients with sexual dysfunction represent the patients with a more extensive and longstanding disease and therefore are more affected by sexual dysfunction.[8] In a recent study of Lindau and colleagues who investigated sexuality and sexual problems in 3005 US adults between 57 to 85 years of age, respondents who rated their health as being poor were more likely to report sexual problems.[21]

Although male patients with midgut carcinoid tumours have increased levels of plasma serotonin, only 7 out of 27 complained of erectile dysfunction. It would be of interest to study the effect of doxazosin and ketanserin in male patients with midgut carcinoid tumours and erectile dysfunction to investigate whether blocking the serotonin receptor inhibits the effect of serotonin on the contraction in the cavernosal tissue, during the erection.[22]

Our data indicate that the use of IFN-alpha may be associated with sexual dysfunction. The influence of IFN-alpha on sexual dysfunction was previously noted in patients with hepatitis C treated with IFN-alpha and was explained by lower testosterone concentrations.[23] Total testosterone concentration were also lower in our patients treated with IFN-alpha, however testosterone concentrations did not differ between patients who reported sexual dysfunction and patients who did not report problems.

Hypogonadism was a frequently occurring condition (in 64% of patients) in a recent study in 48 male patients with advanced cancer.[24] In our population free plasma testosterone concentrations were low (< 300 nmol/L) in about 41% and total testosterone concentrations were below the reference level in about 26% of the male patients. The reference range for free plasma testosterone was obtained in men aged 20 to 54 years while our patients were older and these levels tend to decline during life.[25] The underlying mechanism of low testosterone concentrations is unclear, but next to the influence of IFN-alpha, factors secreted by the carcinoid tumours could play a role. Tachykinins have been shown to inhibit the release of testosterone in vitro.[26] Patients might benefit from testosterone replacement not solely for improvement of sexual function but also for overall well-being.[1, 27]

Ratings on the health related QoL were comparable to those previously reported in patients with midgut carcinoid tumours.[2,3] The global QoL tended to be lower in patients with sexual dysfunction however this was not statistical significant and it can not be ruled out that this is due to more advanced disease.

Overall, our results show that about 30% male patients with midgut carcinoid tumours experience sexual dysfunction, which is comparable to the general population. However, physicians should be aware that sexual dysfunction in patients with midgut carcinoid tumours occurs, especially in case of longstanding disease, with low plasma tryptophan concentrations and during IFN-alpha use.

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Chapter 7

^{123}iodine-Metaiodobenzylguanidine scintigraphy in localising phaeochromocytomas – experience and meta-analysis

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Abstract

Introduction

Recommendations on the use of [123]iodine(I)-metaiodobenzylguanidine (MIBG) scintigraphy in localising phaeochromocytomas vary. We determined the accuracy of ^{123}I-MIBG scintigraphy by evaluating our own ^{123}I-MIBG scans and performing a meta-analysis.

Patients and methods

Between January 1992 and May 2002, the ^{123}I-MIBG scans of consecutive patients suspected of a phaeochromocytoma were re-evaluated. For the meta-analysis, we selected studies with more than five ^{123}I-MIBG scans.

Results

Thirty patients were evaluated. The sensitivity in our own population was 92%, specificity was 100%. Twenty-one articles were selected for our meta-analysis. Overall sensitivity and specificity was 96% and 100% respectively. Sensitivity and specificity for tumours in the adrenal gland was 98% for both. For tumours located outside the adrenal gland sensitivity was 98%. Sensitivity for malignancies was 79%.

Conclusion

^{123}I-MIBG scintigraphy has an excellent sensitivity and specificity in localising phaeochromocytomas except for malignant tumours. ^{123}I-MIBG scintigraphy is superior in localising tumours outside the adrenal gland.

Introduction

Phaeochromocytomas are rare catecholamine-secreting neuroendocrine tumours arising from chromaffin tissue of the adrenal gland or extra-adrenal paraganglia.[1] Phaeochromocytoma arising from the extra-adrenal paraganglia are also named extra-adrenal phaeochromocytomas. About 80-85% of the phaeochromocytomas arise from the adrenal gland, about 15-20% arise from extra-adrenal paraganglia, in the retroperitoneum, in the mediastinum, the pelvic region, and in the head and neck region.[2,3] The suspicion of a phaeochromocytoma is based on clinical signs and symptoms and includes a medical history of hypertension, episodic "spells" (i.e. headache, palpitation, sweating, pallor) or a family history of syndromes known to be associated with phaeochromocytomas.[4-7] Laboratory investigations consist of the demonstration of biochemical excess of catecholamine and/or metabolites in blood and/or urine and should be performed for biochemical confirmation prior to the localisation of the tumour. Localisation of the tumour is necessary for surgical removal. This can be achieved by computed tomography (CT), magnetic resonance imaging (MRI) or metaiodobenzylguanidine (MIBG) scintigraphy. CT or MRI is mandatory for accurate anatomical information, especially since the introduction of laparoscopic techniques.[8,9]

Sensitivity for tumour localisation varies between 87%-100% for CT and 91%-98% for MRI.[10-14] Studies comparing CT and MRI are in favour for MRI.[13-15] Sensitivity is about 20% lower for phaeochromocytomas outside the adrenal gland, compared to phaeochromocytomas in the adrenal gland.[14,16] The specificity of MRI is reported to be higher compared to the specificity for CT for tumours of the adrenal gland, between 88 and 100% for MRI.[4,14,17-19]

Wieland and colleagues developed the use of whole body MIBG scintigraphy in 1980.[20] MIBG is a guanithidine analogue that is structurally similar to norepinephrine and is taken up by adrenergic storage vesicles in the adrenal gland and the paraganglia, thus visualising neuroendocrine tissue. MIBG can be labelled with [131]iodine(I) or [123]I. The sensitivity of ^{131}I-MIBG scintigraphy is limited and ^{123}I-MIBG scintigraphy was introduced to improve the image quality and to increase sensitivity.[21] However, ^{123}I-MIBG is not widely available and more expensive. Recommendations on the use of MIBG scintigraphy in localising a phaeochromocytoma depend on the author. It is preferred as an initial diagnostic localising modality because whole body images can be obtained.[14,22-26] It is recommended additionally to MRI or CT to rule out multiple lesions outside the adrenal

gland and for confirmation of the lesion found on CT or MRI.[15,27-32] Other authors do not recommend to routinely perform a MIGB scintigraphy.[19,33-35] These diverse recommendations have several drawbacks. Most recommendations are based on studies using ^{131}I as a label since this was introduced before the ^{123}I-MIBG scintigraphy and because of better availability of ^{131}I-MIBG in the USA. Furthermore, studies using ^{123}I-MIBG as a label are, because of the rarity of the disease, mostly retrospective and study population is relatively small.

The aim of this study was to determine the diagnostic accuracy of ^{123}I-MIBG scintigraphy in localising a phaeochromocytoma and to propose a diagnostic approach for localisation. We reviewed our ^{123}I-MIBG scans and carried out a meta-analysis of previous studies, investigating the sensitivity and specificity of ^{123}I-MIBG scintigraphy.

Patients and methods

Patients

Between January 1992 and May 2002 all consecutive patients suspected of a phaeochromocytoma at the Department of Endocrinology were studied. Thirty-four patients were included. They had typical signs and symptoms combined with a consistent (>2 occasions) increase of urinary excretion of the fractionated metanephrines (i.e. normetanephrine and metanephrine). Patients whose ^{123}I-MIBG scintigraphy was not available for reanalysis (*n*=1) and patients without a final diagnosis (*n*=3), were excluded. The charts of the remaining 30 patients were retrospectively reviewed for data about age, sex, the presence of a familial syndrome, lab investigations, radiology examinations and therapy. For the gold standard we used the pathology reports, if patients were operated. In those cases where histopathology was lacking, independent physicians were asked to review the case.

Laboratory investigations

The urinary excretion of fractionated normetanephrine and metanephrine was used for biochemical proof of increased catecholamine secretion. Fractionated total normetanephrine and metanephrine were determined using extractive derivatisation and stable isotope gas chromatography with mass fragmentographic detection.[36] Values more than 1.5 times above the upper limit (normal value metanephrine: 33-99 μmol/mol

creatinine, normal value normetanephrine: 64-260 μmol/mol creatinine) were considered abnormal.

123I-MIBG scintigraphy

All MIBG scans were performed using 123I as a label. Injected dose was 185 MBq. Scans were acquired after 24 hours on dedicated gamma camera (DIACAM or MultiSPECT 2, Siemens, Hoffman Estates, IL, USA), in a 256 matrix, zoom factor 1.23, using a 15% window center around I-123 photopeak of 159 keV. The original 123I-MIBG scans were re-evaluated by two experienced observers who were unaware of the clinical circumstances. Each independently rated the intensity the lesions, ranging from 0 to grade 3. A score of 0 represented no uptake, grade 1 low uptake, lower than the liver, grade 2 moderate uptake equal to the liver and grade 3 intense uptake, more than the liver. Grade 2 and 3 were defined as MIBG positive. A consensus was reached if there was a discrepancy between the two observers.

Gold standard

The histopathological diagnosis was considered the gold standard. A distinction was made between a phaeochromocytoma and hyperplasia. Hyperplasia was defined as glands harbouring tumour nodules less than 1 cm. Normalisation of the urinary excretion of the catecholamines metabolites was considered proof of absence of disease. Three independent physicians reviewed the medical charts of patients with persistent elevated urinary excretion of the catecholamine-metabolites in whom no histopathological diagnosis was obtained. They were unaware of the clinical outcome. Their consensus was considered the gold standard.

Search strategy

For the meta-analysis, we searched the literature. We selected studies, on the value of 123I-MIBG scintigraphy, including studies with more than 5 scans. We used the clinical database MEDLINE (PubMed) using the following MESH headings and/or text words: MIBG, metaiodobenzylguanidine, MIBG scintigraphy, localisation, phaeochromocytoma, paraganglioma, sensitivity, and specificity. We also used the references of the identified papers. Only studies published in America or Europe were included. Studies were excluded if data from the same patients were used in more than one publication. We used the paper that included the most patients in such cases.

Statistical analysis

Using the mentioned golden standard for presence and absence of a phaeochromocytoma the MIBG scintigraphy was classified as true positive, true negative, false positive, and false negative on a patients level.

For the meta-analysis, we used information of each ^{123}I-MIBG scintigraphy given in the several studies. Because the number of studies overall, showed no heterogeneity regarding the effect estimates (sensitivity and specificity), we show the results of the pooled-analyses of sensitivity and specificity in the studies using fixed effects models using continuity correction. Fixed effects analysis computes the summary estimates of the sensitivity and specificity with its confidence interval of the individual studies, and Woolf's test for heterogeneity. The method that is presented in the table shows standard meta-analysis estimates and the 95% confidence interval with continuity correction for each study is given. Sensitivity has, by definition, been truncated at 1.0. Sensitivity of ^{123}I-MIBG scintigraphy for phaeochromocytoma inside the adrenal gland, outside the adrenal gland, and for malignant disease has been computed. Meta-analyses were performed using the Rmeta package of the R Project for Statistical Computing (Build 1.8.1).

Results

Patients

Thirty patients were included. Median age was 44 years (range 23-78 years), 12 (40%) were men. Ten patients (33%) were evaluated because of a familial syndrome, 8 because of multiple endocrine neoplasia (MEN) type 2a, two because of Morbus Recklinghausen. Final diagnosis was phaeochromocytoma in 26 patients (87%). In 24 out of 26 patients, there was histopathological proof, in 2 patients the judgement of the reviewers served as the gold standard. Both patients had recurrent disease, 1 patient was not operated because of malignant disease, in the other patient imaging showed no tumour although he had a rise in the urinary excretion of normetanephrines 15 years after the resection of a phaeochromocytoma outside the adrenal gland. The tumour was located in the adrenal gland in 20 of 26 patients (77%), in 4 patients (15%) it was located outside the adrenal gland and 2 patients (8%) had malignant disease.

Four patients (13%) were classified with another diagnosis, 3 patients had a spontaneously normalisation of the urinary excretion of catecholamine-metabolites and remained free of symptoms after a median follow-up of 3 years. The remaining patient was analysed because of abdominal pain, spells and marginally raised urinary excretions of metanephrines. She was operated because of a suspected phaeochromocytoma outside the adrenal gland. During the operation no phaeochromocytoma could be detected and post-operatively the urinary excretion of metanephrines normalised spontaneously.

Sensitivity and specificity of 123I-MIBG scintigraphy in our own population (Table 1)

The 123I-MIBG scintigraphy was false negative in 2 patients. One 123I-MIBG scan did not localise a phaeochromocytoma in the right adrenal gland in a patient with MEN 2a syndrome and bilateral phaeochromocytoma. The tumour in the right adrenal gland had a diameter of 2.4 cm.

Table 1. Results of re-evaluation of 123I-MIBG scintigraphy

Final diagnosis	MIBG true positive	MIBG false negative	Total
Phaeochromocytoma (total)	24	2	26
In adrenal gland	19	1	20
MEN 2a	6	1	7
Unilateral	5	0	5
Bilateral	1	1	2
Recklinghausen	2	0	2
Sporadic	11	0	11
Outside adrenal gland	3	1	4
Malignant	2	0	2
Other diagnosis, no phaeochromocytoma	0	4	4

MEN denotes multiple endocrine neoplasia.

Another false negative scan concerned the patient with the recurrent phaeochromocytoma 15 years after the resection of a phaeochromocytoma outside the adrenal gland. Thereby the sensitivity of the 123I-MIBG in the localisation of a phaeochromocytoma was 92%. There were no false positive results, thereby specificity was 100%. The patient operated on because of a suspected phaeochromocytoma, original had a positive scintigraphy, although the revised scintigraphy was negative.

An additional single photon emission computed tomography (SPECT) view was available in 15 patients. It was of additional value in 2 patients, visualising a tumour in the adrenal gland that was not visualised with the planar view.

Meta-analysis

Using the mentioned search strategy we identified 20 articles studying the sensitivity and/or specificity of the ^{123}I-MIBG scintigraphy. Twelve articles were included in meta-analysis.[12,19,24,28,34,35,37-42] Nine articles were excluded. Three articles from de Graaf and colleagues studied similar patients, the one that studied the most patients, was included in our meta-analysis.[11,33,38] Two studies did not provide information about the final diagnosis.[21,43] Three studies could not be included because no distinction was made between ^{131}I-MIBG and ^{123}I-MIBG scan.[10,44,45] One further study was excluded because phaeochromocytomas as well as non-functioning paragangliomas were included, leaving 12 articles for meta-analysis. The results are shown in Table 2. The overall sensitivity and specificity was 96% and 100% respectively. For phaeochromocytomas in the adrenal gland sensitivity and specificity both were 98%. Sensitivity for tumours located outside the adrenal gland was 98% and sensitivity for malignant disease was 79%.

Table 2. Pooled sensitivity and specificity of ^{123}I-MIBG scintigraphy, including own patients

		Nº observations
Sensitivity [12,19,24,28,34,35,37-42]	96 (94-99)	303
Specificity [12,19,24,28,37,40,41]	100 (99-100)	207
Sensitivity in adrenal gland [12,19,28,34,41,42]	98 (95-100)	133
Specificity in adrenal gland [24,35]*	98 (95-100)	357*
Sensitivity outside adrenal gland [12,19,24,28,42]	98 (91-100)	22
Sensitivity malignancy [35]	79 (37-100)	16

Data are % with 95% confidence interval * Based on numbers of adrenal glands.

Discussion

This is the first meta-analysis concerning the accuracy of ^{123}I-MIBG scintigraphy, confirming that it is an excellent diagnostic tool in localising a phaeochromocytoma with an overall sensitivity and specificity of 96% and 100% respectively.

The accuracy of [123I]-MIBG scintigraphy appears to be superior in the visualisation of phaeochromocytomas located outside the adrenal gland compared to the in the literature reported sensitivity of [131I]-MIBG scintigraphy, CT and MRI. In our meta-analysis sensitivity was 98% for pheochromocytomas located outside the adrenal gland, compared to a sensitivity of 80% for CT and MRI and 64% for [131I]-MIBG scintigraphy.[14,16,19] Although other studies also have reported this high sensitivity for tumours outside the adrenal gland, they were based on very few patients. Our meta-analysis includes 22 observations. We were not informed about possible interfering medication in the separate studies, so the sensitivity of [123I]-MIBG scintigraphy could even be higher. Therefore for patients suspected of phaeochromocytoma, and no tumour localisation on MRI and/or CT one must always perform an [123I]-MIBG scintigraphy to rule out tumours outside the adrenal gland (Figure 1). A complementary MRI or CT after the localisation with [123I]-MIBG scintigraphy is helpful for anatomical information.

Our meta-analysis has a drawback, since we excluded some papers. We found a high specificity for phaeochromocytomas in the adrenal gland. This high specificity of [123I]-MIBG scintigraphy is probably overestimated in patients with MEN type 2a. We excluded the article of de Graaf and colleagues, in which they studied patients with MEN type 2a. The [123I]-MIBG scintigraphy was false positive in 5 out of 6 adrenal glands, 4 of them being classified as hyperplastic glands.[33] Although hyperplasia is probably a precursor of a phaeochromocytoma, there is no evidence that a hyperplastic gland should be operated on.[46,47] In conclusion, for patients with MEN type 2a, because of the risk of visualising a contralateral normal or hyperplastic gland, [123I]-MIBG scintigraphy should probably not be performed (Figure 1).[21,33,35,43]

On the other hand, in patients, without MEN type 2a, we suggest to perform an [123I]-MIBG scintigraphy for confirmation of the tumour detected by MRI or CT. This can exclude the possibility of an incidentaloma and the possibility that the phaeochromocytoma is located outside the adrenal gland. These incidentalomas were found in 1.5% of CT scans in a large survey.[48]

Although in our population [123I]-MIBG scintigraphy showed 1 malignant lesion not visualised with MRI, the diagnostic value of [123I]-MIBG scintigraphy for malignant tumours is disappointing. The sensitivity of 79%, however is based on only 16 patients. Affinity towards [123I]-MIBG appears to be low for malignant tumours because dedifferentiated tumours can lose their ability to accumulate MIBG. In these patients whole body MRI or 6-[^{18}F]-Dopamine positron emission tomography (PET) is an alternative.[49]

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┌─────────────────────────────────────────────────────────────────┐
│   Clinical suspicion and biochemical confirmation of a            │
│                     phaeochromocytoma                             │
└─────────────────────────────────────────────────────────────────┘
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                    ┌───────────────────────┐
                    │      MRI or CT of      │
                    │    the adrenal area    │
                    └───────────────────────┘
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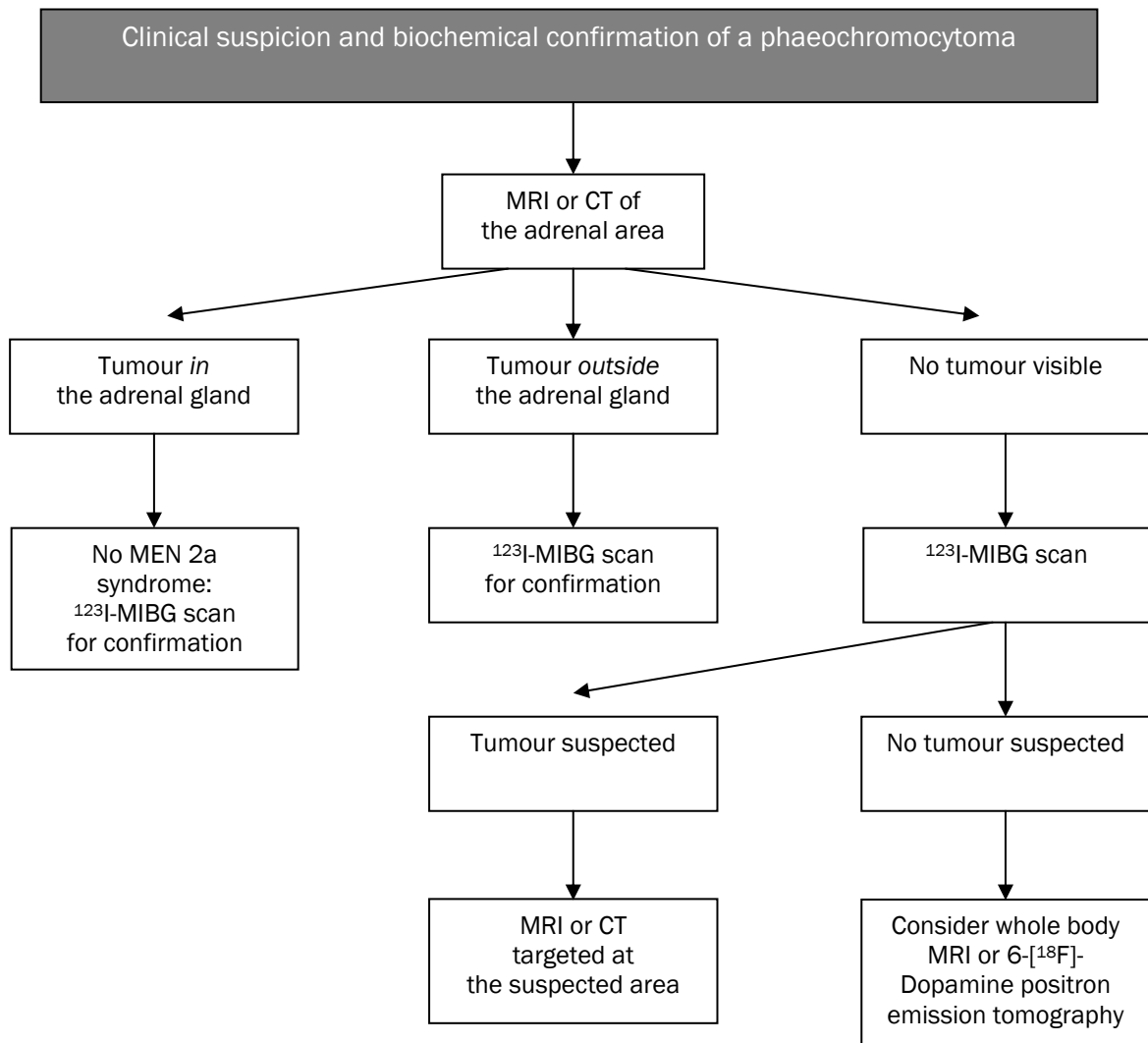
Figure 1. Diagnostic approach in localising a phaeochromocytoma

We conclude that the diagnostic value of ^{123}I-MIBG scintigraphy is excellent. It should always be performed when MRI or CT fails to localise a tumour, which is more likely to occur when the tumour is located outside the adrenal gland. When MRI or CT localises a tumour in the adrenal gland in patients with MEN type 2a, ^{123}I-MIBG scintigraphy should not be performed. The role of ^{123}I-MIBG scintigraphy in malignant disease is disappointing and remains unclear. In summary Figure 1 shows the proposal for imaging studies in localising a phaeochromocytoma.

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Chapter 8

Summary and future perspectives

Summary

Neuroendocrine tumours (NET) are a group of rare tumours, arising from cells of the neuroendocrine system. To this group belong midgut carcinoid tumours originating from the enterochromaffin cells of the bowel and phaeochromocytomas arising from chromaffin cells in the medulla of the adrenal gland and paraganglia.

The aim of this thesis is to evaluate the course and follow-up of patients with NETs focussing on the midgut carcinoid tumours and phaeochromocytomas.

Chapter 1 is a general introduction outlining the content of this thesis.

In **chapter 2** we present an overview of the literature concerning symptoms in patients with the carcinoid syndrome. This syndrome consists of symptoms such as diarrhoea, flushing and carcinoid heart disease. Most of the symptoms are caused by the secretion of biogenic amines or polypeptides, with serotonin being the most important biogenic amine secreted by the tumour. The carcinoid syndrome usually develops at the moment when large amounts of these factors enter the systemic circulation such as in patients with liver metastases. Therapy generally aims at lowering the concentration of secreted factors, but more specific interventions e.g. specific receptor blockers, inhibiting the effect of the secreted factor may also be useful. Disseminated midgut carcinoid tumours can in addition lead to pellagra and psychiatric problems. Pellagra, and maybe also psychiatric symptoms, are due to a depletion of the essential amino-acid tryptophan, competitively consumed by the carcinoid tumour for serotonin synthesis.

Follow-up of patients with disseminated midgut carcinoid tumours includes measuring the urinary excretion of 5-hydroxyindolacetic acid (5-HIAA), plasma chromogranin A (CgA) concentrations, nuclear scans like ^{111}In-octreotide scintigraphy (SRS), computed tomography (CT) and for screening of carcinoid heart disease echocardiography. However the optimal timing of these investigations is not yet totally clear.

In **chapter 3.1** we studied prognostic factors for survival in patients with disseminated midgut carcinoid tumours. Previously identified unfavourable factors for survival are high age, high urinary 5-HIAA concentrations at first visit, high plasma CgA concentrations, the presence of liver or lymph node metastases, the presence of carcinoid heart disease, the tumour size, and the histological grade of differentiation. We analysed 76 patients referred to the University Medical Centre Groningen between 1992 and 2003, with a

median survival from diagnosis of 74 months (95% confidence interval (CI) 61-88 months).

Prognostic factors for poor survival at referral were high age, high gamma-glutamyltransferase levels and high urinary 5-HIAA concentrations. In a multivariate survival analysis with the urinary 5-HIAA concentration as a time dependent variable, the urinary 5-HIAA concentration was an independent prognostic factor for worse survival. (Hazard Ratio (HR) 1.007 (95% CI 1.004-1.010, P=0.000). This implies that patients with persistent low urinary 5-HIAA concentration have favourable outcome. Given the numerous drugs that seem to become available for these tumours it is helpful, in addition to radiological tumour measurements, to have a non-invasive marker that indicates prognosis during follow-up. Thus the urinary 5-HIAA concentration can assist in identifying patients who will benefit from a more intense follow-up and more aggressive management.

Chapter 3.2 is a response to an article of Møller and colleagues addressing the topic of progression of carcinoid heart disease.[1] One conclusion of their study was that somatostatin analogues did not prevent progression of carcinoid heart disease. In a series of 73 patients with the carcinoid syndrome treated in our hospital between 1985 and 2002, we showed that the median urinary 5-HIAA concentrations gradually increased during follow-up and that in our population urinary 5-HIAA concentrations were lower compared to the referral population of Møller and colleagues. There are several data suggesting that serotonin is involved in the development of carcinoid heart disease, and somatostatin analogues inhibit the release of serotonin. Therefore the potential beneficial effects of somatostatin analogues on the prevention of carcinoid heart disease are not precluded in less advanced carcinoid tumours as seen in our hospital.

In **chapter 4** the effects of the functional polymorphisms of the serotonin transporter (*5-HTT*) and Monoamine Oxidase-A (*MAO-A*) gene in patients with disseminated midgut tumours are described. Both genes are involved in the metabolism of serotonin. We hypothesized that, given the role of serotonin, the functional polymorphisms of the *5-HTT* and the *MAO-A* could affect the symptoms of the carcinoid syndrome and survival. One hundred and five patients were genotyped for the promoter region of the *5-HTT* gene (*5-HTTLPR*) and the polymorphic region of the MAO-A gene (upstream variable number of tandem repeats (*uVNTR-MAO-A*)). For this study we reclassified the triallelic *5-HTTLPR* in a biallelic model, according to previous studies; into a short (S', functional less active variant) and long (L') variant.[2]

Patients with the S'S' genotype (*n*=29) of the *5-HTTLPR* had a shorter overall survival, median 69.6 months (95% CI 20.8-118.3 months) compared to the patients with L'L' (*n*=26) and L'S' genotypes (*n*=49) who had a median survival of respectively 91.2 months (95% CI 41.4-140.8 months) and 114.6 months (95% CI 41.4-140.8 months) (P=0.001). No association was observed between the different genotypes of the *5-HTTLPR* or *uVNTR-MOA-A* and symptoms of flushes or diarrhoea at the moment of referral. Seven out of 26 patients (26.9%) with the L'L' genotype developed carcinoid heart disease compared to 4 of the 49 patients (13.8%) with the L'S' and 4 out of 26 patients (8.2%) with the S'S' genotype (P=0.09).

In the multiple logistic regression analysis the S'S' genotype of the 5-HTTLPR, high age and high urinary 5-HIAA concentrations were independent predictors of dying within 7, 8, 9 and 10 years after referral. Patients with the S'S' genotype had an increased risk to die within 10 years after referral compared to patients with the L'S' genotype with an odds ratio (OR) of 4.36 (95% CI 1.33-14.30, P=0.02). The shorter survival in patients with the S'S' variant can be explained by the fact that these patients are probably exposed to high levels free serotonin for a longer period of time. Free serotonin is known for its several acute and chronic, especially cardiovascular harmful effects, like carcinoid heart disease. Genotyping patients with midgut carcinoid tumours for *5-HTTLPR* can help identify patients who should be followed intensely with a more aggressive therapeutic approach.

In **chapter 5** we show the results of a feasibility study. Nine patients with advanced NETs were treated with interferon (IFN)-alpha-2b (2.5 million U/day subcutaneously), and after 2 weeks, intravenous 5-fluorouracil (5-FU) 750 mg/m² (day 2) and oral leucovorin 180 mg/day (day 1 and 2) was added as a 2-weekly cycle. This combination was administered because of promising results obtained by others.[3] The combination resulted in numerous side effects and only 3 patients completed more than 4 cycles. Interestingly serum levels of the angiogenic vascular endothelial growth factor (VEGF) decreased after treatment in 7 out of 8 evaluable patients with a median of 40% namely from a median level of 352 pg/ml to 219 pg/ml. Less toxic combinations should be explored, also in combination with new antiangiogenic drugs, like bevacizumab which already showed antitumour activity in NETs.

In **chapter 6** a study is described which analyses the occurrence of sexual dysfunction in patients with disseminated midgut carcinoid tumours. These patients may be at risk of developing sexual dysfunction as serotonin plays in the brain a role in normal sexual behaviour. Data suggest that patients with midgut carcinoid tumours have serotonergic

dysfunctions in the brain. This is considered to be due to low tryptophan concentration in the brain as a consequence of tryptophan consumption by the tumour and the fact that serotonin does not cross the blood brain barrier. Furthermore serotonin has vasoactive properties that may lead to erectile problems and patients can suffer from pain, fatigue and use medication that can influence the sexual function. In this study 43 patients with disseminated midgut carcinoid tumours, 27 men and 16 women filled in a validated questionnaire for screening sexual dysfunction (QSD). The results were compared to a Dutch reference population. Dysfunction was defined as a sexual problem occurring at least regularly and experienced as at least stressful. Respectively 6 (22.2%), 4 (14.8%), and 7 (25.9%) of the male patients experienced sexual problems regarding arousal, orgasm and erection. One female patient (6.3%) experienced a problem regarding orgasm. Compared to the Dutch reference population, no differences were found.

Male patients with a sexual dysfunction on any of the selected subscales had, compared to those without a sexual dysfunction, a longer duration of disease, namely 95.3 months (range 5.4-314.5 months) versus 18.6 months (range 0.6-167.9 months) (P=0.024), lower mean±SD plasma tryptophan concentrations of respectively 31.5±16.1 µmol/L and 48.9±14.5 µmol/L (P=0.031) and more frequent IFN-alpha use, respectively 4 out of 8 patients (50%) and 2 out of 19 (10.5%) (P=0.044).

In conclusion patients with disseminated midgut carcinoid tumours do not experience sexual dysfunction more often compared to a population based reference population. However, physicians should be aware that sexual dysfunction in patients with midgut carcinoid tumours occurs especially in case of longstanding disease, with low plasma tryptophan concentrations and during IFN-alpha use.

Finally, in **chapter 7** we studied the diagnostic efficacy of [123]iodine(I)-Metaiodobenzylguanidine (MIBG) scintigraphy in patients with a phaeochromocytoma. We evaluated the ^{123}I-MIBG scintigraphy of 30 patients and performed a meta-analysis selecting studies from the literature with more than five ^{123}I-MIBG scans. The sensitivity in our population was 92%, specificity was 100%. Overall sensitivity and specificity in the meta-analysis was 96% and 100% respectively. Sensitivity and specificity for tumours in the adrenal gland was 98% for both. For tumours located outside the adrenal gland the sensitivity was 98%. Sensitivity for malignant phaeochromocytomas however was only 79%. ^{123}I-MIBG scintigraphy is superior over reported accuracy of computed tomography (CT) or magnetic resonance imaging (MRI) in localising tumours outside the adrenal gland.

Future perspectives

During the last decades advances in the diagnostics and treatment of the relatively rare occurring NETs have been made. Patients with midgut carcinoid tumours often present with metastases. In this circumstance cure can hardly ever be achieved. Even when the disease is incurable, the prognosis is relatively good compared to other types of cancer, because of the often low-grade malignant characteristics of these tumours. Prognosis however, varies to a great extent between patients, and survival up to 20 years has been observed.

Several prognostic factors have been identified, but in general (clinical) practice and in treatment guidelines these factors do not seem to influence treatment or the intensity of follow-up of patients with midgut carcinoid tumours. It is interesting to investigate the effect of using combinations of known combined prognostic factors, i.e. patients with the S'S' genotype and urinary 5-HIAA concentration above 20 mmol/mol creatinine, on survival. Better knowledge could support improved evidence based follow-up frequencies for subgroups of patients. Given the rarity of midgut carcinoid tumours, an (inter)national database combined with a serum and tissue bank seems the way to answer these questions. This strategy could be favourable for other rare neuroendocrine tumours.

Prognostic factors, like the urinary 5-HIAA concentration, might become important to identify patients who are eligible for, and who will benefit from, new treatment modalities. Currently several randomised studies are in progress to determine the effect of angiogenesis inhibitors and inhibitors of the mammalian target of rapamycin (mTOR).[4-6] In these studies patients with radiological progressive disease are included, and regression of the identified lesions serves as outcome measure. To identify eligible patients frequent scans are required to prove progression. Since the urinary 5-HIAA concentration is a marker of impaired outcome, using the urinary 5-HIAA concentrations as an inclusion criterium, might make more patients eligible for inclusion

New diagnostic modalities for patients with midgut carcinoid patients, like 6-[^{18}F]-fluoro-L-dihydroxyphenylalanine (^{18}F-DOPA) positron emission tomography (PET), and [^{11}C]-5-hydroxytryptophan (5-HTP) PET with higher sensitivity compared to conventional imaging may play a role in treatment decisions.[7,8] Although ^{18}F-DOPA-PET is already used in patients with phaeochromocytomas, additional studies in both benign and malignant phaeochromocytomas will have to confirm its role.[9] The same accounts for PET scanning

using various different tracers in other NETs, e.g. those NETs occurring in familial syndromes. For instance, if these new imaging techniques show more (extensive) lesions compared to convential imaging techniques, the prognostics value has to be considered before treatment decisions could be based on these findings.

Imaging techniques will also have to focus on carcinoid heart disease. Until now the golden standard for diagnosing carcinoid heart disease is echocardiography. New cardiac imaging techniques like cardiac CT or cardiac MRI are more and more available and perhaps more sensitive in diagnosing carcinoid heart disease, although there are also developments in echocardiography.[10,11]

Patients with midgut carcinoid tumours survive relatively long after diagnosis, interest in the quality of life of the patients is essential. The quality of life in general refers to the patient's ability to enjoy activities in daily life. Many aspects can influence this quality, i.e. specific symptoms, concerns about the future, and factors like sexual function. New developments in anti-cancer treatment but also guidelines for follow-up of patients will have to take into account its effects on the quality of life.

Plasma testosterone concentrations are low in a subgroup of male patients with midgut carcinoid tumours. Supplementation with testosterone might be beneficial in male patients complaining of decreased libido and erectile dysfunction, and perhaps also beneficial for overall well being. However, until now this is not investigated and the underlying mechanism for low testosterone concentrations is not yet elucidated.

Finally, giving the increasing options for diagnosis and treatment of patients with NETs optimal approach will more and more require a multidisciplinary task. There are many specialists involved like medical oncologists, endocrinologists, nuclear medicine specialists, surgeons, pathologists, gastroenterologists, cardiologists, clinical chemists, and psychologists helping patients try to deal with these tumours with such a diversity of problems.

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Chapter 9

Dutch summary - Nederlandse samenvatting

Neuro-endocriene tumoren zijn zeldzame tumoren, die zowel goedaardig als kwaadaardig kunnen zijn. De tumoren zijn opgebouwd uit neuro-endocriene cellen. Gewoonlijk kunnen deze cellen hormoon producerende klieren vormen (bijvoorbeeld bijschildklieren, bijnieren). Daarnaast komen (neuro)endocriene cellen verspreid in verschillende organen voor: het diffuse neuro-endocriene systeem. De cellen zijn op verschillende plaatsen in het lichaam aanwezig en zijn in staat om allerlei stoffen produceren en uit te scheiden. Deze stoffen (zoals vasoactive aminen, polypeptiden en prostaglandines) hebben verschillende effecten op het lichaam. Bij patiënten met neuro-endocriene tumoren komen klachten voor die veroorzaakt worden wanneer de stoffen in grote hoeveelheden uitgescheiden worden. Dit geldt zowel voor goedaardige als kwaadaardige neuro-endocriene tumoren.

Het doel van dit proefschrift is om het beloop en de follow-up van patiënten met neuro-endocriene tumoren te beschrijven. Hierbij hebben we ons gefocust op twee neuro-endocriene tumoren.

Ten eerste de carcinoïde tumoren van de middendarm. Dit zijn kwaadaardige tumoren die ontstaan uit neuro-endocriene cellen, die verspreid in de darm liggen en vaak in staat zijn om onder andere serotonine (vasoactive amine) te produceren.

Ten tweede het feochromocytoom, een (meestal) goedaardige tumor die ontstaat uit neuro-endocriene cellen gelegen in het bijniermerg, die leidt tot een overmaat aan adrenaline en noradrenaline (vasoactive aminen).

In **hoofdstuk 2** wordt een overzicht gegeven van de verschillende symptomen die kunnen optreden bij patiënten met een carcinoïde tumor van de middendarm gegeven. Klachten kunnen ontstaan door de ligging en grootte van de tumor zelf, zoals darmobstructie, maar ook door overproductie van stoffen, voornamelijk serotonine. De combinatie van klachten die hierbij optreedt, wordt carcinoïdsyndroom genoemd. De drie belangrijkste symptomen van dit syndroom zijn diarree, flushes (opvliegers) en carcinoïde hartziekte. Diarree en flushes treden op in ongeveer 80% van de patiënten. De diarree wordt veroorzaak door serotonine en het feit dat bij de resectie van de primaire tumor een gedeelte van de darm wordt weggehaald. De oorzaak van de flushes is niet geheel duidelijke zowel serotonine als andere stoffen; tachykininen, catecholaminen en histamine worden hiervoor verantwoordelijk gehouden. Flushes kunnen worden uitgelokt door het eten van bepaald voedsel of drinken van bijvoorbeeld alcohol. Carcinoïde hartziekte wordt gekenmerkt door

schade aan de hartkleppen, vooral aan de rechterzijde van het hart (de tricuspidalis- en de pulmonalisklep). In deze kleppen treedt bindweefselvorming op (fibrosering), waarbij er zowel een vernauwing als een lekkage van die kleppen kan optreden. Dit kan uiteindelijk tot falen van de rechterhartkamer leiden. Patiënten met carcinoïde hartziekte hebben een slechtere overleving dan carcinoïd patiënten zonder hartziekte. Het vaststellen van carcinoïde hartziekte vindt plaats door middel van echocardiografie. In de toekomst zal onderzocht kunnen worden of nieuwere afbeeldingstechnieken zoals computertomografie (CT) of magnetic resonance imaging (MRI) een plaats hebben in het vaststellen van carcinoïde hartziekte.

Het carcinoïdsyndroom treedt op als er al uitzaaiingen (metastasen) in de lever zijn. Serotonine, uitgescheiden door de tumor, wordt in de lever afgebroken tot 5-hydroxyindolazijnzuur (5-HIAA). Wanneer grote hoeveelheden serotonine (en andere stoffen) in de bloedsomloop (circulatie) terechtkomen, en niet in de lever afgebroken worden, krijgt een patiënt klachten. De overproductie van serotonine door de tumor kan tevens leiden tot een tekort aan tryptofaan, het essentiële aminozuur dat een bouwsteen is voor de aanmaak van serotonine, maar ook belangrijk is voor gewone lichaamsprocessen. Zo is tryptofaan nodig voor de aanmaak van serotonine in de hersenen (serotonine passeert de bloedhersenbarrière niet) en voor de aanmaak van niacine (vitamine B3). Een niacine tekort leidt tot pellagra, een aandoening gekenmerkt door huidafwijkingen, dementie en diarree. Serotonine tekort in de hersenen (door een tryptofaan gebrek in de hersenen) kan leiden tot een verstoring van de slaap, de gemoedstoestand en het libido.

Verschillende laboratorium bepalingen en beeldvormend onderzoek kunnen verricht worden om carcinoïde tumoren van de middendam te diagnosticeren en de uitgebreidheid vast te stellen. Dit kan zowel bij presentatie als gedurende de follow-up. De meest gebruikte laboratoriumbepalingen zijn de concentratie serotonine in trombocyten (voornamelijk bij het diagnosticeren) en de 5-HIAA concentratie in de urine en het chromogranine A gedurende de follow-up. Beeldvormende technieken om de omvang en lokalisatie van uitzaaiingen vast te stellen zijn echografie, CT, MRI, somatostatinereceptorscintigrafie en tegenwoordig met positron emissie tomografie (PET) met verschillende metabole tracers. Verder is het van belang om regelmatig een echocardiogram te verrichten om carcinoïde hartziekte op te sporen. Het optimale moment en het interval waarin deze onderzoeken zouden moeten plaatsvinden is nog niet duidelijk en de meningen van experts op dit gebied verschillen.

In geval van uitgezaaide ziekte is de behandeling meestal is gericht op het verminderen van klachten, het verbeteren van de kwaliteit van leven en het verbeteren van de levensverwachting. Somatostatine analogen verminderen de uitscheiding van de geproduceerde stoffen, zoals serotonine, waardoor klachten verminderen. Naast somatostatine analogen is interferon (IFN)-alfa geregistreerd voor de behandeling van deze tumoren. Van beide middelen is aangetoond dat in ongeveer 70% van de patiënten een vermindering optreedt van de klachten.

De 5-jaarsoverleving van patiënten met een gemetastaseerde carcinoïde tumor van de middendarm is ongeveer 60%. De overleving verschilt aanzienlijk tussen patiënten. Ondanks het feit dat de tumor gemetastaseerd is, zijn er patiënten met een lange (> 20 jaar) overleving. Het identificeren van patiënten met een slechte overleving na diagnose, maar ook gedurende de follow-up zou van invloed kunnen zijn op de frequentie van follow-up en op de beslissing van het starten van nieuwe behandelingen. In **hoofdstuk 3.1** worden prognostische factoren van overleving beschreven, zowel bij presentatie als gedurende de follow-up.

Tussen 1992 en 2003 werden er op de afdeling Medische Oncologie in het Universitair Medisch Centrum Groningen (UMCG) 76 patiënten met een gemetastaseerde carcinoïde tumor van de middendarm behandeld. Gegevens, zoals leeftijd bij diagnose, de aanwezigheid van uitzaaiingen, 5-HIAA concentraties in de urine, leverenzymen (gamma GT en alkalisch fosfatase) bij initiële presentatie en gedurende follow-up werden geanalyseerd. De patiënten hadden een gemiddelde overleving van 74 maanden. Oudere patiënten, patiënten met en een hoog gamma GT en met een hoge 5-HIAA concentratie (> 20 mmol/mol kreatinine) in de urine hadden een slechtere overleving. Patiënten met een 5-HIAA concentratie > 20 mmol/mol kreatinine in de urine bij presentatie hadden een 5-jaarsoverleving van 26%, patiënten met een 5-HIAA concentratie ≤ 20 mmol/mol kreatinine in de urine bij presentatie een 5-jaaroverleving van 72%. Gedurende de follow-up bleek de urine 5-HIAA concentratie een onafhankelijke (onafhankelijk van leeftijd en gamma GT) voorspeller voor overleving te zijn. Patiënten met een persisterende lage urine 5-HIAA concentratie hebben een betere overleving en deze marker kan dus in de praktijk gebruikt worden om patiënten met hoge excretie te identificeren die baat hebben bij een intensieve follow-up en mogelijk nieuwe middelen.

Deze nieuwe middelen zijn voornamelijk gericht op het remmen van vaatnieuwvorming (angiogenese remmers). De behandeling van de patiënten is vaak gebaseerd op ziekte

activiteit gevonden op CT of MRI. Echter de progressie/ernst van ziekte bij patiënten met carcinoïde tumoren van de middendarm is niet altijd met beeldvorming vast te leggen. De 5-HIAA concentratie in de urine zou eveneens als maat voor ziekteactiviteit gebruikt kunnen worden. Met deze marker zouden mogelijk meer patiënten kunnen deelnemen aan studies met nieuwe geneesmiddelen en daar de gunstige effecten van kunnen ondervinden.

Hoofdstuk 3.2 is een reactie op een artikel van Møller (New England Journal of Medicine, 2003). In dit artikel worden factoren beschreven die geassocieerd zijn met de progressie van carcinoïde hartziekte. Er zijn verschillende studies waaruit blijkt dat serotonine betrokken is bij de ontwikkeling van carcinoïde hartziekte. Somatostatine analogen verminderen de afgifte van serotonine door de tumor en kunnen theoretisch dus ook effectief zijn in de preventie van carcinoïde hartziekte. Echter een van de conclusies van Møller was dat somatostatine analogen geen invloed hadden op het voorkomen de progressie van carcinoïde hartziekte.

In 73 patiënten met het carcinoïdsyndroom behandeld in het UMCG tussen 1985 en 2002 toonden we aan dat de gemiddelde 5-HIAA concentratie in de urine steeg gedurende follow-up, maar dat in onze populatie deze 5-HIAA concentraties in de urine lager was vergeleken met de populatie van Møller. Dit betekent dat de conclusie van Møller niet te extrapoleren is naar patiënten met minder vergevorderde ziekte zoals in het UMCG. In deze patiëntengroep zouden somatostatine analogen nog steeds effectief kunnen zijn ter preventie van carcinoïde hartziekte.

In **hoofdstuk 4** wordt het effect beschreven van 2 functionele polymorfismen (een natuurlijk voorkomende variatie van een gen) in het serotonine transporter (SERT) gen en het Monoamine Oxidase-A (MAO-A) gen bij patiënten met een gemetastaseerde carcinoïde tumor van de middendarm. Zowel de SERT als het enzym MAO-A is betrokken bij de afbraak van serotonine tot 5-HIAA. Gezien de belangrijke rol van serotonine bij patiënten met een carcinoïde tumor van de middendarm, zou een functioneel polymorfisme in de genen betrokken bij de afbraak van serotonine, zowel het optreden van symptomen als ook de overleving kunnen beïnvloeden. Het polymorfisme in het SERT gen bestaat uit een korte variant (S: short) en een lange variant (L: long). Het L allel is functioneel actiever en leidt tot meer SERT transcriptie vergeleken met de S variant. Het MAO-A gen is gelegen op het X-chromosoom en heeft vergelijkbaar met het SERT

polymorfisme een actievere (L) en een minder actievere (S) variant. In DNA van 107 patiënten werden gekeken naar het polymorfisme van het SERT en het MAO-A gen.

Patiënten met het SS genotype van het SERT gen (n=29) hadden een kortere mediane overleving, 69,6 maanden vergeleken met patiënten met het LL (*n*=26) en het LS genotype (n=49). Patiënten met het LL en LS genotype hadden een mediane overleving van respectievelijk 91,2 en 114,6 maanden (P=0,001). Patiënten met een SS genotype bleken een verhoogd risico op sterfte te hebben vanaf 7 jaar na verwijzing en gecorrigeerd voor de leeftijd en de 5-HIAA concentratie in de urine. Het risico op overlijden aan de ziekte binnen 10 jaar na verwijzing was 4,36 (odds ratio (OR): 4,36) maal zo groot vergeleken met patiënten met het LS genotype. Deze slechtere overleving bij patiënten met het SS genotype valt te verklaren doordat vrij serotonine langzamer wordt opgenomen en patiënten met het SS genotype daardoor langer bloot staan aan dit schadelijke vrije serotonine. Vrij serotonine is op korte en lange termijn schadelijk vooral voor hart en vaten. Het belangrijkste voorbeeld hiervan is het ontstaan van carcinoïde hartziekte. Er was geen associatie tussen deze verschillende genotypen en klachten van diarree en/of flushes. Er waren tevens geen verschillen in overleving tussen de verschillende genotypen van het MAO-A gen.

Naast de prognostische factoren zoals beschreven in hoofdstuk 3a kan genotypering van patiënten helpen bij het identificeren van de groep patiënten die baat zouden kunnen hebben bij een intensievere follow-up en het eerder voorschrijven van nieuwe middelen. Bovendien ligt het combineren van de verschillende prognostische factoren (bijvoorbeeld, patiënten met het S'S' genotype en een urine 5-HIAA excretie > 20 mmol/mol kreatinine) voor de hand. Gezien de zeldzaamheid van deze tumor leidt het vormen van combinaties natuurlijk tot kleine aantallen. Een (inter)nationale databank, gecombineerd met een serum en weefselbank is noodzakelijk om deze vraagstukken op te lossen.

Hoofdstuk 5 beschrijft de resultaten van een studie naar de haalbaarheid van een behandeling met de combinatie IFN-alfa (2,5 miljoen E/dag subcutaan), gevolgd na 2 weken met intraveneus 5-fluorouracil (5-FU) 750 mg/m^2 (dag 2) en oraal leucovorin 180 mg/dag (dag 1 en 2) in een 2 wekelijkse cyclus. Negen patiënten met gemetastaseerde neuro-endocriene tumoren werden hiermee behandeld. Deze combinatie ging gepaard met veel bijwerkingen en slechts 3 van de 9 patiënten voltooiden meer dan 4 cycli. Echter de serum concentratie van de vasculair endotheliale groei factor (VEGF) daalde

met ongeveer 40% tijdens de behandeling. Uit recente studies blijkt dat carcinoïde tumoren sterk gevasculariseerd zijn en dat VEGF en VEGF receptoren aanwezig zijn in deze tumoren. Momenteel wordt veel onderzoek verricht naar nieuwe angiogenese remmers, zoals bevacizumab. De onderzochte dosering van de combinatie IFN-alfa en 5-FU gaat gepaard met te veel bijwerkingen echter gezien het anti-angiogenetische effect is het de moeite waard om minder toxische doseringen en combinaties te onderzoeken, eventueel in combinatie met de nieuwe angiogenese remmers.

In de behandeling van patiënten met carcinoïde tumoren van de middendarm is kwaliteit van leven een belangrijk aspect, mede gezien de relatief lange overleving. In **hoofdstuk 6** wordt één aspect van deze kwaliteit belicht: het seksueel functioneren. Patiënten met een carcinoïde tumor van de middendarm zouden een vergrote kans kunnen hebben op het ontwikkelen van een seksuele disfunctie. De enige studie waarin dit onderzocht werd, dateert uit de jaren 70. Serotonine in de hersenen is betrokken bij het seksuele gedrag van mensen. Serotonine tekort in de hersenen zou invloed kunnen hebben op het seksuele gedrag van patiënten. Bovendien heeft serotonine vasoactieve eigenschappen die tot erectiele disfunctie bij mannen zouden kunnen leiden. Ook kan de fysieke conditie (pijn, moeheid) en het gebruik van geneesmiddelen het normaal seksueel functioneren beïnvloeden. In deze studie werden 43 patiënten, 27 mannen en 16 vrouwen, met een gemetastaseerde carcinoïde tumor gevraagd om de verkorte vragenlijst voor seksueel disfunctioneren (VSD) in te vullen. De uitkomst hiervan werd vergeleken met de uitkomst van een referentie populatie die diezelfde vragenlijst ingevulde. De referentie populatie bestond uit een steekproef uit de Nederlandse bevolking en werd uitgevoerd door de Rutger Nisso Stichting in verband met het beschrijven van seksueel gedrag bij de Nederlandse bevolking. Seksueel disfunctioneren werd door ons gedefinieerd als een seksueel probleem op de door ons geselecteerde subschalen (opwinding, erectie, orgasme, dyspareunie, ejaculatie en pijn), regelmatig voorkomend en stressvol. Van de 27 mannen hadden er 6 (22,2%), 4 (14,8%), en 7 (25,9%) een seksuele disfunctie betreffende de opwinding, het orgasme en de erectie. Slechts één van de 16 vrouwen had een seksuele disfunctie betreffende het orgasme. Vergeleken met de referentie populatie werden geen verschillen aangetoond.

Mannen met een seksuele disfunctie hadden vergeleken met mannen zonder een seksuele disfunctie een langere ziekteduur (95,3 maanden versus 18,6 maanden; P=0,024). Zij hadden een lager plasma tryptofaan concentratie respectievelijk 31,5

µmol/L en 48,9 µmol/L (P=0,031) en zij gebruikten vaker IFN-alfa, te weten 4 van de 8 patiënten (50%) en 2 van de 19 patiënten (10,5%) (P=0,004). Het plasma testosteron, LH en FSH verschilden niet tussen beide groepen. Wel bleken 7 (25,9%) van de 27 mannen een verlaagde testosteronconcentratie te hebben.

Concluderend komt seksueel disfunctioneren niet vaker voor bij patiënten met een carcinoïde tumor van de middendarm vergeleken met de Nederlandse bevolking. Echter artsen moeten zich wel bewust zijn dat seksueel disfunctioneren, kan optreden bij patiënten vooral bij mannen met al langer bestaande ziekte, met lage tryptofaan concentratie en bij gebruik van IFN-alfa.

Testosteron suppletie zou kunnen helpen bij mannen met een verminderde libido of erectiele disfunctie en misschien ook wel bijdragen aan een verbetering van het welbevinden, hoewel nog niet duidelijk is wat het onderliggende mechanisme is van het verlaagde testosteron gehalte.

In **hoofdstuk 7** wordt de waarde van de ^{123}I-meta-iodobenzylguanidine (MIBG) scintigrafie beschreven bij patiënten met een feochromocytoom. Een feochromocytoom ontstaat uit chromaffiene cellen en is bij de meeste patiënten gelegen in de bijnier. Bij ongeveer 10% van de patiënten is het feochromocytoom buiten de bijnier in een paraganglion gelegen, er wordt dan gesproken van een paraganglioom. Feochromocytomen zijn meestal goedaardig, bij ongeveer 10% van de patiënten is er echter sprake van een maligne feochromocytoom. Voor de diagnose feochromocytoom is het van belang om eerst biochemisch de afwijking vast te stellen. Dit gebeurt door het aantonen van een overmaat aan adrenaline en noradrenaline en de metabolieten hiervan in urine en tegenwoordig ook in plasma. Vervolgens dient beeldvormend onderzoek plaats te vinden. Voor een anatomische lokalisatie wordt een CT of MRI verricht. Ook nucleaire imaging is essentieel voor de lokalisatie van de tumor. MIBG is een analoog van noradrenaline en wordt opgenomen in de neuro-endocriene cellen van het feochromocytoom. Door MIBG te labelen met jodium kan het afgebeeld worden. Deze ^{123}I-MIBG scan heeft als voordeel dat het gehele lichaam kan worden afgebeeld. De sensitiviteit voor het opsporen van feochromocytoom zou mogelijk beter zijn vergeleken met een CT of MRI. Door de zeldzaamheid van patiënten met een feochromocytoom zijn studies die de sensitiviteit en specificiteit onderzoeken vaak klein.

Wij evalueerden de waarde van ^{123}I-MIBG scintigrafie bij 30 patiënten en verrichtten een gestructureerd overzicht (meta-analyse). De sensitiviteit in onze eigen populatie was 92%,

de specificiteit 100%. In de meta-analyse was de sensitiviteit en specificiteit respectievelijk 96% en 100%. De sensitiviteit en specificiteit voor feochromocytomen in de bijnier was 98%. Voor feochromocytomen buiten de bijnier gelegen (paragangliomen) was de sensitiviteit 98%. De sensitiviteit voor maligne feochromocytomen was slechts 79%. Concluderend is de sensitiviteit en specificiteit van de ^{123}I-MIBG scintigrafie beter dan de gerapporteerde sensitiviteit en specificiteit van CT en MRI. Voor patiënten met maligne feochromocytomen is de ^{123}I-MIBG scintigrafie weinig sensitief. Positron emission tomography (PET) met verschillende tracers (zoals 6-[^{18}F]-fluoro-L-dihydroxyphenylalanine (^{18}F-DOPA), wordt op dit moment nog nader onderzocht bij zowel goed- als kwaadaardige feochromocytomen. Dit geldt tevens voor andere neuro-endocriene tumoren. ^{18}F-DOPA-PET is veel belovend en in een eerdere studie bij carcinoïde tumoren van de middendarm zeer sensitief.

Tenslotte zullen, door de nieuwe diagnostische en behandelingsmogelijkheden, steeds meer disciplines betrokken zijn bij de zorg van patiënten met neuro-endocriene tumoren. Een multidisciplinair team van ervaren specialisten, te weten oncologen, endocrinologen, nucleair geneeskundigen, chirurgen, pathologen, gastro-enterologen, cardiologen, klinische chemici en psychologen, is nodig om goede zorg aan deze patiënten te bieden.

List of abbreviations

NET	neuroendocrine tumour
WHO	World Health Organisation
5-HTP	5-hydroxytryptophan
TPH	tryptophan hydroxylase
AADC	aromatic-L-amino acid decarboxylase
IDO	indolamine dioxygenase
TDO	tryptophan dioxygenase
CgA	chromogranin A
5-HIAA	5-hydroxyindolacetic acid
CT	computed tomography
MRI	magnetic resonance imaging
SRS	somatostatin receptor scintigraphy
MIBG	metaiodobenzylguanidine
^{18}F-DOPA	6-[^{18}F]-fluoro-L-dihydroxyphenylalanine
PET	positron emission tomography
IFN	interferon
MEN	multiple endocrine neoplasia
SDH	succinate dehydrogenase
NKA	neurokinin A
SERT	serotonin transporter
5-HTT	serotonin transporter
MAO-A	monoamine oxidase-A
5-HTTPLR	serotonin transporter gene–linked polymorphic region
S	short
L	long
uVNTR	upstream variable number of tandem repeats
VEGF	vascular endothelial growth factor
5-FU	5-fluorouracil
QoL	quality of life
CHD	carcinoid heart disease
TGF	transforming growth factor
HR	hazard ratio

CI	confidence interval
mTOR	mammalian target of rapamycin
GEP-NET	gastroenteropancreatic neuroendocrine tumour,
NPK	neuropeptide K
GI	gastrointestinal
HPLC	high performance liquid chromatography
ALP	alkaline phosphatase
GT	gamma-glutamyltransferase
PCR	polymerase chain reaction
OR	odd ratio
QSD	questionnaire for screening of sexual dysfunction
EORTC	European Organization of Research and Treatment of Cancer
QLQ-C30	quality of life-Core 30 Questionnaire
LH	luteinizing hormone
FSH	follicle stimulating hormone
SHBG	sex hormone-binding globulin

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